Diving Deeper into Cancer Research

The 77th Annual Meeting of the Japanese Cancer Association

September 27th (Thu.) - 29th (Sat.), 2018

Venue

Osaka International Convention Center
RIHGA Royal Hotel Osaka

President

Masaki Mori
Department of Gastroenterological Surgery,
Graduate School of Medicine, Osaka University

(c/o Congress Corporation)
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Program

The 77th Annual Meeting of the Japanese Cancer Association

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9 27(Thu)
Days 1

Room 1 | 5F Large Hall, Osaka International Convention Center

CS1 [English]
Application of Artificial Intelligence (AI) to cancer research and clinical practice 9:00-11:30

Hideo Baba （Dept. Gastroenterological Surg. Grad. Sch. of Life Sci. Kumamoto Univ.）
Jun Sese （AIRC, AIST）

CS2 [English]
Elucidation of tumor microenvironment for new cancer treatments 13:00-15:30

Kohei Miyazono （Dept. Mol. Pathol., Grad. Sch. Med., the Univ. of Tokyo）
Hideyuki Saya （Gene Regulation, IAMR, Keio Univ. Sch. Med.）

Room 2 | 5F Small Hall, Osaka International Convention Center

IS1 [English]
Single cell genomics of cancer 9:00-11:30

Tatsuhiro Shibata （Lab. of Mol. Med., Human Genome Ctr., the Inst. of Med. Sci., the Univ. of Tokyo）
Zemin Zhang （College of Life Sci., Peking Univ.）

IS3 [English]
Genetic and epigenetic aberrations in gastric cancer 13:00-15:30

Atsushi Kaneda （Dept. Mol. Oncology, Grad. Sch. of Med., Chiba Univ.）
Patrick Tan （Programme in Cancer & Stem Cell Biol., Duke-NUS Med. Sch.）

E6 [English]
DNA replication / cell cycle / genomic instability (1) 15:30-16:45

Natsuko Chiba （Can. Biol., IDAC, Tohoku Univ.）

Room 3 | 10F 1003, Osaka International Convention Center

S1 [English]
Metabolic mechanisms in cancer and normal cells 9:00-11:30

Hiroyasu Esumi （Res. Inst. Biomed. Sci, Tokyo Univ. Sci）
Hozumi Motohashi （IDAC, Tohoku Univ.）

LS1 [Japanese]
Innovative environmental infection control measures with low concentration chlorine dioxide gas 11:50-12:40

Morito Monden （Sakai City Hospital Organization （The Cancer Institute Hospital of Japanese Foundation For Cancer Research）

SS1 [Japanese]
Progress in basic research and clinical medicine over the last decade 13:00-15:30

Tadamitsu Kishimoto （Immunol. Frontier Res. Ctr., Osaka Univ.）
Morito Monden （The Japan Med. Sci. Federation）

SP1 [Japanese]
How to survive hard science society 15:30-17:00

Nobuyuki Onishi （Div. Gene Reg. IAMR, Keio Univ. Sch. of Med.）
Kentaro Kajiwara （Dept. Oncogene, RIMD, Osaka Univ.）

Room 4 | 10F 1001, Osaka International Convention Center

E9-1 [English]
DNA methylation / chromatin structure 9:00-10:15

Takehiko Kamijo （Res. Inst. Clin. Oncol. Saitama Cancer Ctr）

E9-2 [English]
Histone modification and epigenome 10:15-11:30

Satoshi Fujii （Path. of Div. EPOC, Natl. Cancer Ctr.）

LS2 [Japanese]
Genetic analysis and its clinical application in malignant lymphomas 11:50-12:40

Takahiro Maeda （Center for Cellular and Molecular Medicine, Kyushu University Hospital）

E17-1 [English]
Anticancer drugs and molecular mechanism 13:00-14:15

Masahiro Yasunaga （Developmental Therap., EPOC, Natl. Cancer Ctr.）

E14-3 [English]
Head and neck cancer 14:15-15:30

Hiroshi Miyata （Dept. Gastroenterological Surg., Osaka International Cancer Institute）

J14-1 [Japanese]
Esophageal cancer 15:30-16:45

Makoto Yamasaki （Dept. Gastroenterological Surg., Grad. Sch. Med., Osaka Univ.）

Room 5 | 10F 1002, Osaka International Convention Center

E12-1 [English]
Innate immunity (1) 9:00-10:15

Kazuhiro Kakimi （Dept. ImmunoTherap., Univ. Tokyo）

E12-2 [English]
Development of novel molecular targeted therapies 10:15-11:30

Shigehisa Kitano （Dept. Exp. Therap., Natl. Cancer Ctr. Hosp.）

LS3 [Japanese]
Cancer associated with inflammatory bowel disesases 11:50-12:40

Yanaga Katsuhiko （Jikei University School of Medicine Department of Surgery）

E16-1 [English]
Signal transduction inhibitor (1) 13:00-14:15

Kohji Noguchi （Div. Chemother., Facult. Pharm., Keio Univ.）

E16-2 [English]
Signal transduction inhibitor (2) 14:15-15:30

Akihiro Tomida （Div. Genome Res., Cancer Chemotherap. Ctr., JFCR）

E16-3 [English]
New molecular targeted agent 15:30-16:45

Kosei Maemura （Dept. Digestive Surg., Kagoshima Univ., Sch. Med. Dent. Sci.）

Room 6 | 10F 1004+1005, Osaka International Convention Center

E14-1 [English]
Hepatocellular carcinoma (1) 9:00-10:15

Akio Saiura （Hepato-Biliary-Pancreatic Surg., Cancer Inst. Hosp.）

E14-2 [English]
Pancreatic cancer (1) 10:15-11:30

Yutaka Takeda （Dept. of Surg., Kansai Rosai Hosp.）

LS4 [English]
Onco-Hu™ Mice for Evaluation of Immuno-oncology Therapeutics 11:50-12:40

Tadashi Kondo （Division of Rare Cancer Research, National Cancer Center Research Institute）

E10-1 [English]
Metastasis and invasion 13:00-14:15

Ryuichi Sakai （Dept. Biochem., Kitasato Univ. Sch. Med.,）

E10-2 [English]
Invasion and metastasis (1) 14:15-15:30

Mayumi Ono （Dept. Pharm. Oncology., Grad. Sch. Pharm. Sci., Kyushu Univ.）

E11-1 [English]
Metabolism / metabolome (1) 15:30-16:45

Koji Okamoto （Natl. Cancer Ctr. Res. Inst., Div. Cancer Differentiation）

Room 7 | 10F 1006+1007, Osaka International Convention Center

E21 [English]
Gene therapy and oncolytic virus therapy (1) 9:00-10:15

Masatoshi Tagawa （Div. Pathol & Cell Ther., Chiba Cancer Ctr. Res. Inst.）

E1-1 [English]
DNA damage and carcinogenic process 10:15-11:30

Dai Nakae （Dept. Nutr. Sci. Food Safety, Facul. Appl. Biosci., Tokyo Univ. Agricul.）

LS5 [Japanese]
Diagnostic approach to proliferation of mesothelial cells - Ancillary studies in limited specimens 11:50-12:40

Kenji Morinaga （Department of Asbestos-Related Health Damage Relief, Environmental Restoration and Conservation Agency）

E1-2 [English]
Process of carcinogenesis (1) 13:00-14:15

Noriko Hosoya （Lab. Mol. Radiol., CDBIM, Grad. Sch. Med., Univ. of Tokyo）

E11-2 [English]
Cell-to-cell interaction (1) 14:15-15:30

Osamu Nagano （Div. Gene Regulation, IAMR, Keio Univ. Sch. of Med.）

J11-1 [Japanese]
Cell-to-cell interaction (2) 15:30-16:45

Yoshiyuki Fujiwara （Dept. Surgery, Tottori University, Faculty of Medicine）

Room 8 | 10F 1008, Osaka International Convention Center

J12-1 [Japanese]
Anticancer agents and effects 9:00-10:15

Kenzaburo Tani （The Inst. of Med. Sci., The Univ. of Tokyo）

J6 [Japanese]
DNA replication / cell cycle / genomic instability (2) 10:15-11:30

Masatoshi Fujita （Dept. Cell. Biochem., Grad. Sch. Pharm. Sci., Kyushu Univ.）

LS6 [Japanese]
Advanced in vivo imaging technology revolutionizing cancer diagnosis and therapies in clinic 11:50-12:40

Koshi Mimori （Department of Surgery, Kyushu University Beppu Hospital）

J21 [Japanese]
Gene therapy and oncolytic virus therapy (2) 13:00-14:15

Hiroshi Fukuhara （Dept. Urol., Kyorin Univ. Fac. Med.）

J1 [Japanese]
Process of carcinogenesis (2) 14:15-15:30

Michihiro Mutoh （Ctr. for Public Health Sci., Natl. Cancer Ctr.）

J10-2 [Japanese]
Cell adhesion / invasion 15:30-16:45

Kaoru Miyazaki （Mol. Pathol. Genetics Div., Kanagawa Cancer Ctr. Res. Inst.）

Room 9 | 10F 1009, Osaka International Convention Center

IS2 [English]
New antibody therapeutics in oncology 9:00-11:30

Yuki Abe （Daiichi Sankyo Co., Ltd. Biologics & Immuno-Oncology Laboratorie）
Maggie Lu （Targeted Drug & Delivery Tech. Div., Biomed. Tech. & Device Res. Laboratories, Industrial Tech. Res. Inst.）

LS7 [Japanese]
Basic study of new molecular target therapy for ER-positive/HER2-negative advanced/metastatic breast cancer 11:50-12:40

Masahiko Watanabe （Kitasato University School of Medicine, Department of Surgery）

IS4 [English]
Application of artificial intelligence for cancer research; integrated analysis of cancer omics data using machine learning and deep learning 13:00-15:30

Ryuji Hamamoto （Natl. Cancer Ctr. Res. Inst.）
Jinhua Yu （Fudan Univ.）

E25 [English]
Data science / AI 15:30-16:45

Masaaki Matsuura （Teikyo Univ. Grad. Sch. of Public Health）

Room 10 | 11F 1101+1102, Osaka International Convention Center

J16-1 [Japanese]
New molecular target (1) 9:00-10:15

Kazuo Shin-ya （BRD, AIST）

J16-2 [Japanese]
New molecular target (2) 10:15-11:30

Mikihiko Naito （Div. Mol. Target & Gene Ther. Products, Natl. Inst. Health Sci.）

LS8 [Japanese]
What to read, How to read. 11:50-12:40

Takashi Joh （Gamagori City Hospital）

J14-2 [Japanese]
Colorectal cancer: prognostic factor 13:00-14:15

Takashi Yao （Dept. Human Path., Juntendo Univ., Grad. Sch. Med.）

J14-3 [Japanese]
Colorectal cancer 14:15-15:30

Ichiro Takemasa （Dept. Surg., Surg. Oncol. & Sci., Sapporo Med. Univ.）

J14-4 [Japanese]
Colorectal cancer: clinical 15:30-16:45

Masataka Ikeda （Div. Lower GI, Hyogo College of Med.）

Room 11 | 12F Conference Hall, Osaka International Convention Center

J8-1 [Japanese]
Cell death / immortalization / cell cycle 9:00-10:15

Hiroyuki Kugoh （Dept. Biomed. Sci., Ins. Regenerative Med., Tottori Univ.）

J10-1 [Japanese]
Angiogenesis 10:15-11:30

Hiroyuki Konno （Hamamatsu Univ. Sch. of Med.）

SP2 [Japanese]
What is the optimal social insurance system for sustainable best practice in cancer care? 15:00-18:00

Hitoshi Nakagama （Natl. Cancer Ctr.）
Masaki Mori （Dept. Gastroenterological Surg., Osaka Univ.）

Room 12 | 12F 1202, Osaka International Convention Center

J17-1 [Japanese]
Anticancer drug and cell death 9:00-10:15

Masaya Imoto （Fac. Sci. Tech. Keio Univ.）

J17-2 [Japanese]
Anticancer drug resistance 10:15-11:30

Toshiyuki Sakai （Dept. Mol. -Target. Cancer Prev., Kyoto Pref. Univ. Med.）

LS9 [Japanese]
Surgical nutrition invasion study aimed at improving prognosis 11:50-12:40

Eigo Otsuji （University Hospital Kyoto Prefectual University of Medicine , Department of Surgery , Division of Digestive Surgery）

J11-2 [Japanese]
Metabolism / metabolome (2) 13:00-14:15

Tetsuo Morita （Dept. Biochem. Grad. Sch., Fukuyama Univ.）

J18-1 [Japanese]
Molecular target therapy 14:15-15:30

Daisuke Okuzaki （Res. Inst. for Microbial Diseases, Osaka Univ.）

J12-2 [Japanese]
Innate immunity (2) 15:30-16:45

Tsukasa Seya （Dept. Pathol. Hokkaido Univ. Sch. Med.）

Room 13 | 3F Korin1, RIHGA Royal Hotel Osaka

S2 [English]
Characteristics of cancer revealed by bio-imaging technology 9:00-11:30

Masaru Ishii （Dept. Immunol. Cell Biol., Osaka Univ. Grad. Sch. Med.）
Etsuko Kiyokawa （Dept. Oncol. Pathol, Kanazawa Med. Univ.）

LS10 [Japanese]
Clinical impact of cancer stemness and immune microenvironment 11:50-12:40

Hideshi Ishii （Osaka University, Graduate School of Medicine, Department of Medical Data Science）

S5 [English]
New developments in cancer research revealed by genome instability 13:00-15:30

Kozo Tanaka （Dept. Mol. Oncol., Inst. Dev. Aging Cancer, Tohoku Univ.）
Hiroyuki Seimiya （Div. Mol. Biother., JFCR Cancer Chemother. Ctr.）

S6 [English]
Epigenome abnormalities and translational research 15:30-18:00

Toshikazu Ushijima （Div. Epigenomics, Natl. Cancer Ctr. Res. Inst.）
Yutaka Kondo （Div. Cancer Biol., Nagoya Univ., Grad. Sch. Med.）

Room 14 | 3F Korin2, RIHGA Royal Hotel Osaka

S3 [English]
The role of intestinal microbiota in cancer progression 9:00-11:30

Hiroshi Kiyono （Int'l Res. & Development Ctr. for Mucosal Vaccines, The Inst. of Med. Sci., The Univ. of Tokyo）
Naoko Ohtani （Grad. Sch. of Med., Osaka City Univ.）

LS11 [English]
Immunomodulatory drugs for multiple myeloma: from bench to bedside 11:50-12:40

Junya Kuroda （Division of Hematology and Oncology, Department of Medicine, Kyoto Prefectural University of Medicine）

S7 [English]
Progress in cancer research through RNA biology 13:00-15:30

Fuyuki Ishikawa （Kyoto Univ., Grad. Sch. of Biostudies）
Hideshi Ishii （Med. Data Sci., Osaka Univ. Grad. Sch. Med.）

S8 [English]
Medical achievements of nucleotide analogs in cancer treatment 15:30-18:00

Satoshi Obika （Grd. Sch. Pharm. Sci., Osaka Univ.）
Nobuhiro Nishiyama （Lab. Chem. Life Sci., Tokyo. Tech.）

Room 15 | 3F Korin3, RIHGA Royal Hotel Osaka

S4 [English]
Cancer invasion and metastasis - A summary and the future 9:00-11:30

Nariaki Matsuura （Osaka InterNatl. Cancer Inst.）
Yoshinori Murakami （Div. Mol. Pathol., Inst. Med. Sci., the Univ. of Tokyo）

LS12 [Japanese]
Toward development of combination cancer immunotherapy 11:50-12:40

Eishi Baba （Department of Comprehensive Clinical Oncology Faculty of Medical Sciences,Kyushu University）

YIA [Japanese]
The Young Investigator Awards Lecture 13:00-15:30

Wataru Yasui （Dept. Mol. Pathol., Hiroshima Univ. Gradatesch. Biomed. Sci.）

J10-3 [Japanese]
Invasion and metastasis (2) 15:30-16:45

Kazuki Nabeshima （Dept. Pathol., Fukuoka Univ. Sch. Med. & Hosp.）

Room 16 | 2F Katsura, RIHGA Royal Hotel Osaka

SST1 [Japanese]
Developments in gastrointestinal cancer treatments 9:00-11:30

Atsushi Ochiai （Exp. Oncol. Res. Clin. Trial Ctr.）
Yoshihiro Kakeji （Div. of Gastrointest Surg, Dept. of Surg, Grad Sch Med, Kobe Univ）

LS13 [Japanese]
Single cell multi-parameter analysis of tumor infiltrating lymphocyte (TIL) 11:50-12:40

Hitoshi Kiyoi （Department of Hematology and Oncology, Nagoya University Graduate school of Medicine）

SST2 [Japanese]
Current status and the future of hepatobiliary and pancreatic cancer research and treatment 13:00-15:30

Masatoshi Kudo （Dept. Gastroenterol & Hepatol, Kindai Univ.）
Mitsuo Shimada （Dept. Surg., Tokushima Univ.）

E14-4 [English]
Hepatocellular carcinoma (2) 15:30-16:45

Michiie Sakamoto （Dept. Pathol., Keio Univ. Sch Med）

Room P(A) | 3F Event Hall, Osaka International Convention Center

P2-1 [English/Japanese]
Animal models for cancer (1) 16:30-17:15

Kazumi Nakano （Grad.Sch. Frontier Sci, The Univ. Tokyo）

P2-3 [English/Japanese]
Animal models for cancer (3) 16:30-17:15

Shunsuke Noguchi （Vet. Radiol. Osaka Pref. Univ.）

P2-5 [English/Japanese]
Animal models for cancer (5) 16:30-17:15

Rieko Ohki （Natl. Cancer Ctr. Res. Inst.）

P4-2 [English/Japanese]
Expression / functional analysis of novel oncogenes / tumor-suppressor genes 16:30-17:15

Tohru Ishitani （IMCR, Gunma Univ）

P5-1 [English]
MicroRNAs (1) [English] 16:30-17:15

Yamin Qian （Dept. Mol. Pathol., Health&Sci., Grad. Sch. Med., Osaka Univ.）

P5-2 [Japanese]
MicroRNAs (2) 16:30-17:15

Chitose Oneyama （Div. Cancer Cell Regulation, Aichi Cancer Ctr. Res. Inst.）

P5-4 [Japanese]
Signal transduction (1) 16:30-17:15

Masayuki Hiraki （Dept. Surg. Itami City Hosp.）

P5-6 [Japanese]
Signal transduction (3) 16:30-17:15

Yosuke Hirotsu （Genome Analysis Center, Yamanashi Central Hospital）

P5-8 [English]
Proliferation (1) [English] 16:30-17:15

Koji Tanaka （Dept. Gastroenterological Surgery, Osaka Univ）

P5-10 [Japanese]
Translation / non-coding RNAs 16:30-17:15

Masahisa Ohtsuka （Osaka Police Hosp., Dept. Surg.）

P8-2 [English/Japanese]
Cell death 16:30-17:15

Yuichi Takiguchi （Dept. Med. Oncol., Grad. Sch. Med., Chiba Univ.）

P12-1 [English/Japanese]
Molecular target therapy (1) 16:30-17:15

Takashi Ishida （Div. Hematology & Oncology, Iwate Med. Univ.）

P14-1 [English/Japanese]
Esophageal cancer (1) 16:30-17:15

Hiroki Sasaki （Dept. Translational Oncol, Natl. Cancer Ctr. Res. Inst.）

P14-3 [English/Japanese]
Esophageal cancer and GIST 16:30-17:15

Masahiko Yano （Dept. Gastroenterol. Surg., Osaka International Cancer Institute）

P14-5 [English/Japanese]
Novel biotherapy and molecular analysis of gastric cancer 16:30-17:15

Daisuke Ichikawa （1st Dept. Surg., Med., Univ. of Yamanashi）

P14-7 [English/Japanese]
Novel biotherapy for gastric cancer: new antibody therapeutics and oncolytic virus therapy 16:30-17:15

Tetsuo Ushiku （Dept. Pathology, Tokyo Univ.）

P14-9 [English/Japanese]
Prognostic biomarkers in gastric cancer 16:30-17:15

Michitaka Fujiwara （Clin. Simulation Ctr., Nagoya Univ. Grad. Sch. of Med.）

P14-11 [English/Japanese]
Epigenetics in gastric cancer 16:30-17:15

Motohiro Hirao （Dept. of Surg. Natl. Hosp. Organization, Osaka Natl. Hosp.）

P14-13 [English/Japanese]
GIST 16:30-17:15

Masaaki Motoori （Dept. Surg., Osaka Ganeral Med. Ctr.）

P2-2 [English/Japanese]
Animal models for cancer (2) 17:15-18:00

Daisaku Yamada （Dept. Gastroenterological Surg., Osaka Internatinal Cancer Inst.）

P2-4 [English/Japanese]
Animal models for cancer (4) 17:15-18:00

Hiroshi Suemizu （Lab. Anim. Res. Dept., CIEA）

P4-1 [English/Japanese]
Novel oncogenes in solid tumor 17:15-18:00

Tatsuo Hata （Dept. Surg., Tohoku Univ., Sch. Med.）

P4-3 [English]
Novel oncogenes / tumor suppressor genes [English] 17:15-18:00

Jun-Ya Kato （Biosci. NAIST）

P5-3 [Japanese]
MicroRNAs (3) 17:15-18:00

Takaaki Masuda （Dept. Surg., Kyushu Univ., Beppu hosp）

P5-5 [Japanese]
Signal transduction (2) 17:15-18:00

Noriaki Kitamura （Bristol-Myers Squibb）

P5-7 [Japanese]
Transcriptional regulation 17:15-18:00

Yoji Andrew Minamishima （Dept. Mol. Cell. Boil., Med. Inst. Bioreg., Kyushu Univ.）

P5-9 [Japanese]
Proliferation (2) 17:15-18:00

Keiji Miyazawa （Dept. Biochem., Univ. Yamanashi）

P8-1 [English/Japanese]
Cell death / DNA replication 17:15-18:00

Takashi Suda （Div. Immnol. & Mol. Biol., Cancer Res. Inst., Kanazawa Univ.）

P8-3 [English/Japanese]
Cell death / cellular senescence 17:15-18:00

Katsuya Ohta （Dept. Gastroenterological Surg., Higashiosaka City Med. Ctr.）

P12-2 [English/Japanese]
Molecular target therapy (2) 17:15-18:00

Kazunori Kato （Dept. Biomed. Eng., Toyo Univ.）

P14-2 [English/Japanese]
Esophageal cancer (2) 17:15-18:00

Yoshifumi Baba （Dept. of gastroenterological Surg., Kumamoto Univ.）

P14-4 [English/Japanese]
Esophageal cancer (3) 17:15-18:00

Hiromi Kataoka （Dept. Gastroenterology & Metabolism, Nagoya City Univ., Grad. Sch. Med.）

P14-6 [English/Japanese]
Clinical practice in gastric cancer 17:15-18:00

Tsuyoshi Etoh （Dept. Gastroenterological & Pediatric Surg., Oita Univ. Faculty of Med.）

P14-8 [English/Japanese]
Cell-to-cell interaction in gastric cancer 17:15-18:00

Yoshinori Fujiwara （Kawasaki Med. Sch., Dept. Digestive Surg.）

P14-10 [English/Japanese]
Pathological features in gastric cancer 17:15-18:00

Shunsuke Kagawa （Minimally Invasive Therapy Ctr, Okayama Univ. Hosp.）

P14-12 [English/Japanese]
Translational research in gastric cancer and GIST 17:15-18:00

Tsuyoshi Takahashi （Dept. Surg, Osaka Univ. Sch. Med）

P14-14 [English/Japanese]
Molecular analysis of cervical cancer and uterine sarcomal in clinical senario 17:15-18:00

Takuma Fujii （Dept. OB（GYN Fujita Health Univ., Sch. Med.）

Room P(B) | 7F Lobby/701+702, Osaka International Convention Center

P14-15 [English/Japanese]
Molecular analysis of uterine cancer 16:30-17:15

Yutaka Ueda （Dept. Obstet. Gynecol., Osaka Univ., Grad. Sch. Med.）

P14-17 [English/Japanese]
Acute myelocytic leukemia 16:30-17:15

Momoko Nishikori （Dept. Hematol（Oncol, Kyoto Univ.）

P14-19 [English/Japanese]
Malignant lymphoma 16:30-17:15

Kohei Yamamoto （Dept. Comprehensive. Pathol., Tokyo Med. & Dent. Univ.）

P14-21 [English/Japanese]
Head and neck cancer (1) 16:30-17:15

Yohei Miyagi （Kanagawa Ca Ctr Res Inst）

P14-23 [English/Japanese]
Head and neck cancer (3) 16:30-17:15

Nobuhiko Oridate （Dept. Otolaryngology-Head & Neck Surg., Yokohama City Univ, SchMed）

P14-16 [English/Japanese]
Molecular and cellular characteristics of ovarian cancer 17:15-18:00

Shingo Miyamoto （Dept. Obstet. Gynecol. Fukuoka. Univ. Faculty of Med.）

P14-18 [English/Japanese]
CML and post-transplant leukemia / lymphoma 17:15-18:00

Yoshikane Kikushige （1st Dept. Int. Med. Kyushu Univ.）

P14-20 [English/Japanese]
Adult T-cell leukemia / lymphoma and multiple myeloma 17:15-18:00

Yasuhide Hayashi （Inst. Physiol. Med., Jobu Univ.）

P14-22 [English/Japanese]
Head and neck cancer (2) 17:15-18:00

Takashi Saku （Fukuoka Dent. College）

Room P(C) | 8F Lobby/801+802, Osaka International Convention Center

P14-26 [English/Japanese]
Head and neck cancer (5) 16:30-17:15

Takahito Fukusumi （ORL-HNS. Med. Osaka Univ.）

P15-1 [English/Japanese]
Diagnostic biomarker and prognostic factors 16:30-17:15

Kazufumi Honda （Dept. Cancer Early Detection, Natl. Cancer Ctr.）

P15-3 [English/Japanese]
Cancer screening 16:30-17:15

Hiroshi Fujiwara （Dept. Personalized Cancer Immunother., Mie Univ.Grad. Sch.Med.）

P15-5 [English/Japanese]
Genetic diagnosis (1) 16:30-17:15

Shugo Suzuki （Dept.Exp.Path.Tumor.Biol., Nagoya City Univ.）

P14-24 [English/Japanese]
Head and neck cancer (4) 17:15-18:00

Masashi Shiiba （Dept. Med. Oncology, Grad. Sch. Med., Chiba Univ.）

P14-25 [English/Japanese]
Head and neck cancer (6) 17:15-18:00

Yuichiro Koma （Div. Pathol., Kobe Univ., Grad. Sch. Med.）

P15-2 [English/Japanese]
Novel cancer diagnostic tools and treatments (1) 17:15-18:00

Kiyotaka Shiba （Div. Prot. Engin., Cancer Inst, , JFCR）

P15-4 [English/Japanese]
Screening assay 17:15-18:00

Fumito Yamazaki （Dept. of Clin. Genomics, Natl. Cancer Ctr. Res. Inst.）

P15-6 [English/Japanese]
Genetic diagnosis (2) 17:15-18:00

Toyomasa Katagiri （Div. Genome Med., Inst. Genome Res., Tokushima Univ.）

Room P(D) | 10F 1010+10-1&2, Osaka International Convention Center

P15-7 [English/Japanese]
Diagnostic imaging (1) 16:30-17:15

Nakanishi Hayao （Lab. Path. & Clin. Res. Aichi Cancer Ct.）

P15-9 [English/Japanese]
Diagnostic imaging (3) 16:30-17:15

Fumiaki Isohashi （Dept. Radiation Oncology, Osaka Univ., Sch. Med.）

P15-11 [English/Japanese]
New biomarker (1) 16:30-17:15

Masatoshi Inoue （Dept. Surg., Kindai Univ. Nara Hosp.）

P15-8 [English/Japanese]
Diagnostic imaging (2) 17:15-18:00

Hirofumi Hanaoka （Gunma Univ. Grad. Sch. Med.）

P15-10 [English/Japanese]
Pathological diagnosis 17:15-18:00

Daisuke Matsubara （Dept. Path., Jichi Med. Univ.）

Room P(E) | 12F Lobby, Osaka International Convention Center

P15-13 [English/Japanese]
New biomarker (3) 16:30-17:15

Takeshi Tomonaga （Lab. Proteome Res., Natl. Inst. Biomed. Innov. Health Nutl.）

P17-2 [English]
Natural anticancer compounds (2) [English] 16:30-17:15

Naohiro Nishida （Dept. Frontier Sci. for Cancer & Chemother., Osaka Univ.）

P17-4 [English]
Anticancer drug resistance (1) [English] 16:30-17:15

Yukinori Kurokawa （Dept. Gastroenterological Surg., Osaka Univ.）

P17-6 [English/Japanese]
Anticancer drug resistance (3) 16:30-17:15

Yoshihiro Torimoto （Oncology Ctr. Asahikawa Med. Univ. Hosp.）

P17-8 [English]
Mechanism of action and resistance of anticancer drugs (1) [English] 16:30-17:15

Ken Kato （Natl. Cancer Ctr. Hosp.）

P17-10 [English/Japanese]
Drug delivery system (1) 16:30-17:15

Shigeru Marubashi （Dept. Hepato-Biliary-Pancreatic & Transplant Surg., Fukushima Med. Univ.）

P15-12 [English/Japanese]
New biomarker (2) 17:15-18:00

Ryohei Kawabata （Dept. Surg., Osaka Rosai Hosp.）

P17-1 [English]
Natural anticancer compounds (1) [English] 17:15-18:00

Satoshi Shibata （Dept. Mol. Pathol., Osaka Univ. Grad. Sch. Med., Div. Health. Sci.）

P17-3 [Japanese]
Natural anticancer compounds (3) 17:15-18:00

Takehiro Noda （Dept. Gastroenterol. Surg., Osaka Univ.）

P17-5 [English/Japanese]
Anticancer drug resistance (2) 17:15-18:00

Yasuo Saijo （Dept. Med. Oncol., Niigata Univ., Sch. Med.,）

P17-7 [English/Japanese]
Anticancer drug resistance (4) 17:15-18:00

Jun Inoue （Dept. Mol. Cytogent., Med. Res. Inst., Tokyo Med. & Dent. Univ.）

P17-9 [English/Japanese]
Mechanism of action and resistance of anticancer drugs (2) 17:15-18:00

Daisuke Sakai （Dept Frontier Science for Cancer and Chemotherapy, Osaka Univ.）

P17-11 [English/Japanese]
Drug delivery system (2) 17:15-18:00

Jun Fang （Dept. Microbiol. & Oncol., Faculty Pharm. Sci., Sojo Univ.）

Room P(F) | 3F Lobby, RIHGA Royal Hotel Osaka

P17-12 [English/Japanese]
Synthetic anticancer compounds 16:30-17:15

Tomoya Kishimoto （Dept. Surg., Yao Municipal Hosp.）

P18-1 [English/Japanese]
Chemosensitivity (1) 16:30-17:15

Naoyuki Nishiya （Div. Integ. Info., Dept. Clin. Pharm., Iwate Med. Univ. Sch. Pharm.）

P18-3 [English/Japanese]
Anticancer drug and side efficacy and toxicity 16:30-17:15

Masayuki Shiota （Res. Sprt. Platf., Osaka City Univ., Grad. Sch. Med.）

P17-13 [English/Japanese]
Clinical experience and supportive care 17:15-18:00

Kazuhiro Noma （Dept. Gastroenterological Surg., Okayama Univ. Med. Sch.）

P18-2 [English/Japanese]
Chemosensitivity (2) 17:15-18:00

Yoshifumi Iwagami （Dept. Gastroenterological Surg., Grad. Sch. Med., Osaka Univ.）

P19 [English/Japanese]
Novel cancer diagnostic tools and treatments (2) 17:15-18:00

Takafumi Nakamura （Grad. Sch. of Med. Sci., Tottori Univ.）

9 28(Fri)
Days 2

Room 1 | 5F Large Hall, Osaka International Convention Center

CS3 [English]
Regulation of tumor immunity and evolution of cancer treatments 9:00-11:30

Yusuke Nakamura （Dept. Clin. Oncol, Univ. of Chicago）
Yutaka Kawakami （Div. Cell. Signal., Inst. Adv. Med. Res., Keio Univ. Sch. Med.）

SP3 [Japanese]
Development of Cancer Genomic Medicine Platform in Japan 13:00-15:30

Hiroyuki Mano （Natl. Cancer Ctr. Res. Inst.）
Yuichiro Doki （Dept. Gastroenterological Surg. Osaka Univ.）
Koichi Goto （Natl. Cancer Ctr. Hosp. East, Dept. Thracic Oncol.）

SP4 [Japanese]
Joint symposium of the Japan Society of Human Genetics (JSHG), Japanese Society for Genetic Counseling (JSGC), and Japanese Society for Familial Tumors (JSFT): Genetic counseling 15:30-18:00

Kaori Muto （IMS, the Univ. of Tokyo）
Teruhiko Yoshida （Dept. Gen. Med. Serv., Natl. Cancer Ctr. Hosp.）

Room 2 | 5F Small Hall, Osaka International Convention Center

ML1 [Japanese]
Morning Lectures 1 8:00-8:50

Yusaku Nakabeppu （Div. Neurofunc. Genomics, Med. Inst. Bioreg. Kyushu Univ.）

IS5 [English]
The emerging role of exosome in carcinogenesis 9:00-11:30

Takahiro Ochiya （Natl. Cancer Ctr. Res. Inst.）
Tang-Long Shen （Natl. Taiwan Univ.）

IS7 [English]
Beyond current immune-checkpoint inhibitors 13:00-15:30

Toshihiko Torigoe （Dept. Path., Sapporo Med. Univ.）
Junho Chung （Cancer Res. Inst., Seoul Natl. Univ.）

SS2 [Japanese]
Women scientists in cancer research (WSCR symposia) 15:30-18:00

Ai Kotani （Tokai Univ. Inst. Med. Sci. Dept. Hematol Malignancy（Dept. Hematol & Oncol., Tokai Univ. Sch. Med.）
Sachiko Tsukita （Grad. Sch. of Front. Biosci., Grad. Sch. of Med., Univ. of Osaka）

Room 3 | 10F 1003, Osaka International Convention Center

ML2 [Japanese]
Morning Lectures 2 8:00-8:50

Chikashi Ishioka （Dept. Clin. Oncol., IDAC, Tohoku Univ.）

AACR1 [English]
Cancer Microbiome: exploring the roles and mechanisms of microbial communities in tumorigenesis 9:00-11:30

Eiji Hara （Dept. Mol. Microbiol., Res. Inst. for Microbial Diseases, Osaka Univ.）
Andrew T. Chan （Clin. & Translational Epidemiology Unit, Massachusetts General Hosp.）

LS14 [English]
Digital Genomics applied to Liquid Biopsies 11:50-12:40

Shinzaburou Noguchi （Department of Breast and Endocrine Surgery, Osaka University Graduate School of Medicine））

S11 [English]
Molecular target therapy under precision medicine 13:00-15:30

Yoshihiko Maehara （Kyushu Central Hosp. of the Mutual Aid Association of Public Sch. Teachers）
Takashi Kohno （Div. Genome Biol, Natl Cancer Ctr Res Inst.）

SP5 [Japanese]
Meet your good collaborators 15:30-17:00

Masamitsu Konno （Dept. CFS Grad. Sch. Med. Osaka Univ.）
Hiroyasu Kidoya （Dept. Signal Transduction, RIMD, Osaka Univ.）

Room 4 | 10F 1001, Osaka International Convention Center

ML3 [Japanese]
Morning Lectures 3 8:00-8:50

Takashi Kanematsu （Nagasaki City Hosp. Organization）

E7 [English]
Genomic analysis 9:00-10:15

Masahito Kawazu （Div. Cell. Sig., Natl. Cancer Ctr. Res. Inst.）

E3-1 [English]
Virus, bacteria infection, inflammation and cancer (1) 10:15-11:30

Masashi Fukayama （Dept. Pathol. Grad. Sch. of Med. Univ. of Tokyo）

LS15 [Japanese]
Clinical implication for whole-genome sequencing in human cancers 11:50-12:40

Mitsuo Shimada （Department of Surgery, Tokushima University）

E3-2 [English]
Virus, bacteria infection, inflammation and cancer (2) 13:00-14:15

Akinori Takaoka （Div. Signaling in Cancer & Immunol., Inst. for Genet. Med., Hokkaido Univ.）

E15-1 [English]
New biomarker for digestive cancers 14:15-15:30

Kikuya Kato （Lab. Med. Genomics, Nara Inst. Sci. Tech.）

E15-2 [English]
New biomarker / liquid biopsy 15:30-16:45

Hiroshi Inoue （Dept. Surg., Eikoh Hosp.）

Room 5 | 10F 1002, Osaka International Convention Center

ML4 [Japanese]
Morning Lectures 4 8:00-8:50

Keiichi Nakayama （Dept. Mol. Cell. Biol., Med. Inst. Bioreg., Kyushu Univ.）

E14-5 [English]
Brain tumor 9:00-10:15

Motomasa Furuse （Dept. NeuroSurg., Osaka Med. College）

E14-6 [English]
Genetic analysis and new treatment of hematological malignancies 10:15-11:30

Mineo Kurokawa （Dept. Hematol. Oncol., Grad. Sch. Med., Univ. Tokyo）

LS16 [Japanese]
Capture and Recovery of Circulating Tumor Cells Using Biocompatible Materials 11:50-12:40

Yuko Kitagawa （Department of Surgery, Keio University, School of Medicine,）

E14-9 [English]
Breast cancer 13:00-14:15

Hirotaka Iwase （Dept. Breast & Endocrine Surg. Kumamoto Univ.）

E14-10 [English]
Molecular characteristics of gynecologic cancer; from carcinogenesis to immune circumstances 14:15-15:30

Kiyoshi Yoshino （Dept. Ob&Gyn. Univ. of Occupational & Environmental Health）

E14-11 [English]
Colorectal cancer 15:30-16:45

Satoshi Nagayama （Dept. Gastroenterological Surg., Cancer Inst. Hosp., JFCR）

Room 6 | 10F 1004+1005, Osaka International Convention Center

ML5 [Japanese]
Morning Lectures 5 8:00-8:50

Toshiyoshi Fujiwara （Dept. Gastroenterol. Surg., Okayama Univ. Grad. Sch. Med.）

E11-3 [English]
Cancer stem cell (1) 9:00-10:15

Kazuhito Naka （Dept. Stem Cell Biol., Res Ins Radiation Biol & Med., Hiroshima Univ.）

E11-4 [English]
Cancer stem cell (2) 10:15-11:30

Yoshihiro Kawasaki （IQB., Tokyo Univ.）

LS17 [Japanese]
Early detection of cancer by liquid biopsy 11:50-12:40

Kenichi Matsubara （Professor Emeritus, Osaka University）

E2-1 [English]
Gene-manipulated animal models 13:00-14:15

Hiroshi Seno （Dept. Gastroenterol. & Hepatol., Kyoto Univ. Grad. Sch. Med.）

E2-2 [English]
Animal models for cancer (1) 14:15-15:30

Satoshi Nishizuka （Div. Biomed. R&D., Inst. Biomed. Sci., Iwate Med. Univ.）

E5-1 [English]
Cancer specific signal transduction (1) 15:30-16:45

Naoto Tsuchiya （Lab. Mol. Carcinogenesis, Natl. Cancer Ctr. Res. Inst.）

Room 7 | 10F 1006+1007, Osaka International Convention Center

ML6 [Japanese]
Morning Lectures 6 8:00-8:50

Hiroshi Yokozaki （Div. Pathol., Dept. Pathol., Kobe Univ. Grad. Sch. Med.）

J5-1 [Japanese]
Cancer specific signal transduction (2) 9:00-10:15

Takashi Matozaki （Div. Mol. Cell. Sig. Kobe. Univ. Grad. Sch. Med.）

J5-2 [Japanese]
MicroRNAs in cancer progression (1) 10:15-11:30

Masahiko Kuroda （Dept. Mol. Pathol. Tokyo Med. Univ.）

LS18 [Japanese]
Expectation for new technology and Premonition of the cancer immunotherapy 11:50-12:40

Yoshihiro Kakeji （Division of Gastrointestinal Surgery, Department of Surgery, Graduate School of Medicine, Kobe University）

J3 [Japanese]
Virus, bacteria infection, inflammation and cancer (3) 13:00-14:15

Tetsuya Tsukamoto （Dept. Diag. Path., Fujita Health Univ. Sch. Med.）

J11-3 [Japanese]
Cancer stem cell (3) 14:15-15:30

Tomoaki Tanaka （Dept. of Mol. Diag., Chiba Ionic., Grad. Sch. Med.）

J11-4 [Japanese]
Cancer stem cell (4) 15:30-16:45

Yohei Shimono （Dept. Biochem., Fujita Health Univ.）

Room 8 | 10F 1008, Osaka International Convention Center

ML7 [Japanese]
Morning Lectures 7 8:00-8:50

Hideki Wanibuchi （Mol. Path., Grad. Sch. Med., Osaka City Univ.）

J15-1 [Japanese]
New biomarker (1) 9:00-10:15

Yasushi Shintani （Dept. Gen. Thoracic. Surg., Osaka Med. Univ., Sch. Med.）

J15-2 [Japanese]
New biomarker (2) 10:15-11:30

Shingo Dan （Div. Mol. Pharmacol, Cancer Chemother. Ctr. of JFCR）

LS19 [Japanese]
Cancer metabolic disorder diagnosed by photodynamic technology 11:50-12:40

Kazuhiro Yoshida （Department of Surgical Oncology, Gifu University, Graduate School of Medicine）

J14-7 [Japanese]
Pancreatic cancer (4) 13:00-14:15

Tomoo Kosuge （Sangenjaya Dai Ichi Hosp.）

J14-8 [Japanese]
Liver cancer 14:15-15:30

Tomoharu Yoshizumi （Dept. Surg. & Sci., Kyushu Univ.）

E14-12 [English]
Biliary tract cancer 15:30-16:45

Shogo Kobayashi （Dept. Gastroenterol. Surg., Osaka Univ.）

Room 9 | 10F 1009, Osaka International Convention Center

JWSA [Japanese]
JCA Women Scientists Award 8:00-8:30

Mari Kannagi （Dept. ImmunoTherap., Tokyo Med. & Dent Univ.）

IS6 [English]
Emerging roles of RUNX genes 9:00-11:30

Kinuko Mitani （Dept. Hematology & Oncology, Dokkyo Med. Univ.）
Suk-Chul Bae （Dept. Biochem., Chungbuk Natl. Univ.）

LS20 [Japanese]
Clinical question of the systemic chemotherapy for colorectal cancer 11:50-12:40

Hideo Baba （Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University）

IS8 [English]
Application of epidemiological knowledges into personalized medicine for cancer prevention in Asia 13:00-15:30

Keitaro Matsuo （Div. Mol. & Clin. Epidemiology, Aichi Cancer Ctr. Res. Inst.）
Youlin Qiao （Dept. Cancer Epidemiology, Cancer Inst. & Hosp., Chinese Academy of Med. Sci., Peking Union Med. Sci.）

J12-3 [Japanese]
Antitumor effector cells 15:30-16:45

Kiyoshi Yoshimura （Showa Univ. Dev. of Immuno Oncology）

Room 10 | 11F 1101+1102, Osaka International Convention Center

ML8 [Japanese]
Morning Lectures 8 8:00-8:50

Shinji Tanaka （Dept. Mol. Oncol., Tokyo Med. Dent. Univ.）

J4-1 [Japanese]
Cancer related genes 9:00-10:15

Keishi Yamashita （Kitasato Univ., Sch. Med.）

J4-2 [Japanese]
Oncogenes and tumor-suppressor genes (1) 10:15-11:30

Issei Imoto （Risk Assessment Ctr., Aichi Cancer Ctr. Hosp.）

LS21 [English]
Clinical Utility and Outcome of Guardant360 in Cancer Patients in Asia 11:50-12:40

Hisahiro Matsubara （Chiba University Graduate School of Medicine, Department of Frontier Surgery）

J14-9 [Japanese]
genetic abnormalities of hematological malignancies 13:00-14:15

Akifumi Takaori-Kondo （Dept. Hematol. （Oncol., Grad. Sch. of Med., Kyoto Univ.）

J14-10 [Japanese]
Hematological malignancies: pathogenesis, treatment and drug resistance 14:15-15:30

Masashi Sanada （Dept. Advanced Diagnosis, Clin. Res. Ctr., Nagoya Med. Ctr.）

J14-11 [Japanese]
Molecular analysis for development and survival mechanisms of gynecologic malignancies 15:30-16:45

Satoru Kyo （Dept. Obstet. Gynecol., Shimane Univ., Faculty of Med.）

Room 11 | 12F Conference Hall, Osaka International Convention Center

ML9 [Japanese]
Morning Lectures 9 8:00-8:50

Hiroyuki Aburatani （Genome Sci., Res. Ctr. Adv. Sci. Tech., the Univ. of Tokyo）

E14-7 [English]
Pancreatic cancer (2) 9:00-10:15

Masahiro Tanemura （Pept. Surg., Osaka Police Hosp.）

J14-5 [Japanese]
Pancreatic cancer (3) 10:15-11:30

Toru Kitagawa （Kyowakai Med. Corporation）

PD [Japanese]
Human papilloma virus (HPV) vaccines in Japan; current problems and future directions 15:30-18:00

Tetsuo Noda （Cancer Inst. of JFCR）
Tomotaka Sobue （Div. Environ Med., Osaka Univ. Sch. Med.,）

Room 12 | 12F 1202, Osaka International Convention Center

ML10 [Japanese]
Morning Lectures 10 8:00-8:50

Hiroki Kuniyasu （Dept. Mol. Pathol., Nara Med. Univ.）

E14-8 [English]
Novel biotherapy and molecular mechanism 9:00-10:15

Yasushi Toh （Dept. Gastroenterol. Surg., Nat'l Kyushu Cancer Ctr.）

J14-6 [Japanese]
Molecular mechanism and diagnosis of peritoneal dissemination in gastric cancer 10:15-11:30

Shuji Takiguchi （Nagoya City Univ. Hosp. Dept. Gastroenterological Surg.）

LS22 [Japanese]
Significance of 'inner' immunity at anti-cancer therapies 11:50-12:40

Shogo Kobayashi （Osaka University, Graduate School of Medicine, Department of Gastroenterological Surgery）

J14-12 [Japanese]
Genetic analysis of gastric cancer 13:00-14:15

Hiroyuki Sugihara （Div. Mol. Diagn. Pathol., Dept. Pathol., Shiga Univ. Med. Sci.）

J19 [Japanese]
Radiation / photodynamic / thermal therapy 14:15-15:30

Kazuhiko Ogawa （Dept. of Radiat Oncol, Osaka Univ. Scool of Med.）

J24 [Japanese]
Cancer epidemiology 15:30-16:45

Isao Miyashiro （Cancer Control Ctr., Osaka InterNatl. Cancer Inst.）

Room 13 | 3F Korin1, RIHGA Royal Hotel Osaka

ML11 [Japanese]
Morning Lectures 11 8:00-8:50

Toshinari Minamoto （Div. Transl. Clin. Oncol., Cancer Res. Inst., Kanazawa Univ）

S9 [English]
An update on hereditary tumors 9:00-11:30

Yoshio Miki （Dept. Mol. Genet., Inst. Med. Sci., Tokyo Med. & Dent. Univ.）
Naohiro Tomita （Div. Lower GI Surg. Dept. Surg. Hyogo Col. Med.）

LS23 [Japanese]
New applications for Clinical Sequencing 11:50-12:40

Junichi Mineno （TAKARA BIO INC. Bioindustry Business Unit）

S12 [English]
Animal models in cancer research 13:00-15:30

Takuro Nakamura （Div. Carcinogenesis, Cancer Inst., JFCR）
Akira Suzuki （Div. Mol. & Cell. Biol., Kobe Univ. Grad. Sch. Med.）

S13 [English]
Frontier of basic research and clinical practice targeting individualized medicine 15:30-18:00

Yuko Kitagawa （Dept. Surg. Keio Univ. Hosp.）
Kazuto Nishio （Dept. Genome Biol, Kindai Uni., Sch. Med.）

Room 14 | 3F Korin2, RIHGA Royal Hotel Osaka

ML12 [Japanese]
Morning Lectures 12 8:00-8:50

Shinichiro Motohashi （Dept. Med. Immunol. Grad. Sch. Med. Chiba Univ）

IC1 [Japanese]
Introduction Course for Current Cancer Research 1 9:00-9:35

Hirofumi Yamamoto （Det. Mol Path, Osaka Univ.）

IC2 [Japanese]
Introduction Course for Current Cancer Research 2 9:35-10:10

Kiyoko Kato （Ob&Gy Dept. Kyushu Univ., Sch. Med.）

IC3 [Japanese]
Introduction Course for Current Cancer Research 3 10:10-10:45

Meinoshin Okumura （Dept. General Thoracic Surg., Osaka Univ. Grad. Sch. of Med.）

IC4 [Japanese]
Introduction Course for Current Cancer Research 4 10:45-11:30

Shinzaburo Noguchi （Dept. Breast Endocrine Surg., Osaka Univ. Sch. Med.）

LS24 [Japanese]
Tissue Biopsy and Liquid Biopsy: Detection and analysis of drug resistance in lung cancer 11:50-12:40

Masatoshi Soejima （Bio-Rad Laboratories K.K）

IC5 [Japanese]
Introduction Course for Current Cancer Research 5 13:00-13:35

Masahiko Watanabe （Dept. Surg. Kitasato Univ., Sch. Med.）

IC6 [Japanese]
Introduction Course for Current Cancer Research 6 13:35-14:10

Hisahito Matsubara （Dept. Frontier Surg., Chiba Univ., Grad. Sch. Med.）

S14 [English]
Inflammation and tumorigenesis 14:10-16:40

Tetsuo Takehara （Gastroenterology & Hepatology, Osaka Univ. Grad. Sch. Med.）
Masanori Hatakeyama （Dept. Microbiol, Grad. Sch. Med, Univ. Tokyo）

Room 15 | 3F Korin3, RIHGA Royal Hotel Osaka

ML13 [Japanese]
Morning Lectures 13 8:00-8:50

Masakazu Toi （Breast Surgery. Kyoto Univ. Med.）

S10 [English]
Overview of cancer genome research and new challenges 9:00-11:30

Johji Inazawa （Dept. Molec. Cytogenet, Med. Res. Inst, Bioresouce Res. Ctr., TMDU）
Hidewaki Nakagawa （RIKEN IMS）

LS25 [Japanese]
Precision cancer biology by target capture sequencing 11:50-12:40

Yutaka Suzuki （Laboratory of Systems Genomics, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo）

S15 [English]
Cancer and cellular senescence signaling 13:00-15:30

Shigeomi Shimizu （Pathol. Cell Biol., Med. Res. Inst., TMDU）
Makoto Nakanishi （Dept. Cancer Cell Biol. IMS. The Univ. of Tokyo）

J2 [Japanese]
Animal models for cancer (2) 15:30-16:45

Takayuki Nakagawa （Lab. Vet. Surg., Dept. Agri. Life Sci., The Univ. Tokyo）

Room 16 | 2F Katsura, RIHGA Royal Hotel Osaka

MV1 [Japanese]
The JCA-Mauvernay Award Lecture 8:00-8:50

Ryuzo Ueda （Dept. Tumor Immunol., Aichi Med. Univ. Sch. Med.）

SST3 [Japanese]
New insights and treatments for hematopoietic malignancy 9:00-11:30

Yuzuru Kanakura （Dept. Hematol. Oncol., Osaka Univ. Grad. Sch. Med.）
Atsushi Hirao （Div. Mol. Gen., Cancer Res. Inst. Kanazawa Univ.）

LS26 [Japanese]
Treatment Strategy for 2nd-line mCRC 11:50-12:40

Naohiro Tomita （Division of Lower GI Surgery, Department of Surgery, Hyogo College of Medicine）

SST4 [Japanese]
New treatments for skin cancer 13:00-15:30

Ichiro Katayama （Dept. Dermatol. Osaka. Univ. Sch. Med.）
Heiichiro Udono （Dept. Immunol., Okayama Univ. Grad. Sch. Med.）

J14-13 [Japanese]
Other cancers 15:30-16:45

Yae Kanai （Dept. Path., Keio Univ. Sch. Med.）

Room P(A) | 3F Event Hall, Osaka International Convention Center

P1-1 [English/Japanese]
Cell culture (1) 16:30-17:15

Masumi Tsuda （Dept. Cancer Path., Faculty of Med., Hokkaido Univ.）

P1-3 [English/Japanese]
DNA damage 16:30-17:15

Kumiko Ogawa （Path., Natl. Inst. Health Sci.）

P1-5 [English/Japanese]
Process of carcinogenesis (2) 16:30-17:15

Katsumi Imaida （Onco-Path., Kagawa Univ., Fac. Med.）

P1-7 [English/Japanese]
Radiation carcinogenesis and oxidative stress (1) 16:30-17:15

Yoichiro Kusunoki （Mol. Biosci., Radiat. Effects Res. Found.）

P1-9 [English/Japanese]
DNA damage and carcinogenic process 16:30-17:15

Kiyoshi Miyagawa （Lab. Mol. Radiol., Grad. Sch. of Med., The Univ. of Tokyo）

P4-5 [English/Japanese]
p53-related genes (1) 16:30-17:15

Yasushi Sasaki （Biol., Ctr. Med. Education, Sapporo Med. Univ.）

P4-7 [English/Japanese]
p53-related genes (2) 16:30-17:15

Kiyotsugu Yoshida （Dept. Biochem., Jikei Univ. Sch. Med.）

P6-2 [English/Japanese]
Cell cycle / genomic instability 16:30-17:15

Moriya Iwaizumi （Dept. Lab. Med., Hamamatsu Univ. Sch. Med.）

P7-1 [English]
Development of novel device / tool in genomic analysis [English] 16:30-17:15

Kentaro Semba （Dept. Life Sci & Med. Biosci, Waseda Univ.）

P7-3 [English]
Comprehensive genomic analysis / whole genome / exome sequencing in solid tumor [English] 16:30-17:15

Yataro Daigo （Ctr. for Antibody & Vaccine, Inst. Med. Sci, Univ. Tokyo）

P7-5 [English/Japanese]
Genomic analysis in solid tumor 16:30-17:15

Yuko Murakami-Tonami （Dept. Clin. Lab. Med., Juntendo Univ. Grad. Sch. Med.）

P10-2 [English/Japanese]
Angiogenesis / Extracellular matrix 16:30-17:15

Ai Takemoto （Dept. Exp. Chemother., Cancer Chemother. Ctr., JFCR）

P10-4 [English/Japanese]
Cell adhesion / invasion (2) 16:30-17:15

Kazuyuki Itoh （Res. Inst. Nozaki Tokushukai）

P10-6 [English/Japanese]
Cell adhesion / invasion (4) 16:30-17:15

Masami Suganuma （Grad. Sch. Sci. Engi. Saitama. Univ.）

P10-8 [English/Japanese]
Extracellular matrix 16:30-17:15

Yasuhiko Kitadai （Pref. Univ. Hiroshima）

P10-10 [English]
Invasion and metastasis (1) [English] 16:30-17:15

Atsushi Osoegawa （Dept. Surg. & Sci., Grad Sch. Med. Sci., Kyushu Univ.）

P10-12 [Japanese]
Invasion and metastasis (3) 16:30-17:15

Tetsuya Kodama （Dept. Biomed. Engineering, Tohoku Univ.）

P11-1 [English/Japanese]
Metabolism / metabolome (1) 16:30-17:15

Naotsugu Haraguchi （Dept. Gastroenterological Surg., Grad. Sch. of Med., Osaka Univ.）

P11-3 [English/Japanese]
Metabolism / metabolome (3) 16:30-17:15

Masaaki Miyo （Dept of Surg, Kinan Hosp.）

P12-3 [English]
Cancer immunity (1) [English] 16:30-17:15

Sadamu Homma （Div. Oncol. Jikei Univ., Sch. Med.）

P1-2 [English/Japanese]
Cell culture (2) 17:15-18:00

Toshio Imai （Ctr. Anim. Div., Natl. Cancer Ctr. Res. Inst.）

P1-4 [English/Japanese]
Process of carcinogenesis (1) 17:15-18:00

Reo Maruyama （Project for Cancer Epigenomics, Cancer Inst., JFCR）

P1-6 [English/Japanese]
Detection and assessment of carcinogens 17:15-18:00

Satoru Takahashi （Dept. Exp. Pathol. Tumor Biol., Nagoya City Univ. Sch. Med.）

P1-8 [English/Japanese]
Radiation carcinogenesis and oxidative stress (2) 17:15-18:00

Kunihiko Sakumi （Div. Neurofunctional Genomics, Med. Inst. Bioreg., Kyushu Univ.）

P4-4 [English/Japanese]
Oncogenes and tumor-suppressor genes 17:15-18:00

Hiroki Nagase （Chiba Cancer Cent. Res. Inst.）

P4-6 [English/Japanese]
Cancer related genes 17:15-18:00

Masuyuki Noguchi （Div. Cancer Biol, Inst for Genetic Medicine, Hokkaido Univ.）

P6-1 [English/Japanese]
DNA repair / genomic instability 17:15-18:00

Hiroshi Itoh （Dept. Mol. Pathol., Yamaguchi Univ., Grad. Sch. Med.）

P6-3 [English/Japanese]
DNA repair 17:15-18:00

Akira Tomokuni （Osaka International Cancer Inst.）

P7-2 [English/Japanese]
Genomic analysis in hereditary / familial disease 17:15-18:00

Tomohiko Ohta （Dept. Translational Oncol. St. Marianna Univ. Grad. Sch. Med）

P7-4 [English/Japanese]
Genomic analysis for tumor microenvironment in solid tumor 17:15-18:00

Yasuhiro Minami （Div. Cell Physiol., Kobe Univ., Grad. Sch. Med.）

P10-1 [English/Japanese]
Angiogenesis 17:15-18:00

Yasufumi Sato （Dept. Vasc. Biol., IDAC, Tohoku Univ.）

P10-3 [English/Japanese]
Cell adhesion / invasion (1) 17:15-18:00

Yasunori Okada （Pathophysiol., Juntendo Univ. Sch. Med.）

P10-5 [English/Japanese]
Cell adhesion / invasion (3) 17:15-18:00

Shiro Suetsugu （NAIST）

P10-7 [English/Japanese]
Transcription and gene expression (1) 17:15-18:00

Genichiro Ishii （Div. Path. EPOC Natl. Cancer Ctr.）

P10-9 [English/Japanese]
Transcription and gene expression (2) 17:15-18:00

Susumu Itoh （Lab. of Biochem., Showa Pharm. Univ.）

P10-11 [Japanese]
Invasion and metastasis (2) 17:15-18:00

Motoko Shibanuma （Cancer Cell Biol., Showa Univ., Sch. Pharm.）

P10-13 [Japanese]
Invasion and metastasis (4) 17:15-18:00

Tomoya Yamaguchi （Dept. Cancer Biol., Grad. Sch. Med. Sci., Kumamoto Univ.）

P11-2 [English/Japanese]
Metabolism / metabolome (2) 17:15-18:00

Ichiro Izawa （Dept. Nutr. Sci., Nagoya Univ. Arts Sci.）

P11-4 [English/Japanese]
Metabolism / metabolome (4) 17:15-18:00

Tetsuo Mashima （Div. Mol Biother, Cancer Chemother. Ctr, JFCR）

Room P(B) | 7F Lobby/701+702, Osaka International Convention Center

P12-5 [Japanese]
Cancer immunity (2) 16:30-17:15

Takuro Saito （Dept. Surg., Osaka Police Hosp.）

P14-27 [English/Japanese]
Hepatocellular carcinoma (1) 16:30-17:15

Hidenori Ojima （Dept. Path., Keio Univ. Sch. Med.）

P14-29 [English/Japanese]
Hepatocellular carcinoma (3) 16:30-17:15

Eisaku Kondo （Div. Mol. Cell. Pathol., Niigata Univ. Grad. Sch. Med.）

P14-31 [English/Japanese]
Surgical treatment of hepatobiliary pancreatic cancers (1) 16:30-17:15

Keisuke Tateishi （Dept. Gastroenterology, The Univ. of Tokyo Hosp.）

P12-4 [Japanese]
Tumor antigens and immunity 17:15-18:00

Hiroyuki Kishi （Dept. Immunol., Grad. Sch. Med. Pharm. Sci., Univ. Toyama）

P12-6 [Japanese]
Cancer immunity (3) 17:15-18:00

Koichi Kawamoto （Kinki Regional Bureau of Health & Welfare）

P14-28 [English/Japanese]
Hepatocellular carcinoma (2) 17:15-18:00

Tadafumi Asaoka （Dept. Gastroenterological Surg., Grad. Sch. of Med., Osaka Univ.）

P14-30 [English/Japanese]
Biomarkers for hepatobiliary pancreatic cancers 17:15-18:00

Shunsuke Kato （Dept. Clin Oncol, Juntendo Univ. Grad. Sch. Med.）

P14-32 [English/Japanese]
Surgical treatment of hepatobiliary pancreatic cancers (2) 17:15-18:00

Hiroshi Wada （Dept. Surg., Osaka InterNatl. Cancer Inst.）

Room P(C) | 8F Lobby/801+802, Osaka International Convention Center

P14-33 [English/Japanese]
Breast cancer (1) 16:30-17:15

Hiroko Yamashita （Breast Surg., Hokkaido Univ. Hosp.）

P14-35 [English/Japanese]
Breast cancer (3) 16:30-17:15

Takayuki Kinoshita （Natl. Cancer Ctr. Hosp.）

P14-37 [English/Japanese]
Urothelial cancer (1) 16:30-17:15

Masayuki Takahashi （Dept. Urology, Tokushima Univ. Grad. Sch. Biomed. Sci.）

P14-39 [English/Japanese]
Renal cell carcinoma (1) 16:30-17:15

Masahiro Nozawa （Dept. Urol., Kindai Univ., Faculty of Med.）

P14-34 [English/Japanese]
Breast cancer (2) 17:15-18:00

Hitoshi Tsuda （Dept. Basic Pathol., Natl. Def. Med. Coll.）

P14-36 [English/Japanese]
Breast cancer (4) 17:15-18:00

Goro Kutomi （Dept. Surg., Sapporo Med. Univ.）

P14-38 [English/Japanese]
Urothelial cancer (2) 17:15-18:00

Wataru Obara （Dept. Urology, Iwate Med. Univ.）

P14-40 [English/Japanese]
Renal cell carcinoma (2) 17:15-18:00

Masayuki Nakagawa （Dept. Urology, Kagoshima Univ. Grad. Sch. Med. Dent. Sci.）

Room P(D) | 10F 1010+10-1&2, Osaka International Convention Center

P14-41 [English/Japanese]
Renal cell carcinoma (3) 16:30-17:15

Fumiya Hongo （Dept. Urol., Kyoto Pref. Univ. of Med.）

P14-43 [English/Japanese]
Renal cell carcinoma (5) 16:30-17:15

Eri Arai （Dept. Pathol., Keio Univ. Sch. Med.）

P14-45 [English/Japanese]
Prostate cancer (2) 16:30-17:15

Takahiro Kojima （Dept. Urology, Univ. of Tsukuba）

P14-42 [English/Japanese]
Renal cell carcinoma (4) 17:15-18:00

Kei Ishibashi （Dept. Urology, Fukushima Med. Univ. Sch. of Med.）

P14-44 [English/Japanese]
Prostate cancer (1) 17:15-18:00

Takeo Kosaka （Dept. Urology, Keio Univ. Sch. of Med.）

Room P(E) | 12F Lobby, Osaka International Convention Center

P16-1 [English/Japanese]
Signal transduction inhibitor (1) 16:30-17:15

Daizo Koinuma （Dept. Mol. Pathol., Grad. Sch. Med., The Univ. Tokyo）

P16-3 [English/Japanese]
Lung cancer kinase inhibitor 16:30-17:15

Masakiyo Sakaguchi （Dept. Cell Biol., Okayama Univ. Grad. Sch. Med. Dent. Pharm. Sci.）

P16-5 [English/Japanese]
Signal transduction inhibitor (2) 16:30-17:15

Siro Simizu （Dept. Applied Chem., Fac. Sci. Tech., Keio Univ.）

P16-7 [English/Japanese]
New targeted therapy (1) 16:30-17:15

Kensuke Kojima （Dept. Hematol. Resp. Med. & Oncol., Saga Univ.）

P21-1 [English/Japanese]
Gene therapy and oncolytic virus therapy (1) 16:30-17:15

Tomoki Makino （Dept. Gastroenterological Surg., Grad. Sch. of Med., Osaka Univ.）

P22 [English/Japanese]
Medical care of progressive cancer 16:30-17:15

Shogo Ehata （Dept. Mol. Path., Grad. Sch. Med., Univ. Tokyo）

SSP [English/Japanese]
Survivor Scientist Program 17:05-18:00

Hiroshi Harada （Lab. of Cancer Cell Biol., Grad. Sch. Biostudies, Kyoto Univ.）

P14-46 [English/Japanese]
Prostate cancer (3) 17:15-18:00

Masahito Watanabe （Dept. Urol., Aichi Med. Univ., Sch. Med.）

P16-2 [English/Japanese]
Cell death / synthetic lethal target 17:15-18:00

Shinichiro Hasegawa （Tondabayashi Hosp. Surg. Dept.）

P16-4 [English/Japanese]
New target screening 17:15-18:00

Jun Koseki （Grad. Sch. Med., Osaka Univ.）

P16-6 [English/Japanese]
Signal transduction inhibitor (3) 17:15-18:00

Etsu Tashiro （Fac. of Sci. & Tech., Keio Univ.）

P16-8 [English/Japanese]
New targeted therapy (2) 17:15-18:00

Akinobu Hamada （Div. Mol. pharm., Natl. Cancer Ctr. Res. Inst.）

P21-2 [English/Japanese]
Gene therapy and oncolytic virus therapy (2) 17:15-18:00

Tomoyuki Nishikawa （Gene Therapy Sci., Sch. of Med., Osaka Univ.）

Room P(F) | 3F Lobby, RIHGA Royal Hotel Osaka

P23-2 [English/Japanese]
Natural products / dietary factors (1) 16:30-17:15

Keiji Wakabayashi （Grad. Div. Nutritional & Environmental Sci. Univ. Shizuoka）

P25-1 [English/Japanese]
Data science / AI (1) 16:30-17:15

Masato Morikawa （Dept. Mol. Pathol., Grad. Sch. Med., Univ. Tokyo）

P23-1 [English/Japanese]
Natural products 17:15-18:00

Naoki Yoshimi （Path. &Onco., Grad. Sch. Med., Univ. of the Ryukyus）

P23-3 [English/Japanese]
Natural products / dietary factors (2) 17:15-18:00

Masumi Suzui （Dept. Mol. Toxicol. Nagoya City Univ. Grad. Sch. Med. Sci.）

P25-2 [English/Japanese]
Data science / AI (2) 17:15-18:00

Hidetaka Eguchi （Diagnos. & Ther. Intractable Diseases, Juntendo Univ. Grad. Sch. Med.）

9 29(Sat)
Days 3

Room 1 | 5F Large Hall, Osaka International Convention Center

AACR2 [English]
Cancer metabolism 9:00-11:30

Tatsuhiko Furukawa （Dept. Mol. Onc, Kagoshima Univ., Grad. Sch. Med. Dent. Sci.）
Alec C. Kimmelman （New York Univ. Langone Health, New York）

TYPL [Japanese]
The Tomizo Yoshida Prize Lecture 12:55-14:25

Masaki Mori （Dept. Gastroenterological Surg., Osaka Univ.）

MNPL [Japanese]
The Mataro Nagayo Prize Lecture 12:55-14:25

Fuyuki Ishikawa （Kyoto Univ., Grad. Sch. of Biostudies）

CS4 [English]
Significance of cancer stem cells as therapeutic target 14:35-17:05

Koichi Akashi （Dept. Med. & Biosystemic Sci., Faculty of Med., Kyushu Univ.）
Nobuyuki Takakura （Dept. Signal Transduction, RIMD, Osaka Univ.）

Room 2 | 5F Small Hall, Osaka International Convention Center

ML14 [Japanese]
Morning Lectures 14 8:00-8:50

Satoru Miyano （IMS, the Univ. of Tokyo）

IS9 [English]
Universal health coverage (UHC) and cancer control 9:00-11:30

Tetsuo Noda （Cancer Inst. of JFCR）
Thomas Cueni （InterNatl. Federation of Pharm. Manufacturers & Association）

IS11 [English]
Role of innate immunity and tumor microenvironment in cancer progression 13:40-16:10

Masanobu Oshima （Div. Genetics, Cancer Res. Inst., Kanazawa Univ.）
Brendan John Jenkins （Hudson Inst. of Med. Res., Monash Univ.）

Room 3 | 10F 1003, Osaka International Convention Center

ML15 [Japanese]
Morning Lectures 15 8:00-8:50

Toshirou Nishida （Natl. Cancer Ctr. Hosp., Dept. Surg.）

S16 [English]
Signal transduction analysis for cancer profiling 9:00-11:30

Makoto Noda （Mol. Oncol., Kyoto Univ. Grad. Sch. Med.）
Akira Kikuchi （Dept. Mol. Biol. & Biochem., Grad. Sch. Med., Osaka Univ.）

LS27 [Japanese]
New Biomarker for Cancer Immunotherapy pioneered by Precision medicine 11:50-12:40

Tetsuya Mitsudomi （Department of Surgeons, School of Medicine, Kinki University）

S19 [English]
Liquid biopsy paves the way for next-generation medicine 13:40-16:10

Koshi Mimori （Dept. Surg., Kyushu Univ., Beppu Hosp.）
Hidetoshi Eguchi （Dept. Gastroenterol. Surg, Osaka Univ., Grad. Sch. Med.）

Room 4 | 10F 1001, Osaka International Convention Center

ML16 [Japanese]
Morning Lectures 16 8:00-8:50

Keiya Ozawa （Div. Immuno-Gene & Cell Ther）

MVA [English]
JCA-Mauvernay Awards Session 9:00-11:30

Hitoshi Nakagama （Natl. Cancer Ctr.）
Takashi Takahashi （Div. Mol. Carcinog., Nagoya Univ. Sch. Med.）

LS28 [English]
The development of future therapies for multiple myeloma in the era of increasing role of immunotherapy 11:50-12:40

Yusuke Nakamura （Cancer Precision Medicine Center of JFCR）

E5-2 [English]
MicroRNAs in cancer progression (2) 13:40-14:55

Hidetoshi Tahara （Dept. Cell & Mol. Biol., Grad. Sch. of Biomed. & Health Sci., Univ. of Hiroshima）

E5-3 [English]
Transcriptional regulation 14:55-16:10

Taro Yamashita （Dept. Gen. Med., Kanazawa Univ. Hosp.）

Room 5 | 10F 1002, Osaka International Convention Center

ML17 [Japanese]
Morning Lectures 17 8:00-8:50

Yutaka Kondo （Div. Cancer Biol, Nagoya Univ., Grad. Sch. Med.）

E12-3 [English]
Tumor antigens and immunity 9:00-10:15

Yasuharu Nishimura （Dept. Immunogenet., Grad. Sch. Med. Sci., Kumamoto Univ.）

E20 [English]
Regenerative medicine 10:15-11:30

Akira Shimamoto （Field of Regenerative Med. Res. Faculty of pharm. Sci. Sanyo-Onoda City Univ.）

E17-2 [English]
Drug delivery system (1) 13:40-14:55

Yasuhiro Matsumura （Div. Dvelopmental Therap., EPOC, Natl Cancer Ctr）

E15-3 [English]
Radiation / photodynamic therapy and novel cancer diagnostic tool 14:55-16:10

Masahiko Koizumi （Dept. Med. Phys. Eng., Osaka Univ., Sch. Med. Health Sci.）

Room 6 | 10F 1004+1005, Osaka International Convention Center

ML18 [Japanese]
Morning Lectures 18 8:00-8:50

Kenkichi Masutomi （Natl. Cancer Ctr.）

E4-1 [English]
Novel oncogenes / tumor suppressor genes (1) 9:00-10:15

Hirofumi Arakawa （Div. Cancer Biol., Natl. Cancer Ctr. Res. Inst.）

E4-2 [English]
Oncogenes and tumor-suppressor genes (2) 10:15-11:30

Ryoji Yao （Dept. Cell Biology., JFCR-Cancer Inst.）

LS29 [English]
Uncovering the complexity of RNA with NGS 11:50-12:40

Toshiyoshi Fujiwara （Gastroenterological Surgery at Okayama University）

E4-3 [English]
Oncogenes and tumor-suppressor genes (3) 13:40-14:55

Takashi Tokino （Sapporo Med. Univ., Med. Genome Sci.）

E14-13 [English]
Pediatric cancer (1) 14:55-16:10

Junko Takita （Dept. Pediatrics, Univ. Tokyo）

Room 7 | 10F 1006+1007, Osaka International Convention Center

ML19 [Japanese]
Morning Lectures 19 8:00-8:50

Masakazu Yashiro （Mol. Onc. & Therap., Osaka City Univ.）

E12-4 [English]
Antitumor effector cells and their induction 9:00-10:15

Hitoshi Kiyoi （Dept. Hematol. & Oncol. Nagoya Univ., Sch. Med.）

E12-5 [English]
Antitumor effector cells and their inhibition 10:15-11:30

Yoshiki Akatsuka （Dept. Immunol（Cell. Immunol, Nagoya Univ. Grad. Sch. Med.）

LS30 [Japanese]
CAT (Cancer-associated thrombosis) 11:50-12:40

Shoji Natsugoe （Department of Digestive Surgery, Breast and Thyroid Surgery, Kagoshima University Graduate School of Medicine）

J15-3 [Japanese]
Liquid biopsy and pathology 13:40-14:55

Akashi Ooi （Dept. Mol. Cell. Pathol., Kanazawa Univ.）

J4-3 [Japanese]
Novel oncogenes / tumor suppressor genes (2) 14:55-16:10

Masatoshi Kitagawa （Dept. Mol. Biol. Hamamatsu Univ. Sch. Med.）

Room 8 | 10F 1008, Osaka International Convention Center

IAL [English]
JCA International Award Lecture 8:00-8:50

Yasuhito Yuasa （URA Div., Tokyo Med. & Dent. Univ.）

J7-1 [Japanese]
Genomic analysis in gastroenterological disease 9:00-10:15

Kohichiroh Yasui （Dept. Gastroenterol. Hepatol., Kyoto. Pref. Univ. Med.）

J7-2 [Japanese]
Familial tumor related genes 10:15-11:30

Yoichi Furukawa （Inst. Med. Sci., Tokyo Univ.）

LS31 [Japanese]
Pharmacokinetic analysis to predict the clinical effect of new anticancer agents in preclinical study 11:50-12:40

Yasuyuki Seto （University of Tokyo, Graduate School of Medicine, Gastrointestinal Surgery）

J14-19 [Japanese]
Pediatric cancer (2) 13:40-14:55

Tatsuro Tajiri （Dept. Pediatric Surg., Grad. Sch. of Med. Sci., Kyoto Pref. Univ. of Med.）

J7-3 [Japanese]
Genomic analysis in Japanese population 14:55-16:10

Hiromi Sakamoto （Dept. Clin. Genomics, Natl. Cancer Ctr. Res. Inst.）

Room 9 | 10F 1009, Osaka International Convention Center

ML20 [Japanese]
Morning Lectures 20 8:00-8:50

Michiaki Unno （Dept. Surg., Tohoku Univ.）

IS10 [English]
Molecular basis of therapy resistance for development of next generation drugs 9:00-11:30

Ryohei Katayama （Div. Exp. Chemother., Cancer Chemother. Ctr., JFCR）
Pieter Eichhorn （Cancer Sci. Inst. of Singapore）

LS32 [Japanese]
Optimal treatment Strategy based on Pan-Asian Adapted ESMO Consensus Guidelines for mCRC 11:50-12:40

Taroh Satoh （Department of Frontier Science for Cancer and Chemotherapy, Osaka University Graduate School of Medicine）

IS12 [English]
Novel therapeutic strategy for primary and metastatic tumors in the central nervous system 13:40-16:10

Seiji Yano （Div. Med. Oncology, Cancer Res. Inst., Kanazawa Univ.）
Byoung Chul Cho （Div. Med. Oncology, Yonsei Cancer Ctr.）

Room 10 | 11F 1101+1102, Osaka International Convention Center

ML21 [Japanese]
Morning Lectures 21 8:00-8:50

Mitsugu Sekimoto （Dept. Surg., Osaka Natl. Hosp.）

J14-14 [Japanese]
Colorectal cancer: cancer immunity 9:00-10:15

Masahiko Shibata （Dept. Advanced Cancer Immunotherapy, Fukushima Med. Univ.）

J14-15 [Japanese]
Colorectal cancer 10:15-11:30

Masayuki Ohue （Dept. Gastroenterological Surg., Osaka InterNatl. Cancer Inst.）

LS33 [English]
The dog as a human cancer model 11:50-12:40

Shimpei Nishikawa （TRAC (Translational Research Unit for Small Animal Cancer), Core Clusters for Research Initiatives of Yamaguchi University）

J13 [Japanese]
Growth factors / cytokines 13:40-14:55

Kunio Matsumoto （Cancer Res. Inst., Kanazawa Univ.）

J17-3 [Japanese]
Drug delivery system (2) 14:55-16:10

Eishi Ashihara （Dept. Clin. & Translational Physiol. Kyoto Pharm. Univ.）

Room 11 | 12F Conference Hall, Osaka International Convention Center

ML22 [Japanese]
Morning Lectures 22 8:00-8:50

Hidenori Inohara （Dept. Otolaryngol-Head & Neck Surg., Osaka Univ. Sch. Med.）

J14-16 [Japanese]
The pathogenesis and the development of novel therapies for bone and soft tissue tumors 9:00-10:15

Makoto Endo （Dept. Orthop. Surg., Kyushu Univ.）

J9-1 [Japanese]
DNA methylation 10:15-11:30

Hiromu Suzuki （Dept. Mol. Biol., Sapporo Med. Univ., Sch. Med.）

LS39 [English]
Biological considerations in Colorectal Cancer~Primary Tumor location and Consensus Molecular Subtype~ 11:50-12:40

Xundi Xu （Department of Gastroenterology, Central South University, China）

J9-2 [Japanese]
Histone modification 13:40-14:55

Keiko Shinjo （Div. Cancer Biol., Nagoya Univ Grad. Sch. Med.）

J12-4 [Japanese]
Cancer immunity 14:55-16:10

Hiroaki Ikeda （Dept. Oncology, Nagasaki Univ., Grad. Sch. Biomed.）

Room 12 | 12F 1202, Osaka International Convention Center

ML23 [Japanese]
Morning Lectures 23 8:00-8:50

Hiroki Yamaue （2nd Dept. Surg., Wakayama Med. Univ.）

J14-17 [Japanese]
Novel targets for lung cancer therapy 9:00-10:15

Yasuhiko Nishioka （Dept. Respir. Med. & Rheumatol, Grad. Sch. Biomed. Sci, Tokushima Univ.）

J14-18 [Japanese]
Cutting edge of lung cancer research 10:15-11:30

Yoshitaka Sekido （Aichi Cancer Ctr. Res. Inst., Div. Cancer Biol.）

LS34 [Japanese]
Recent advances in the molecular pathogenesis of B-cell lymphomas 11:50-12:40

Koichi Akashi （Department of Medicine and Biosystemic Science, ）

J14-20 [Japanese]
Prostate cancer 13:40-14:55

Motohide Uemura （Dept. Urol., Osaka Univ. Grad. Sch. Med.）

J14-21 [Japanese]
Non-prostate genitourinary cancer 14:55-16:10

Seiichi Mori （Japanese Foundation for Cancer Res., CPM Ctr.）

Room 13 | 3F Korin1, RIHGA Royal Hotel Osaka

ML24 [Japanese]
Morning Lectures 24 8:00-8:50

Yasuhiro Kodera （Dept. Gastroenterol Surg., Nagoya Univ.）

S17 [English]
Current status and prospects in translational research 9:00-11:30

Atsushi Ohtsu （Natl. Cancer Ctr. Hosp. East）
Shoji Natsugoe （Dept. Digestive Surg., Breast & Thyroid Surg., Kagoshima Univ., Sch. Med.）

LS35 [Japanese]
Current Status and Future Perspectives of Carbon Ion Radiotherapy 11:50-12:40

Hidetoshi Eguchi （Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University）

S20 [English]
Revolution in basic research and clinical practice by genome editing 13:40-16:10

Takashi Takahashi （Div. Mol. Carcinog., Nagoya Univ. Sch. Med.）
Tomoji Mashimo （GERDC（IEXAS, Med, Osaka Univ.）

Room 14 | 3F Korin2, RIHGA Royal Hotel Osaka

ML25 [Japanese]
Morning Lectures 25 8:00-8:50

Naoya Fujita （Cancer Chemother Ctr., JFCR）

IC7 [Japanese]
Introduction Course for Current Cancer Research 7 9:00-9:35

Tomotaka Sobue （Div. Environ Med, Osaka Univ. Sch. Med.）

IC8 [Japanese]
Introduction Course for Current Cancer Research 8 9:35-10:10

Eiji Miyoshi （Dept. Mol. Biochem. & Clin. Invest. Osaka Univ. Grad. Sch. Med.）

IC9 [Japanese]
Introduction Course for Current Cancer Research 9 10:10-10:45

Hisashi Wada （Clin. Res. in Tumor Immunol.）

IC10 [Japanese]
Introduction Course for Current Cancer Research 10 10:45-11:20

Hiroaki Kataoka （Dept. Path., Facul. Med., Univ. Miyazaki）

LS36 [Japanese]
Immuno-oncology & Molecular Imaging~Molecular mechanisms of immune checkpoint molecules and chimeric antigen receptor, CAR~ 11:50-12:40

Norio Nonomura （Department of Urology, Osaka University Graduate School of Medicine）

IC11 [Japanese]
Introduction Course for Current Cancer Research 11 13:40-14:15

Hideaki Tahara （Advanced Clin. Res. Ctr., Inst. Med. Sci., the Univ. of Tokyo）

IC12 [Japanese]
Introduction Course for Current Cancer Research 12 14:15-14:50

Eiichi Morii （Dept. Pathol, Osaka Univ. Grad. Sch. Med.）

IC13 [Japanese]
Introduction Course for Current Cancer Research 13 14:50-15:25

Shoji Nakamori （Osaka Natl. Hosp.）

Room 15 | 3F Korin3, RIHGA Royal Hotel Osaka

ML26 [Japanese]
Morning Lectures 26 8:00-8:50

Taroh Satoh （Dept. Frontier Sci. for Cancer & Chemother. Osaka Univ. Grad. Sch. of Med.）

S18 [English]
Radiation oncology in cancer research and treatment 9:00-11:30

Hiroyuki Kuwano （Dept. General Surg. Sci. Gunma Univ., Grad. Sch. of Med.）
Hiroshi Harada （Lab. of Cancer Cell Biol., Grad. Sch. Biostudies, Kyoto Univ.）

LS37 [Japanese]
Topics of cancer immunology: Lessons learned from immune checkpoint blockade 11:50-12:40

Hideaki Shimada （Department of Surgery, Toho University Graduate School of Medicine）

YSA [English]
The Young Scientist Award Lecture 14:10-16:40

Yasufumi Kaneda （Gene Ther. Sci., Gr. Sch. Med., Osaka Univ.）

Room 16 | 2F Katsura, RIHGA Royal Hotel Osaka

MV2 [Japanese]
The JCA-Mauvernay Award Lecture 8:00-8:50

Tomoki Naoe （Nagoya Med. Ctr.）

SST5 [Japanese]
Recent progress of urologic oncology 9:00-11:30

Norio Nonomura （Dept. Urology, Osaka Univ., Grad. Sch. Med.）
Yasuhisa Fujii （Dept. Urol, Tokyo Med. Dent. Univ.）

LS38 [Japanese]
RamDA-seq: Single-cell full-length total RNA sequencing method to uncover expression and full-length structure of total RNA 11:50-12:40

Akira Watanabe （Center for iPS Cell Research and Application, Kyoto University）

SST6 [Japanese]
Advances in treatments for lung cancer 13:40-16:10

Tetsuya Mitsudomi （Thorac. Surg., Kindai Univ. Fac. Med.）
Yuichi Ishikawa （Pathol. Div., JFCR Cancer Inst.）

Room P(A) | 3F Event Hall, Osaka International Convention Center

P3-1 [English]
Virus, bacteria infection, inflammation and cancer (1) [English] 16:00-16:45

Naoko Kamiya （Dept. Microbiol., Grad. Sch. Med., Univ. Tokyo）

P3-3 [English]
Virus, bacteria infection, inflammation and cancer (3) [English] 16:00-16:45

Dai Iwakiri （Nat.Cent.Child Health and Dev.）

P3-5 [Japanese]
Virus, bacteria infection, inflammation and cancer (5) 16:00-16:45

Futoshi Okada （Div. Pathol. Biochem., Tottori Univ. Facul. Med.）

P4-8 [English/Japanese]
Translational research in colorectal cancer 16:00-16:45

Sonshin Takao （Tanegashima Med. Ctr.）

P4-10 [English/Japanese]
Cancer related genes / metabolome 16:00-16:45

Hiroshi Fukamachi （Dept. Mol. Oncol., Tokyo Med. Dent. Univ.）

P9-2 [English]
Histone modification (1) [English] 16:00-16:45

Satoshi Yamashita （Divison of Epigenomics, Natl. Cancer Ctr. Res. Insitute）

P9-4 [Japanese]
DNA methylation (2) 16:00-16:45

Yuji Masuda （Dept. Genome Dynamics, Res. Inst. Environ. Med., Nagoya Univ.）

P9-6 [Japanese]
Histone modification (2) 16:00-16:45

Yoshiyuki Watanabe （Div. Gastroenterol., St. Marianna Univ. Sch. Med.）

P9-8 [Japanese]
Epigenetics and others 16:00-16:45

Yasuhito Nannya （Dept. Pathol. & Tumor Biol., Kyoto Univ.）

P11-6 [English/Japanese]
Cancer stem cell (2) 16:00-16:45

Yukinari Kato （Dept. Antibody Drug Development, Tohoku Univ. Grad. Sch. Med.）

P11-8 [English/Japanese]
Cancer stem cell (4) 16:00-16:45

Hiroko Oshima （Div. Genetics, Cancer Res. Inst., Kanazawa Univ.）

P11-10 [English/Japanese]
Cell culture (3) 16:00-16:45

Tohru Kiyono （Div. Carcinogenesis & Cancer Prevention, Natl Cancer Ctr. Res. Inst.）

P11-12 [English/Japanese]
Cell-to-cell interaction (1) 16:00-16:45

Hiroshi Shima （Div. Chemother., Miyagi Cancer Ctr. Res. Inst.）

P11-14 [English/Japanese]
Glycosylation and glycosyltransferase 16:00-16:45

Naoki Itano （Faculty Life Sci., Kyoto Sangyo Univ.）

P12-8 [English/Japanese]
Innate immunity (2) 16:00-16:45

Kota Iwahori （Dept. Clin. Res. Tumor Immunol., Osaka Univ., Sch. Med.）

P12-10 [English/Japanese]
Other immunotherapies (1) 16:00-16:45

Takashi Masuko （Cell Biol. Lab., Sch. Pharm ., Kindai Univ.）

P12-12 [English/Japanese]
Antitumor effector cells and their induction (1) 16:00-16:45

Shin Kaneko （Ctr. for iPS Cell Res. & Application (CiRA), Kyoto Univ.）

P12-14 [English/Japanese]
Cancer vaccine (1) 16:00-16:45

Tsutomu Takeda （OCICC）

P13-1 [English/Japanese]
Factors regulation growth and differentiation 16:00-16:45

Hugh Colvin （Kagawa Prefectural Central Hospital）

P13-3 [English/Japanese]
TGF-β / Smad, others 16:00-16:45

Yasumichi Inoue （Cell Signal., Grad. Sch. Pharm., Nagoya City Univ.）

P3-2 [English]
Virus, bacteria infection, inflammation and cancer (2) [English] 16:45-17:30

Hironori Yoshiyama （Dept. Microbiol., Shimane Univ., Sch. Med.）

P3-4 [Japanese]
Virus, bacteria infection, inflammation and cancer (4) 16:45-17:30

Kiichiro Tsuchiya （Dept. Gastroenterol., Tokyo Med. Dent. Univ.）

P3-6 [Japanese]
Virus, bacteria infection, inflammation and cancer (6) 16:45-17:30

Masao Matsuoka （Dept. Hematol, Rheumatol, Inf. Dis., Kumamoto Univ.）

P4-9 [English/Japanese]
Signaling of tumor-suppressor genes / novel diagnostic modality 16:45-17:30

Takeshi Urano （Dept. Biochem., Shimane Univ., Sch. Med.）

P9-1 [English]
DNA methylation (1) [English] 16:45-17:30

Takashi Sakatani （Dept. Diagnostic Path., Nippon Med. Sch. Hosp.）

P9-3 [English]
Chromatin structure, others [English] 16:45-17:30

Yoshimasa Saito （Div. Pharmacotherapeutics Keio Univ. Faculty of Pharm.）

P9-5 [Japanese]
DNA methylation (3) 16:45-17:30

Mamoru Uemura （Dept. Surg., National Hospital Organization Osaka National Hospital）

P9-7 [Japanese]
Chromatin structure 16:45-17:30

Yoshimitsu Akiyama （Dept. Mol. Oncology, Tokyo Med. & Dentl. Univ.）

P11-5 [English/Japanese]
Cancer stem cell (1) 16:45-17:30

Takuichiro Hide （Dept. NeuroSurg., Kitasato Univ. Sch. of Med.）

P11-7 [English/Japanese]
Cancer stem cell (3) 16:45-17:30

Hideaki Ijichi （Dept. Clin. Nutri. Ther., The Univ. of Tokyo., Sch. Med.）

P11-9 [English/Japanese]
Cancer stem cell (5) 16:45-17:30

Tsukasa Okuda （Dept. Biochem. Molec. Biol., Kyoto Pref. Univ. Med.）

P11-11 [English/Japanese]
Cell culture (4) 16:45-17:30

Yoshiyuki Rikitake （Lab. Med. Pharma., Kobe Pharm. Univ.）

P11-13 [English/Japanese]
Cell-to-cell interaction (2) 16:45-17:30

Koichi Furukawa （Dept. Biomed. Sci., Chubu Univ. Coll. Life Sci.）

P12-7 [English/Japanese]
Innate immunity (1) 16:45-17:30

Shoichi Hazama （TR & Develop. Therap. against Cancer, Yamaguchi Univ.）

P12-9 [English/Japanese]
Dendritic cells / antigen-presenting cells 16:45-17:30

Shinichi Kageyama （Immuno-Gene Ther. Mie Univ. Grad. Sch. Med.）

P12-11 [English/Japanese]
Other immunotherapies (2) 16:45-17:30

Mamoru Harada （Dept. Immunol., Shimane Univ. Facult. Med.）

P12-13 [English/Japanese]
Antitumor effector cells and their induction (2) 16:45-17:30

Kazunori Aoki （Dept. Immune Med. Natl. Cancer. Ctr. Res. Inst.）

P12-15 [English/Japanese]
Cancer vaccine (2) 16:45-17:30

Yoshihiro Hayakawa （Inst. Nat. Med., Univ. Toyama）

P13-2 [English/Japanese]
Cytokines 16:45-17:30

Masao Saitoh （Ctr. for Med. Sci., Grad. Sch. Med., Univ. of Yamanashi）

Room P(B) | 7F Lobby/701+702, Osaka International Convention Center

P14-48 [English/Japanese]
Skin tumor and endocrine tumor 16:00-16:45

Toru Takano （Dept. Metab. Med., Osaka Univ.）

P14-50 [English/Japanese]
Other Cancers 16:00-16:45

Kiyoshi Yanagisawa （Div. Mol. Car., Nagoya Univ. Grad. Sch. Med.）

P14-52 [English/Japanese]
Colorectal cancer: pathology 16:00-16:45

Chu Matsuda （Dept. Gastroenterological Surg. Osaka Univ.）

P14-54 [English/Japanese]
Colorectal cancer (1) 16:00-16:45

Yozo Suzuki （Dept. Gastroenterol. Surg. Osaka Police Hosp.）

P14-47 [English/Japanese]
Brain tumor 16:45-17:30

Toshihiko Wakabayashi （Dept. Neurosurg., Nagoya Univ., Sch. Med.）

P14-49 [English/Japanese]
Brain tumor and others 16:45-17:30

Atsushi Natsume （Dept. Neurosurg., Nagoya Univ. Sch. Med.）

P14-51 [English/Japanese]
Colorectal cancer: prognostic factor (1) 16:45-17:30

Hidekazu Takahashi （Dept. Gastroenterological Surg., Osaka Univ. Grad. Sch. of Med.）

P14-53 [English/Japanese]
Colorectal cancer: prognostic factor (2) 16:45-17:30

Taishi Hata （Dept. GI Surg. Grad. Sch. of Med., Osaka Univ.）

Room P(C) | 8F Lobby/801+802, Osaka International Convention Center

P14-56 [English/Japanese]
Colorectal cancer: clinical study 16:00-16:45

Norihisa Saeki （Okinawa Pref College Nursing）

P14-58 [English/Japanese]
Colorectal cancer: metastasis of CRC 16:00-16:45

Hiroki Ochiai （Surg., Kitasato Univ., Kitasato Inst. Hosp.）

P14-60 [English/Japanese]
Colorectal cancer (2) 16:00-16:45

Mutsumi Fukunaga （Dept. Surg., Hyogo Prefectural Nishinomiya Hospital）

P14-62 [English/Japanese]
Biliary tract cancer (2) 16:00-16:45

Kunihito Gotoh （Dept. Gastroenterol. Surg., Osaka Univ., Sch. Med.）

P14-55 [English/Japanese]
Colorectal cancer (2) 16:45-17:30

Noriyuki Nishimura （Dept. Pediatrics, Kobe Univ. Grad. Sch. Med.）

P14-57 [English/Japanese]
Colorectal cancer: chemotherapy 16:45-17:30

Kazumasa Minami （Dept. Radonc., Osaka Univ., Grad. Sch. Med.）

P14-59 [English/Japanese]
Colorectal cancer (1) 16:45-17:30

Kazuhiro Morishita （Dept. of Med Sci., Fac. of Med., Univ. of Miyazaki）

P14-61 [English/Japanese]
Biliary tract cancer (1) 16:45-17:30

Tetsuo Ajiki （Dept. Surg., InterNatl. Clin. Cancer Res. Ctr., Kobe Univ.）

Room P(D) | 10F 1010+10-1&2, Osaka International Convention Center

P14-64 [English/Japanese]
Pancreatic cancer (1) 16:00-16:45

Koji Umeshita （Div.Health Sci., Osaka Univ.Med.Sch.）

P14-66 [English/Japanese]
Pancreatic cancer (3) 16:00-16:45

Atsushi Miyamoto （Dept. Surg., Natl. Hosp. Organization Osaka Natl. Hosp.）

P14-63 [English/Japanese]
Biliary tract cancer (3) 16:45-17:30

Shinichi Aishima （Path. & Microbiol., Saga Univ.）

P14-65 [English/Japanese]
Pancreatic cancer (2) 16:45-17:30

Yoshiki Murakami （Dept. Hepatology, Osaka City Univ）

P14-67 [English/Japanese]
Pancreatic cancer (4) 16:45-17:30

Yasuhiko Tomita （Dept. Pathol. Int. Univ. Health Welfare Sch. Med.）

Room P(E) | 12F Lobby, Osaka International Convention Center

P14-68 [English/Japanese]
Pancreatic cancer (5) 16:00-16:45

Hirofumi Akita （Dept. of Gastroenterological Surg., Osaka Univ. Grad. Sch. of Med.）

P14-70 [English/Japanese]
Pancreatic cancer (7) 16:00-16:45

Tadafumi Asaoka （Dept. Gastroenterological Surg., Grad. Sch. of Med., Osaka Univ.）

P14-72 [English/Japanese]
Lung cancer (2) 16:00-16:45

Takashi Kijima （Div. Resp. Med., Dept. Int Med., Hyogo College of Med.）

P14-74 [English/Japanese]
Lung cancer (4) 16:00-16:45

Takeshi Yoshida （Dept. Med. Oncology, Kindai Univ. Faculty of Med.）

P14-76 [English/Japanese]
Osteosarcoma 16:00-16:45

Tadashi Hasegawa （Dept. Surg. Pathol., Sapporo Med. Univ. Sch. Med.）

P14-78 [English/Japanese]
The development of novel therapies for soft tissue sarcomas 16:00-16:45

Yoshiyuki Suehara （Dept. Orthopedic Surg., Juntendo Univ.）

P14-69 [English/Japanese]
Pancreatic cancer (6) 16:45-17:30

Hidenori Takahashi （Dept. Surg., Osaka International Cancer Institute）

P14-71 [English/Japanese]
Lung cancer (1) 16:45-17:30

Tatsuro Okamoto （Dept. Thoracic & Breast Surg., Oita Univ.）

P14-73 [English/Japanese]
Lung cancer (3) 16:45-17:30

Nagio Takigawa （Dept. General Int. Med. 4, Kawasaki Med. Sch.）

P14-75 [English/Japanese]
Lung cancer (5) 16:45-17:30

Izumi Nagatomo （Dept. Respiratory Med. and Clin. Immunol., Osaka Univ., Grad. Sch. Med.）

P14-77 [English/Japanese]
Lung cancer (6) 16:45-17:30

Masakuni Serizawa （Drug Discovery & Development Div. Shizuoka Cancer Ctr. Res. Inst.）

P14-79 [English/Japanese]
The pathogenesis for soft tissue sarcomas 16:45-17:30

Miwa Tanaka （Div. Carcinogenesis, Cancer Inst, JFCR）

Room P(F) | 3F Lobby, RIHGA Royal Hotel Osaka

P24-1 [English/Japanese]
Cancer epidemiology (1) 16:00-16:45

Motoki Iwasaki （Natl. Cancer Ctr., Ctr. Pub. Hlth. Sci., Div. Epi.）

P24-2 [English/Japanese]
Cancer epidemiology (2) 16:45-17:30

Yusuke Takahashi （Dept. Surg., Osaka InterNatl. Cancer Institite）

Public Open Seminar | Hall A+B, Knowledge Capital Congrès Convention Center, Second Basement, North Building, Grand Front Osaka

CL [Japanese]
Public Open Seminar 15:30-17:50

Masaki Mori （Dept. Gastroenterological Surg. Osaka. Univ.）
Takuro Nakamura （Div. Carcinogenesis, Cancer Inst, JFCR）

Abstract

The 77th Annual Meeting of the Japanese Cancer Association

The Young Investigator Awards Lectures

Cancer Science Young Scientists Award Lectures

English Oral Sessions

Japanese Oral Sessions

Poster Sessions

Luncheon Seminars

Survivor Scientist Program

Public Open Seminar

Opening

CS Associate Editors' Meeting

Councilors' Meeting

General Assembly / Award Ceremony / Award Lectures

[CS1-1] CS1 [English]
Application of Artificial Intelligence (AI) to cancer research and clinical practice

2018 / 9 / 27 (Thu) 9:00-11:30 Room 1/5F Large Hall, Osaka International Convention Center Room 1

Hideo Baba / Dept. Gastroenterological Surg. Grad. Sch. of Life Sci. Kumamoto Univ.、Jun Sese / AIRC, AIST

Artificial intelligence (AI) is rapidly spreading in society, and news on artificial intelligence is reported daily. Applications of AI to cancer research and clinical practice are also highly anticipated because AI can find new targets of drugs, detect cancerous shades from X-ray/CT images, suggest personalized treatment from complete physical examination and so on. In this symposium, we invited five cutting-edge researchers, and the talks cover various applications such as prognosis prediction, cancer tumor detection and surgical care.
Furthermore, their contents are not only in basic research but also in real clinical and surgical applications. With these talks, we would like to discuss what AI can do for cancer patients shortly.

CS1-1
[Keynote] Intelligence, Autonomy and Connectivity in Future Surgery

Peter C Kim
Sch. of Engineering & Sci.

The benefits of less traumatic and enabling nature of minimally invasive and robot assisted surgery clearly represent a distinct transition point for surgery from an analog era to a digital realm. However, the impact and benefits of digital surgery are not fully realized given the current adoption rates only in 10-30 % range for any complex surgery. Newer generations of surgical technologies powered by machine learning, supra-human vision and dexterity however will however bridge the gap and usher in fully digital age improving patient safety and outcomes and enhancing surgeon and patient experience. Concepts and examples of how digital apps, machine vision, learning and intelligence are revolutionizing current surgery into proactive, predictive and personalized care through intelligence and connectivity. An example of Smart Tissue Autonomous Robot (STAR) will illustrate impact and benefits of intelligence and autonomy in improving surgical outcomes. This collaborative approach promises that future surgery can be improved with better outcomes, less complications, and open access to optimal surgical techniques, thus 'democratizing' surgery and creating a value for all patients.

▲TOP

CS1-2

An AI-based scoring system precisely predicts overall survival of breast cancer patients

Keiichi Nakayama
Dept. Mol. Cell. Biol. Med. Inst. Bioreg., Kyushu Univ.

Cancer is the leading cause of death in developed countries, with methods to better stratify susceptible individuals being actively pursued. Although many prognosis-predicting molecular scores for breast cancer have been developed, they are unable to predict overall survival and are applicable to only limited disease subtypes. Here we have comprehensively developed a complete atlas of prognostic genes based on an integrated meta-analysis of one of the largest assembled breast cancer cohorts. Artificial intelligence (AI)-based approaches established a universal molecular prognostic score (mPS) that relies on the expression status of only 23 genes and is independent of any clinical information. This scoring system is almost universally applicable to breast cancer patients, and it outperforms conventional clinical stage classification. We expect that our AI-based unbiased approach will not only facilitate appropriate treatment selection for breast cancer patients but also provide molecular insight into the complex nature of this disease.

CS1-3

Development of a Real-time Endoscopic Image Diagnosis Support System Using Deep Learning Technology in Colonoscopy

Masayoshi Yamada
Endoscopy Div., Natl. Cancer Ctr. Hosp., Div. Mol. Modification & Cancer Biol., NCC Res. Inst., Advanced Intelligence Project Ctr., RIKEN, Biostatistics Div., Natl. Cancer Ctr.

Co-author：Yutaka Saito[1]、Shigemi Yamada[2]、Hiroko Kondo[2]、Aya Kuchiba[3]、Ryuji Hamamoto[4]
[1]Endoscopy Div., Natl. Cancer Ctr. Hosp.、[2]Div. Mol. Modification & Cancer Biol., NCC Res. Inst., Advanced Intelligence Project Ctr., RIKEN、[3]Biostatistics Div., Natl. Cancer Ctr.、[4]Div. Mol. Mod. Cancer Biol., Natl. Cancer Ctr. Res. Inst., Cancer Transl. Res. Team, RIKEN Ctr. for AIP project

Background: Development of a real-time robust detection system for colorectal neoplasms will significantly reduce the risk of missed lesions during colonoscopy. We aimed to develop an artificial intelligence (AI) system that automatically detects early signs of colorectal cancer during a colonoscopy.Methods: A training data set of colonoscopic still and video images with a total of 5000 images of 2116 colorectal cancers or precursor lesions and 134,983 images of without lesions were provided to build a deep learning algorithms to learn features of the disease. Diagnostic accuracy and area under the receiver-operating characteristic curve (AUC) of this algorithm as well as processing speed were measured by validation set of 4,840 images.Results: The system achieved a 97.3% (95%CI 95.9-98.3) detection success rate and 99.0% (95%CI 98.6-99.2) specificity. The AUC was 0.975 (95%CI 0.964-0.986). The sensitivity were 98.0% in a polypoid sub-group, and 93.6% in a non-polypoid sub-group. The system analyzed all 4,840 images in 106.0 s (average, 21.9 ms/image). Conclusions: We have developed a real-time endoscopic image diagnosis support system using deep learning technology.

CS1-4

Clinical Sequence with Artificial Intelligence to Interpret Whole Genome Sequence and Multi Omics Data

Seiya Imoto
HIC, Inst. Med. Sci., Univ. Tokyo

Since 2011, we have been establishing systems for clinical sequence for cancers. Using technologies of next generation sequencing, we can have the information of several to tens thousands of mutations occurred in cancer genomes of a patient. To utilize this information to clinical site, interpretation for such huge number of mutations is a critical bottleneck. PubMed stores more than 25 million papers' abstracts. Furthermore, the number of papers, if we only focus on cancer research, rapidly increases; more than two hundred thousand every year. No one can read all, it is obviously beyond human ability. Therefore, in 2015, we started to use an artificial intelligence including IBM Watson for Genomics, and installed in our center in order to make clinical sequence more accurate, comprehensive and faster. In the presentation, we will introduce our achievements on cancer big data analysis and AI-based clinical sequence.

▲TOP

CS1-5

Predicting prognosis from MR images and genomic features in glioma with versatile machine-learning approaches

Jun Sese
AIRC, AIST, Div. Mol. Mod. Cancer Biol., Natl. Cancer Ctr. Res. Inst.

Co-author：Risa Kawaguchi[1]、Masamichi Takahashi[2]、Mototaka Miyake[3]、Koichi Ichimura[4]、Ryuji Hamamoto[5]、Yoshitaka Narita[2]、Manabu Kinoshita[6]
[1]AIRC, AIST, Div. Mol. Mod. Cancer Biol., Natl. Cancer Ctr. Res. Inst.、[2]Dept. NeuroSurg. & Neuro-Oncol., Natl. Cancer Ctr.、[3]Dept. Diagnostic Radiology, Natl. Cancer Ctr. Hosp.、[4]Div. Brain Tumor Translational Res., Natl. Cancer Ctr. Res. Inst.、[5]Div. Mol. Mod. Cancer Biol., Natl. Cancer Ctr. Res. Inst.、[6]Dept. NeuroSurg. Osaka InterNatl. Cancer Inst.

Preoperative prediction of prognosis of a patient with lower grade glioma (LrGG) and glioblastoma (GBM) can change the treatment strategy. Several genetic mutations and epigenetic silencing are known to be diagnostic markers or prognostic factors. However, these genetic statuses can be observed only after surgery. The magnetic resonance images (MRI) show the radiological features of tumor preoperatively, while the tumor classification from the images is not straightforward. Hence, a computational method to suggest the progression and prognosis from the images has been anticipated. We here introduce our radio-epigenomics project to predict genomic/epigenomic status (IDH mutation, MGMT methylation, etc.) of glioma patients from their MRI by machine-learning/deep-learning analysis. We used T1-weighted images (WI) with or without administration of contrast media, T2-WI and fluid-attenuated inversion recovery (FLAIR) sequences as well as genomic features captured from 93 GBM and 76 LrGG patients in National Cancer Center Japan. We show the current achievements by traditional feature-based machine-learning approaches as well as deep-learning approach with automatic feature extraction.

▲TOP

[CS2-1] CS2 [English]
Elucidation of tumor microenvironment for new cancer treatments

2018 / 9 / 27 (Thu) 13:00-15:30 Room 1/5F Large Hall, Osaka International Convention Center Room 1

Kohei Miyazono / Dept. Mol. Pathol., Grad. Sch. Med., the Univ. of Tokyo、Hideyuki Saya / Gene Regulation, IAMR, Keio Univ. Sch. Med.

Tumor microenvironments are highly heterogenous, consisting of cancer cells as well as various types of stromal cells, including fibroblasts, immune cells, and endothelial cells, and pericytes. Interaction between cancer cells and stromal cells play critical roles in cancer progression, and numerous studies on targeting tumor microenvironments for treatment of cancer have recently been reported. In this core symposium, Dr. Kinzler gives a plenary lecture, discussing the genetic landscape of human cancer, and its influence on tumor microenvironment and immunotherapy. In the symposium, the roles of TGF-beta signaling on tumor microenvironment, using a TGF-beta receptor inhibitor, will be discussed from various aspects. Heterogeneity of cancer-associated fibroblasts will be also discussed through the identification of some functional markers that define mesenchymal stem cells. Finally, tumor angiogenesis and lymphangiogenesis play important roles in tumor development, and identification of molecular markers for vascular endothelial cell markers and their functions will be presented.

CS2-1

[Keynote] The genetic landscape of human cancer as it relates to the microenvironment and therapy

Kenneth W Kinzler
The Johns Hopkins Kimmel Cancer Ctr.

The genetic landscape of a tumor can influence the tumor microenvironment and its therapeutic response. This is especially apparent in the response of tumors with high mutational burdens to immunotherapy. In this context, I will discuss the therapeutic implications of the genetic landscape of human cancers, the therapeutic benefit of immunotherapy for tumors with defective DNA mismatch repair and approaches for enhancing the immunotherapeutic responses of tumors with low mutational burden.

▲TOP

CS2-2

Targeting TGF-β signal: A key strategy to overcome resistance to cancer therapy

Seong Jin Kim
Precision Med. Res. Ctr., AICT, Seoul Natl. Univ.

TGF-β signaling inhibitors have shown promise in blocking the TGF-β-mediated tumor progression and metastasis, and enhancing antitumor immunity. Immune checkpoint inhibitors (ICI) have shown unprecedented clinical activity in several types of cancer. However, several ICIs largely target 'inflamed' tumor types. Increased TGF-β in the tumour microenvironment represents a primary mechanism of immune evasion. 'Stromal excluded' and 'immune desert' tumors are still areas where there is a huge unmet need. Stromal signature regulated by TGF-β pathway is one of the major mechanisms of tumor immune surveillance, leading to resistance to ICI. Moreover, TGF-β responsive signatures (TBRS) of stromal cells have been associated with poor prognosis. In this talk, I will talk about the recent progress of clinical trial of vactosertib, a potent, highly selective, oral inhibitor of TGF-β type I receptor (TGFBRI) that has shown promise as a drug candidate for the treatment of various solid tumors and hematological malignancies. *This work was supported by a KHIDI grant (HI14C2640) funded by the Korea government.

CS2-3

A role of undifferentiated mesenchymal stem cells in cancer progression

Atsushi Enomoto
Dept. Pathol., Nagoya Univ. Grad. Sch. Med.

Co-author：Masahide Takahashi
Dept. Pathol., Nagoya Univ. Grad. Sch. Med.

One of the features of cancers is the proliferation of cancer-associated fibroblasts (CAFs) that results in profound fibrosis in the stroma. Previous studies have shown that the production of extracellular matrix by CAFs leads to the stiffening of cancer tissues, which makes cancer cells more malignant to spread out to adjacent tissues and metastasize. CAFs also produce a number of growth factors to promote cancer cell proliferation and tumor angiogenesis. Recent studies on genetically engineered mouse models, however, have pointed out the possibility that there are diverse populations of CAFs, and some of them have a function to restrain, but not promote, cancer progression. We have been interested in the identification of functional markers that define mesenchymal stem cells (MSCs), and found that undifferentiated MSCs constitute a population of CAFs that restrain cancer progression. We also found that CAF diversity is also involved in the response of cancers to chemotherapies. In the symposium, we will discuss the clinical relevance of those findings, which will help us understand the tumor microenvironment and develop new approaches to treat cancer patients.

CS2-4

Roles of TGF-β signals during the progression of oral squamous carcinoma cells

Tetsuro Watabe
Dept. Biochem., Grad. Sch. Med. Dent. Sci., TMDU

Transforming growth factor-β (TGF-β) is abundant in tumor microenvironment and has been implicated in progression of multiple types of epithelial cancer,.While TGF-β elicits multiple effects on epithelial cancer cells, it remains to be elucidated whether there is a link between increased migration and cell cycle arrest induced by TGF-β. Here, we utilized SAS oral squamous cell carcinoma (OSCC) cells carrying Fluorescent ubiquitination-based cell cycle indicator (Fucci) system in order to study this correlation at a single cell level. We found that TGF-β induced cell cycle arrest and increased motility in SAS-Fucci cells. Interestingly, the SAS-Fucci cells residing in G1 phase were more motile than cells residing in S/G2/M phase suggesting a correlation between TGF-β-dependent cell cycle progression and migration. These results were confirmed by HSC4 OSCC cells carrying Fucci system. Furthermore, cDNA microarray analyses revealed that this elevated migration is not caused by TGF-β-induced EMT. These findings suggest that OSCC cells under cell cycle arrest are prone to metastasize, and can be a novel target of cancer therapy.

▲TOP

CS2-5

Discovery of vascular endothelial stem cell population and its impact on tumor angiogenesis

Nobuyuki Takakura
Dept. Signal Transduction, Res. Int. Microbial Disease, Osaka Univ.

Neo-vessel formation in adult is usually induced by sprouting angiogenesis from pre-existing blood vessels. During sprouting angiogenesis, at least three different types of endothelial cells (ECs) emerge to generate functional blood vessels, i.e., tip, stalk and phalanx cells. In embryo, arterial, venous, and lymphatic ECs are derived from mesoderm by distinct molecular mechanism and moreover, ECs should adapt tissue specific blood vessels such as liver sinusoid and glomerulus in kidney. These, including tip, stalk, and phalanx phenotype, indicate that ECs in vascular system need to acquire phenotypical and functional diversity. We previously reported that ECs in side population fraction (SP-ECs) by Hoechst method show highly proliferative property; however, stemness of those SP-ECs has not been elucidated. Recently, based on the profiling SP-ECs, we identified molecular markers to isolate vascular endothelial stem cells (VESCs) and this enabled us to understand the hierarchy of ECs and mechanism of rapid vascular regeneration. In this session, we will show the detail of VESCs and discuss its impact to consider the regulation of tumor angiogenesis.

CS2-6

Mechanisms of induction of cancer stem cells in the bone microenvironment ; involvement of drug resistance

Mitsuru Futakuchi
Nagasaki Univ. Grad. Sch. Biomed. Sci., Dept. Path., Nagasaki Univ. Hosp.

Previously, we have demonstrated that the ratio of cancer stem cells (CSCs), was higher in the bone microenvironment (ME) than those in the subcutaneous ME by our animal models. To identify the mechanisms for the induction of CSCs in the bone ME, we examine the effects on the induction of CSCs in the bone ME of therapeutic agents used for bone metastasis.Treatment of bone modifying agents, targeting osteoclasts, did not change the ratio of CSCs in the bone ME as well as subQ ME. Treatment of TGF β signaling inhibitor, significantly reduced the ratio of CSC in the bone ME. Interestingly, inappropriate treatments of chemotherapy targeting prostate cancer cells, significantly increased the ratio of CSCs in the bone ME in our model using androgen independent or dependent prostate cancer cells.In conclusion, our results indicated that bone MI would be good “niche” for CSCs, and TGF β signaling could be involved in the induction of CSCs in the bone MI. Because inappropriate treatment may induce the CSCs in the bone MI, anti-tumor agents combined with agents targeting CSC in the bone MI may plays a key role to eliminate the drug resistant bone metastasis of prostate or breast cancer.

▲TOP

[IS1-1] IS1 [English]
Single cell genomics of cancer

2018 / 9 / 27 (Thu) 9:00-11:30 Room 2/5F Small Hall, Osaka International Convention Center Room 2

Tatsuhiro Shibata / Lab. of Mol. Med., Human Genome Ctr., the Inst. of Med. Sci., the Univ. of Tokyo、Zemin Zhang / College of Life Sci., Peking Univ.

Understanding of tumor heterogeneity that may cause therapy resistance and metastasis is one of the critical issues in cancer biology and treatment. Since cancer tissues is a complex eco-system consisted of heterogeneous tumor cells including so-called cancer stem cells and other cell types such as immune cells, endothelial cells and stromal cells, molecular analysis at the single cell resolution would be essential. Recent rapid advances in single-cell genomics by a combination of single cell isolation/bar-coding, high-throughput sequencing/immuno-detection, and bioinformatics tools have enabled us to explore the cellular and functional diversity of each cell in cancer tissues. However, the single cell genomics technology is still in immature and further progress would be expected. In this international session, we invited six expert and active researchers in this field and would like to discuss the current status of single cell analytic technologies, data integrity and validation and future directions.

IS1-1

Single cell analysis of the tumor microenvironment

Zemin Zhang
BIOPIC & College of Life Sci., Peking Univ., Beijing, China

We performed deep single-cell RNA sequencing on thousands of single T cells isolated from peripheral blood, tumor and adjacent normal tissues from multiple cancer patients. The transcriptional profiles of these individual cells, coupled with assembled TCR sequences, enabled us to identify T cell subsets based on their molecular and functional properties, and delineate their developmental trajectory. Specific subsets such as exhausted CD8+ T cells and Tregs were preferentially enriched and potentially clonally expanded in HCC, and we identified signature genes for each subset. We have now expanded this study to include additional types of tumor and leukocytes, and found distinct patterns of clonal expansion, functional states and transition processes. Such compendium of transcriptome data provides valuable insights and a rich resource for understanding the immune landscape in cancers.

▲TOP

IS1-2

Single-cell gene expression analysis in cancer microenvironment

Shinichi Hashimoto
Grad. Sch. Med. Sci., Kanazawa Univ.

The phenotypic heterogeneity of cancer cells and their surrounding normal cells was observed in cancer tissue. In addition, cancer development is influenced by differential microenvironmental conditions such as hypoxia, immune infiltration. Recently, we have developed novel strategy of single-cell transcriptome analysis(Nx1-seq) for thousands of single cells to identification of different cell types in these conditions. In this study, we applied Nx1-seq analysis to characterize complex heterogeneous samples in the cancer tissue in the mammary carcinoma mouse model. Breast cancer is the most common of the gynecologic malignancies in the world. In spite of the high and increasing incidence of mammary carcinoma, these processes include tumor promotion and metastasis are unclear. In addition, novel molecular biomarkers are needed to assist clinical decisions. The Nx1-seq data showed the distinction of the cancer cell state with tumorigenicity, stem like cells and infiltrated leukocytes in the mammary carcinoma tissue and the metastatic region. Finally, single cell transcriptome analysis is a powerful approach for characterizing and understanding cellular diversity in cancer tissues.

IS1-3

The slow-growing sub-population of Lgr5-positive colon tumor stem cells is resistant to an anti-cancer drug treatment

Daisuke Shiokawa
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Co-author：Hirokazu Ohata、Koji Okamoto
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In this study, we analyzed the heterogeneity of Lgr5-positive stem cells of colitis-induced colon tumors in mice in order to understand the molecular mechanisms of colon carcinogenesis and drug resistances. Lgr5-positive stem cell populations from tumors were single-cell-sorted and their gene expression profiles were determined by RNA-seq. By processing the gene expression data by hierarchical clustering and a dimensionality reduction with t-SNE, we dissected the Lgr5-positive tumor epithelium into two different groups with distinct gene expression profiles. Gene Set Enrichment Analysis revealed that the two populations differ in their proliferation status and represented cycling and slow-growing stem cells. Importantly, the slow-growing stem cells showed a stronger tumor-initiating activity in a xenograft model. In accordance, we identified slow-growing stem cell populations in human PDX colon cancers by single-cell qPCR. Intra-peritoneal administration of Camptothecin significantly reduced the tumor volumes. Interestingly, the slow-growing stem cells were enriched in the remaining tumor tissues, indicating that they are resistant to the drug-treatment.

IS1-4

Understanding tumor microenvironment by single cell analysis

Woong-Yang Park
Samsung Genome Inst., Samsung Med. Ctr., Seoul, Korea

Co-author：Hae-Ock Lee、Dong-Hyun Park
Samsung Genome Inst., Samsung Med. Ctr., Seoul, Korea

Clinical responses to anticancer therapeutics underlie tumor heterogeneity. The range of tumor heterogeneity is widely variable in cancer patients. Although we can estimate the tumor heterogeneity in bulk tumor tissues, single cell analysis will provide more accurate information about the subclonality, tumor evolution as well as tumor microenvironment. Through single cell transcriptome profile, we could figure out the immune cell population in cancer tissue, which might determine the response to immunotherapy. We can also investigate stromal cells interacting with cancer and immune cells. We will discuss about the landscape of tumor microenvironment in various cancer types.

▲TOP

IS1-5

Single-cell RNA-seq reveals intratumor heterogeneity and interaction networks in nasopharyngeal carcinoma

Fan Bai
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Co-author：Shanzhao Jin、Ruoyan Li
Sch. of Life Sci., PKU, Biodynamic Optical Imaging Ctr., PKU

Nasopharyngeal carcinoma (NPC) is a common cancer with high prevalence in southern China and southeast Asia, highly associated with Epstein-Barr virus (EBV) infection. Previous studies have investigated the recurrent genomic alterations and aberrant regulatory programs of NPC. However, a detailed portrait of the interaction networks among EBV, malignant cells and immune cells is lacking. To gain a deeper insight into the cellular population and interaction networks in NPC, we applied SMART-seq2 and 10X Genomics chemistry to ~20k single cells from 8 NPC patients. Large-scale CNVs based on single cell expression profiles was analyzed and we revealed variable extent of intra-tumor heterogeneity among NPC tumors. Latent and lytic genes of EBV were detected mostly in malignant cells. Interesting, we deciphered several novel gene expression meta-signatures among malignant cells across NPC cases. Overall, our findings provide a comprehensive portrait of cellular ecosystem of NPC, revealing the unique interaction networks among EBV, malignant cells and immune cells at single-cell resolution.

IS1-6

Mechanisms of the clonal evolution of MDS as revealed by single-cell sequencing

Masahiro Nakagawa
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Co-author：Ryosaku Inagaki[1]、Yasuhito Nannya[2]、June Takeda[2]、Akinori Yoda[2]、Ayana Kon[2]、Tetsuichi Yoshizato[2]、Hideki Makishima[2]、Seishi Ogawa[2]
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The development and progression of myeloid malignancies are shaped by multiple rounds of acquisition of new driver mutations and subsequent clonal selection. However, the functional impacts of these mutations or combination/order of mutations on disease progression is poorly understood. Recently, we have developed a novel platform of single-cell sequencing that enabled simultaneous measurement of gene mutations and expression profiles at a single-cell level. Copy number (CN) status is also determined based on gene expression levels. Using this platform, we analyzed longitudinal samples from a patient with myelodysplastic syndromes (MDS) who was progressed to secondary acute myeloid leukemia (sAML). Mutations and CN status were successfully determined in hundreds of cells from distinct lineages at different time points, where mutations/CN abnormalities (CNAs) were tightly associated with unique expression signatures, allowing for the analysis of the impacts of different mutations/CNAs and their combinations on differentiation/proliferation of cells. In conclusion, single-cell sequencing provides a powerful platform to understand clonal evolution and intratumor heterogeneity of MDS.

▲TOP

[IS3-1] IS3 [English]
Genetic and epigenetic aberrations in gastric cancer

2018 / 9 / 27 (Thu) 13:00-15:30 Room 2/5F Small Hall, Osaka International Convention Center Room 2

Atsushi Kaneda / Dept. Mol. Oncology, Grad. Sch. of Med., Chiba Univ.、Patrick Tan / Programme in Cancer & Stem Cell Biol., Duke-NUS Med. Sch.

Cancer arises through accumulation of genetic and epigenetic aberrations. Recent comprehensive analyses have revealed that cancer is stratified into several molecular subtypes, exhibiting unique genetic and epigenetic profiles, which might be associated with its background environment and tumorigenic pathway. The tumorigenic environment of the stomach includes bacteria, virus, inflammation related with gastric reflux or the remnant stomach after gastrectomy, etc., and gastrointestinal cancer can also be stratified into several molecular subtypes through genomic and epigenomic analyses. In the International Session 3 “Genetic and epigenetic aberrations in gastric cancer”, we will discuss comprehensive genomic/epigenomic profiles of gastric cancer, functional genomic strategies, and genomic/epigenomic alterations related with the environments e.g. microbiota, virus, and the remnant stomach, which will help us uncover genesis of gastric cancer and establish new genomic/epigenomic therapeutic strategies.

IS3-1

Genomic and Epigenomic Alterations in Gastric Cancer

Patrick Tan
Programme in Cancer & Stem Cell Biol., Duke-NUS Med. Sch., Biomed. Res. Council, A*STAR, Cancer Sci. Inst. of Singapore, NUS

Gastric cancer remains a leading cause of global cancer mortality. Although the age-adjusted incidence of gastric cancer is declining, the absolute number of gastric cancer cases remains high due to overall aging of the patient population, particularly in Asia. A significant number of gastric cancer cases are also diagnosed at late clinical stages, where disease treatment is challenging due to high molecular heterogeneity and tumor evolution. In this presentation, I will describe recent work attempting to identify early steps in gastric cancer development, which may represent opportunities for early diagnosis and improve our understanding of stomach carcinogenesis. I will also present our attempts to identify new driver events in gastric cancer previously missed in earlier genomic analysis.

▲TOP

IS3-2

Genetic and epigenetic features of highly methylated subgroups of gastric cancer

Atsushi Kaneda
Dept. Mol. Oncol, Grad. Sch. Med., Chiba Univ.

Gastric epithelium acquires epigenomic aberrations to show unique phenotypes depending on pathogens. DNA methylome analysis stratified gastric cancer into several molecular subgroups, which exhibited different genetic features. The most apparent features included absence of TP53 mutation in highly methylated subgroups with MLH1 methylation or infection of Epstein-Barr virus (EBV), but its presence in the other subgroups. The MLH1-methylated cases all showed activating mutation of specific oncogene and specific frameshift mutation of genes in critical signal, which lead to bypass of senescence without TP53 mutation. The EBV(+) cases did not show these critical genetic mutations, but the extensive epigenomic alterations contributed to tumorigenesis. First, extensive DNA hypermethylation through TET2 repression silenced tumor suppressor genes. Second, enhancer activation by particular transcription factors upregulated groups tumorigenic genes. Third, loss of CTCF binding due to DNA hypermethylation altered interaction of the surrounding regions. Moreover, EBV episome was found to bind specific heterochromatin regions, leading to activation of silenced enhancers of tumorigenic genes.

IS3-3

Epigenomic and microbiota alterations in gastric cancer

Jun Yu
The Chinese Univ. of Hong Kong

Through the use of screening tools such as CpG island array and expression array, we have identified numerous novel tumor suppressor genes mediated by epigenetic dysregulation which are related to the disease initiation, progression and prognosis (PCDH10, DKK3, ZNF545, ADAMTS9, etc). In particular, we developed non-invasive diagnostic strategies of cell-free methylated DNA in blood as tumor-specific detection. The blood methylated RNF180 DNA detection kit has been established. Using bioinformatic tools, we integrated somatic mutational profiles and clinicopathologic information from 544 gastric cancers, and redefined gastric cancer into regular (86.8%) and hypermutated (13.2%) subtypes. A novel mutational signature can predict patient prognosis in regularly-mutated gastric cancer. We have demonstrated the microbial compositional changes and interactions across stages of gastric carcinogenesis. The significant enrichments and network centralities suggest the important roles of P. stomatis, D. pneumosintes, S. exigua, P. micra and S. anginosus in GC progression. Stronger interactions among gastric microbes were observed in Helicobacter pylori-negative samples.

IS3-4

Defined Life style and Germline Factors Predispose Asian Populations to Gastric Cancer

Akihiro Suzuki
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Gastric cancer (GC) is one of the leading causes of cancer mortality throughout the world, with the highest incidences in east-Asia. Although the somatic genetics of GC has extensively been characterized by the recent advances of cancer genome sequencing, the germline and environmental effects on GC and its ethnic differences have poorly been understood. Here, we performed genomic scale trans-ethnic analysis of 531 GC cases, by integrating east-Asian and public GC data sets. We discovered that a subgroup was strongly contributed by Signature 16 of COSMIC, whose contributions consisted of approximately 35% of somatic SNVs, and that almost all the patients of this cluster (15/16=93.8%) harbored a well-known inactive ALDH2 allele (rs671-AA or AG) and the Asian ethnic background. In addition There is one distinct GC subclass with clear alcohol-associated mutation signature and strong Asian specificity, and almost all the cases in the subclass are attributable to alcohol intake behavior and Asian-specific ALDH2 defective allele. This result revealed uncharacterized impacts of germline variants and their interplays with life styles in the high incidence areas.

▲TOP

IS3-5

Functional genomic strategies to identify therapeutic opportunities in gastrointestinal cancers

Ron Firestein
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[1]Ctr. for Cancer Res., Hudson Inst., Dept. Mol. & Translational Sci., Monash Univ.、[2]Dept. Res. Path., Genentech Inc.

Large scale efforts to systematically characterize the cancer genome have led to comprehensive annotation of the structural variations and mutations in cancer; nevertheless, complementary approaches are required to understand the functional consequences of these alterations. We are using integrative and functional genomic strategies to interrogate the dysregulated cancer genome and epigenome to identify new cancer pathways and therapeutic targets. In this talk, I will describe recent progress from our lab that illustrates the potential power of such approaches to dissect critical nodes of tumor dependencies and identify key oncogenic regulators in gastrointestinal malignancies.

IS3-6

DNA Methylation Genome-Wide Analysis in Remnant Gastric Cancer

Kiichi Sugimoto
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Purpose: Due to the importance of chronic inflammation in the carcinogenesis of gastric malignancies, we hypothesized that DNA promoter hypermethylation would play a critical role in the carcinogenesis of primary gastric cancer (PGC) and remnant gastric cancer (RGC). Methods: We investigated the genome-wide DNA methylation patterns of PGC and RGC tissues from 48 patients using the Infinium HumanMethylation450 Beadchip assay. The results were validated by qMSP in separate, independent cohorts.Results: We found that in our training cohort of 48 patients, genes from the gastric tissues identified by the Infinium HumanMethylation 450 Beadchip clustered into high and low methylation groups on multivariate analysis (p=0.004, OR=12.33). PGCs contributed significantly to the high methylation group suggesting that the promoter methylation status in PGC is higher than that in RGC. Supporting this conclusion was the finding that in a separate qMSP analysis in a test cohort, the gene A had significantly higher DNA promoter methylation in cancer tissues in the validation PGC tissues than in RGC. Conclusion: This study demonstrated that promoter methylation status in PGC is higher than in RGC.

▲TOP

[E-1049] E6 [English]
DNA replication / cell cycle / genomic instability (1)

2018 / 9 / 27 (Thu) 15:30-16:45 Room 2/5F Small Hall, Osaka International Convention Center Room 2

Natsuko Chiba / Can. Biol., IDAC, Tohoku Univ.

E-1049

Proper ATM activation mediated by lysine methyltransferases EHMT1/2 upon DNA damage: relevance to senescence and cancer

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In aging process, genomes are attacked by numerous genotoxic stresses, and thus DNA repair systems are essential for the genome integrity. Persistent DNA damage response (DDR) is activated in senescent cells, yet their precise activating mechanisms remain elusive. Here we identified euchromatic histone-lysine N-methyltransferase 1 (EHMT1) and EHMT2 as novel regulators of MDC1, which is a pivotal chromatin adaptor in DDR, via its methylation at lysine 45 facilitated by ATM signalling. This regulatory modification promotes the accumulation of activated ATM on damaged chromatin, including dysfunctional telomere, through their interaction. Given that EHMT1 and EHMT2 are downregulated in senescent cells, dysregulation of activated ATM might be required for survival of replicative senescent cells, which possess eroded telomeres and decompacted chromatin. On the other hand, EHMT2 is often overexpressed and associated with poor prognosis in several cancers. We would like to discuss the implication of our recent findings with relevance to senescent phenotype, and their possibility as therapeutic target against cancer.

▲TOP

E-1050

Splicing inhibitors with antitumor activity induce G2/M arrest through various mechanisms depending on the concentration

Daisuke Kaida
Dept. Gene Exp. Reg., Univ. of Toyama Sch. Med.

Pladienolide-B (Pla-B), a potent anti-tumor compound, is a splicing inhibitor that causes cell cycle arrest both at G1 and G2/M phases. However, the molecular mechanism of G2/M arrest remains unknown. In this study, we found that 10 ng/ml of Pla-B induced G2 phase arrest with no apparent cell shape changes. In addition, 10 ng/ml of Pla-B decreased M-phase cyclins, Cyclin A2 and Cyclin B1. Lower concentration of Pla-B (5 ng/ml) increased rounded cells, which seems to be in prometapahase with spindle formation defect. Treatment with further lower concentration of Pla-B (2 ng/ml) caused M-phase arrest with spindle formation, suggesting that 2 ng/ml of Pla-B inhibits chromosome separation. These results suggest that Pla-B inhibits cell cycle progression in G2/M phase through various mechanisms depending on the concentration of Pla-B. This finding will contribute to the development of a novel anti-cancer drug targeting splicing machinery. We will discuss the detailed molecular mechanisms of G2/M phase arrest by Pla-B treatment.

E-1051

Telomere shortening in stroma cells of recurrent pancreatic cancer

Yoko Matsuda
Dept. Path., Tokyo Metropolitan Geriatric Hosp.

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[1]Dept. Path., Tokyo Metropolitan Geriatric Hosp.、[2]Geriatric Path., Tokyo Metropolitan Inst. of Gerontology

Aims: This study aimed to investigate the clinicopathological features associated with recurrent pancreatic cancer. Methods: We performed quantitative fluorescent in situ hybridization to determine telomere length using pathological specimens from 12 patients who received a surgical resection of pancreatic cancer and underwent an autopsy. Results: Histological grading (well, moderately, and poorly differentiated), and mucin, p53, and SMAD4 expression levels were similar in the surgically resected pancreatic cancers and recurrent cancers at autopsy. We did not find dedifferentiation of pancreatic cancers in the samples from the recurrent cases in the present study, which suggests that pancreatic cancer has an aggressive phenotype even in its early stage. Stroma cells from autopsies showed telomere shortening while cancer cells maintained telomere length. Telomere length of cancer cells was negatively correlated to p53 expression. Conclusion: Pancreatic cancer cells can maintain telomere length because of telomerase expression while stroma cells cannot maintain telomere length. The aggressive phenotype of pancreatic cancer might be related to its poor prognosis.

E-1052

Genetic inactivation of ATRX can induces ATM dependent DNA damage response in neuroblastoma (NB) cells

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ATRX is a tumor suppressor gene, and its recurrent somatic mutations associated with stage 4 NB patients. But our knowledge about ATRX mutation in NB tumorigenecity is still limited. Previously, ATRX knockout (KO) clones in NB cells by CRISPR/Cas9 system was established and interestingly, the KO clones proliferate slowly compared to control group and become differentiated. Microarray based GSEA analysis showed that gene-set related to DNA double-strand break (DSBs) repair, DNA damage response, negative cell cycle regulation, G2M checkpoint and, p53 pathway activation were induced in ATRX KO cells. By in vitro analysis, ATRX loss results in augmentation of gamma-H2AX, a canonical marker for DSBs, indicate the accumulation of endogenous DNA damage in ATRX KO cells. Furthermore, accumulation of DNA damage results in activation of the ATM/Chk2/p53 pathway, leading to cell cycle arrest. We also observed the positive correlation between ATRX mutation and ATM/p53 pathway inactivation in high-risk NB by multiomics database analysis, indicating that ATM/p53 pathway inactivation is required for ATRX mutation-related NB tumorigenesis.

▲TOP

E-1053

Effects of 5-Aminosalicylic Acid on apoptosis-induced by Hyperthermia in Human Oral Squamous Cell Carcinoma cells

Rohan Moniruzzaman
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An anti-inflammatory drug; 5-aminosalicylic acid (5-ASA) has shown a potent anticancer effect against colorectal cancer. Hyperthermia (HT) allowing for effective and sustained targeted delivery of anti-cancer agents. However, there is no study conducted to investigate the anti-tumor effects of combination therapy between 5-ASA and HT. Here, we evaluated the potential of 5-ASA on HT-induced apoptosis using human oral squamous cell carcinoma (OSCC) cells. Induction of apoptosis was synergistically enhanced when 5-ASA used in combination with HT compared to either treatment alone. It was confirmed by Annexin V-FITC/PI staining using flow cytometry. We also found 5-ASA markedly enhanced HT-induced intracellular reactive oxygen species (ROS) generation. In addition, the loss of MMP, intracellular calcium ions concentration, expression of several apoptotic-related proteins including caspase family and FAS-receptor, and ER stress marker were increased following combined treatment with 5-ASA and HT than alone. In conclusion, the present study demonstrates the first evidence that 5-ASA in combination with HT could represent an effective therapeutic approach for the treatment of human OSCC.

E-1054

TERT-ADAR1 interaction: heterochromatin regulation mediated by RNA editing

Marco Ghilotti
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RNA editing is a kind of posttranscriptional modification that is conserved across species. Adenosine (A) to inosine (I) conversion is the most prevalent type of RNA editing in mammals; the A-to-I editing is mediated by adenosine deaminase acting on RNA (ADAR), which specifically deaminates adenosine on double-stranded RNA (dsRNA) to inosine. We have reported that human telomerase reverse transcriptase (TERT) has an RNA-dependent RNA polymerase (RdRP) activity and synthesizes dsRNA. This activity is enriched in mitotic phase and since dsRNA is selectively edited by ADAR1, we hypothesized an interaction between TERT and ADAR1 mediated by TERT-RdRP activity products. To investigate functional association of TERT-RdRP activity with ADAR1, we performed in vitro RdRP assay and found that wild type ADAR1 increases the RdRP products generated by TERT, suggesting a functional role of ADAR1 enzymatic activity to enhance TERT-RdRP activity. Interestingly, suppression of ADAR1 expression as well as inhibition of the TERT-RdRP activity upregulated heterochromatic transcription, indicating biological significance of the TERT-ADAR1 interaction in maintenance of heterochromatin in human cells.

▲TOP

[S1-1] S1 [English]
Metabolic mechanisms in cancer and normal cells

2018 / 9 / 27 (Thu) 9:00-11:30 Room 3/10F 1003, Osaka International Convention Center Room 3

Hiroyasu Esumi / Res. Inst. Biomed. Sci, Tokyo Univ. Sci、Hozumi Motohashi / IDAC, Tohoku Univ.

Cancer metabolism has generated a lot of excitement in the past few years. The discovery of mutations in the metabolic enzymes isocitrate dehydrogenase (IDH) 1 and 2 genes in several types of gliomas have connected cancer genetics and biochemistry and invigorated the “well-established” field of biochemistry. A word “oncometabolite” was invented describing a unique metabolite that is produced by cancer cells. Together with rapid technical advances in the analytical devices, novel oncometabolites have been described, and their contributions to carcinogenesis and cancer evolution are now under active investigation. Intervention of the metabolic pathways is now considered as one of the promising therapeutic strategies. In addition, metabolism has turned out to be an important regulatory node for an efficient immunothrerapy of cancers. This symposium focuses on the new aspects of cancer metabolism as well as metabolic regulation in the immune cells that are responsible for the anti-cancer immunity.

S1-1
Multi-omics Analysis of Colorectal Cancer Metabolism

Tomoyoshi Soga
Inst. Adv. Biosci., Keio Univ., AMED-CREST

Cancer cells alter their metabolism for the production of precursors of macromolecules. However, the control mechanisms underlying this reprogramming are poorly understood. Here, we show that metabolic reprogramming of colorectal cancer is caused chiefly by aberrant MYC expression. Multi-omics-based analyses of paired normal and tumor tissues from 275 patients with colorectal cancer revealed that global alterations in metabolite and metabolic gene expression levels occur at the adenoma stage of carcinogenesis, in a manner not associated with specific gene mutations involved in colorectal carcinogenesis. We found that MYC expression induced at least 215 metabolic reactions. Further, MYC negatively regulated the expression of genes involved in mitochondrial biogenesis and maintenance but positively regulated genes involved in DNA and histone methylation. Knockdown of MYC in colorectal cancer cells reset the altered metabolism and suppressed cell growth. Moreover, inhibition of MYC target pyrimidine synthesis genes such as CAD, UMPS and CTPS blocked cell growth, and thus they are potential targets for colorectal cancer therapy. [Ref] K. Satoh, et al. PNAS, 2017, 114, E7697-E7706.

▲TOP

S1-2

Metabolic Alterations Promotes Tumor Progression under Amino Acids deprivation

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Tumor microenvironments such as hypoxia and nutrient starvation are critical for tumor progression; however, the role of the amino acids deprivation in cancer cells under is not fully established. Here we show that decreased phosphatyldyl-ethanolamine (PE) biosynthesis by down-regulation of phosphate cytidylyltransferase 2 (PCYT2), the rate-limiting enzyme for PE biosynthetic pathway, are regulated by amino acid deprivations. Comprehensive metabolomics approach revealed that an pro-tumor metabolite PEtn was the most accumulated metabolites in cancer cells under starvation by down-regulation of PCYT2. Exogenous administration of PEtn increased cell growth under starvation in vitro and tumor growth in vivo. Moreover, inhibition of PCYT2 accumulated PEtn and stimulated tumor growth. In contrast, overexpression of PCYT2 suppressed accumulation of PEtn and tumor growth. Furthermore, down-regulation of PCYT2 associated with accumulation of PEtn was observed in tumor tissues of breast cancer patients leading to poor prognosis. Together, these results suggest the down-regulation of PCYT2 and accumulation of PEtn leading to tumor progression through amino acids deprivation.

S1-3

The roles of sphingosine kinases for metabolic regulations in breast cancer cells

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A pleiotropic bioactive lipid mediator, sphingosine-1-phosphate (S1P), produced by sphingosine kinases (SphK1 and SphK2) regulates many physiological and pathological processes. Previously we demonstrated that S1P and SphKs play important roles in breast cancer progression in animal models (Cancer Res 2018). Moreover, we have recently reported that expression of SphK1 associates with worse prognosis of breast cancer patients (J Surg Res 2017). Based on these findings, we hypothesized that SphKs regulate cancer cell-specific metabolism, which related to the cancer cell proliferation and survival. Proliferation assays revealed significantly less proliferation of SphK1 knockout (KO) E0771 cells compared to the control cells. On the other hand, SphK2KO E0771 cells showed significantly more proliferation than the control. Capillary electrophoresis time-of-flight mass spectrometry analysis revealed the metabolomics profiles of both SphK1KO and SphK2KO E0771 breast cancer cells, which were dramatically changed in the glycolysis pathway and TCA cycle compared to the control cells. Our data suggests that SphKs play an important role in cancer specific metabolism.

S1-4

O-GlcNAcylation Signal Mediates Proteasome Inhibitor Resistance in Cancer Cells by Stabilizing NRF1

Hiroki Sekine
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Co-author：Keito Okazaki、Hozumi Motohashi
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Cancer cells often heavily depend on the ubiquitin-proteasome system (UPS) for their growth and survival. Irrespective of their strong dependence on the UPS, cancer cells, except for multiple myeloma, are resistant to proteasome inhibitors. A major cause of this resistance is the "proteasome bounce-back response" mediated by NRF1, a transcription factor that coordinately activates proteasome subunit genes. To identify new targets for efficient suppression of UPS, we explored nuclear proteins cooperating with NRF1 and obtained O-GlcNAc transferase (OGT)/Host Cell Factor C1 (HCF-1) complex. O-GlcNAcylation catalyzed by OGT was essential for NRF1 stabilization and consequent upregulation of proteasome subunit genes. Meta-analysis of breast and colorectal cancers revealed positive correlations in the protein abundances of OGT and proteasome subunits. OGT inhibition successfully enhanced anti-tumorigenic activity of a proteasome inhibitor. Our study has clarified a novel linkage between O-GlcNAcylation and UPS function and suggested OGT as a therapeutic target for proteasome inhibitor-resistant cancers.

▲TOP

S1-5

Ferroptosis in Cancer Research

Shinya Toyokuni
Dept. Pathol Biol Responce, Grad. Sch. Med., Nagoya Univ.

Iron is a fundamental metabolic element, but excess iron is associated with carcinogenesis. Ferric nitrilotriacetate-induced renal cancers in wild-type rats reveal similar genetic alterations to those in humans. Ferroptosis is defined as a form of regulated necrosis, characterized by lipid peroxidation through high iron/sulfur(antioxidants) ratio. Considering that cancer cells retain more catalytic Fe(II) than non-tumorous cells, many carcinogenesis models, including asbestos-induced mesothelioma, suggest that cancer is a state of iron addiction with ferroptosis-resistance. Non-thermal plasma (NTP) is a novel physical technique that can directly load oxidative stress, where HNE may be a marker. We found that NTP exposure is highly dependent on Fe(II) in situ, causing cancer cell-specific ferroptosis, which was associated with autophagy activation and lysosome genesis. Lastly, we discuss the effects of phlebotomy as cancer prevention and the use of NTP as novel cancer therapy. References: Toyokuni S et al., FRBM 108: 610, 2017; Shi L et al., FRBM 108: 904, 2017; Stockwell BR, et al. Cell 171: 273, 2017.

S1-6

PPAR-induced fatty acid oxidation in T cells ameliorates the antitumor activity of PD-L1 blockade

Kenji Chamoto
Dept. Imm. Genom., Schol. Med., Kyoto Univ.

Co-author：Tasuku Honjo
Dept. Imm. Genom., Schol. Med., Kyoto Univ.

Although cancer immunotherapy has shown potential for a wide range of cancer patients, its efficacy is limited partly because of the insufficiency of effector cytotoxic T lymphocytes (CTLs) due to their loss via apoptosis. We previously demonstrated that mitochondrial activation chemicals had synergistic effects with a PD-1-blockade antibody in a mouse tumor model. In the current study, we examined the molecular mechanism of the synergistic effects of bezafibrate, a peroxisome proliferator-activated receptor (PPAR) agonist, which was shown to enhance the tumoricidal effects of PD-1 blockade. Bezafibrate activated mitochondrial activities by modifying the overall metabolic state of CTLs, and promoted the proliferation of effector CTLs from naive T cells. In addition, bezafibrate induced augmentation of fatty acid oxidation and expression of carnitine palmitoyl transferase 1 (Cpt1), which upregulates Bcl2 expression to prevent apoptosis in killer T cells. Together, these results indicate that bezafibrate increases the number of functional CTLs by activating mitochondrial metabolism and cellular metabolism, leading to enhanced anti-tumor immunity during PD-1 blockade.

▲TOP

[LS1] LS1 [Japanese]

Innovative environmental infection control measures with low concentration chlorine dioxide gas

2018 / 9 / 27 (Thu) 11:50-12:40 Room 3/10F 1003, Osaka International Convention Center Room 3
：Taiko Pharmaceutical Co., Ltd

Morito Monden / Sakai City Hospital Organization / The Cancer Institute Hospital of Japanese Foundation For Cancer Research

LS1

Innovative environmental infection control measures with low concentration chlorine dioxide gas

Takashi Shibata
Japan Chlorine Dioxide Industry Association

No Abstract

▲TOP

[SS1-1] SS1 [Japanese]
Progress in basic research and clinical medicine over the last decade

2018 / 9 / 27 (Thu) 13:00-15:30 Room 3/10F 1003, Osaka International Convention Center Room 3

Tadamitsu Kishimoto / Immunol. Frontier Res. Ctr., Osaka Univ.、Morito Monden / The Japan Med. Sci. Federation

この10年癌研究は基礎、臨床共に飛躍的に進展し、その成果は今新しい癌治療として利用されている。
その第1は癌の免疫治療の進展でありチェックポイント療法とCAR-T治療である。癌の治療法として外科治療や化学療法と並んであるいはそれ以上に現在免疫治療が重要であることが認識されるようになった。
もう1つは癌細胞の遺伝子を詳細に解析することにより分子標的治療も生まれたし、1つ1つの癌に最も適した化学療法を行うという方向になってきた。外科治療においても患者に大きな負担をもたらさない内視鏡治療が主流になりつつある。
この特別シンポジウムではこれらの問題について現在我が国の第1人者であり世界的にもよく知られた演者の先生方に語ってもらう予定である。

SS1-1
Cancer immunotherapy by PD-1 blockade

Tasuku Honjo
Kyoto Univ. Inst. for Advanced Study

Although immunotherapy by PD-1 blockade dramatically improved the survival rate of cancer patients, further improvement of the efficacy is required to reduce a fraction of less sensitive patients. In mouse models of PD-1 blockade therapy we found that tumor-reactive cytotoxic T lymphocytes (CTLs) in draining lymph nodes (DLNs) carry increased mitochondria mass and more reactive oxygen species (ROS). We showed that ROS generation by ROS precursors or indirectly by mitochondrial uncouplers synergized the tumoricidal activity of PD-1 blockade by expansion of effector/memory CTLs in DLN and within the tumor. These CTLs carry not only activation of mTOR and AMPK, but also increment of their downstream transcription factors such as PGC-1 and T-bet. Furthermore, direct activators of mTOR, AMPK or PGC-1 also synergized the PD-1 blockade therapy whereas none of above mentioned chemicals alone had any effects on tumor growth. These findings will pave a way to develop novel combinatorial therapies with PD-1 blockade.

▲TOP

SS1-2

CAR T cell therapy

Naoki Hosen
Dept. Cancer Stem Cell Biol., Osaka Univ. Sch. Med.

Chimeric antigen receptor (CAR) T cell therapy is a novel immunotherapy. A CAR combines antigen-binding domains derived from the antibodies with the signaling domains of the TCR ξ chain and additional costimulatory domains from receptors such as CD28 or 41BB. CARs are transduced into T cells ex vivo, creating expandable antigen-specific CAR T cells. On the basis of dramatic efficacy in clinical trials, CAR T cells targeting CD19 have recently been approved by the U.S. Food and Drug Administration (FDA) for treatment of B cell leukemia and lymphoma. Although serious cytokine release syndrome (CRS) associated with CAR T cell therapy was a major concern, tocilizumab has been proved to suppress CRS without impairing anti-tumor effect. Now many researchers aims to expand CAR therapy to other types of cancers. However, appropriate target antigens are lacking in many cancers. Recently, we discovered that the active conformer of an integrin could serve as a specific therapeutic target for multiple myeloma (MM), suggesting that more cancer immunotherapeutic targets can be identified in cell surface proteins that undergo conformational changes.

SS1-3

Chromosome rearrangements and molecularly targeted therapy

Hiroyuki Mano
Natl Cancer Ctr. Res Inst.

Cancer-specific chromosome anomaly was first discovered in Philadelphia, USA in 1959. Philadelphia chromosome thus named is t(9;22) specifically detected in chronic myeloid leukemia, and juxtaposes BCR gene to ABL1 tyrosine kinase gene. Following this discovery, a plethora of disease-specific chromosome rearrangements have been described in hematological malignancies. These transformation mechanisms had been widely thought to be hematological disorder-specific until the discovery of TMPRSS2-ERG fusion gene in prostate cancer in 2005 and that of EML4-ALK fusion gene in lung adenocarcinoma in 2007. Our discovery of the latter gene was especially important, since the product is a fusion-type tyrosine kinase just like BCR-ABL1. ALK inhibitors have thus been developed, and one of such compounds, crizotinib, became approved in US FDA in 2011, only 4 years after the discovery. Extensive research also took place to find other fusion-type kinases in solid tumors, indeed leading to the isolation of fusion products of ROS1, RET, NTRK, FGFR and others. Thus, our understanding on the role of fusion oncogenes in solid tumors has been drastically changed in the last decade.

SS1-4

On the Origin of Human Cancer

Seishi Ogawa
Dept. Pathology & Tumor Biology, Kyoto Univ.

Cancer is thought to comprise a heterogeneous population of neoplastic cells having a complex hierarchical structure in terms of gene mutations. According to recent studies, the entire cancer hierarchy by itself might be further embedded in higher level hierarchies that are recursively generated by multiple rounds of clonal selections, where acquisition of driver mutations plays a central role. Thus, before a cancer develops, many independent, precancerous populations of clones are presumed to be present within apparently normal tissues. In fact, clonal outgrowth in physiologically normal tissues has recently been reported in blood and skin, in which common driver mutations of myeloid malignancies or skin cancers found in aged healthy individuals are implicated in the development of respective cancers. However, it is poorly understood how those clones evolve from early adult life to the end of life span in terms of their frequency and size and how its dynamics is affected by environmental/genetic factors to contribute to cancer. In this session, recent finding on early development of leukemia and other solid cancer will be presented and discussed.

▲TOP

SS1-5

A 10 years progress in minimal invasive surgery

Seigo Kitano
Oita Univ.

It has been 30 years since endoscopic surgery was introduced in Japan. As someone who have directly been involved in establishing evidences, development of various new devices and procedures, as well as medical education in emerging Asian countries, I would like to present some of my experience in the last 10 years of laparoscopic surgery. For early gastric cancer surgery, laparoscopy has been shown to be one of the preferred options since it was introduced in 1991. Moreover, its positive outcome has also been shown to be equal to open surgery even for moderately advanced cancer. In colorectal cancer, laparoscopic surgery has been an important option in each stage. Bariatric surgery has become conspicuous as a new field for laparoscopy. We have also seen introduction of new devices and their great improvements in the last 10 years. Furthermore, the surgical skill qualification system established by JSES has been introduced for various countries as a leading country of endoscopic surgery. Therefore, we have been active in international cooperation with Asian countries such as Thailand, Russia, and Saudi Arabia, all of which have active support by the governments.

▲TOP

[SP1-1] SP1 [Japanese]
How to survive hard science society

2018 / 9 / 27 (Thu) 15:30-17:00 Room 3/10F 1003, Osaka International Convention Center Room 3

Nobuyuki Onishi / Div. Gene Reg. IAMR, Keio Univ. Sch. of Med.、Kentaro Kajiwara / Dept. Oncogene, RIMD, Osaka Univ.

本特別企画『研究格差社会をどう生きるか」は、がん研究に限らず様々な領域にて世界をリードする若手研究者がどのようにして独自のフィールドを生み出してきたか、さらに研究社会に生じつつある「格差」を若手がどう生き抜いていくべきかを議論する聴衆参加型のパネルディスカッション企画です。
パネリストとして、近年注目度が高い研究分野（ゲノム・がん不均一性、人工知能・ビッグデータ、疫学、バイオイメージング）において世界の第一線で活躍中の新進気鋭の若手演者が登壇し、各分野における「研究格差」の現状を説明します。その後、クラウドシステムを駆使した全聴衆が参加する大討論会にて、「研究格差社会」を打破するためのbrainstormingを試みます。本企画の演者は癌学会の参加者全員です。研究格差社会から派生する研究費、ジョブハント、共同研究など皆が抱える様々な問題の解決策を模索しましょう！
刻々と変化する研究情勢の中、我々がん研究者がどのよう方向に進むことで活路が見いだせるのか、日本のがん研究の未来も含めて考えてみましょう。

SP1-1

Speaker：Kazuhiro Aoki

1979年8月生まれ。A型。
2002年名古屋大学理学部物理学科卒業、2004年大阪大学医学系研究科医科学修士修了、2007年大阪大学大学院医学系研究科博士課程を修了、博士（医学）取得。京都大学大学院生命科学研究科研究員、助教、さきがけ研究員（兼任）、講師、京都大学大学院医学研究科特任准教授を経て、2016年自然科学研究機構　岡崎統合バイオサイエンスセンター/基礎生物学研究所教授。2018年4月から岡崎統合バイオサイエンスセンターが生命創成探究センターに改組し、所属変更。

得意なこと：生細胞イメージング、とくにFRETイメージングなど蛍光タンパク質を利用したプローブの開発と応用が得意です。最近は、光遺伝学的な手法による細胞内シグナル伝達系の操作にも取り組んでいます。Ras-ERK経路やPI3K-Akt-mTOR経路とそれらの表現型が研究対象です。最近は細胞周期の研究も始めました。癌細胞がもつ頑健性と脆弱性のバランスに興味があります。

▲TOP

SP1-2

Statistical Genetics and my research

Yukinori Okada
Dept. Stat. Genet., Grad. Sch. Med. Osaka Univ.

Statistical genetics is a research field that evaluates causality of human genetic variations on diseases, using statistical and bioinformatics approaches. Recent developments of sequencing technologies have provided human disease genome data of hundreds of thousands of the subjects, and successfully identified comprehensive catalogues of genetic susceptible loci. However, little is known regarding how to develop methodology to integrate large-scale human genome data with diverse biological resources, to which statistical genetics should contribute. We have developed such methods and applied to a pioneering example of large-scale genetic association studies on a variety of human complex traits. As our efforts to develop young researchers, we hold 'Summer School of Statistical Genetics in Osaka University'.

SP1-3

Atsuo T. Sasaki
University of Cincinnati, College of Medicine: Dept. of Internal Medicine　Associate Professor

GTPエネルギー代謝の研究をしています。GTPギークとして研究を続けるなか、GTPエネルギーのセンサーを最近発見し、GTPエネルギー代謝は癌の増殖維持に特に重要であることを見いだし、発見したセンサーの先に広がるGTPエネルギー代謝機構の存在と細胞増殖との関係を、世界に先駆けて明らかにしています。

SP1-4

A. Etsuo Susaki
The Univ. of Tokyo

「研究格差社会をどう生きるか」とのことですが、研究は尖って（格差を作って）ナンボというのが一番重要な部分だと思いますので、「研究で格差を作ってどう生きるか」というのが要するに本シンポジウムの趣旨だと私は思っています。どれだけ尖れるか、というのは、「問題設定×戦略×高い専門性と技術×メタ視点とゼロベース思考×深い議論×没頭できる時間とお金×他の専門家を巻き込めるコミュ力とバイタリティ×運」あたりの掛け算の結果でしょうか。尖りかけな時期はわりと死に物狂いです。尖った後はもっとそうだと思います。失敗のパターンに比べると成功のルートはとても多いそうなので、私の事例がどれほど参考になるか（そもそも成功事例になるのか）わかりませんが、今の成果が幸いにも得られた環境や要素を振り返りながら、思い当たることをいくつか紹介してみたいと思います。

▲TOP

SP1-5

Interdisciplinary collaboration among young investigators from public health viewpoint

Hirokazu Takahashi
Natl. Cancer Ctr., Div. Cancer Statistics Integration

Co-author：Kota Katanoda[1]、Taichi Shimazu[2]、Tomohiro Matsuda[3]、Itsuro Yoshimi[4]、Eiko Saito[1]、Megumi Hori[1]、Atsushi Goto[5]、Yosuke Uchitomi[6]
[1]Natl. Cancer Ctr., Div. Cancer Screening Assessment & Management、[2]Natl. Cancer Ctr., Div. Prevention、[3]Natl. Cancer Ctr., Ctr. for Cancer Registries、[4]Natl. Cancer Ctr., Div. Tobacco Policy Res.、[5]Natl. Cancer Ctr., Div. Epidemiology、[6]Natl. Cancer Ctr., Ctr. for Public Health Sci.

公共政策の実践においては、根拠に基づく政策立案（evidence based policymaking）や体系的・横断的な検討方法の確保に加え、科学的根拠を創出・伝達する役割間の連携を持続的に行うことが不可欠となる。特にがん対策は、科学的根拠の進歩が速く、受け手のニーズも多様化していることから、新たな対策を生み出し実践するためには、さまざまな分野における知識と経験が集積することが望まれる。国立がん研究センターにおいては、がんに特化した臨床及び研究・事業・教育を行っているが、分野横断的に議論する場を設ける試みにより、公衆衛生科学に関する新たなプロジェクトが生まれている。本シンポジウムでは、そのプロセスついて紹介するとともに、将来的に分野の垣根を超えた研究・事業の体制構築を見据えた、多角的な議論を行いたい。
In the practice of public policy, it becomes indispensable that sustainable collaboration between roles of create and disseminate a scientific evidence, in addition to ensuring evidence based policy making and cross-sectional examination methods. Especially for cancer control, scientific evolution advances quickly and recipient's needs are diversifying, so it is desirable that knowledge and experience accumulated in various fields in order to create and practice for new counter measures. At the National Cancer Center, we are conducting clinical and basic research, projects and education specialized in cancer, based on this setting new projects from public health viewpoint are born by attempts to establish a cross-sectional discussion of young investigators. We will introduce the process, and would like to make a multifaceted discussion with a view to make a structure beyond boundaries of each fields in the future.

SP1-6

Hirohiko Niioka
Osaka Univ.

新岡は現在、深層学習を用いて病理画像やCT画像など様々な医療画像の分類や解析を行う研究に従事している。具体的な応用として、例えば、iPS細胞などの分化やクオリティーなどをモニタリングする深層学習アルゴリズムを開発し、再生医療用の細胞培養の自動化およびコスト削減に関する研究や、CT画像から癌細胞の浸潤度を予測する研究を行なっている。また、新規顕微鏡による病理画像データベース構築と深層学習を組み合わせ、HE染色に代わる新たな病理診断技術の構築や、イメージング時のノイズを削減およびイメージングに必要な時間を短縮する深層学習アルゴリズムの開発に携わる。さらに、AI人材育成に関しても精力的であり、所属部署における社会人教育プログラムに携わる一方で、阪大内外でAIメディカル研究会を立ち上げて主に医学部や工学部の学生を対象として人工知能に関する勉強会を開催している。AIメディカル研究会ではkaggleなどの国際コンペティションでメダルを獲得する学生を輩出しつつ、高校生を対象としたプログラミングの教育イベントを開催している。

SP1-7

Itoshi Nikaido
RIKEN

筑波大学 グローバル教育院 ライフイノベーション学位プログラム 生物情報領域 教授 (協働大学院)

▲TOP

[E-1001] E9-1 [English]
DNA methylation / chromatin structure

2018 / 9 / 27 (Thu) 9:00-10:15 Room 4/10F 1001, Osaka International Convention Center Room 4

Takehiko Kamijo / Res. Inst. Clin. Oncol. Saitama Cancer Ctr

E-1001

Glutamine induced transcriptional regulation in cancer cells metabolism

Muyassar Anwar
Genome Sci. RCAST Univ. of Tokyo

Co-author：Hiroyuki Aburatani[1]、Tsuyoshi Osawa[2]
[1]Gen. Sci. Div., RCAS, Univ. of Tokyo、[2]Nutriomics Oncl. RCAST Univ. of Tokyo

Cancer cells within solid tumors are often exposed to extreme microenvironments, such as hypoxia, acidosis and nutrient deprivation. However, effects of glutamine under nutrient starvation on gene expressions in cancer cells are not fully understood. Here we examined how glutamine affects transcriptional regulation in cancer cells using comprehensive genomic analysis. We examined mRNA expression and histone modification to reveal glutamine responsive genes under nutrient starvation medium when supplemented with 4mM of glutamine. We predicted transcriptional regulators for glutamine inducible genes under nutrient starvation by integration of transcriptome analysis and motif analysis of H3K27Ac marks. Under growth rich (control), nutrient starvation and nutrient starvation supplemented with glutamine, motif analysis suggested potential transcriptional regulators for glutamine responsive genes. Taken together, glutamine deprivation affects gene expressions through glutamine-responsive transcription factors in cancer cells.

▲TOP

E-1002

Cooperative epigenetic remodeling by TET2/NRAS mutation drives myeloid transformation and MEK inhibitor sensitivity

Hiroyoshi Kunimoto
Dept. Hematol., Yokohama City Univ., Sch. Med.

Co-author：Hideaki Nakajima
Dept. Hematol., Yokohama City Univ., Sch. Med.

Ten-Eleven-Translocation 2 (TET2) and NRAS mutations are frequently mutated and often co-occur in myeloid malignancies. However, how these mutations cooperate to cause myeloid leukemia is not fully understood.
To assess if Tet2 loss and Nras mutation cooperate in myeloid transformation, we generated mice with both disease alleles in hematopoietic cells ($Tet2^{-/-}Nras^{G12D}$). These mice developed lethal chronic myelomonocytic leukemia-like disease in vivo. In western blot and phospho-flow analysis, we confirmed greater MAPK output in $Tet2^{-/-}Nras^{G12D}$ cells compared to WT or single mutant cells. RNA sequencing and bisulfite sequencing of $Mac1^{+}$ myeloid cells revealed silencing of multiple Dusp/Spry family members, negative regulators of MAPK signaling, in $Tet2^{-/-}Nras^{G12D}$ cells and validated Spry2 as a key epigenetic target in $Tet2^{-/-}Nras^{G12D}$ leukemia. Of note, $Tet2^{-/-}Nras^{G12D}$ leukemic mice, TET2-silenced NRAS-mutant human leukemia cells and TET2/NRAS double-mutant leukemia patient samples all showed higher sensitivity to MEK inhibition compared to NRAS single mutant cells, demonstrating this approach may have value in leukemia patients with concurrent TET2/NRAS mutations.

E-1003

Methylica: A GUI tool for independent component analysis of methylome data

Hiromitsu Araki
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Co-author：Takashi Ito
Dept. Biochem., Kyushu Univ., Grad. Sch. Med. Sci.

Independent component analysis (ICA) is a widely used blind source separation method to decompose a complex mixture of signals from multiple sources. It is a popular method to analyze ECG, EEG and fMRI data. In the field of oncology, it has been applied to expression microarray data to identify independent components (ICs) which are interpreted as transcriptional modules, including those representing cancer subtypes. ICA quantifies contributions of individual genes to each IC as loadings. Conversely, functions of high loading genes for an IC enable biological interpretation of the IC. Since no study, to our knowledge, has applied ICA to methylome data, we developed Methylica, a GUI tool to perform ICA of whole-genome bisulfite-seq (WGBS) and Infinium 450K data. Methylica not only conducts ICA-based clustering of samples, which often outperforms other clustering approaches, but helps users to identify potential methylation markers for cancer subtypes, stages, prognosis and so forth. We will present applications of Methylica to two public datasets (i.e., breast cancer Infinium 450K data and medulloblastoma WGBS data) to demonstrate its utility in cancer epigenomics.

E-1004

Repression of TET genes and enhancement of DNMT activity are critical for induction of aberrant DNA methylation

Hideyuki Takeshima
Div. Epigenomics, Natl. Cancer Ctr. Res. Inst.

Co-author：Tohru Niwa[1]、Harumi Yamada[1]、Satoshi Yamashita[2]、Toshikazu Ushijima[2]
[1]Div. Epigenomics, Natl., Cancer Ctr. Res. Inst.、[2]Div. Epigenomics, Natl. Cancer Ctr. Res. Inst.

Chronic inflammation is deeply involved in the development of human cancers by inducing aberrant DNA methylation, and the methylation induction has been shown to be closely associated with expression of Il1b, Tnf, and Nos2. However, the molecular mechanisms by which these cytokines induce aberrant methylation remain unclear. Here, we show 1) that Tet methylcytosine dioxygenases (Tet) genes, Tet1, Tet2, and Tet3, involved in DNA demethylation, were repressed by NF-kB signaling, downstream of IL-1β and TNF-α, via up-regulation of specific miRNAs, such as miR-20a, miR-26b, and miR-29c, and 2) that exposure to nitric oxide, produced by Nos2, enhanced enzymatic activity of DNA methyltransferases (DNMTs). In cultured cells, TET repression by overexpression of one of these miRNAs did not induce aberrant methylation, while triple knockout of TET genes induced aberrant methylation at thousands of genomic loci. The number of hypermethylated loci became larger in combination with exposure to nitric oxide. These results suggested that, in human tissues, a vicious combination of TET repression and increased DNMT activity biologically induce aberrant DNA methylation.

▲TOP

E-1005

Epigenetic disruption of adipogenic regulators in dedifferentiated liposarcoma

Hironori Takamatsu
Div. Epigenomics, Natl. Cancer Ctr. Res. Inst., Dept. Orthopaedic Surg., Keio Univ. Sch. Med.

Co-author：Naoko Hattori[1]、Naofumi Asano[2]、Naoko Iida[1]、Eisuke Kobayashi[3]、Robert Nakayama[4]、Morio Matsumoto[4]、Masaya Nakamura[4]、Akira Kawai[3]、Toshikazu Ushijima[1]
[1]Div. Epigenomics, Natl. Cancer Ctr. Res. Inst.、[2]Dept. Orthopaedic Surg., Keio Univ. Sch. Med.、[3]Dept. Musculoskeletal Oncol., Natl. Cancer Ctr. Hosp.、[4]Dept. Orthop. Surg., Keio Univ., Sch. Med.

Well-differentiated liposarcomas (WDLPS) and dedifferentiated liposarcomas (DDLPS) develop as a result of a disturbance in adipogenic differentiation, but the epigenetic alterations underlying liposarcomagenesis are unknown. Here, we investigated DNA methylation profiles of 6 WDLPS, 10 DDLPS, and 6 normal adipose tissue (AT) samples using the Infinium EPIC. Principal component analysis (PCA) clearly classified the samples into 3 groups: AT, WDLPS, and DDLPS. The former two groups were distributed closely, while DDLPS sparsely. Genomic regions (n = 193) that highly contributed to the PCA classification and were aberrantly methylated in DDLPS contained large numbers of PPAR γ binding sites (6.2%: whole genome 1.1%) and super-enhancers (22.2%: whole genome 7.6%) in pre- and mature-adipocytes. Among the 193 regions, the promoter regions of 10 genes were marked with H3K27ac in adipocytes, and at least 2 genes (STAT5A and METTL7A) involved in adipogenesis were repressed in DDLPS. METTL7A was re-expressed after combined treatment with DAC and HDAC inhibitor. These results indicated that suppression of adipogenic genes by epigenetic alterations was involved in DDLPS tumorigenesis.

E-1006

A Novel Diagnostic Method to Detect Aberrant DNA Methylation in cfDNA of Pancreas Cancer Patients

Keiko Shinjo
Div. Cancer Biol., Nagoya Univ. Grad. Sch. Med.

Co-author：Keisuke Katsushima[1]、Genta Nagae[2]、Hiroyuki Aburatani[3]、Kenji Yamao[4]、Yutaka Kondo[1]
[1]Div. Cancer Biol., Nagoya Univ. Grad. Sch. Med.、[2]Genome Sci. Div., Res. Cent. Adv. Sci. Tech., Univ. Tokyo、[3]Gen. Sci. Div., RCAS, Univ. of Tokyo、[4]Aichi Cancer Ctr. Dept. Gastroenterology

Cancer cells release cell-free DNA (cfDNA) into the blood that can be used to diagnose cancer and monitor treatment response of cancer. In order to improve the survival of pancreatic cancer patients, development of non-invasive detection biomarkers has been challenged. DNA methylation has been considered as a promising biomarker for cancer. Performing genome-wide DNA methylation analysis in 38 pancreatic cancers in conjunction with public database information, we identified five markers with high sensitivity and specificity (91% and 100%, respectively) in a validation cohort (n=46). Using these markers, we performed methylation analysis in cfDNA of pancreatic cancer patients. Since the methylation detection rate was not high enough by the conventional bisulfite sequencing with next generation sequencer, we developed a sensitive method based on a methyl-CpG binding protein enrichment strategy and found that DNA methylation was stably detected even in 1ng of DNA. This method allowed us to detect at least one among the five markers in all cfDNA from pancreas cancer patients tested (n=13). Our new sensitive method may be a useful diagnostic tool for pancreas cancer.

▲TOP

[E-1007] E9-2 [English]
Histone modification and epigenome

2018 / 9 / 27 (Thu) 10:15-11:30 Room 4/10F 1001, Osaka International Convention Center Room 4

Satoshi Fujii / Path. of Div. EPOC, Natl. Cancer Ctr.

E-1007

Enhancing the efficacy of liver cancer immunotherapy by specific inhibition of histone deacetylase 8

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Accumulating evidence is underscoring the fundamental importance of epigenetic regulation in tumor immune evasion. We have previously elucidated a critical role of histone deacetylase 8 (HDAC8) in hepatic carcinogenesis. Here, we aim to investigate the therapeutic potential of a HDAC8-specific inhibitor PCI-34051 in preclinical hepatocellular carcinoma (HCC) models. PCI-34051 significantly reduced HCC tumorigenicity in immunocompetent but not immunodeficient mice. Immune profiling revealed specific reduction in tumor-infiltrating regulatory T cells, which was associated with significant increase in CD8+ T cells. Notably, combined PCI-34051 and anti-PD-L1 treatment resulted in complete tumor eradication in all of the co-treated mice. Moreover, the combination therapy promoted long-term survival, which was associated with elevated CD8+ T effector and central memory cells. Our data suggest that selective chromatin modifications by HDAC8 alter the tumor immune surveillance program and demonstrate the potential of rational combinatorial epigenetic immunotherapy to fully unleash T-cell responses, leading to long-term remission of HCC.
This work is supported by the RGC CRF (C4017-14G).

▲TOP

E-1008

Aberrant active-enhancers associated with downregulation of HDAC1-RFP complex overcome chemoresistance in glioblastoma

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RET finger protein (RFP) plays a pivotal role in the acquisition of chemoresistance via formation of a complex with NF-Y and HDAC1, producing specific enhancer activity. We hypothesized that chemoresistance mediated by RFP may result from aberrant deacetylation of H3K27 and dysregulation of novel cis-regulatory (active) enhancers. We found that the combination of RFP depletion and TMZ markedly suppressed the growth of glioma cells and extended the survival time of intracranial tumor-bearing mice compared to that of TMZ alone. ChIP-seq and RNA-seq analyses revealed that RFP depletion weakened a significant number of enhancers, and diminished RNA with functions related to mitosis, DNA replication and cell cycle. Further, the transcriptomes of FOXO1 and TBP2 were significantly increased, while that of PARPBP was decreased, resulting in induction of ROS and cell death. This study suggests that RFP contributes to chemoresistance via aberrant deacetylation of H3K27 and dysregulation of RFP-associated active-enhancers in glioma and that the combination of targeting RFP and TMZ has potential as an effective novel treatment strategy for lethal glioma.

E-1009

A novel epigenetic mechanism revealed tumor associated angiogenesis

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Angiogenesis plays a key role for solid tumor progression. Although VEGF (Vascular Endothelial Cell Growth Factor) signal is the most important for angiogenesis, precise molecular mechanism has been largely unknown. To clarify the epigenetic mechanism about angiogenesis, we conducted ChIP-seq with H3K4me3, H3K27me3, H3K27ac, and H2AK119Ub antibodies and mRNA-seq 15, 60 min after VEGF stimuli on HUVECs (Human Umbilical Vein Endothelial Cells). As a result, we discovered the early response transcription factors (such as EGR3) have rich repressive marks before stimulation. Under stimuli within only 15 min, these genes transited to the bivalent state (H3K4me3 and H3K27me3 double positive) and their expressions and modifications were inhibited by knockdown of one of COMPASS complex proteins. In addition, non-canonical polycomb repressive complex 1 (ncPRC1) regulated these early response genes activation. In vivo, knockdown of this protein inhibited angiogenesis and macrophage infiltration, which lead to the reduction of tumor size. Together all, we demonstrated a novel epigenetic mechanism that bivalent transcription factors have critical roles in tumor associated angiogenesis.

E-1010

Translational approach to target epigenome abnormality in gastrointestinal cancer stem cells

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Given that epigenetic alterations make a treatment of heterogeneous tumor difficult, we have studied histone modifications and found the Jaird1B with Jumonji domain, a demethylation enzyme at H3K4, is involved in growth and metastasis of gastrointestinal cancer. The data show Jaird1B plays a role in maintenance of cancer stemness, i.e., a resistant phenotype for chemo- and radiation-therapy. We performed drug discovery from mutually non-exclusive points; computational structural based analyses and high throughput screening. As the former, we performed molecular dynamics simulation under water phase environment at 310 [K]. Based on thermodynamically behavior of Jaird1B under an organism environment, anti-tumor drug candidate compounds were obtained from a library with 5 million compounds. As the latter, we performed animal experiments and molecular profiling as well as in vitro targeting study to narrow down lead compounds, which were extended further with chemical synthesis in Osaka University. Taken together, our translational approach allowed the identification of precise common structure of inhibitory compounds against Jaird1B to target gastrointestinal cancer stem cells.

▲TOP

E-1011

E2F6 Functions as a ceRNA and a Transcriptional Repressor to Promote Stemness and Immunoevasion in Ovarian Cancer

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In this study, we tested our previous mathematical model of the role of estrogen, and the transcriptional repressor E2F6, in ovarian carcinogenesis. Estrogen treatment of ovarian surface epithelial cells upregulated E2F6 and c-KIT, but downregulated the tumor suppressor miR-193a. In vitro and in vivo studies confirmed E2F6's role in miR-193a epigenetic silencing, via recruitment of EZH2 and ceRNA. E2F6 or EZH2 depletion de-repressed miR-193a, and opposed cancer stemness, by alleviating repressive chromatin. We also identified PBX1, a transactivator of the immunosuppressive cytokine IL-10, as another miR-193a target and E2F6 ceRNA. Importantly, differentiation of THP-1 human monocytic cells with conditioned media from E2F6 3'-UTR-overexpressing cells resulted in the formation of tolerogenic DC and subsequent inhibition of T-cell proliferation, while this inhibition was reversed by anti-IL-10 antibody or EZH2 inhibitor. Finally, patients with higher miR193a promoter methylation had poorer survival, compared to others. Our results suggest that an inhibitable, estrogen-mediated E2F6 ceRNA network epigenetically silences miR-193a, promoting ovarian cancer stemness and immunoevasion.

E-1012

Transcriptional Regulatory Program Controlled by LMO1 in Neuroblastoma

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Neuroblastoma is an embryonal tumor of the peripheral sympathetic nervous system. Overexpression of the transcription factor LMO1 and the polymorphisms within this gene are associated with the susceptibility to develop neuroblastoma. LMO1 has been implicated as an oncogene in T-cell acute lymphoblastic leukemia (T-ALL); however, transcriptional targets regulated by LMO1 in neuroblastoma cells are poorly understood. Here we identify genes and pathways controlled by LMO1 and MYCN in neuroblastoma cells. ChIP-seq analysis demonstrated that LMO1, GATA3 and MYCN frequently co-occupy regulatory elements. RNA-seq analysis after LMO1 knockdown showed that genes regulated by LMO1 in neuroblastoma cells were distinct from those in T-ALL cells. LMO1 directly activates the expression of ASCL1, which was also coordinately regulated by GATA3 and MYCN. Blockage of a putative enhancer element of ASCL1 gene by CRISPR-dCas9 technology downregulated ASCL1 expression. Knockdown of ASCL1 inhibited neuroblastoma cell growth. ASCL1 expressions were associated with the inferior survival of primary neuroblastoma cases. Our results indicated that ASCL1 is a critical downstream of LMO1 in neuroblastoma.

▲TOP

[LS2] LS2 [Japanese]
Genetic analysis and its clinical application in malignant lymphomas

2018 / 9 / 27 (Thu) 11:50-12:40 Room 4/10F 1001, Osaka International Convention Center Room 4
：Eisai Co., Ltd

Takahiro Maeda / Center for Cellular and Molecular Medicine, Kyushu University Hospital

LS2

Genetic analysis and its clinical application in malignant lymphomas

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No Abstract

▲TOP

[E-1055] E17-1 [English]
Anticancer drugs and molecular mechanism

2018 / 9 / 27 (Thu) 13:00-14:15 Room 4/10F 1001, Osaka International Convention Center Room 4

Masahiro Yasunaga / Developmental Therap., EPOC, Natl. Cancer Ctr.

E-1055

Targeting RSPO3 Reduces Stem Cell Function in RSPO3-Fusion-positive Colon cancer and RSPO3 high Lung cancer

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Recent studies have revealed that the R-spondins (RSPOs) can mediate with Lgr4 and Lgr5 proteins/Frizzled/LRP receptor complexes as an independent (noncanonical) control of the Wnt pathway. Several lines of evidence supported that RSPOs play a positive role in the regulation of Wnt/beta catenin signaling. We have identified an anti-RSPO3 antibody (DBPR117; hB1) using a rational design approach. DBPR117 was identified as an ideal candidate to be developed as a therapeutic antibody. DBPR117 was well characterized in a variety of assays including the binding assays, in vitro bioassays, in vivo PDX (patient-derived xenograft), lung cancer or colon cancer CDX (cell line-derived xenograft) models. DBPR117 is capable of binding specifically to the human RSPO3 with novel amino acid sequences in the complementary determining regions (CDRs). DBPR117 showed efficacy in human colon and lung cancers with RSPO3 fusion/overexpression. We are currently evaluating whether antagonizing RSPO3 by DBPR117 would synergize with anti-PD-L1 antibody to combat cancers using syngeneic murine models.

▲TOP

E-1056

Isolation of ketomycin from Streptomyces as an inhibitor of 2D and 3D invasion of human breast carcinoma cells

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Metastasis inhibitors without cellular toxicity should be useful for anticancer agents with limited toxicities. Then, we are screening cellular migration inhibitors of low molecular weight from microbial culture filtrates. In the present research, we report the inhibitory activity of ketomycin on breast cancer cell migration and invasion with mechanistic analysis. After the screening of several hundred broths, we isolated ketomycin from the culture filtrate of Streptomyces as an inhibitor of cancer cell migration. It inhibited migration and invasion of breast carcinoma MDA-MB-231 and MCF-7 cells without toxicity. Human tumor metastasis PCR array showed ketomycin inhibits MMP-9 and MMP-11 expressions in MDA-MB-231 cells. Then, knockdown of each protein lowered the migration and invasion. It also inhibited the cellular NF-kappa B activity. It also inhibited 3D invasion of MDA-MB-231 cells without toxicity. The structure of ketomycin is comparatively simple and may become a candidate for anti-metastasis agents.

E-1057

Chem-seq: a platform to evaluate the oncotherapeutic potentials of minor-groove-binding pyrrole-imidazole polyamides

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Pyrrole-imidazole ("PI") polyamides are motif-specific DNA minor groove binders that can strike at oncotargets deemed "undruggable" at the protein level, e.g. Ras. While extensive studies illuminate the potential of PI polyamides at modifying the cancer epigenome and disrupting oncogenes, their short recognition motifs can imply the presence of multiple binding targets, thus highlighting the need for evaluating the impact of whole-genome binding for bedside translation. This presentation discusses our current development of a "Chem-seq" platform to address the genome-wide effect of PI polyamides via affinity-based IonTorrent sequencing, expression microarrays and computational analysis. Chem-seq can be used to identify a PI polyamide's binding targets in vitro from sequencing data to reveal the underlying biochemical changes, and to infer subsequently the possible phenotypic changes in vivo via the prediction of side effects from gene expression profiling by machine learning. The use of this platform allows us to evaluate PI polyamide candidates with increased throughput and confidence that may hopefully accelerate the bedside use of these molecules as cancer therapeutic agents.

E-1058

Preclinical activity of apalutamide (ARN-509) in genetically engineered mouse models of Pten-deficient prostate cancer

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Apalutamide (Apa) is an oral nonsteroidal androgen receptor (AR) antagonist that is currently undergoing late-stage clinical development for the management of castration-resistant prostate cancer (CRPC). Here, we examine the preclinical activity of Apa in castration-naïve and castration-resistant mouse prostate cancer (PCa) models disease and delve into the molecular mechanisms underlying CRPC progression. In an early-stage efficacy model of Pten-deficient PCa, Apa significantly reduced tumor burden by 33.5% (P=0.002) in castration-naive mice but was ineffective in mouse CRPC. Notably Apa enhanced survival in models of advanced Pten/P53-deficient PCa. Molecular studies showed elevated PI3K-AKT signaling after treatment with apalutamide in CRPC. In vitro studies using mouse PCa cell lines showed synergistic responses with apalutamide and AKT inhibition (GSK-690693). Combination therapy in Pten-KO mice showed enhanced antitumor activity compared to single drug. Our data show that apalutamide is active in mouse preclinical models and support further investigation for developing rational treatment combinations for the management of advanced PCa.

▲TOP

E-1059

Biological and immunological mechanisms underlying metastatic tumor burst

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Distant recurrence following metastasis is a major cause of cancer-associated death because of the refractoriness. Interfering spread and re-growth of the metastatic tumor cells must be greatly helpful for improving prognosis of cancer patients. However, little is known about the molecular mechanisms underlying the refractoriness of the disseminated dormant cancer cells. In this study, we found that the metastatic tumor cells are polyploidy under a tranquil state, and once receiving a treatment stress, aggressively generate its progeny cells in response to treatment stress for surviving. Also, these polyploid giant cells expand type 2 innate lymphoid cells (ILC2), and accelerate the progeny production in response to the produced IL13 leading to hyperprogression of the metastatic tumors. These suggest that evolutionary transformation of the metastatic tumor cells with genomic instability is extrinsically facilitated toward metastatic burst. We finally identified a specific molecule by utilizing cDNA microarray for targeting the metastatic polyploid tumor cells.

E-1060

Pemetrexed resistance irrelevant to nucleotide synthesis is linked with up-regulated AMP-activated protein kinase

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Pemetrexed (PEM) inhibits DNA and RNA synthesis and primarily targets enzymes involved in the synthesis, TS, GARFT and DHFR. Elevation of the enzyme activities in tumors is thus linked with PEM resistance. PEM also stimulates AMP-activated protein kinase (AMPK) and mTOR pathways since the secondary target is AICART, aminoimidazolecarboxamide ribonucleotide formyltransferase. We established 2 kinds of PEM-resistant mesothelioma cells which did not elevate the relevant 3 primary targets, and found that these cells increased phosphorylated levels of AMPK, AKT, p53 and the mTOR target p70S6K in comparison with those of the respective parent cells. PEM treatments also augmented phosphorylation of AMPK, AKT, p53 and p70S6K in most of the cases. An AMPK activator increased the autophosphorylation and PEM resistance, and an AMPK inhibitor decreased the PEM resistance. In contrast, an inhibitor for AKT or p70S6K did not influence the resistance, and increased endogenous p53 levels did not affect the PEM sensitivity. These data collectively suggested that constitutive activation of AMPK was associated with PEM resistance which was independent of the enzymes involved in nucleotide synthesis.

▲TOP

[E-1061] E14-3 [English]
Head and neck cancer

2018 / 9 / 27 (Thu) 14:15-15:30 Room 4/10F 1001, Osaka International Convention Center Room 4

Hiroshi Miyata / Dept. Gastroenterological Surg., Osaka International Cancer Institute

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E-1061

Inactivation of BDH2 promotes proliferation and metastasis of nasopharyngeal carcinoma via iron retention

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3-hydroxybutyrate dehydrogenase type 2 (BDH2) is well known to catalyze a rate-limiting step in the biogenesis of the mammalian siderophore, regulating intracellular iron metabolism. In the present study, we found that the transcription and translationexpression of BDH2 was significantly downregulated in nasopharyngeal carcinoma (NPC). Ectopic expression of BDH2 inhibited NPC cells proliferation and colony formation. In addition, we confirmed that BDH2 suppresses the migration and invasion of NPC cells by reversing the epithelial mesenchymal transition. Furthermore, overexpression of BDH2 decreased the growth and metastasis of NPC cells by reducing intracellular iron level, the same effect seen on applying iron chelator. Our findings suggest that BDH2 may be a candidate tumor-suppressor gene in NPC. Decreasing intracellular iron or iron supplement could be an effective therapeutic approach for NPC.

▲TOP

E-1062

NOTCH4 - HEY1 pathway induces epithelial mesenchymal transition in head and neck squamous cell carcinoma

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Background: Recently, several comprehensive analyses for head and neck squamous cell carcinoma (HNSCC) gene mutations were examined using the high-throughput next generation sequencings. In these analyses, NOTCH1 mutation is found at 10-15% rate. This rate is higher than previously considered. After this finding, NOTCH pathway is provided more interest in their functions. However, the complete diversity of NOTCH receptor function and the relationship with the downstream genes in HNSCC is not well understood.
Methods: We analyzed the relationship between NOTCH and its downstream gene expression using the cancer genome atlas (TCGA) data set. To explore the functional role, we performed in vitro EMT related assays.
Results: a NOTCH downstream gene, HEY1 is specifically up-regulated in HNSCC compared with normal tissues. NOTCH4 is most significantly related to HEY1 activation in comparison to other NOTCH receptors. Furthermore, NOTCH4 and HEY1 expression were associated with EMT phenotypes as well as increased invasion and cell migration.
Conclusion: In HNSCC, the NOTCH4-HEY1 pathway is specifically up-regulated and promotes EMT.

E-1063

Interferon-stimulated gene 15 promotes lymph node metastasis via interacting Rac1 in oral squamous cell carcinoma cells

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Lymph node metastasis is the main factor of poor prognosis in oral squamous cell carcinoma (OSCC) patients. Using proteomic analysis, we found that interferon-stimulated gene 15 (ISG15) was the highest expressed protein related to lymph node metastasis. Abundant ISG15 was also observed in the microarray datasets and immunohistochemical of OSCC patients. In the orthotopic xenograft model, ISG15 knockdown decreased tumor lymphangiogenesis and lymph node metastasis. Similarly, ISG15 knockdown diminished cell migration, invasion and transendothelial migration in OSCC cells. Especially, ISG15-induced cell migration was in an intracellular ISGylation-independent manner and associated with membrane protrusions. Ectopic expression of ISG15 increased Rac1 activity and knockdown of Rac1 impaired ISG15-enhanced migration. Moreover, ISG15 was co-localized and interacted with Rac1 in the region of membrane protrusions by immunofluorescence and proximity ligation asays. Immunoprecipitated ISG15 interacted with Rac1, especially Rac1-GDP form. Our study indicated that ISG15 promotes cell migration and lymph node metastasis via regulation of Rac1 activity and physical interaction with Rac1.

E-1064

Classification of HPV-associated oropharyngeal cancer by definition of DNA methylation epigenotypes

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The incidence of oropharyngeal squamous carcinoma (OPSCC) associated with Human Papilloma Virus (HPV) is dramatically increasing, mainly due to infection of HPV 16. Whereas HPV-associated OPSCC patients significantly correlate with better prognosis, 36% patients show worse prognosis. To stratify OPSCC into distinct molecular subtypes, reflecting these different clinicopathological features, we performed genome-wide DNA methylation analysis by Infinium 850k using 89 OPSCC samples. Hierarchical clustering analysis classified OPSCC into four epigenotypes. Analysis of methylome data of 170 OPSCC including 81 cases of the Cancer Genome Atlas (TCGA), revealed that HPV(+) OPSCC samples correlated with higher methylation accumulation ($P<1\times10\text{-}5$, Fisher's exact test), and that high methylation subtype significantly correlated with better prognosis (P=0.004, log-rank test). Target exon sequencing analysis showed that the prevalence of TP53 and CDKN2A mutations were significantly higher in HPV(-) OPSCC than HPV(+) OPSCC ($P<1\times10\text{-}5$, Fisher's exact test). It is indicated that OPSCC is stratified into several molecular subtypes reflecting distinct genetic and epigenetic features and prognosis.

▲TOP

E-1065

Inactivation of ACAT1 involved in the ketogenesis promote the proliferation and metastasis of nasopharyngeal carcinoma

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Altered metabolism is considered as a hallmark of cancer. Here we investigated expression of Acetyl-Coenzyme A acetyltransferase 1 (ACAT1), an enzyme associated with ketogenesis, was downregulated in NPC in contrast with normal nasopharyngeal epithelium (NNE). Overexpression of ACAT1 suppressed the proliferation and colony formation of NPC cells in vitro and decreased tumorigenicity in vivo. In addition, overexpressing ACAT1 inhibited the motility of NPC cells by reversing epithelial-mesenchymal transition (EMT), with upregulation of E-cadherin and downregulation of β-Catenin, but no significance with Vimentin. Further, we found that one member of ketone bodies, β-hydroxybutyrate, were increased in NPC cell overexpressed ACAT1. The growth and migratory capacity of NPC cells were decreased by β-hydroxybutyrate, in a dose dependent manner. Taken together, our findings demonstrate the inactivation of ACAT1 in NPC, which associates with abnormal production of ketone bodies. This may play a crucial role in the progression of NPC.

E-1066

Proteomic analysis of papillary thyroid carcinoma to identify cancer biomarkers

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Papillary thyroid carcinoma (PTC) is one of the most common malignant tumors of the thyroid glands. It tends to grow slowly but is associated with a poor prognosis when metastasis happens. To better understand basic mechanisms of tumor development and identify potential new biomarkers of PTC, we examined protein expression profiling in clinical PTC tissue and matched normal thyroid tissue using fluorescence two-dimensional difference gel electrophoresis (2D-DIGE). To identify the proteins, peptide mass fingerprinting via MALDI-TOF mass spectrometry was carried out. Using these strategies, 85 up-regulated or down-regulated proteins were found in PTC. Imaging software determined 26 proteins to be differentially expressed at the two-fold (or greater) level. Among them, some proteins were selected for further analyses. Western blotting and immunohistochemical staining showed a higher expression of those proteins in clinical PTC tissue compared to normal thyroid tissue. We are presently investigating those roles in PTC. Proteomic analysis of PTC using 2D-DIGE and mass spectrometry could provide novel potential biomarkers and insights into global pathophysiologic changes in PTC.

▲TOP

[J-1049] J14-1 [Japanese]
Esophageal cancer

2018 / 9 / 27 (Thu) 15:30-16:45 Room 4/10F 1001, Osaka International Convention Center Room 4

Makoto Yamasaki / Dept. Gastroenterological Surg., Grad. Sch. Med., Osaka Univ.

J-1049

Exome sequence analysis of carcinomatous and sarcomatous elements of an esophageal carcinosarcoma

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Carcinosarcoma (CS) is a rare malignant neoplasm that comprises both carcinoma element (CE) and sarcoma element (SE). In contrast to epithelial mesenchymal transition found in cancer metastasis, sarcomatous cells of the CS have been considered irreversible. In this study, next generation sequencing was performed in both elements of an esophageal CS to elucidate the histogenesis of the CS. A target sequencing using an originally-designed esophageal cancer panel revealed that two mutations (*TP53 Y220C* and (*CDKN2A D14fs*) were present in both elements, whereas *TP53 R280T* was present only in CE, validated by digital PCR. An exome sequence demonstrated that mutations were more frequently observed in the CE than those of the SE (216 and 131 mutations, respectively). Of these, 41 somatic mutations were present in both elements. Copy number variantations (CNVs) were more frequently observed in the SE than those of the CE (571 and 190 regions, respectively). Our results suggested that both elements of the esophageal CS were originated from a single cell, and subsequently branched to carcinoma with frequent mutations and sarcoma with frequent CNVs.

▲TOP

J-1050

Role of FAP-positive cancer-associated fibroblasts in the esophageal squamous cell carcinoma microenvironment

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The tumor microenvironment, which consists of non-cancer cells such as cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs), was reported to promote several cancers, including esophageal squamous cell carcinoma (ESCC). However, the role of CAF in ESCC remains to be elucidated. First, we found the intensity of α SMA and FAP expression in the stroma of ESCC correlated with the clinicopathological features and disease-free survival. We co-cultured human bone marrow-derived mesenchymal stem cells (MSCs) with ESCC cells and confirmed the induction of FAP expression. These FAP-positive MSCs (which we defined as CAF-like cells) promoted the cell growth and migration of ESCC cells and peripheral blood mononuclear cell-derived macrophage-like cells. CAF-like cells induced the M2 polarization of macrophage-like cells. CAF-like cells secreted more CCL2, IL6 and CXCL8 than the MSCs and the silencing of FAP in CAF-like cells attenuated these cytokine secretions. These cytokines promoted the migration of tumor cells and macrophage-like cells. These findings indicate that FAP-positive CAFs are responsible for the tumor promotion and immunosuppression.

J-1051

Expression and role of Leucine-Rich Repeat-Containing protein 8A in esophageal squamous cell carcinoma

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【Background】Leucine-Rich Repeat-Containing protein 8A (LRRC8A) was reported as the main protein of anion channel responsible for regulatory volume decrease. However, the expression and role remain unclear. In this study, we investigated the clinicopathological significance of LRRC8A expression and role in esophageal squamous cell carcinoma (ESCC).【Methods】An immunohistochemical (IHC) analysis was performed on 64 patients with ESCC who underwent curative surgery. We carried out knockdown using siRNA to evaluate the role of LRRC8A in ESCC.【Results】An IHC revealed that high expression of LRRC8A was significantly correlated with pT and pN. The 5-year overall survival rate of patients with high LRRC8A expression was significantly lower than that of patients with low expression (44.9% vs 83.4%, p =0.002). Multivariate analyses showed that high expression was an independent poor prognostic factor (HR =3.763, p =0.006). Knockdown of LRRC8A induced G0/G1 cell cycle arrest and apoptosis and inhibited proliferation, migration and invasion in vitro.【Conclusions】 The LRRC8A expression is related to worse prognosis in ESCC patients and regulates tumor progression. .

J-1052

GSTO2, a novel tumor suppressor gene, regulates ERK signaling pathway in esophageal squamous cell carcinoma

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We performed integrative transcriptome and methylome sequencing in esophageal squamous cell carcinoma (ESCC) and found down-regulation of glutathione S-transferase Omega 2 (GSTO2) in association with DNA hypermethylation (Oncotarget, 8:84434, 2017). Using surgically resected tissues of 62 cases, we confirmed that mRNA and protein expression of GSTO2 were significantly decreased in ESCC compared to that in normal tissues. Treatment of ESCC cell lines with 5-aza-2'-deoxycytidine, a DNA-methyltransferase inhibitor, induced GSTO2 transcription. Forced overexpression of GSTO2 in ESCC cell lines inhibited cell growth and colony formation in vitro. GSTO2-transfected cells formed smaller tumors in nude mice than mock-transfected cells. Furthermore, we found that phosphorylation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) was reduced in GSTO2-transfected cells compared to that in mock-transfected cells. These results indicate that the expression of GSTO2 is suppressed in ESCC by aberrant DNA hypermethylation, which contributes to the promotion of the ESCC growth. Modification of ERK1/2 pathway is a possible gene function of GSTO2 as a tumor suppressor.

▲TOP

J-1053

Auto-antibodies against p53 or NY-ESO-1 are useful biomarkers in patients with esophageal squamous cell carcinoma

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BACKGROUND AND AIM: Serum p53 antibodies have been developed and approved as blood test for monitoring esophageal squamous cell carcinoma. We focused on serum auto-antibodies against p53 and NY-ESO-1. PATIENTS AND METHODS: Serum samples of patients with esophageal SCC were obtained before surgery. Cut-off values were fixed using mean+3SD of values of healthy controls. Changing pattern of serum p53 antibodies titers was also assessed during postoperative follow-up. RESULTS AND DISCUSSION: Positive rates of serum antibodies were 18% for p53, 31% for NY-ESO-1. Positive rates of these antibodies in healthy controls were 0%. Combination assay improved positive rates without increased false positive rates as follows; p53+NY-ESO-1=40%. Although some patients with extremely-high antibody titer for p53 persistently positive even after curative surgery, changing patterns of serum titers seemed to be associated with clinical outcome. Changing pattern of serum auto-antibodies may have adding information to conventional serum markers. CONCLUSIONS:Although the positive rates of single serum auto-antibody were still relatively low, combination assay with plural auto-antibodies may be useful.

J-1054

Tumor long interspersed nucleotide element-1 methylation level and immune response in esophageal cancer

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Evidence suggest that the abundance of immune cells might be associated with a favorable prognosis in cancer patients. Since molecular alterations in esophageal cancer including long interspersed nucleotide element-1 (LINE-1) hypomethylation have been associated with clinical outcome, we hypothesized that tumor LINE-1 methylation level might be associated with immune response to esophageal cancer.Using a database of 292 resected esophageal cancers, we evaluated the relationship of tumor LINE-1 methylation level with lymphocytic reaction patterns and T-cell densities.We found a positive association of tumor LINE-1 methylation level with peritumor lymphocytic reaction (P=0.01). Compared with LINE-1 hypermethylation cases, LINE-1 hypomethylation cases showed significantly lower peritumoral lymphocytic reaction [univariable odds ratio (OR) 0.32, 95% confidence interval (CI) 0.16-0.64, P=0.009; multivariable OR 0.32, 95% CI 0.15-0.65, P=0.01].Tumor LINE-1 methylation level might be an independent predictor of peritumoral lymphocytic reaction, suggesting a possible role of LINE-1 methylation level in enhancing immune response in tumor microenvironment during esophageal carcinogenesis.

▲TOP

[E-1013] E12-1 [English]
Innate immunity (1)

2018 / 9 / 27 (Thu) 9:00-10:15 Room 5/10F 1002, Osaka International Convention Center Room 5

Kazuhiro Kakimi / Dept. ImmunoTherap., Univ. Tokyo

E-1013

Blockade of myeloid-derived suppressor cell-intrinsic cell cycle-related kinase amplifies T cell immunity

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Myeloid-derived suppressor cell (MDSC) comprises a heterogeneous population of immature myeloid cells that induces the exhaustion of anti-tumor immune responses. We have previously demonstrated that tumor cells could promote immune escape via MDSC. While targeting tumor cell-MDSC crosstalk can blunt T cell activity, new approach of driving differentiation of MDSC into antigen presentation cell, which is crucial for T cell priming and activation, may elicit even greater anti-tumor immunity. We found that the latest CDK member, cell cycle-related kinase (CCRK), is over-expressed in cancer patient-derived and cytokine-induced MDSC. Knockdown of CCRK suppressed MDSC proliferation and induced MDSC differentiation into antigen presenting macrophage, which amplified T cell responses in vitro and in vivo. Mechanistically, CCRK activated STAT3/E4BP4 signaling to deregulate the balance of IL-10/IL-12 and induce arginase I for maintenance of immune suppression. Our data delineate a molecular mechanism underlying tumor immune evasion and reveal a novel targetable kinase for cancer immunotherapy. Acknowledgement: This project is supported by the RGC-CRF (C4017-14G).

▲TOP

E-1014

Dysregulated IL-18 critically drives multiple myeloma progression by generating an immunosuppressive milieu

Kyohei Nakamura
QIMRB Med. Res. Inst.

The complex interplay between inflammation and immunity creates the immunosuppressive tumor microenvironment. Given that myeloma progression is tightly associated with tissue injury in the bone marrow (BM) environment, we hypothesized that the inflammasome-mediated sterile inflammation might shape the pro-inflammatory myeloma milieu. Indeed, mice deficient for IL-18 or Nlrp1 were remarkably protected from myeloma progression. Mechanistically, the inflammasome-derived IL-18 gave rise to myeloid-derived suppressor cells (MDSC), leading to generation of the immunosuppressive BM niche. A global transcriptome analysis of the immune microenvironment in 73 patients at diagnosis strongly supported the negative impact of IL-18-driven MDSC on anti-myeloma T cell responses. Strikingly, high levels of BM IL-18 were independent prognostic determinant of poor prognosis in 152 patients. Finally, our preclinical studies suggested that therapeutic blockade of IL-18 could be a potential therapeutic approach. Together, dysregulated IL-18 is a key driver for the vicious cycle of inflammation and immunosuppression in the myeloma niche. Non-member collaborators: Ludovic Martinet and Mark Smyth.

E-1015

Suppression of STING signaling in KRAS-LKB1 mutant lung cancer

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KRAS-driven lung cancers frequently inactivate the tumor suppressor genes TP53 and/or STK11/LKB1. These co-mutations define different tumor subclasses with increasing clinical relevance. Specifically, KRAS-LKB1 (KL) mutant lung cancers are aggressive, lack PD-L1 expression, and fail to respond to immune checkpoint blockade. The mechanistic basis for this impaired immunogenicity, despite the high mutational load of KRAS mutant lung cancers, remains obscure. Here we report that LKB1 loss results in silencing of STING expression and insensitivity to cytoplasmic dsDNA sensing. Ectopic expression of STING in KL cells promotes engagement of IRF3 downstream of TBK1 and impairs cellular fitness, in contrast to KRAS-TP53 (KP) mutant cells. This finding relates to the pathologic accumulation of cytoplasmic dsDNA in KL cells associated with their mitochondrial dysfunction and defective autophagy. Thus, silencing of STING enables cells to avoid these negative consequences of LKB1 inactivation during oncogenesis. Since LKB1 loss is an obligate component of this treatement refractory subtype, developing strategies to re-induce STING signaling may have important therapeutic consequences.

E-1016

NK cells control tumor-promoting function of neutrophils

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It has been widely recognized that cancer-associated inflammation regulates tumor progression. NK cells are recognized as direct antitumor effectors, however, the ability of NK cells to control cancer-associated inflammation, which facilitates tumor progression, remains unknown. In this study, we demonstrate that NK cells control tumor-promoting inflammation through functional modification of neutrophils. NK cells control the tumor-promoting function of neutrophils through an IFN γ -dependent mechanism. Tumor progression in an NK cell-depleted mice is diminished when the IL17A-neutrophil axis is absent. In the absence of NK cells, neutrophils acquire a tumor-promoting phenotype, characterized by up-regulation of VEGF-A expression, which promotes tumor growth and angiogenesis. A VEGFR inhibitor which preferentially suppressed tumor growth in NK cell-depleted mice was dependent on neutrophils. Furthermore, the systemic neutropenia caused by an anti-metabolite treatment showed an anti-cancer effect only in mice lacking NK cells. Collectively, our present results suggest that NK cells control the tumor-promoting and angiogenic function of neutrophils.

▲TOP

E-1017

Boosting fatty acid oxidation by PPAR signal activation enhances CTL longevity and the efficacy of PD-1 blockade

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PD-1 blockade immunotherapy has dramatically ameliorated the survival rate of cancer patients. However, loss of effector cytotoxic T lymphocytes (CTLs) due to terminally differentiation-induced apoptosis has plagued its efficacy. We previously reported that bezafibrate, an agonist of PGC-1 α /PPAR complexes, had synergistic effects with PD-1 blockade in mouse tumor model. Here we examined the molecular mechanism of the synergistic effect of bezafibrate with PD-L1 blockade. Bezafibrate treatment was accompanied by metabolic reprogramming in CTLs, where means of energy generation was shifted from glycolysis to oxidative phosphorylation. As a result fatty acid oxidation (FAO) and mitochondrial spare respiratory capacity, which supports cell survival, of CTL were also augmented. Eventually we discovered that number of CTLs was maintained due to upregulation of Cpt1a, a critical enzyme for FAO, and Bcl2, which prevent overactivation-induced apoptosis of cells in bezafibrate co-treatment. In conclusion, regulation of metabolic reprogramming by bezafibrate results in the increase of CTL longevity to enhance anti-tumor immunity during PD-L1 blockade.

E-1018

Immune-mediated antitumor effects of a pan PI3K inhibitor ZSTK474

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Inhibition of the PI3K signal, an attractive target for cancer immunotherapy, decreases immune suppressive cells including regulatory T cells (Tregs), thereby activating anti-tumor immunity in animal models. Here we explored whether ZSTK474, a selective PI3K inhibitor, activated anti-tumor immune responses and augmented the anti-tumor effect by PD-1 blockade. In murine models, ZSTK474 administration decreased Tregs, resulting in activation of the anti-tumor immune responses against NY-ESO-1-expressing CMS5a (CMS5a-NY-ESO-1) via augmenting $CD8^+$ T-cell responses in combination with PD-1 blockade. We next examined memory T-cell responses and found that mice treated with combination of ZSTK474 and PD-1 blockade were resistant to re-challenge with CMS5a-NY-ESO-1 and parental CMS5a, but not by PD-1 blockade alone, suggesting efficient generation of memory T cells against both immunogenic and non-immunogenic antigens by treatment with the combination. Together, we propose that ZSTK474 activates anti-tumor immunity not only by decreasing Tregs but also promoting memory T-cell responses against wide ranges of tumor antigens, indicating a promising combination with PD-1 blockade.

▲TOP

[E-1019] E12-2 [English]
Development of novel molecular targeted therapies

2018 / 9 / 27 (Thu) 10:15-11:30 Room 5/10F 1002, Osaka International Convention Center Room 5

Shigehisa Kitano / Dept. Exp. Therap., Natl. Cancer Ctr. Hosp.

E-1019

Telomelysin as an immunotherapy sensitizing gastrointestinal tumors to anti-PD-1 antibody

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Anti-programmed death-1 antibody (PD-1 Ab) has improved clinical outcomes for patients with various types of cancer. However, the benefit is limited to small population with rich tumor infiltrating lymphocytes. We previously developed a telomerase-specific oncolytic adenovirus, Telomelysin (OBP-301). A phase I clinical trial of combination therapy with radiation is ongoing for patients with esophageal cancer. Here, we analyzed whether our established RGD fiber-modified Telomelysin (OBP-502) could sensitize tumors to PD-1Ab. OBP-502 killed CT26 murine colon cancer cells and PAN02 murine pancreatic cancer cells in vitro and induced the ATP and HMGB1 release. In vivo subcutaneous tumor models, OBP-502 significantly induced CD8 positive cells and vaccination with OBP-502-treated cells effectively inhibited tumor engraftment. Combination of OBP-502 and PD-1 Ab significantly suppressed tumor growth. In addition, in a bilateral CT26 mice model, combination therapy showed significant therapeutic effects at the non-treatment site as well as treatment site. These results proved that our telomerase-specific oncolytic adenovirus sensitizes gastrointestinal tumors to PD-1 Ab.

▲TOP

E-1020

Interim immunostaining results from phase I study of pre-operative combination therapy with mogamulizumab and nivolumab

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Clinical trial of pre-operative combination therapy with mogamulizumab(moga) and nivolumab(nivo) (NCT02946671) was conducted for 12 pts with solid cancers of lung(n=4), esophagus(n=3) and kidney(n=5) by Apr 30, 2018. Double immunostaining of CCR4 and FOXP3 using sets of paraffin sections of pre-operative biopsy specimens and surgical tissues was performed to evaluate depletion effect of tumor infiltrated eTregs with moga. The numbers of double positive cells(CCR4+FOXP3+) in surgical tissues (avg. 13.5/mm^2) were notably lower than those in pre-operative biopsy specimens (avg. 1.5/mm^2) in all cases, which indicate that the tumor infiltrated eTregs were decreased in about 1/9 by moga. eTreg depletion effect was comparable in cohort 1 (n=6, moga:0.1mg/kg, nivo:1.0mg/kg) and cohort 2 (n=6, moga:0.3mg/kg, nivo:1.0mg/kg). It is considered that moga dose of 0.1mg/kg is enough amount for depletion of eTreg in tumor tissues. Although notable eTreg(CCR4+FOXP3+) depletion in the tumor tissues was observed, CCR4-FOXP3+ cells were not decreased so much in almost cases. Further studies on characterization of the CCR4-FOXP3+ populations are necessary for improvement of anti-tumor effects of moga.

E-1021

Comparison of anapocosis cell death induced by anti-pan mouse MHC class I mAb, and anti-pan HLA class II mAb.

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Previously, we reported that rat anti-pan mouse MHC class I mAb induced cell death in activated mouse lymphocytes, T cell clones and lymphoma cells but not normal resting lymphocytes. And we reported that mouse anti-pan HLA class II mAb induced cell death in lymphoma cells and aggravated IL2-independent Adult-T cell leukemia cell (ATL) cell lines but not normal human lymphocytes or mild IL-2-independent ATL cell lines.　These monoclonal antibodies (mAb) induced cell death is dependent of cytoskeleton and independent of complements, ADCC and caspases. Treatment with these mAbs induced the formation of large pores on the surface of target cells within 30 min. We named the cell death induced by cytolytic anti-pan MHC antibodies as anapocosis. Anapoco means holes in Japanese.

E-1022

The 5-FU and Lantana camara Resulted G1 Arrest and Cell Death Induction on HeLa and T47D

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The cervical and breast cancer are the leading cause of death in women. 5-Fluorouracil (5-FU) is one of the chemotherapy agents often used in cervical cancer therapy. it usually combined with other chemotherapeutic agents to increase its effectiveness. The combination of 5-FU and EET treatment on both cells resulted in additive to moderate synergistic effects.The prolong treatment exhibited worse results than a single one. It is presumed that during the longer treatment the 5-FU effect predominates in the proliferation kinetics inhibition. The 5-FU-EET trigger accumulation of T47D cancer cells in sub-G1 phase which cause cell death through apoptotic mechanism. The accumulated increasing is 36% higher than a single 5-FU. However it does not provide a similar effect on HeLa. The 5-FU-EET treatment results in cell accumulation in G1 phase on both cells, but more pronounced in HeLa cells, which is >70%. The EET treatment has additive to synergistic effect as a 5-FU co-chemotherapeutic agent against cervical and breast cancer. The effect is thought to occur due to G1 arrest which will trigger induction of cell death through apoptotic mechanism.

▲TOP

E-1023

Co-administration strategy to enhance bioavailability of sorafenib by modulating cytochrome P450 3A and P- glycoprotein

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Most of HCC patients with liver dysfunction cannot tolerate long-term chemotherapy. Therefore, therapy strategies with high efficiency and low toxicity is needed. Sorafenib is metabolized via cytochrome P450 (CYP) 3A and transported by P-glycoprotein (P-gp), which can inhibit the penetration of drugs into targeted tissues and limit the drug accumulation. Therefore, inhibition of CYP 3A and P-gp might be a viable strategy to enhance bioavailability and overcome drug resistance. Schisantherin A and Schisandrin are main active ingredients of S.sphenanthera, which possess liver-protecting, anti-inflammatory activities. We found both Schisantherin A and Schisandrin could inhibit the activity of CYP450 and P-gp. The co-administration of sorafenib with Schisantherin A and Schisandrin enhanced the sorafenib cellular accumulation and reduced its ratio, better than that of verapamil. Compared with Schisantherin A, Schisandrin had better suppression effect on CYP450 and P-gp under the same concentration. These results indicated that co-administration strategy might be a useful tool for enhancing the drug response and reducing drug resistance.

E-1024

Heterogeneous response to the blockade of BMP pathway in combination with RAS/MEK inhibition in colorectal cancer

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Co-author：Masahiro Inoue
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Inter-tumor heterogeneity is one of the major obstacle both for developing therapeutics and selecting appropriate treatment for individuals. We have been working on the inter-tumor heterogeneity by using a panel of CTOS (cancer tissue-originated spheroid) lines, which are prepared from some hundreds of patients. In this study, we seek to clarify heterogeneous nature of the response to BMP pathway in colorectal cancer using CRC CTOS panel. Inhibition of BMP signaling by LDN193189 (LDN) suppressed the growth of a portion of CTOS lines in a growth factor (GF) reduced medium, while none of the lines responded to BMP inhibition in a GF rich medium. A KRAS mutant line in which the growth is suppressed by LDN in vitro although no inhibitory effect was observed in vivo. LDN, however, noticeably enhanced the effect of the combination treatment (cetuximab and trametinib) in vivo. The result suggests that tumor environment also affects heterogeneous response to LDN, and the suppression of the support from microenvironment-derived GFs potentially sensitize to inhibition of BMP in a portion of the patients.

▲TOP

[LS3] LS3 [Japanese]
Cancer associated with inflammatory bowel disesases

2018 / 9 / 27 (Thu) 11:50-12:40 Room 5/10F 1002, Osaka International Convention Center Room 5
：MIYARISAN PHARMACEUTICAL CO.,LTD

Yanaga Katsuhiko / Jikei University School of Medicine Department of Surgery

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LS3

Cancer associated with inflammatory bowel disesases

Mizushima Tsunekazu
Osaka University, Graduate School of Medicine, Department of Gastroenterological Surgery

No Abstract

▲TOP

[E-1067] E16-1 [English]
Signal transduction inhibitor (1)

2018 / 9 / 27 (Thu) 13:00-14:15 Room 5/10F 1002, Osaka International Convention Center Room 5

Kohji Noguchi / Div. Chemother., Facult. Pharm., Keio Univ.

E-1067

Identification of URST1 as a biomarker and therapeutic target for lung cancer

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To identify biomarker and therapeutic targets for lung cancer, we screened genes that overexpressed in the majority of lung cancer by using our original gene expression profile database. During this process, we identified up-regulated in solid tumor 1 (URST1) as a candidate. Western blotting showed that URST1 was expressed in the majority of lung cancer cells and URST1 levels increased at G2/M of cells. Immunohistochemical staining showed that URST1 expression was observed in 231 (64.5%) of 358 NSCLCs (non-small cell lung cancer) that had undergone curative surgery. In addition, high level of URST1 expression was associated with poor prognosis for NSCLC patients (P = 0.0003, by log-rank test). Multivariate analysis confirmed strong expression of URST1 was an independent prognostic factor. Suppression of URST1 expression by siRNA or treatment with small molecule inhibitor specific for URST1 activity suppressed the growth of lung cancer cell lines partly through G2/M arrest and subsequent cell death. Our data suggest that URST1 is a possible prognostic biomarker and therapeutic target for lung cancer.

▲TOP

E-1068

STXBP4 Regulates APC/C-Mediated p63 Turnover and is a Novel Biomarker in Lung Squamous Cell Carcinoma

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p63 plays a pivotal role in basal-epidermal gene expression and epithelial maintenance. δN isoform of p63 is frequently overexpressed from metaplasia to severe dysplasia and in more advanced stages of squamous carcinogenesis. We previously reported that Syntaxin-binding protein 4 (STXBP4) drives tumor growth in a δNp63-dependent manner. We demonstrated the mechanisms that APC/C plays a role in ubiquitin-mediated turnover of δNp63 and that STXBP4 suppresses the ubiquitination. APC/C-resistant version of δNp63α (RL7-δNp63α) inhibit the terminal differentiation process in 3D organotypic cultures ,suggesting the context-dependent pro-oncogenic roles. To clarify the biological and the oncogenic roles of Stxbp4 and δNp63 in lung SCC, we performed a genome-wide transcriptome analysis (RNA-seq) using NGS in clinical samples of lung SCC patients followed by gene functional analysis and found that PDGF pathway is a key downstream mediator of STXBP4 function. Thus, our study indicate that Stxbp4 plays a crucial role in the development of tumors, suggesting it may be a relevant therapeutic target for patients with lung SCC. (Collaborator: Prof. Carol Prives, Columbia University, NY, USA)

E-1069

RBPJ could be a new therapeutic target for refractory solid neuroendocrine type tumors

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The biological significance of recombination signal binding protein for immunoglobulin-kappa-J region (RBPJ) was investigated in small cell lung cancer (SCLC) and pancreatic cancer (PC) which were neuroendocrine type tumors, and whether RBPJ could be a new therapeutic target for SCLC and PC was investigated. SCLC and PC cell lines were used as target cells. Suppression of RBPJ significantly decreased invasiveness through inhibition of MMP-2 and MMP-9 expressions in SCLC and PC. Suppression of RBPJ significantly decreased proliferation in vitro in SCLC and PC.Tumor volume in mice injected with RBPJ-inhibited PC cells was significantly lower than that in control mice. Signaling from RBPJ was through Hh and Notch signaling pathways in SCLC and PC. Chemosensitivities of gemcitabine and CDDP in RBPJ-inhibited SCLC cells were significantly lower than those in control cells. These results suggest that RBPJ could be a new therapeutic target for refractory solid neuroendocrine type tumors. However, we should take care of the combinational use of the other chemo agent, because chemosensitivity may decrease in RBPJ inhibited SCLC cells.

E-1070

AXL confers intrinsic resistance to osimertinib and the emergence of tolerant cells

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The third generation EGFR tyrosine kinase inhibitor osimertinib has shown marked efficacy in patients with EGFR mutated lung cancer (EGFR-LC). Some patients, however, show intrinsic resistance and insufficient response, although the mechanisms are not fully understood. This study showed that osimertinib adversely stimulates AXL, activated AXL associates with EGFR and HER3 to maintain cell survival and induces the emergence of cells tolerant to osimertinib. AXL inhibition reduced the viability of AXL-overexpressing EGFR-LC cells exposed to osimertinib. In the cell line- and patient-derived xenograft models of AXL-overexpressing EGFR-LC, the combined treatment of osimertinib with an AXL inhibitor remarkably regressed tumors and delayed tumor re-growth than osimertinib alone. IHC analysis of clinical specimens revealed high expression of AXL was associated with low response rate to EGFR-TKIs. These results indicate the pivotal role of AXL in the intrinsic resistance and the emergence of osimertinib-tolerant cells. Combined treatment with osimertinib and an AXL inhibitor may prevent intrinsic resistance and the emergence of drug tolerant cells.

▲TOP

E-1071

Epithelial-to-mesenchymal transition as an independent mechanism of ALK inhibitor resistance in EML4-ALK lung cancer

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Mutations in the ALK gene are detectable in ~40% of ALK-rearranged lung cancers resistant to ALK inhibitors. While epithelial-to-mesenchymal transition (EMT) is a mechanism of resistance to various targeted drugs, its involvement in ALK-inhibitor resistance is largely unknown. We found that both ALK L1196M mutation and EMT were concomitantly detected in a single crizotinib-resistant lesion in an ALK-rearranged lung cancer patient. Digital PCR analyses combined with microdissection revealed that the ALK L1196M mutation was predominantly detected in epithelial type tumor cells, indicating that mesenchymal phenotype and ALK mutation can co-exist as independent mechanisms. Pre-clinical experiments with crizotinib-resistant lung cancer cells showed that EMT associated with decreased miR-200c and increased ZEB1 expression causes cross resistance to new generation ALK inhibitors. Pre-treatment with the histone deacetylase (HDAC) inhibitor can overcome the resistance by reverting EMT, in vitro and in vivo, indicating that HDAC inhibitor, followed by a new generation ALK inhibitor may be useful to circumvent resistance due to intra-tumor heterogeneity of resistance mutations and EMT.

E-1072

Activation of lysosomal mediated cell death in the course of autophagy via mTORC-1 suppression

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Alteration in lysosomal functions and turnover are common in cancer cells, which promotes growth and invasion. However, excessive lysosomal accumulation triggers lysosomal cell death. In this study, we found that suppression mTORC-1 kinase by novel inhibitor S22 leads to the activation of both autophagy and lysosomal biogenesis in pancreatic cancer line Mia PaCa-2. S22 inhibited the fusion between autophagosomes and lysosomes in these cells, thereby inducing the accumulation of autophagosomes and lysosomes. Inhibition of lysosomal function and not autophagy completely rescued S22 mediated cell death as pre-treatment with ammonium chloride and bafilomycin, inhibitors of lysosomal acidification, prevents S22 mediated death whereas, early autophagy inhibitors 3-methyladenine, wortmannin had no effect. Knockdown of Atg5 or Atg7 effectively diminished autophagy but enhanced lysosomal function and cell death. Further, TFEB was found necessary for S22 mediated lysosomal function as its knockdown abrogates lysosomal biogenesis and cell death. It was concluded that during mTOR-c1 inhibition by S22, it is the excessive lysosomal flux and not autophagy that triggered cancer cell death.

▲TOP

[E-1073] E16-2 [English]
Signal transduction inhibitor (2)

2018 / 9 / 27 (Thu) 14:15-15:30 Room 5/10F 1002, Osaka International Convention Center Room 5

Akihiro Tomida / Div. Genome Res., Cancer Chemotherap. Ctr., JFCR

E-1073

A novel therapeutic option for synovial sarcoma using alpha-radiolabeled FZD10 antibody

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Synovia sarcoma (SS) is a rare but aggressive soft-tissue sarcoma commonly occurs in young adults. Local recurrence and metastasis of SS result in poor prognosis and the establishment of an effective therapeutic option is desired. Frizzled homolog 10 (FZD10), the transmembrane protein highly expressed in SS tumor but not in most normal tissues, is expected as a therapeutic target against SS. Recently, the advantages of alpha-radioimmunotherapy (alpha-RIT) using radioisotope(RI)-labeled antibody emitting alpha-particles have been shown in treating metastatic cancer. Alpha-particle can be specifically delivered to and effectively kill the cancer cells sparing normal tissues. Astatine 211 (At211) is one of the attractive RIs because of the 100% alpha-particle emission and appropriate half-life.In this study, we verified the tumor accumulation of At211-labeled anti-FZD10 antibody ([At211]anti-FZD10 Ab) and performed preclinical alpha-RIT to SS tumor xenograft model mice in order to investigate the antitumor effect. Our results provided the proof of concept that alpha-RIT using [At211]anti-FZD10 Ab has high potential as a novel therapeutic option against SS.

▲TOP

E-1074

ZY0511, a novel, potent and slective LSD1 inhibitor, exhibits potent anticancer activity via Ddit4/mTOR pathway

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Yan Li, Zeping Zuo, Lei Tao, Yang Zhou, Shengyong Yang, Yinglan Zhao. Lysine specific demethylase1 (LSD1) is a promising target for cancer therapy. The present study describes the discovery and biological activity of a novel, potent, orally bioavailable, well-tolerated LSD1 inhibitor, ZY0511. ZY0511 was developed via CADD, de novo synthesis and HT screening. It inhibited LSD1 activity with IC50 1.4 nM, and proliferation of human cancer cells with IC50 0.2-4.8 μM. It induced S phase cell cycle arrest and reduced colony formation in A2780 and HCT116 (high LSD1 expression). mRNA seq results identified change of genes after ZY0511 treatment. ZY0511 significantly upregulated ddit4 and ddit4 depletion blocked effect of ZY0511. Crucially, ZY0511 triggered ddit4 expression through altering H3K4 methylation of its promoter by LSD1 inhibition, thus suppressed mTORC1 activities. Oral administration of ZY0511 to nude mice tumor xenografts significantly prevented growth of HCT116 and A2780 tumors without detectable toxicity. Taken together, ZY0511 shows therapeutic potential for solid tumors and warrants further investigation. Ddit4 could be used as a predictive biomarker of LSD1 inhibitor.

E-1075

Ginsenoside compound K inhibits NF-κB by targeting Annexin A2

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Ginsenoside compound K(C-K), a major metabolite of ginsenoside exhibits anti-cancer activity in various cancer cells and animal models. A cell signaling study has shown that C-K inhibited NF-κB pathway in human astroglial cells and liver cancer cells. However, the molecular targets of C-K and the initiating events were not elucidated. In order to explore the molecular target of C-K for its inhibitory effect on NF-κB, both molecular docking and thermal shift assay were performed with C-K and NF-κB related proteins, which positively conformed the interaction between Annexin A2 and C-K. This interaction prevented the interaction between Annexin A2 and NF-κB p50 subunit and their nuclear co-localization, which attenuated the activation of NF-κB as well as the expression of its downstream genes, followed by the activation of caspase 9 and 3. In addition, the over expression of Annexin A2-K320A, a C-K binding-deficient mutant of Annexin A2, rendered cells to resist C-K treatment, indicating that C-K exert its cytotoxic activity mainly by targeting Annexin A2. This study for the first time revealed a cellular target of C-K and the molecular mechanism for its anti-cancer activity.

E-1076

PIM inhibition reduces tumor growth and improves survival in mouse advanced castration-resistant prostate cancer

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PIM serine/threonine kinases are overexpressed in various cancers and are correlated with human prostate cancer progression. We previously evaluated the antitumor activity of the pan-PIM kinase inhibitor AZD1208 and showed that it could effectively suppress tumor growth in early-stage mouse models of castration-naïve and castration-resistant Pten-deficient prostate cancer. To fully determine the therapeutic potential of PIM inhibition we examined the therapeutic efficacy of AZD1208 in a mouse model of advanced prostate using clinically relevant endpoints. Additionally, since AKT and PIM kinases modulate survival processes by the phosphorylation of common substrates, we also examined the treatment combination with the pan-AKT inhibitor, AZD5363. Treatments with AZD1208 suppressed CRPC growth and prolonged overall survival times in mice with Pten/P53-prostate tumors. Combined PIM/AKT inhibition provided a mild improvement in overall survival in this setting. This study provides evidence to support further investigation of into the mechanisms driving tumor survival and develop strategies that include PIM and AKT inhibition for the management of human advanced prostate cancer.

▲TOP

E-1077

Preclinical evaluation of the multi tyrosine kinase inhibitor TAS-115 in mouse Pten-deficient prostate cancer

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The tumor microenvironment (TME) is comprised of various cell types that can contribute to malignancy. Here we use preclinical mouse models of prostate cancer to show that TAS-115, a multi-kinase inhibitor suppresses prostate tumor growth by acting on the TME. Pten/Trp53-double knockout (Pten/P53-DKO) mice were used to evaluate the antitumor activity of TAS-115, respectively. Four weeks of treatment with TAS-115 suppressed prostate tumor growth in by 22.6% and reduced cancer cell proliferation by 28.6%. Immunohistochemical analysis showed inhibition of phosphorylated-ERK in both epithelial and stromal cells and reduced levels of phosphorylated-CSF1-R in tumor infiltrating immune cells in mice treated with TAS-115. In a castration-resistant Pten/P53-DKO cancer model 2 of 6 (33.3%) mice treated with TAS-115 showed reduced tumor burden. qRT-PCR-based analysis of immune-responsive genes showed greater response in TAS-115 castration-resistant tumors. TAS-115 also decreased tumor neovascularization in both models. Overall our studies show that TAS-115 is capable of suppressing prostate tumor growth by acting primarily on the TME.

E-1078

A Drosophila platform to generate novel kinase inhibitor leads

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Kinase inhibitor drugs have demonstrated benefits for some patients but often at the cost of significant toxicity and dosing problems. Here, we report a multidisciplinary approach for developing improved drug analogs within a whole animal context using the fruit fly Drosophila. We combine chemical and genetic modifier screening with computational modeling to develop new analogs of the approved kinase inhibitor sorafenib within an established Drosophila model for RET-dependent medullary thyroid carcinoma (MTC). Resulting tumor calibrated inhibitors (TCIs) have reduced activity towards ‘anti-targets’ MKNK and RAF therefore enhanced Ras pathway inhibition, and exhibit strongly improved therapeutic index in whole animal fly and human MTC xenograft models. Applying this platform to a variety of cancers leads can provide a rational path forward for the development of new classes of high efficacy/low toxicity drugs.

▲TOP

[E-1079] E16-3 [English]
New molecular targeted agent

2018 / 9 / 27 (Thu) 15:30-16:45 Room 5/10F 1002, Osaka International Convention Center Room 5

Kosei Maemura / Dept. Digestive Surg., Kagoshima Univ., Sch. Med. Dent. Sci.

E-1079

Autotaxin inhibition suppresses colon cancer growth and metastasis

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Autotaxin (ATX) is secreted enzyme that produces Lysophosphatidic acid (LPA), which stimulates LPA receptors to mediate multiple pathological effects that are associated with cancer development. The present work investigated whether the inhibition of ATX could decrease colon cancer growth and metastasis. Cre/loxP gene knockout strategy was used to delete the ATX gene in mouse. The deletion of ATX decreased the incidence of tumors in AOM/DSS-induced colorectal carcinogenesis mouse model (number:13.3±1.13 vs 20.4±1.77, $p<0.05$) (size:18.6±2.12 mm vs 30.3±0.56 mm, $p<0.05$). Nude mice implanted with HCT116 on their flanks were treated with ATX inhibitor (PF-8380). PF-8380 treatment reduced tumor growth (419±107.0 mm^3 vs 717±73.4 mm^3, $p<0.05$). Nude mice implanted with HCT116 in their spleens were treated with PF-8380 to investigate liver metastasis. PF-8380 reduced the incidence of liver metastasis (17 % vs 83 %, $p<0.05$), supported by the evidence that many oncogenic genes were suppressed in PCR array analysis. Our results showed the importance of ATX in promoting colon cancer development. ATX inhibition could provide a novel therapeutic approach in colon cancer.

▲TOP

E-1080

Drug repositioning by in vivo functional genomic screens using PDX models in colorectal cancer

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Molecular targeted therapy options that are clinically available for colorectal cancer (CRC) are very limited. Therefore, there is an urgent need to identify novel, effective targeted therapy to improve outcome for patients with CRC. To this end, we developed an integrated in vivo genomic and in vitro pharmacologic screening approach using CRC patient-derived xenografts (PDXs). We screened each model in vivo with a shRNA library targeting 200 genes specifically belonging to FDA-approved targeted therapies. One of the benefits of using this library is the direct correspondence of target genes with clinically available drugs. To prioritize the genetic results with the highest chances of being impactful, we decided to incorporate a high-throughput compounds screen design using a custom clinical drug library as a second filtering criteria. Through this integrated approach, we were able to identify novel target therapies and validate them as effective therapies for CRC. This approach enables us to identify clinically available drugs and rapidly evaluate these drugs for CRC. This approach also includes proof of principle insights into a drug repositioning in a precision medicine manner.

E-1081

Anti-melanoma effect of CDK inhibitor and its combination strategy with BRAF inhibition

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Although BRAF inhibitors show significant clinical responses in melanoma patients, it fails to eradicate tumor in almost all patients because melanoma cells often acquire resistance against BRAF inhibitors. Therefore, a strategy to overcome such resistance to BRAF inhibitors is required for successful melanoma therapy. In this study, we focused on a use of CDK inhibitor, dinaciclib, which shows its clinical efficacy in different cancers including melanoma. We demonstrate that dinaciclib exerts growth inhibition, cell cycle arrest, and induction of apoptosis on melanoma cells. BAK is indispensable for dinaciclib-induced melanoma cell apoptosis, and it is dependent on MCL1 reduction. A BRAF inhibitor, vemurafenib, induces apoptosis through BAX, and the combination of dinaciclib with vemurafenib synergistically inhibited melanoma cell growth through inducing both BAK- and BAX-dependent apoptosis. Collectively, our results suggest a combination of BRAF and CDK inhibitors can be an attractive strategy for treating melanoma.

E-1082

Effects of flavopiridol on cholangiocarcinoma cells

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Flavopiridol is a cyclin dependent kinase (cdk) inhibitor and has been reported as an effective antitumor agent in several cancers. We aimed to investigate the effect of flavopiridol on liver fluke-associated cholangiocarcinoma (CCA) which is a lethal bile duct cancer in Northeastern Thailand. The present study demonstrated flavopiridol suppressed CCA cell proliferation using MTT assay. Flow cytometry showed an increase in the sub G1 population of flavopiridol treated CCA cells. Flavopiridol also induced caspase-dependent apoptosis. Morover, flavopiridol inhibited tumor formation in xenograft mouse model. The tumor weight of flavopiridol treated group was significantly lower than control group. These results suggest that flavopiridol could be a potential antitumor agent for the treatment of CCA.

▲TOP

E-1083

Aspartate beta-hydroxylase modulates senescence via GSK3beta in hepatocellular carcinoma

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Introduction:Aspartate beta-hydroxylase (ASPH) is an enzyme overexpressed in human HCC. In this study, we demonstrated that inhibition of ASPH guides HCC cells into senescence.Methods:We performed shRNA-mediated knockdown in human HCC cells, and alterations of cell proliferation, colony formation and senescence were evaluated in vitro. We analyzed phosphorylation of GSK3beta as a potential mechanism for activating senescence. Overexpression of GSK3beta co-immunoprecipitation experiments were performed.Results:Knockdown of ASPH significantly reduced cell proliferation, colony formation, and induced senescence. We found that p-GSK3beta and p16 expressions were increased, and cyclin D1 and PCNA decreased after inhibition of ASPH. In addition, overexpression of GSK3beta reversed the phenotype and reduced expression of cell cycle inhibitors that was previously improved by ASPH-knockdown. These findings suggest that ASPH-related senescence goes through a GSK3beta mediated pathway. Co-immunoprecipitation experiments revealed that GSK3beta bound to ASPH which inhibited its binding to AKT and p38.Conclusions:We identified that inhibition of ASPH induced senescence through p-GSK3beta in HCC.

E-1084

Diacylglycerol kinase alpha inhibitor exerts bifunctional antitumor effects

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Diacylglycerol kinases (DGKs) are lipid kinases which transform diacylglycerol into phosphatidic acid and play important roles in intracellular signal transduction. It is reported that diacylglycerol kinase alpha (DGKa), an isozyme of DGKs, works to promote the proliferation and to suppress of the apoptosis in cancer cells. Additionally, DGKa induces to anergy statement in T lymphocytes. In this study, we investigated if a DGKa inhibitor (agent A) have bifunctional antitumor effects not only directly to cancer cells but through tumor immunity on hepatomas. Agent A suppressed the proliferation in human and murine hepatoma cell lines. The inhibitor improved the production of IL-2 and granzyme B in CD8+ T cells from OT-1 mice and of IL-2 in CD4+ T cells from OT-2 mice under stimulation by OVA. In vivo study, Agent A suppressed the tumor size in the liver of tumor bearing mice which injected intrasplenically murine hepatoma cells. Then, the amount of tumor infiltrating T cells and the production of IFN-gamma from CD8+ T cells in the liver were elevated. The DGKa inhibitor suppressed the proliferation of hepatoma and activated T cells, therefore it is expected for new anticancer drug.

▲TOP

[E-1025] E14-1 [English]
Hepatocellular carcinoma (1)

2018 / 9 / 27 (Thu) 9:00-10:15 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Akio Saiura / Hepato-Biliary-Pancreatic Surg., Cancer Inst. Hosp.

E-1025

Silencing of tumor suppressor lGFBP4 constitutes EZH2-driven epigenetic reprogramming in hepatocarcinogenesis

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Aberrant H3K27 trimethylation mediated by EZH2 is a hallmark in hepatocellular carcinoma (HCC), but the underlying mechanistic effects remain incompletely understood. Using ChIP-seq and RNA-seq, we identified the genomic loci silenced by Ezh2/H3K27me3 in HBx-transgenic mouse-derived HCCs, including *Igfbp4* whose ablation significantly correlated with poor survival of HCC patients. Subsequent functional assays revealed the strong growth- and invasion-suppressive effects of IGFBP4, which was associated with transcriptomic alterations leading to deregulation of multiple signaling pathways. Mechanistically, IGFBP4 promoted AKT/EZH2 phosphorylation to attenuate H3K27me3-mediated silencing, forming a reciprocal feedback loop that directed a transcription factor network crucial for liver homeostasis. Of translational significance, the in vivo tumorigenicity of *IGFBP4I*-silenced HCC cells was effectively suppressed by pharmacological inhibition of EZH2, but not AKT. These collective data uncover IGFBP4 as a novel hepatic tumor suppressor, and shed light into the mechanistic basis of EZH2-targeted drugs for HCC treatment. Acknowledgement: this work is supported by the RGC CRF (C4017-14G).

▲TOP

E-1026

Overactivation of a tumor suppressor protein P53 in hepatocytes promotes hepatocarcinogenesis

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Aim: To clarify the impact of P53 activation, which is known to be observed in the liver in chronic liver disease patients, on hepatocarcinogenesis
Methods: Mice harboring $Kras^{LSL\text{-}G12D}$ and Alb-Cre alleles ($Kras^{G12D}$ mice) were used for liver cancer mouse model. To induce hepatocyte-specific activation of P53, KrasG12D mice were crossed with mice harboring floxed alleles of Mdm2, a negative regulator of P53.
Results: Mdm2 deletion induced liver injury and increased tumorigenesis rate in $Kras^{G12D}$ mice in 4 months old (100% vs 18.2% in Mdm2-wild littermates). Upregulation of pro-apoptotic molecules (p16, p21, bax, noxa) and positive TUNEL staining indicated accelerated apoptosis of non-tumorous hepatocytes. Positive β-galactosidase staining and upregulation of inflammatory cytokines (tnf, ccl2, il-1β) suggested senescence-associated secretory phenotype in non-tumorous hepatocytes. Further deletion of P53 in Mdm2-deficient $Kras^{G12D}$ mice downregulated pro-apoptotic molecules and inflammatory cytokines, improved liver injury, and suppressed carcinogenesis (54.5% vs 100% in P53-wild littermates).
Conclusion: P53 activation in hepatocytes promoted liver injury and hepatocarcinogenesis.

E-1027

Activation of TRPM8 Promoted to Hepatocarcinogenesis through Abnormalities of Mitochondrial and Gene Regulation

Xundi Xu
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The TRPM8 channel is the primary molecular transducer of cold somatosensation in humans. In this study we aimed at investigation of its effect on hepatocarcinogenesis. HCC samples were analyzed by IHC and WB. TRPM8 knock out mouse were treated with DEN. Colony formation, flow cytometric analysis, mitochondrial function and chip seq were applied in vitro assay. Xenograft model were performed. TRPM8 was overexpressed in HCC tissue compared with its nontumor counterpart. Compared with wild type, TRPM8 knock out mouse induced with DEN indicated that tumor number of lesions, proliferation demonstrated by Ki 67, Brdu, AgNOR staining were reduced. The antagonist of TRPM8 intervention can inhibit growth rate, ROS, glutamine level, calcium efflux, membrane potential and oxygen comsumption rate of mitochondrial. RT-PCR analysis indicated that TRPM8 ablation can downregulate expression of SLC25A25, Sirt7, RNA polymerase Ⅱ. Finally, xenograft model induced by HepG2 cell treated by antagonist of TRPM8, can significantly inhibit growth of tumor.TRPM8 exert effects in hepatocarcinogenesis through abnormalities of mitochondrial and gene regulation modulated by Slc25a25-Sirt7 signaling.

E-1028

Withdrawn

No Abstract

▲TOP

E-1029

Targeting galectin-1 suppresses fibrosis-promoted hepatocellular carcinoma through disrupting SERPINB2-JNK feedback loop

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Hepatocellular carcinoma (HCC) ususally develops with severe liver fibrosis (cirrhosis) and the activation of hepatic stellate cells (HSCs) is critical for both liver fibrosis and HCC progression. Here, we reported that galectin-1 (Gal-1), a β-galactoside binding lectin, is highly expressed in fibrotic liver and carcinoma-associated stroma and is correlated with the poor prognosis of HCC. Targeting Gal-1 in HSCs suppressed HSC activation, experimental liver fibrosis and pro-inflammatory cytokine expression (IL-6, IL-1β, IL-8, and CCL2), which consequently inhibited HSC-promoted HCC cell invasion, migration and stem-like cell properties. Mechanism studies showed that, upon TNF-α stimulation, intracellular Gal-1 transcriptionally upregulated SERPINB2 which activated the JNK and c-Jun/ATF2 mediated pro-inflammatory cytokine expression. Reversely, JNK activation also increased SERPINB2 expression to maintain its signaling. Therefore, we conclude that targeting Gal-1 attenuates liver inflammation and fibrosis-promoted HCC by disrupting the positive feedback loop between SERPINB2 and JNK/c-Jun/ATF2 signaling cascade in HSCs.

E-1030

The molecular subclass which reflect the HCC stemness is associated with high recurrence rate and the tumor malignancy

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Background: Hepatocellular carcinoma (HCC) is the second most lethal cancer in the world and is highly recurrent even after curative surgery. We aimed to reveal the association between HCC molecular subclass and HCC recurrence, or BALAD score consisting of the value of Bilirubin, Albumin, AFP-L3, AFP, and DCP.Method: The 147 patients who underwent curative surgery for initial HCC was enrolled in this study. HCC subclass (S1: TGF-b activation and inflammation, S2: stemness and S3: b-catenin activation) was used for analysis.Result: The patients were separated into S1- (n=47) and S2- (n=36) and S3- (n=64) subclass, and the early recurrence rate within two years was significantly higher in S2 subclass (61%, p=0.002) compared with the other subtypes (S1: 23%, S3: 42%). The recurrence rate and overall survival rate in the patients with high-BALAD score was significantly worth than that of the patients with low-BALAD score (p=0.0003 and p<0.0001, respectively). The S2 subclass was significantly and strongly associated with high-BALAD score (p<0.0001). Conclusion: S2 subclass which reflect the stemness of HCC was associated with high early recurrence rate and high-BALAD score.

▲TOP

[E-1031] E14-2 [English]
Pancreatic cancer (1)

2018 / 9 / 27 (Thu) 10:15-11:30 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Yutaka Takeda / Dept. of Surg., Kansai Rosai Hosp.

E-1031

Vasohibin-2 plays an essential role in invasion and metastasis of pancreas cancer

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We have isolated vasohibin-1 (VASH1) and vasohibin-2 (VASH2), and are studying their roles. We have revealed that VASH2 is not expressed in normal cells but overexpressed in various cancer cells, and promotes not only tumor angiogenesis but also accumulation of CAFs and EMT of cancer cells. We recently noticed that pancreas cancer patients with higher VASH2 expression exhibited poorer prognosis, and thus focused on pancreas cancers. Knockdown of Vash2 in KPC mouse-derived pancreas cancer cells did not alter their proliferation, but significantly reduced their migration and invasion in vitro. When those cells were inoculated in mice, tumor growth was slightly reduced. However, when those cells were injected from the tail vein, metastasis was markedly diminished. We then crossed KPC mice with our Vash2(LacZ/LacZ) mice. Incidence of PDAC was same between KPC mice and KPC/Vash2 null mice. However surprisingly, invasion and metastasis were completely absent in KPC/Vash2 null mice. Thus, although VASH2 gene is not a driver, it appears to be essential for invasion and metastasis of highly metastatic pancreas cancer. Underlying mechanism will be discussed in the symposium.

▲TOP

E-1032

BRG1/SOX9 axis is critical for acinar cell-derived pancreatic tumorigenesis

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Chromatin remodeler BRG1 is silenced in over 10% of human pancreatic ductal adenocarcinomas (PDA). However, the role of BRG1 in pancreatic intraepithelial neoplasia (PanIN)-derived PDA originated from acinar cells remains elusive. Here, we found that exclusive elimination of Brg1 in acinar cells of Ptf1a-Cre^{ER}; Kras^{G12D}; Brg1$^{f/f}$ (KBC) mice impairs the formation of acinar-to-ductal metaplasia (ADM) and PanIN, while PDA formation was inhibited in the presence of p53 mutation. BRG1 bound to the Sox9 promoter regions to regulate its expression and was critical for recruitment of upstream regulators, including PDX1, to the Sox9 promoter and enhancer in acinar cells. SOX9 expression was downregulated in BRG1-depleted ADMs/PanINs. Notably, Sox9 overexpression canceled this PanIN-attenuated phenotype in KBC mice. Furthermore, Brg1-deletion in established PanIN by using a dual recombinase system resulted in regression of the lesions in mice. Finally, BRG1 expression correlated with SOX9 expression in human PDAs. In summary, BRG1 is critical for PanIN initiation and progression through positive regulation of SOX9. Thus, the BRG1/SOX9 axis is a potential target for PanIN-derived PDA.

E-1033

Presence of viable-peritoneal tumor cells in peritoneal lavage fluid is a prognostic factor in pancreatic cancer

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Peritoneal lavage cytology (CY) is used widely in the staging of pancreatic cancer (PC). The aim of this study was to evaluate use of a modified telomerase-specific adenovirus (TelomeScan F35) in rapid detection of viable peritoneal tumor cell (v-PTC) dissemination of PC. Patients with resectable histologically proven pancreatic ductal adenocarcinoma were enrolled. Peritoneal lavage fluid was harvested just after a laparotomy. Half of the fluid was examined by cytology with papanicolau staining and MOCK-31 immunostaining and the remaining half was used to detect v-PTC by TelomeScan F35. Among 30 patients, 3 were CY+, other 6 were positive by TelomeScan F35 (v-PTC+). All 30 patients underwent a surgical resection. 1 patient was double positive (CY+/v-PTC+), and postoperative peritoneal recurrence early occurred after resection. 2 were CY+, but v-PTC-, and no peritoneal recurrence were observed. Other 5 were CY-, but v-PTC+, and one of these 5 patients early occurred peritoneal recurrence. Remaining 22 patients (CY-/v-PTC-) were observed with neither local recurrence nor distant metastasis. In conclusions, the TelomeScan based v-PTC detection may be an independent prognostic factor.

E-1034

The immunological role of pancreatic cancer-associated fibroblasts to construct an immunosuppressive microenvironment

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Pancreatic cancer has a highly immunosuppressive tumor microenvironment (TME), possibly due to a reactive desmoplastic stroma. Pancreatic cancer associated fibroblasts (CAFs) are a heterogeneous population of activated fibroblasts in the stroma, whose immunological functions still remain unclear. Here, we examined the role of CAFs to build the TME in pancreatic cancer. First, we established original 15 CAF cell lines derived from surgical specimens. Then, based on our hypothesis that CAFs recruit immune suppressive cells into the tumor tissue, we performed migration assays using peripheral blood mononuclear cells including regulatory T cells. The assay showed that culture supernatants of 4 out of examined 7 CAF cell lines induced a significantly higher number of blood cells to migrate, compared with those of 2 pancreatic cancer cell lines. Furthermore, we also found that 8 of examined 9 CAF cell lines expressed immunosuppression-related molecule FAP by FACS analyses. Together these results suggest that CAFs may be important contributors to an immunosuppressive TME, indicating that the disruption of CAF function may open a new perspective for immune therapy to pancreatic cancer.

▲TOP

E-1035

Inhibition of CD110 suppresses liver metastasis of pancreatic cancer

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Thrombopoietin (TPO) is primarily produced by the liver, which regulates the production of platelets. Its receptor, CD110, was reported as an organ-specific marker for liver metastasis of colorectal cancer. In our analysis of pancreatic cancer patients, CD110 expression in cancer cells was associated with poorer prognosis and recurrence of liver metastasis. To investigate the functional role of CD110 in the liver metastasis of pancreatic cancer.We performed both short-term liver colonization assay and long-term experimental liver metastasis formation assay. In short-term liver colonization assay, knock down of CD110 significantly decreased liver colonization of pancreatic cancer cells.In long-term experimental liver metastasis formation assay, shCD110 significantly reduced liver metastasis in a time dependent manner.In the immunohistochemical analyses, we found the decrease in p-ERK1/2 and Ki67 expression in liver metastases in shCD110 group. These data suggest that TPO-CD110 axis promotes the malignant behaviors of pancreatic cancer cells, especially liver metastasis. TPO-CD110 axis may be one of the potential candidates for treatment of pancreatic cancer liver metastases.

E-1036

Biological role of PODXL1 in invasion and metastasis of Pancreatic ductal adenocarcinoma

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Podocalyxin-like 1 (PODXL1) is an anti-adhesive transmembrane protein belonging to the CD34 family which has been reported to be associated with an aggressive tumor phenotype and poor prognosis in cancers of several origins. In this study, we report the biological role of PODXL1 in progression of pancreatic invasive ductal adenocarcinoma (PDAC), especially in invasion and metastasis of PDAC cells.Knockout of PODXL1 gene in three different human PDAC lines, MiaPaCa-2, AsPC1 and Panc-1, almost abrogated metastatic lesion in liver-metastatic mouse model in vivo. Molecular analysis revealed that PODXL1 functioned as a critical activator to multiple cytokine receptors expressed on cancer cells which are regulated by the ligands derived from the tumor microenvironment, and their interaction facilitated PDAC invasion. Expression of PODXL1 was strongly detected on cancer cells at the invasive front of the PDAC tissues, where coincidently expressed specific cytokine receptor that critically mediates cancer metastasis in vivo. Thus, we identify the novel aspect of PODXL1 and its biological role on PDAC as one of the most intractable malignancies.

▲TOP

[LS4] LS4 [English]
Onco-Hu™ Mice for Evaluation of Immuno-oncology Therapeutics

2018 / 9 / 27 (Thu) 11:50-12:40 Room 6/10F 1004+1005, Osaka International Convention Center Room 6
：Charles River Laboratories Japan, Inc.

Tadashi Kondo / Division of Rare Cancer Research, National Cancer Center Research Institute

LS4

Onco-Hu™ Mice for Evaluation of Immuno-oncology Therapeutics

Janine Low-Marchelli
JAX Mice, Clinical & Services, The Jackson Laboratory

No Abstract

▲TOP

[E-1085] E10-1 [English]
Metastasis and invasion

2018 / 9 / 27 (Thu) 13:00-14:15 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Ryuichi Sakai / Dept. Biochem., Kitasato Univ. Sch. Med.,

E-1085

The C5a-C5a receptor system is associated with cancer promotion and is a possible therapeutic target

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A leukocyte chemoattractant C5a is a cancer microenvironment factor. Cancer cells from the patients' tissues expressed C5a-receptor (C5aR) at rates different in organs (CCR 2013). The high C5aR expression of patients' cancer cells was associated with high rates of vascular invasion and metastasis, and advanced clinical stages and poor prognosis of the patients with stomach, breast, kidney or urinary tract cancer. C5a enhanced C5aR-positive cancer cell motility accompanying conversion of RhoA-GDP to RhoA-GTP and invasiveness via the ERK and PI3 kinase pathways and depending on increase of mainly MMP-8 secretion. C5a generated by the immune-complex-induced complement activation in the wild mouse skin enhanced invasion and tumor growth of C5aR-positive homologous cancer cells inoculated into the skin site, together with recruitment of MDSCs which suppress the CD8+ T-cell antitumor response. Human C5aR-positive cancer cells, treated with C5a and injected intravenously, formed 3-fold more nodules in the nude mouse lungs than C5aR-negative cells. These results indicate cancer promotion by the C5a-C5aR system and suggest that blocking this system may be a useful therapeutic strategy.

▲TOP

E-1086

Nrf2 Activation Drive Macrophages Polarization And Cancer Cell Epithelial-Mesenchymal Transition During Interaction

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Co-author：Yuji Morine、Tetsuya Ikemoto、Satoru Imura、Syuichi Iwahashi、Yu Saito、Mitsuo Shimada
Dept. Surg.

The M2 tumor-associated macrophages inhibits the anti-tumor inflammation, increases angiogenesis and promotes tumor progression. Nuclear factor-like 2 (Nrf2) not only modulates the angiogenesis but also plays the anti-inflammatory role; however, the role of Nrf2 in the cancer cell and macrophages interaction is not clear. In this study, we found that cancer cells could induce an M2-like macrophage characterized by up-regulation of CD163, Arg1 and VEGF, and down-regulation of IL-1b and IL-6 through Nrf2 activation. The Nrf2 activation of macrophages was correlated with elevated intracellular reactive oxygen species which induced by cancer cells derived lactate. Cancer cells educated macrophages could activate Nrf2 of the cancer cells, in turn, to increase cancer cells epithelial-mesenchymal transition (EMT) through paracrine VEGF. These findings suggest that Nrf2 plays the important role in the cancer cells and macrophages interaction. Macrophage Nrf2 activation by cancer cell-derived lactate skews macrophages polarization towards an M2-like phenotype and educated macrophages activated Nrf2 of the cancer cells to promote EMT of cancer cells.

E-1087

Crumbs3a enhances receptor kinase mediated phosphor-signaling, and promote colon cancer progression

Hidekazu Iioka
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Co-author：Ken Saito、Eisaku Kondo
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Crumbs3a (Crb3a) is one of the epithelial cell polarity regulator which is necessary for the characterization of the apical plasma membrane in normal epithelial cells. While Crb3a functions as a tumor suppressor in mammalian epithelial cells in vitro, only limited evidence available to Crb3a function in the progression of human cancer. Therefore, we investigated Crb3a expression and function in human cancer cells, and consequentially, Crb3a strongly expressed especially in adenocarcinoma tissue. We established Crb3a knockout (KO) colon cancer cell lines, and those cell lines displayed reduction in malignant tumor cell behaviors in vitro and in vivo. In addition, we performed proteomic analysis and identified receptor kinases as novel binding partner of Crb3a. The downstream phosphor-signaling could be activated using recombinant ligand protein, and Crb3a deficient cells displayed decreased phosphorylation of downstream substrate. Taken together, Crb3a might increase the susceptibility of specific receptor kinase to its ligand to promote cancer cell migration.

E-1088

Claudin-2 activates LKB1-AMPK signals, thereby inducing cell-cycle arrest and autophagy in liver cancer cells

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We previously presented that the expression of CLAUDIN (CLDN)-2, a leaky tight junction protein, was robustly up-regulated in WNT signal-engineered liver cancer cells (Liver Int 2013), and that the increased expression contributed to their high tumorigenicity in nude mice. Indeed, the CLDN-2-overexpressing Hep3B cell clones exhibited higher proliferative activity and tumorigenicity and the CLDN-2-silencing cell clones did lower. However, such phenomenon was not always universal. Namely, CLDN-2-overexpressing HLF cell clones demonstrated extremely low proliferative activity and no tumorigenicity. The molecular basis for it included activation of the tumor-suppressive LKB1 and its downstream signal relay involving AMPK, p53, and p21, being coupled with G1 cell-cycle arrest. In addition, the cells showed conversion of LC-Ⅰ to LC-Ⅱ, a marker of autophagy, in immunoblot analysis. Recent report has demonstrated a gatekeeper action of LKB1 in cell proliferation (Cell Reports 2018). Taken together, the findings obtained from this study suggested a novel tumor-suppressive action of CLDN-2 involving LKB1 in the CLDN-2-negative neoplastic cells.

▲TOP

E-1089

Studies on the mechanism of RUNX3 induced metastasis via VEGFC and CNTN1 in gastric cancer

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RUNX3 is a member of the Runt domain-containing transcription factor family and has been shown to function as a tumor suppressor in multiple types of cancers. However, RUNX3 also behaves as an oncogene in some cases: Hingorani's group showed Runx3 induces metastasis in pancreas. HGC-27, one of gastric cancer cell lines with high expression of RUNX3, exhibited reduced migration and invasion properties in vitro assay after RUNX3 was deleted. When HGC-27 cells were inoculated into spleen of NSG mouse, the cells strongly metastasized to liver. In contrast, metastatic ability of HGC-27 was dramatically reduced after RUNX3 was deleted, although HGC-27 cells with or without RUNX3 show similar growth property in cell culture conditions. Furthermore, we found expression of Vascular endothelial growth factor C (VEGFC) and Contactin 1 (CNTN1), which are major players in metastasis independently, were downregulated after RUNX3 was deleted in HGC-27. Interestingly, TCGA clinical cancer data showed higher expression of RUNX3 in late stage of gastric cancer compared to early stage. We are currently studying how RUNX3 regulates VEGFC and CNTN1 to induce metastasis in gastric cancer.

E-1090

ACTL6a promotes metastasis and predicts poor prognosis of prostate cancer via regulation of YAP and cancer stemness

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Using Micro-Western Array (MWA), a high-throughput Western blotting platform, and 434 antibodies targeting epigenetic regulation, we identified expression of 144 proteins significantly changes during the acini morphogenesis (a differentiation procedure) of prostate epithelial cells in 3D culture. ACTL6a, encoding an SWI/SNF subunit linked to stem cell and progenitor cell function, decreases most during the prostate cell differentiation, suggesting that it may be a novel oncogene in PCa. Tissue array reveals that PCa patients with high ACTL6a expression have worse survival rate. Knockdown of ACTL6a suppresses migration and invasion of PCa cells as determined by transwell assay, inhibits metastasis of prostate tumors in orthotopic model in nude mice, as well as reduces ALDH+ PCa cancer stem cells population. MWA analysis indicates that knockdown of ACTL6a reduces YAP and proteins in Hippo pathway, CD44, Sox2, Nanog, KLF4, HDAC1, HDAC3, and MMPs. Overexpression of YAP rescues the suppressive effect of ACTL6a on cancer metastasis. Our finding suggests that ACTL6a promotes metastasis and predicts poor prognosis of prostate cancer via regulation of YAP-Hippo pathway and cancer stemness.

▲TOP

[E-1091] E10-2 [English]
Invasion and metastasis (1)

2018 / 9 / 27 (Thu) 14:15-15:30 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Mayumi Ono / Dept. Pharm. Oncology., Grad. Sch. Pharm. Sci., Kyushu Univ.

E-1091

A small molecule ligand of VCP inhibits accelerated fibroblast migration by cancer cells

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Carcinoma Associated Fibroblasts (CAFs) are fibroblasts activated by surrounding cancer cells. CAFs show enhanced cell migration, which play an important role in cancer metastasis. In the last meeting, we demonstrated the enhanced migration of NIH3T3 fibroblasts when they were cultured in the presence of MCF7 breast cancer cells. Human fibroblasts showed similar phenomenon even when they were co-cultured with cancer cells other than MCF7.
In the present study, we have screened about 16,000 compounds of RIKEN NPDepo chemical library for inhibitors of the enhanced NIH3T3 migration in the presence of MCF7. We identified RKN8733 as an inhibitor of enhanced cell migration. Using RKN8733-immobilized beads, we found RKN8733 specifically binds to D1 domain of valosin-containing protein (VCP), a member of AAA+ protein family. The D1 domain of VCP is important for its oligomerization. The silencing of VCP by siRNA in NIH3T3 but not in MCF7 inhibited the enhanced migration of co-cultured NIH3T3. These results indicate that MCF7 activates the migration of NIH3T3 through VCP oligomerization via its D1 domain. (Collaborators: Yasumitsu Kondoh and Kaori Honda in RIKEN Chemical Biology)

▲TOP

E-1092

EMP1 signaling promotes cancer invasiveness and metastasis

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Metastatic prostate cancer is frequently led to fatality, and thus, understanding of the underlying mechanism is important to reduce it. We found that the expression of epithelial membrane protein 1 (EMP1) was upregulated in prostate cancer cells by contacting with surrounding stroma cells. EMP1 has four transmembrane domains. Overexpression of EMP1 in LNCaP cells (EMP1-LNCaP cells) enhanced cell motility and invasiveness, which was also observed in other types of cancer cells. After the orthotopic injection of EMP1-LNCaP or parental LNCaP cells into the prostate of nude mice, lymph nodes and lung metastases occurred only by the injection of EMP1-LNCaP cells. We next identified copine-III as a novel molecule binding to the intracellular region of EMP1. The EMP1-copine-III complex induced phosphorylation of Src and Vav2, a guanine nucleotide exchange factor for Rac1, to activate Rac1, leading to the enhanced cancer cell migration and invasion. As clinical implications, increased EMP1 expression was observed in the samples from the patients with higher Gleason scores. These results suggest the significance of EMP1 in cancer invasiveness and metastasis.

E-1093

Metformin suppresses cholangiocarcinoma cell migration/invasion in association with inhibition of mTOR and FAK pathways

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Cancer metastasis is one of the hallmarks of aggressive cancer which correlates with the poor prognosis of patients. Cholangiocarcinoma (CCA) is an aggressive malignancy with poor prognosis. Metformin (Met), a first-line antidiabetic drug, has been suggested to reduce risk of some cancers. The present study, we found that Met inhibited migration in CCA cells, KKU-100 and KKU-M156, as assessed by wound healing assay. Met also suppressed cell invasion of KKU-452 and KKU-M156 cells by transwell chamber assay. Cisplatin, a standard drug treatment in CCA, used alone or in combination with Met exhibited antiproliferation, but Cis showed modest effects on migration and invasion. The antimigration and antiinvasion effects of Met may be related to the suppression of mTOR and FAK signalings. The role of FAK in migration was affirmed by using PF573228, a selective FAK inhibitor. PF573228 markedly inhibited cell migration in CCA cells similar to Met. These results provide an evidence that Met may be an effective agent for suppression of cancer metastasis. Acknowledgement. This work was supported by grant-in-aid from Faculty of Medicine, Khon Kaen University, Thailand.

E-1094

Migration of Pancreatic Cancer cell BxPC-3 is suppressed by ROS

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Background: In the micro-environment of cancer, it has been suggested that various kinds of stress including oxidative stress have influence on cancer metastasis. Here we evaluate the effect of reactive oxygen species (ROS) on cancer cell migration in vitro using pancreatic cancer cell line BxPC-3.Methods: Migration of BxPC-3 cells was evaluated by TAXIScan, a cell dynamics assay device based on optical images. Hydrogen peroxide was mixed with chemoattactive reagent FBS. Cell proliferation was evaluated by WST-1 assay method.Results: The migration of BxPC-3 cells was suppressed by hydrogen peroxide in the dose dependent manner (0 to 0.0003%). On the contrary, the cell proliferation was not influenced by the same concentration of hydrogen peroxide.Discussion: These data suggest that ROS suppress the migration of pancreatic cancer cells. We also demonstrated previously the suppression of chemotaxis in superoxide production defective myeloid leukemia cell lines. ROS in cancer micro-environment may have a negative effect on cancer cell migration.(Non-member contributors: Masumi Itadani and Futoshi Kuribayashi)

▲TOP

E-1095

Cancer-associated mesothelial cells as a potential therapeutic target in epithelial ovarian cancer

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Based on the “seed and soil” theory, we have been investigating interaction of epithelial ovarian cancer cells (EOCs: seed) and cancer-associated mesothelial cells (CAMs: soil). This study illustrates how they promote tumor microenvironment with innovative experimental models and various in silico omics analysis.We established an in vitro peritoneal metastatic invasion model and visualized fluorescent labeled-cell behavior by 3D-construction, which demonstrated that EOCs followed CAMs and invaded into extracellular matrix. We also found acquired platinum-resistance in EOCs co-cultured with CAMs. Transcriptome analysis of the EOCs sorted after co-culturing suggested PI3K/Akt pathway activated by the stimulation of CAMs had a substantial effect in EOCs. In addition, proteome analysis of CAMs and EOCs was used to explore the upstream of the signal activation. The versatile omics analysis revealed fibronectin produced by CAMs could be one of the cause of PI3K/Akt pathway activation in EOCs, which is confirmed by in vitro co-culture analysis.Our results suggest that targeting CAM in peritoneal metastatic niche can be a novel therapeutic strategy in advanced epithelial ovarian cancer.

E-1096

The roles of tumor microenvironment in cancer metastasis

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Tumor microenvironment is heavily involved in carcinogenesis, but its precise roles in metastasis remain elusive. (1) Interstitial lung disease (ILD) is considered a prognosis factor for lung cancer. Bleomycin-induced interstitial fibrosis promoted the metastases of Lewis lung carcinoma cells to the lymph nodes or contralateral lungs in an orthotopic model and conferred poorly differentiated histology in a genetically engineered mouse model of NSCLC. Mechanistic studies using the models and clinical data of postoperative stage I NSCLC patients indicated the role of ILD-associated microenvironment in cancer progression. Therapeutic implications of the study will be discussed. (2) Tumor microenvironment often induces EMT in cancer cells. An in vivo shRNA library screen identified HNRNPLL encoding a pre-mRNA splicing factor as a colorectal cancer metastasis suppressor gene. HNRNPLL suppressed invasion of colon cancer cells partly by regulating the alternative splicing of CD44 pre-mRNA. HNRNPLL was downregulated in colorectal cancer cells undergoing EMT and in those at the invasion front of clinical samples. Control mechanisms for HNRNPLL expression during EMT will also be discussed.

▲TOP

[E-1097] E11-1 [English]
Metabolism / metabolome (1)

2018 / 9 / 27 (Thu) 15:30-16:45 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Koji Okamoto / Natl. Cancer Ctr. Res. Inst., Div. Cancer Differentiation

E-1097

Characterization of the roles of leukemia specific ALDH1A2 isoform in T-cell acute lymphoblastic leukemia

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T-cell acute lymphoblastic leukemia (T-ALL) is a hematological malignancy resulted from the leukemic transformation of T-cell precursors. Aberrant expression of transcription factors including *TAL1/ SCL* is the most frequent genetic abnormality in T-ALL. We previously reported that TAL1 and its regulatory partners coordinately regulate its downstream targets. One of high-confident targets directly regulated by TAL1 is *ALDH1A2*, which is involved in the retinoic acid signaling pathway by catalyzing retinaldehyde into retinoic acid. Interestingly, T-ALL expressed a specific isoform of ALDH1A2 in which a N-terminal sequence is truncated, which likely possess an attenuated enzymatic activity. The expression of *ALDH1A2* is significantly higher in TAL1-positive T-ALL subgroup as compared to other subgroups. Genetic knockdown and overexpression experiments indicated that ALDH1A2 isoform 3 promotes cell growth, proliferation and survival. Metabolome analysis suggested that ALDH1A2 is likely regulating the glycolytic pathway. Furthermore, our study indicated that ADLH1A2 has a protective role from the production of reactive oxidative species.

▲TOP

E-1098

Inhibition of phosphoglycerate dehydrogenase reduces neuroblastoma growth and arginine deiminase expands its application

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Background
The prognosis of neuroblastoma (NBL) remains poor, and novel treatments are demanded.
Methods
RNA sequencing and DNA methylation analysis were performed in 34 NBL specimens. The open datasets were also analyzed. We extracted PHGDH and ASS1 as targets to conduct functional analyses. The effects of knock down of PHGDH and its inhibitor, CBR-5884, on NBL cell lines were assessed. We also examined the effect of recombinant arginine deiminase (rADI) in combination with CBR-5884. Metabolome analysis in NBL cells was further performed.
Results
High expression with hypermethylation of gene body of PHGDH were observed in cases with MYCN amplification and cases with 11q deletion having especially worse prognosis. Inhibition of PHGDH inhibited growth of NBL cells with high expression of PHGDH. rADI enhanced the effect of CBR-5884 on cells with low expression of PHGDH and ASS1. Metabolome analysis showed rADI enhances serine synthesis, suppresses Warburg effect, and induces greater reactions to CBR-5884 in NBL cells with low expression of PHGDH and ASS1.
Conclusion
PHGDH is the promising target for high-risk NBL. rADI changes tumor metabolism and expands application of PHGDH inhibitor.

E-1099

Strategies for overcoming the metabolic flexibility of glioma stem cells

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Several lines of evidence have shown that malignant tumors can adjust their metabolism to ensure growth in hypoxic conditions. Tumor cells that can thus adapt are usually also resistant to conventional therapies and display characteristics of cancer stem cells. For malignant gliomas, such cells can achieve metabolic flexibility by selective use of glucose transporters or alternate fuels. However, targeting individual aspects of this flexibility has not been sufficient to inhibit tumor propagation. Here we have interrogated the adaptation process which allows glioma stem cells (GSCs) to survive hypoxia, using a syngeneic implantation model based on murine genetically-induced GSCs. We show that GSCs have a high energetic demand, independent of their proliferation rate. While they can vary in their ability to accommodate changes in oxygen supply, the resilient fractions do so partially through adjustments in the amino-acid metabolism. The adaptation process is quick, reversible and can occur repeatedly. We also discuss the prevention and inhibition of such metabolic flexibility, along with complementary strategies required to target GSCs residing in both hypoxic and normoxic areas.

E-1100

Cancer stem-like properties and drug resistance are dependent on the mitochondrial enzyme of One-carbon metabolism

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Tumor recurrence is thought to be attributable to drug resistant and cancer stem-like cells. Here, we uncover the critical role of folate-mediated one-carbon (1C) metabolism involving mitochondrial methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) and its downstream purine synthesis pathway. MTHFD2 knockdown greatly reduced tumorigenesis and stem-like properties, which were associated with the purine nucleotide deficiency, and caused marked accumulation of 5-aminoimidazole carboxamide ribonucleotide (AICAR). Lung cancer cells with acquired resistance to the targeted drug gefitinib exhibited increased stem-like properties and enhanced expression of MTHFD2 that plays important roles in cell growth, drug resistance, and stem-like properties. Treatment of the gefitinib-resistant cancer cells with AICAR reduced its stem-like properties and restored the drug sensitivity. Thus, we provide evidence that the MTHFD2-mediated mitochondrial 1C metabolism is critical for cancer cell growth, for drug resistance and for cancer stem-like properties. Since drug-resistant or cancer stem-like cells are dependent on MTHFD2, therapies targeting MTHFD2 can eradicate tumors.

▲TOP

E-1101

COP1-Trib1 targets ACC1 for degradation and protects leukemic cells from metabolic stress in acute myeloid leukemia

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Tumor Cell Biol., Div. Biol. Sci., Nara Inst. Sci. Tech.

COP1 is an E3 ubiquitin ligase that functions in many biological responses in mammals. COP1 exerts its ability through its substrates by direct interactions and with the aid of adaptor proteins, which confers diversity and specificity to the COP1's activity. Putative substrates of COP1 suggest that COP1 is involved in cell growth, differentiation, and cell metabolism. We previously reported that the COP1-Trib1 complex targets C/EBPalpha for degradation, and its overexpression specifically induces acute myeloid leukemia (AML) in a mouse model. In this study, we identified acetyl-CoA carboxylase 1 (ACC1), a regulator of lipid metabolism, as a novel target of the COP1-Trib1 complex. Trib1, an adaptor pseudokinase, directly binds to ACC1 and the COP1-Trib1 complex polyubiquitinates ACC1 for degradation. In murine bone marrow culture, knockdown of ACC1 facilitated the COP1-Trib1-induced growth-promoting activity, and reduced the level of reactive oxygen species (ROS) in the cell. These data imply that the COP1-Trib1 complex targets not only C/EBPalpha but also ACC1 for degradation, thereby protecting the leukemia-initiating cells from metabolic stress during the progression to AML.

E-1102

Glycogen synthase kinase (GSK) 3β induces protooncogenic autophagy in colon cancer

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We have previously demonstrated that various cancers depend on aberrant GSK3β for their proliferation, invasion and resistance to therapy (Cancer Sci 2016;103:1363-72). We have recently discovered that GSK3β participates in aerobic glycolysis (Warburg's effect) and attenuates the oxidative phosphorylation pathway for energy production. Here focusing on autophagy recognized as desperate energy source for cancer cells surviving in a hostile tumor microenvironment, we studied the integrative role of GSK3β in energy and nutrient metabolism. Proliferation of colon cancer cells (SW480, HCT116, LoVo) was decreased with accumulation of LC3-II by treatment with hydroxychloroquine, indicating that the colon cancer cells depend on autophagy for survival and proliferation. A specific GSK3β inhibitor (AR-A014418) also attenuated autophagy activity and cell proliferation. This was associated with decrease in expression of the transcription factors (ATF6, XBP-1, FOXO3A) that are reported to participate in autophagy. These results suggest that deregulated energy and nutrient metabolism underpins the tumor-promoting roles of GSK3β in colon cancer.

▲TOP

[E-1037] E21 [English]
Gene therapy and oncolytic virus therapy (1)

2018 / 9 / 27 (Thu) 9:00-10:15 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Masatoshi Tagawa / Div. Pathol & Cell Ther., Chiba Cancer Ctr. Res. Inst.

E-1037

Adenovirus vectors expressing highly multiplex double-nicking guide RNAs in vivo: high efficiency and low off-target

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Although safety is essential for all therapies, off-target is always a serious problem in the application of genome editing. However, the double-nicking (DN) method using Cas9 nickase and two guide RNAs (gRNAs) for one cleavage drastically decreases off-target compared to usual Cas9/gRNA cleavage, because recognition of 20-nucleotudes is doubled. Also, simultaneous two or more cleavages efficiently disrupt the target gene, since it causes irreversible deletion. To fulfill both requirements simultaneous expression of four gRNAs are desirable. We established a method for construction of replication-deficient adenovirus vectors (AdVs) containing even eight gRNA-expressing units. Surprisingly, once obtained, these vectors could sequentially be amplified with no detectable loss of the gRNA-expressing units and were sufficient for mouse experiments. We have constructed these eight-gRNA AdVs targeting viral DNAs of HBV and HPV integrated in the chromosome of liver and cervical cancers, respectively. Also, we are constructing AdVs simultaneously disrupt two genes responsible for cancer. These data will be presented. We will supply of these order-made vectors in collaboration basis.

▲TOP

E-1038

Genetic regulation of RUNX2-cancer stem cell marker X axis in CRPC-NE cells

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Although Runt-related transcription factor 2 (RUNX2) is notorious for metastatic prostate cancer progression, its function is not yet identified in castration-resistant neuroendocrine prostate cancer (CRPC-NE). To investigate RUNX2 function in CRPC-NE, we analyzed the clinical datasets and found that one of cancer stem cell factor (Gene “X”) was upregulated in RUNX2 high expression cases. We also found the silencing RUNX2 induced apoptosis in CRPC-NE cells through decreasing X expression. In ChIP assay, we revealed RUNX2 binds to the consensus RUNX2 binding sequence (5’-TGTGGT-3’) located in X regulatory region. shRNA-mediated RUNX2 knockdown decreases X expression and induces apoptosis in the CRPC-NE cell line PC-3, we examined the efficacy of our novel drug Chlorambucil-conjugated Pyrrole Imidazole Polyamide (Chb-M’), which specifically binds to the consensus RUNX binding sequence to inhibit RUNX2 target genes. Under Chb-M’ treatment, PC-3 growth was highly suppressed (IC50 = 620 nM) through gene X inhibition; this effect was also confirmed in vivo. Taken together, we propose RUNX2-X axis can be a novel therapeutic target for CRPC-NE.

E-1039

Assessments for prediction of bystander effect in HSV-tk/GCV gene therapy

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Background: Enzyme/prodrug gene therapy using stem cells as cellular vehicles is a feasible treatment strategy for malignant gliomas. With a need for tools to seek out further therapeutic effectiveness in herpes simplex virus-thymidine kinase/ganciclovir system (HSV-tk/GCV system), we tried to establish predictive assessment methods for bystander effect measuring functional gap junction using high-content analysis. Methods: With the use of two rat glioma cell lines (GS-9L, C6), functional gap junction between the same cell lines was assessed by parachute assay and scrape loading/dye transfer assay (SL/DT assay) using high-content analysis. Then, the strength of in-vitro bystander effect was assessed by MTT assay at 5 μ g/ml GCV for 7days using HSV-tk gene transduced cells (GS-9Ltk, C6tk). Results: Functional gap junction was higher in GS-9L than in C6 in both studies. Bystander effect was stronger in GS-9Ltk/GS-9L than in C6tk/C6. Conclusion: Assessments of functional gap junction using high-content analysis were consistent with in-vitro bystander effect. This result may allow these methods to be refined as predictive tools for bystander effect in stem-cell based gene therapy.

E-1040

Novel safe and effective oncolytic virotherapy by miRNA-regulation

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In the past two decades, oncolytic viruses represent a new class of therapeutic agents to cancer treatment. We have demonstrated that coxsackievirus B3 (CVB3) was a potent and novel oncolytic agent with direct lysis of human non-small cell lung cancer cells (NSCLC) previously. However, due to cardiac tissue and exocrine pancreas are among the most relevant targets for CVB3 infection, CVB3 could cause viral myocarditis and pancreatitis. To improve its safety profile, we inserted microRNA target sequence to the 3’UTR, 5’UTR or both of these regions of the CVB3 genome. All viruses elicited massive viral lysis of tumor cells in vitro and in vivo. Human tumor-bearing nude mice treated with wild type virus showed significantly increased serum levels of AST, ALT, LDH and amylase, but recombinant viruses did not. There were no genetic mutations observed in target sequences in 5’ UTR in vitro after 10 serial passages, which indicated that the microRNA target sequences inserted into 5’ UTR is more stable than those into 3’ UTR.Collectively, our current results suggested newly engineered CVB3 targeting miRNA could become a candidate for safer and more effective oncolytic virus therapy.

▲TOP

E-1041

A Wee1 kinase inhibitor enhances replication and infectivity of oncolytic adenoviruses in p53-deficient cells

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Infectivity of adenoviruses (Ad) is greater in the G2 and M phases of the host cell cycle than in the G1 and S phases. We examined whether a Wee1 kinase inhibitor MK-1775, which induces cell death through aberrant mitotic arrest, could further enhance the cytotoxicity of oncolytic Ad driven by the survivin promoter (Ad-Sur). MK-1775 treatment increased apoptosis, expression of Ad hexon and production of Ad-Sur progenies in p53-deficient cells such as MeT-5A, EHMES-1 and AsPC-1, which was confirmed by knockdown of Wee1 with siRNA. Meanwhile, the levels of hexon expression and Ad-Sur replication were unchanged by MK-1775 treatment in three p53 wild-type cell lines, including NCI-H226 cells. EGFP-expressing Ad showed that MK-1775 treatment increased Ad infectivity in MeT-5A cells but decreased it in NCI-H226 cells. Knockdown of p53 with siRNA in NCI-H226 cells augmented the replication, the infectivity and the cytotoxicity of Ad in the presence of MK-1775. These results suggest that Wee1 inhibition enhances the cytotoxicity of oncolytic Ad selectively in p53-deficient cells.

E-1042

Therapeutic potential of LNP-mediated delivery of miR-634 for cancer therapy

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MicroRNAs (miRs) are endogenous small non-coding RNAs negatively regulate gene expression by interfering with the translation or stability of target transcripts. Some miRs can target multiple oncogenes and may be useful as a therapeutic agent for cancer therapy. The development of drug delivery system (DDS) is critical for the implementation of miR-based therapeutics. We have demonstrated that the enforced expression of miR-634 effectively induced the apoptosis by concurrently and directly targeting genes associated with mitochondrial homeostasis, anti-apoptosis, antioxidant ability, and autophagy, in cancer cells. Here, we validated the therapeutic potential of the lipid-nanoparticle (LNP)-mediated DDS of miR-634 for cancer therapy. First, we confirmed that overexpression of miR-634 induced the apoptosis in cell lines from various types of cancer, including pancreatic cancer and hepatocellular carcinoma. In xenograft tumors of BxPC3 cells, a pancreatic cancer cell line, in mice, the intravenous administration of the LNP incorporated miR-634 significantly induced tumor growth inhibition. Thus, these findings suggest the potency of LNP-mediated miRNA delivery for cancer therapy.

▲TOP

[E-1043] E1-1 [English]
DNA damage and carcinogenic process

2018 / 9 / 27 (Thu) 10:15-11:30 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Dai Nakae / Dept. Nutr. Sci. Food Safety, Facul. Appl. Biosci., Tokyo Univ. Agricul.

E-1043

Effect of the radiation therapy on the genome-wide mutation profile of the cell line model

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There has been little report on what type and how many mutations the radiation therapy causes to the cancer genome. In order to investigate the effect of radiotherapy on cancer genome, we established an experimental system that irradiates cancer cell lines with fractinated irradiation (60Gy/20fr) by radiotherapy machine. The cells were placed on polyethylene plates as a bolus and irradiation dose was calculated by clinical treatment planning system (pinnacle).The esophageal cancer cell line KYSE-450 was irradiated at a dose of 60Gy in 20 fractions and the irradiated cells were colonized and 4 clones were sequenced by Hiseq X, mapping was performed using iSAAC-01.15.02.08, mutation was called by Mutect 2 and annotation was performed by SnpEff ver.4.3. We found that 8000 to 12000 new SNVs and 900 to 1300 indels are identified on the each genome. Interestingly, location of the SNVs was biased at the chromosome level, and it was found around the specific gene such as c-Myc. Furthermore, we investigate CNA by Control FREEC and revealed that deletion occurred over a wide range on multiple chromosomes however amplification was hardly observed.

▲TOP

E-1044

Genome-editing methods for research and therapy of genes

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CRISPR/Cas9 or TALEN tool has been used to introduce a targeted genomic double-strand break in a locus of interest; however, there are several problems. One is that the replacement of a designed sequence is difficult due to a lesser frequency of homologous recombination-directed DSB repair than precise and imprecise non-homologous end-joining (NHEJ), which occurs at the target site, even in the presence of the homologous template. The second problem is that these tools cleave off-target sequences, which are similar to the target sequence, to cause imprecise NHEJ at their off-target sites. Such unexpected alterations may provide injury side-effects to patients receiving gene therapy. In the case of genetic research, they may cause different phenotype from the ones caused by the target gene alteration. The third problem is that a CRISPR/Cas9 or TALEN nuclease have to be order-made for every targeted site of the patients' genome and of the genome of model organisms, which is laborious and expensive. We have challenged to these problems.

E-1045

Asbestos exposure as a possible cause of ovarian carcinogenesis

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Background: Epidemiologic studies indicate that asbestos exposure is associated with an increased risk of ovarian cancer. However, the mechanisms by which asbestos cause the disease are not established. We performed this study to evaluate the involvement of crocidolite asbestos in cancer initiation of ovarian cancers. Methods: After incubation of immortalized human ovarian surface epithelium cell (HOSE1C) with crocidolite, reactive oxygen species (ROS) activity was detected with CM-H2DCFDA, and expression of γ H2AX was examined in immunocytochemistry(ICC) and western blot(WB) to detect DNA double strand break. The expression of γ H2AX was also examined in ovaries taken from Wistar rats 4 weeks after crocidolite exposure by intrabursal injection. Results: HOSE1C showed elevated levels of ROS after incubation with crocidolite. Additionally, the cells showed higher expression of γ H2AX. Furthermore, ovaries from the rats with crocidolite exposure showed higher rate of γ H2AX positive cells (control 0.2% vs crocidolite 2.5%, p=0.036). Conclusion: Our findings support a potential cancer-initiating role of crocidolite in ovarian cancers carcinogenesis.

E-1046

Effect of APE1 knockdown on deletion mutation induced by abasic site analogue

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Background: An abasic site, an abundant form of DNA damage, reportedly induces substitution mutations in human cells. Previously, we showed that an abasic site analogue (tetrahydrofuran) induces a large deletion mutation. In this study, we knocked down APE1, the major abasic site repair enzyme, and examined the knockdown effect on the deletion mutation induced by the abasic site analogue.
Methods: The oligodeoxyribonucleotide containing tetrahydrofuran was synthesized. A shuttle vector containing the analogue was constructed and transfected into human U2OS cells with knocked-down APE1. The replicated plasmid DNA was recovered and mutations in the supF gene were analyzed.
Results: The frequency of large deletion mutation induced by the abasic site analogue was slightly increased.
Conclusions: The results obtained in this study indicated that the deletion mutation induced by the abasic site analogue is only slightly influenced by the APE1 knockdown in human cells.
Acknowledgments: Dr. Yasuo Komatsu of National Institute of Advanced Industrial Science and Technology (AIST) synthesized the oligodeoxyribonucleotide containing tetrahydrofuran.

▲TOP

E-1047

Transcription factor, MED1 regulates homologous recombination and maintains genome stability

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Background Homologous recombination (HR) repair is major repair pathway of DNA double-strand breaks and therapeutic biomarker of PARP inhibitors. We aimed to identify novel HR factors and analyze their roles in DNA repair. Method Two independent genome-wide siRNA screenings were performed to identify novel HR genes. We assessed the complex formation with BRCA1 by immunoprecipitation. We further analyzed their roles in DNA damage response by immunofluorescence, comet assay, and HR assay. To analyze cell cycle regulation, we examined flow cytometry and BrdU assay. Result We identified MED1 as candidate of novel HR factor. Immunoprecipitation showed the complex formation between BRCA1 and MED1, and luciferase assay indicated MED1 regulated transcriptional activity of BRCT domain. HR assay showed HR deficiency by MED1 knockdown. In these cells, the recruitment of HR factors at DNA damage sites were severely impaired, and the repair of DSB were significantly delayed. Depletion of MED1 induced disruption of intra-S and G1 phase checkpoint. Conclusion MED1 plays an important role as the guardian of genome stability. Its alterations can be the therapeutic target based on synthetic lethal.

E-1048

Identification of informative microsatellite markers on chromosome 3p in Japanese patients

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In this study, we considered 321 microsatellite markers present on the chromosome 3p to distinguish their characteristic heterozygosity or homozygosity in Japanese patients. Sixteen normal tissues from Japanese patients were used in a PCR-based microsatellite analysis. The microsatellite markers were selected from the NCBI database with supplementary mapping information. The chosen 321 microsatellite markers were linked to each chromosomal region on the chromosome 3p (3p12-26.3), thereby covering the loci of target genes. Among the 321 microsatellite markers, 312 were detectable in the lung and liver tissues, and blood of Japanese patients. Among the 321 microsatellite markers, 312 (97%) were detectable in the lung, and liver tissues, and blood of Japanese patients. Moreover, 47 of 312 (15%) were identified as available markers for microsatellite analysis in Japanese patients. Informative microsatellite markers in 3p are fewer in Japanese patients compared with the patients from Europe and America. These results suggest that the utilization of this Japanese genome characteristic might be more effective in identifying the candidate tumor suppressor gene loci in the chromosome 3p.

▲TOP

[LS5] LS5 [Japanese]
Diagnostic approach to proliferation of mesothelial cells - Ancillary studies in limited specimens

2018 / 9 / 27 (Thu) 11:50-12:40 Room 7/10F 1006+1007, Osaka International Convention Center Room 7
：Environmental Restoration and Conservation Agency

Kenji Morinaga / Department of Asbestos-Related Health Damage Relief, Environmental Restoration and Conservation Agency

LS5
Diagnostic approach to proliferation of mesothelial cells - Ancillary studies in limited specimens

Kenzo Hiroshima
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No Abstract

[E-1103] E1-2 [English]
Process of carcinogenesis (1)

2018 / 9 / 27 (Thu) 13:00-14:15 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Noriko Hosoya / Lab. Mol. Radiol., CDBIM, Grad. Sch. Med., Univ. of Tokyo

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E-1103

HIF1α expression against against TGFβ-induced EMT in lung cancers

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Although the stabilized expression of HIF1α protein has been postulated as a hallmark of the response to hypoxia, a recent study suggested that the stabilization of HIF expression could inhibit dissociation of cadherin complex at the cell membrane in endothelial cells, resulting in attenuation of acute lung injury. This study is aimed to determine the role of stabilized HIF1α expression on TGFβ-induced EMT in lung cancer cells. By using lung cancer cells treating with HIF1α stabilizers or carrying doxycycline-dependent HIF1α deletion or point mutants, we investigated the role of stabilized HIF1α expression on TGFβ-induced EMT in lung cancer cells. Persistent hypoxia and TGFβ stimulation lost the stabilized HIF1α expression, yielding EMT. Dox-induced stabilized HIF1α expression inhibited TGFβ-induced EMT, independent of de novo VEGF-A expression. De novo HIF1αΔODD expression did not directly yield fibronectin expression, accompanied by no apparent dissociation of β-catenin/ E-cadherin complex.Our results indicated that HIF1α protein expression with ΔODD domain might be additional therapeutic target to overcome tumor microenvironment in lung cancer cells.

▲TOP

E-1104

Zebrafish in vivo imaging reveals unknown behaviors of oncogenic cells during primary tumorigenesis

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Tumor is believed to arise from a single oncogenic cell, which has the abnormal activities of oncogenes (e.g. Ras, β-catenin) or tumor-suppressor genes (e.g. p53, Smad4). In addition, mutation accumulation is thought to drive tumor formation. However, the behaviors of single oncogenic cells in living tissue and the molecular mechanisms how mutation accumulation promotes the initial tumorigenesis are poorly understood. To examine them, we analyzed the artificially introduced oncogenic cells with single or double mutations, in zebrafish. When cells with oncogenic Ras mutation are introduced into zebrafish skin, the cells lose cell-adhesion activity and consequently escape from the skin. However, cell with Ras/p53-double mutation activate inflammatory signaling and thereby form heterogeneous tumor-like cell mass including surrounding normal cells. On the other hand, artificially introduced β-catenin-hyperactivated oncogenic cells immediately undergo apoptosis through activating Smad-ROS signaling, while β-catenin-hyperactivated cells survive in Smad4-deficient tissues. Thus, additional mutation promotes the viability and tumorigenic activity of oncogenic cells in living tissue.

E-1105

Withdrawn

No Abstract

E-1106

IL-11 is a novel marker of stromal fibroblasts that promote tumors in a murine model of colitis-associated cancer

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Cancer-associated stromal fibroblast (CAF)s play a crucial role in the development of tumors, however, the markers of CAFs are not fully understood. Interleukin (IL)-11 is a member of IL-6 family of cytokines and has been shown to be highly expressed in inflammatory bowel diseases and colitis-associated cancers (CAC). Although IL-11 has been reported to promote the development of CAC in both human and mice, it is unclear which types of cells produce IL-11 under such conditions. To address this issue, we generated IL-11-Enhanced Green Fluorescent Protein (EGFP) reporter mice, in which expression of Il11 was monitored by EGFP. We found that EGFP+ IL-11-producing cells accumulated around damaged epithelial cells in the colon and IL-11-producing cells were positive for stromal markers such as Thy1 and podoplanin, but not EpCAM in a colitis model in mice. Moreover, similar IL-11-producing cells accumulated around colon tumors in a CAC model in mice. Furthermore, IL-11-producing CAFs accumulated in the intestinal polyps of ApcMin/+ mice. Thus, IL-11+ CAFs appear in response to tissue injury and might promote the development of tumors. Therefore, IL-11 might be a good marker for CAFs.

▲TOP

E-1107

Establishment of cancer cell models derived from human iPS cells based on mitochondrial complex II deficiency

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Oxidative stress plays a pivotal role in the differentiation and proliferation of cells and programmed cell death, and may cause oncogenesis and aging. Studies of the role of oxidative stress in oncogenesis have mainly employed human cancer cell lines. However, such cancer cell lines show characteristics that differ from those of the original tissue and exhibit resistance to oxidative stress by harboring various mutations. Therefore, cancer cell models that accurately reflect the influence of oxidative stress are required. The SDHC gene encoding mitochondrial succinate dehydrogenase complex subunit C has been identified as causing familial paragangliomas, and programmed cell death accompanying increased endogenous oxidative stress was observed in Tet-mev-1 mice overexpressing mutant SDHC (I69E) protein. In the present study, we report the construction of human iPS cell lines exhibiting a change in endogenous ROS production due to regulation of the expression of mutant SDHC or mitochondrial-targeted human catalase, or both. Using these cell lines, we have established cancer cell models derived from human iPS cells and clarified the endogenous role of ROS in oncogenesis.

E-1108

The impact of TNF- α (TNF) on hepatocarcinogenesis related with continuous hepatocyte apoptosis

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Background and Aim: TNF is multifunctional cytokine, inducing cell death and proliferation. In the present study, we investigated the role of TNF in hepatocarcinogenesis related with continuous hepatocyte apoptosis.Methods and Results: Hepatocyte specific knockout mice of Mcl-1 (L-Mcl-1 KO) induced continuous hepatocyte apoptosis with serum ALT increase and elevation of hepatic CD68, MCP1 and TNF mRNA expression levels at 6 weeks of age. 100% (16/16) of these mice developed hepatic tumorigenesis at 1.5 years of age. TNF deficiency in L-Mcl-1 KO significantly decreased tumor incidence rate to 50% (11/22) at 1.5 years of age. TNF deficiency in L-Mcl-1 KO at 6 weeks of age significantly reduced the serum ALT level while it did not affect hepatic CD68 nor MCP1 mRNA level. For in vitro experiments, we treated TNF into CL2 cells, murine hepatocyte cell line, with apoptotic stimuli. Administration of TNF into CL2 cells further decrease cell viability compared with non-treated cells. This decrease of cell viability was not suppressed by caspase inhibitor.Conclusion: TNF might induce hepatic tumorigenesis related with continuous hepatocyte apoptosis mediating non-apopotic hepatocyte death.

▲TOP

[E-1109] E11-2 [English]
Cell-to-cell interaction (1)

2018 / 9 / 27 (Thu) 14:15-15:30 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

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E-1109

Mesenchymal stem cells respond to matrix stiffness to promote breast cancer growth via prosaposin secretion

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Mesenchymal stem cells (MSCs) differentiate into cancer associated fibroblasts (CAFs) in response to chemical stimuli, such as growth factors, and thereby promote cancer progression. However, the contribution of mechanical stimuli, such as stiffness of the ECM, to MSCs in cancer is poorly understood. In this study, we revealed that MSCs show CAF-like phenotypes and promote breast cancer growth in response to mechanical stimuli. On a stiff substrate, MSCs treated with conditioned media from cancer cells expressed increased levels of alpha smooth muscle actin, a marker of CAFs, compared to the MSCs on a soft substrate. Conditioned medium from MSCs cultured on a stiff substrate, but not a soft substrate, increased growth of breast carcinoma cells. The soluble factor prosaposin was highly secreted from the MSCs on a stiff substrate, and the addition of recombinant prosaposin increased proliferation and survival of breast carcinoma cells. These results suggest that increased stiffness of the ECM in the tumor microenvironment induces differentiation of MSCs to CAFs, and as a result, triggers growth of breast cancer via prosaposin secretion.

▲TOP

E-1110

Autophagy of hepatic stellate cells promotes liver carcinogenesis and growth of liver tumors

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Background: Autophagy promotion of liver cancer cells promotes tumor growth, but the influence of autophagy of hepatic stellate cells (HSC) on the tumor growth is not elucidated.
Method: HepG2, Hep3B, Huh7 were used as human hepatoma cells and LX2 was used as a human HSC. LX2 expressing the GFPLC3RFPLC3dG probe was created and autophagy was examined. NOG mice were co-transplanted with LX2 and hepatoma. GFAP-Atg7 KO mice Atg7-deficient in HSC were created. NASH originated liver cancer was induced by streptozotocin and a high fat diet to this mouse.
Result: Autophagy of LX2 was accelerated, α SMA mRNA expression was elevated, and the number of hepatoma increased when LX2 was co-cultured with hepatoma. Knockdown of Atg7 of LX2 using siRNA reduced α SMA and IL6 mRNA expression, and suppressed hepatoma proliferation.Co-transplantation of Atg7KO LX2 with hepatoma in NOG mice suppressed the tumor growth compared to tumors co-transplanted with control LX2..
GFAP-Atg7 KO mice were created and NASH originated liver cancer was induced. The tumor growth was higher in control mice than in GFAP-Atg7 KO mice.
Result: Autophagy of HSC promotes carcinogenesis and tumor growth of liver cancer.

E-1111

Roles of ganglioside GD3 in the regulation of microenvironment of gliomas

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To clarify functions of GD3 in gliomas, our group used GD3-expressing mice (WT) and GD3 synthase (GD3 S) knockout mice (KO) based on RCAS/Gtv, a system to generate glioma-bearing mice. Glioma-bearing KO mice showed a longer survival rate and slower tumor growth than WT mice. Our RT-qPCR data showed ganglioside GD3 expressed on primary-cultured glioma cells induced higher levels of inflammatory cytokines and chemokines such like IL-6, TNF- β 1, CCL2 and CCL7 compared with GD3-lacking primary-cultured cells.We also performed immunohistochemistry assay, showing that activated microglia formed a dense zone surrounding tumor masses in WT. In turn, in KO sections, activated microglia were localized in tumor tissues. In tumor sections derived from WT mice, most glioma-associated microglia (GAMs) showed M2-like phenotypes that could promote glioma growth. Thus, different expression levels of cytokines and chemokines that can chemo-attract microglia/macrophages to recruit into tumor niche and polarize microglia/macrophages into M1-like or M2-like phenotypes were demonstrated.

E-1112

E-cadherin-coating enhances cancer stem-like properties and induces mesenchymal features in colon cancer cells

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[Background & Aim] Cancer stem cells (CSCs) are considered as the novel target of cancer therapies. However, CSC research is facing a difficulty because CSCs are only a small subset of tumor cells. Therefore, it is essential to develop an efficient tool for enriching the CSC population. E-cadherin Fc domain fusion protein matrix (E-cad-Fc) is reported to maintain the embryonic stem cells as the undifferentiated status. Thus we postulate that E-cad-Fc might have a potential for facilitating the CSC population. [Methods & Results] We used ZsGreen-degron ODC (ODC-Degron) system as a CSC model and examined whether CSC properties would be enhanced when the ODC-Degron transduced cells are cultured on E-cad-Fc coated plates. As a result, we found E-cad-Fc suppressed the proteasome activity and facilitated CSC-like properties including increased stem cell marker expression, chemo-resistance and sphere formation. Furthermore, E-cad-Fc induced mesenchymal properties in the ODC-Degron transduced cells. [Conclusion] E-cad-Fc could be an efficient material to enrich the CSC population.

▲TOP

E-1113

Treatment of peritoneal dissemination based on the unique microenvironment in peritoneal cavity

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Peritoneal dissemination is the commonest type of metastasis in abdominal cancers. Peritoneal cavity contains several types of cells which can mediate direct contact. Here, we show two cellular events which could be novel targets for treatment. Intraperitoneal mesenchymal cells (PMC) with CD90(+) CD45(-) phenotype possess stem characteristics and differentiate into myofibroblasts with TGF- β stimulation. The PMC strongly enhance the migration of co-cultured MKN45 in "piggybacked" manner. Intraperitoneal (IP) co-injection of PMC enhances the metastasis of MKN45 on peritoneum in nude mice. Dasatinib strongly inhibits the growth of PMC in vitro and significantly suppressed peritoneal tumor formation with reduced fibrous stroma. Human low density neutrophils (LDN) in postoperative peritoneal lavages produce massive neutrophil extracellular traps (NETs) which can effectively entrap tumor cells. IP co-transfer of the LDN augments peritoneal metastasis of MKN45, which is suppressed by degradation of NETs with DNAse I. Recently, we have shown that IP taxane is highly effective peritoneal dissemination. Dasatinib and DNAse may be additional therapeutic targets for this dismal condition.

E-1114

GPNMB is exposed on the surface of breast cancer cells and induces stem cell-like properties

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Glycoprotein nmb (GPNMB) is a type I transmembrane protein and highly expressed in breast cancer cells. We previously demonstrated that GPNMB contributes to the initiation and malignant progression of breast cancer through induction of epithelial-mesenchymal transition (EMT). It is known that EMT is associated with not only cancer invasion but also acquisition of cancer stem cell (CSC) properties. However, the function of GPNMB in CSC has yet to be elucidated. We found that GPNMB expression is enriched in the three-dimensional (3D) cultured spheres compared to the 2D monolayer cultured cells together with CSC markers and EMT-inducing transcription factors (EMT-TFs). Furthermore, the 3D cultures induced cell surface expression of GPNMB on the limited numbers of cells. Therefore, we isolated cell surface-GPNMBhigh and -GPNMBlow cells from the spheres. The cell surface-GPNMBhigh cells expressed high levels of CSC markers and EMT-TFs, had significantly higher stem cell frequency, and showed no detectable levels of proliferation markers. These findings suggest that GPNMB is exposed on the surface of dormant breast cancer cells and contributes to the acquisition of CSC-like properties.

▲TOP

[J-1055] J11-1 [Japanese]
Cell-to-cell interaction (2)

2018 / 9 / 27 (Thu) 15:30-16:45 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Yoshiyuki Fujiwara / Dept. Surgery, Tottori University, Faculty of Medicine

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J-1055

Adipocytes contribute peritoneal metastasis formation in gastric cancer microenvironment

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Introduction: In gastric cancer, released cancer cell from stomach attached to peritoneal mesothelial cells. As the result, peritoneal metastatic nodules occur in omentum and mesentery which contain rich adipocytes. In this study, we clarified whether adipocytes enhanced gastric cancer progression. **Methods**: Adiponectin in the omentum from resected specimen was measured. Matured adipocyte derived from 3T3-L1 was co-cultured with gastric cancer cell. **Results**: Adiponectin in the fat tissue adjacent primary tumor revealed lower expression in the patients with high T stage than with low T stage. Adipocytes co-cultured with gastric cancer cell showed decreased expression of adiponectin. PPARγ and C/EBPα as differential marker of adipocytes also decreased. On the other hand, expression of IL-6 and PAI-1 as SASP (Senescence-associated Secretory Phenotype) factors were increased in the co-cultured adipocytes. These cells also showed high expression of αSMA as EMT marker and FAP (fibroblast-activated protein) as CAFs (cancer-associated fibroblasts) marker. **Conclusion**: These results suggest that transformed adipocytes in cancer microenvironment involve formation of peritoneal metastasis.

▲TOP

J-1056

A novel antibody for Mac-2 binding protein, 19-8H mAb can recognize tumor-associated macrophages

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It has been well known that Mac-2bp (Mac-2 binding protein) is markedly increased in the serum of cancer patients. We previously found that fucosylation was dramatically increased in Panc-1 cells, showing anti-cancer agent resistance, and Mac-2bp was one of target proteins for fucosylation. In the present study, we generated anti fucosylated Mac-2bp monoclonal antibody, 19-8H mAb with our original screening methods and investigated its availability for clinical implication. The 19-8H mAb recognized characteristic structure of Mac-2bp in native, but not denatured conditions. Interestingly, immunohistochemistry showed that positive staining of 19-8H mAb was observed in cancer-associated macrophages in pancreatic /liver cancer tissues. In co-culture system of pancreatic cancer cells and macrophages, cell staining of 19-8H mAb in macrophages was increased with co-culture and the addition of 19-8H mAb inhibited the polarity of macrophages. These results suggest that 19-8H mAb is a potential tool for novel cancer therapy as a suppressor for the formation of tumor microenvironment.

J-1057

Significance of Annexin A1 expression in renal cell carcinoma

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Despite recent advances in treatment of renal cell carcinoma (RCC), prognosis of metastatic RCC remains poor. Therefore new prognosis markers for RCC are required. Annexin A1 (Anxa1) is a calcium-dependent phospholipid-binding protein, being overexpressed in some tumors such as head & neck and esophageal cancer. However, expression of Anxa1 in RCC is not well documented. We employed immunohistochemistry and determined the levels of Anxa1 in specimens from RCC patients (n=26) by anti-Anxa1 antibody, which revealed significantly shorter recurrence-free survival of Anxa1-positive RCC patients compared to that of Anxa1-negatives. Anxa1 gene knocked-down of RCC line Caki-1 cells using shRNA showed significant attenuation in cell proliferation (MTS assay), motility (scratch assay), invasiveness (matrigel invasion assay) and adhesion (adhesion assay), compared to control Caki-1 cells. These results collectively suggest that Anxa1 could be a marker for poor prognosis of RCC.

J-1058

The impact of IDH expression on cell differentiation status in TGF-beta-induced EMT in liver cancer cells

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Cellular energy metabolism is closely related to a differentiation status of cells. We found that isocitrate dehydrogenases (IDHs), mitochondrial TCA circuit enzymes, are downregulated in undifferentiated liver cancer cells compared with differentiated ones. In order to know a possible role of IDH on cancer cell differentiation, the impact of IDH expression on differentiation status was examined using TGF-β-induced EMT, a typical dedifferentiation model of cancer cells, in hepatoma cells. TGF-β induced mesenchymal phenotype characterized by E-cadherin downregulation and vimentin upregulation in well-differentiated hepatoma cells. Marked decrease of expression in hepatic genes such as ALB and AFP indicated that TGF-β-induced EMT was accompanied by cell-dedifferentiation. Three IDH subtypes, IDH1, IDH2, and IDH3 were significantly downregulated in this process. Additionally, overexpression of IDH subtypes restored the expression of E-cadherin downregulated by EMT. These data suggested that IDH plays a role in the maintenance of differentiated status of hepatoma cells. IDH restoration may be a possible strategy for preventing cancer cell dedifferentiation.

▲TOP

J-1059

Elucidation of novel mechanism of liver metastasis of colon cancer through metabolome analyses of cancer stem cells

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In many types of solid tumors, cancer stem cells (CSC) represent a small fraction of cancer cells and are associated with malignant traits of cancer such as tumorigenesis and metastasis. Elucidation of the biological nature of CSCs will be important to understand the refractory traits of cancer and to devise new strategies for cancer therapy. In an attempt to identify biochemical alterations associated with metastatic traits of cancer, we compared metabolic profiles of spheroids established from CSCs derived from non-metastatic cancers and metastatic foci. We found that Kynurenine was up-regulated in spheroids derived from metastasized liver. In accordance, levels of TDO2, an enzyme responsible for Kynurenine production, were higher in metastatic CSCs than in non-metastatic ones. TDO2 over-expression promoted liver metastasis and suppressed accumulation of immune cells around the metastatic lesion. These results indicate that TDO2 plays an important role in liver metastasis though Kynurenine production.

J-1060

Functional Heterogeneity in Activated Fibroblasts Created by Extracellular Vesicles

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Cancer-associated fibroblasts (CAFs) are the key players in the cancer microenvironment and could be the potential therapeutic target for cancer. However, one of the issues is that CAFs are heterogeneous, and their subpopulation with distinct functions have also been recognized as the major obstacle to targeting CAFs in diagnosis and therapy. While the molecular bases of CAF subpopulations and functions have been reported, it is still obscure how CAF heterogeneity is governed by cancer cells. Here, we showed the important role of extracellular vesicles (EVs) on the forming of CAF heterogeneity. Highly-metastatic diffuse-type gastric cancer (DGC) cells favorably formed the distinct subpopulation of activated fibroblasts by EVs. Low-metastatic DGC cells had less capacity of the education for fibroblast. Additionally, we found that various miRNAs in EVs from highly-metastatic DGC cells regulated the chemokine-producing fibroblast subpopulations. Our findings suggest that the cellular crosstalk between high-metastatic DGC and fibroblasts via EVs contributes to the inflammatory chemokine induction, establishing the appropriate tumor microenvironment toward the metastasis.

▲TOP

[J-1001] J12-1 [Japanese]
Anticancer agents and effects

2018 / 9 / 27 (Thu) 9:00-10:15 Room 8/10F 1008, Osaka International Convention Center Room 8

Kenzaburo Tani / The Inst. of Med. Sci., The Univ. of Tokyo

J-1001

JMJD2A regulates the sensitivity of anticancer drugs via regulation of CCDC8 expression in metastatic gastric cancer

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No useful predictor of chemotherapy for metastatic gastric cancer (MGC) is presently available. We have selected 15 candidate predictor genes by genome-wide association study using gastric cancer tissues of responders and non-responders to S-1, cisplatin and docetaxel (DCS) therapy. Knockdown experiments of these 15 genes with siRNA revealed that JMJD2A is the best predictor for response to these drugs. When IC50s of DCS against gastric cancer cell line MKN45 that was knocked down by siRNA (MKN45/JMJD2A-si) were analyzed by WST assay, they were significantly higher than those in the control cells. Subsequently, we investigated JMJD2A expression in the biopsy specimens from 29 patients with MGC before treatment by immunohistochemistry. There was a significant correlation between immunostaining score and reduction rate of the tumors after treatment with DCS. Expression array analysis of MKN45/JMJD2A-si showed significantly suppressed mRNA level of CCDC8, a co-apoptotic gene related to DNA damage, compared to control cells. Thus, our data strongly suggest that JMJD2A cooperating with CCDC8 is a novel functional factor of DCS therapy in MGC.

▲TOP

J-1002

Genome-wide association study to identify novel biomarkers for trastuzumab-induced cardiotoxicity

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To identify a novel genetic marker(s) determining the risk of trastuzumab-induced cardiotoxicity, we carried out a genome-wide association study (GWAS) using 50 cases (with trastuzumab-induced cardiotoxicity) and 218 controls (showing no sign of trastuzumab-induced cardiotoxicity). Top 100 single nucleotide polymorphisms (SNPs) which revealed smallest P value in GWAS were further examined using replication samples consisted of 52 cases and 159 controls. The combined analysis of the screening and replication study indicated that two SNPs were possibly associated with trastuzumab-induced cardiotoxicity (SNP A; $P_{combined} = 7.98 \times 10^{-5}$ and SNP B; $P_{combined} = 7.07 \times 10^{-6}$). SNP A was located in intron 4 of a transcriptional co-activator gene on chromosome 10 and, SNP B was an intergenic SNP on chromosome 4. This finding provides new insights into personalized trastuzumab therapy for patients with human epidermal growth factor receptor 2 (HER2)-positive cancer.

J-1003

Time-series analysis on the process of acquiring tamoxifen resistance in breast cancer cells

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The process of acquiring drug resistance in cancer cells can be understood as a process of rewiring of molecular network stimulated by the change of extracellular environment. We tried to elucidate the molecular mechanism contributing to the process of acquiring drug resistance by measuring the gene expression pattern and the distribution of the protein expression in the tamoxifen (TAM)-treated cell population. Estrogen receptor positive human breast cancer MCF-7 cells were cultured under a continuous exposure of TAM for 12 weeks. TAM inhibited the growth of MCF-7 at 3 weeks of exposure, and this inhibiting effect was partially canceled from 5 weeks to 8 weeks. We also measured mRNA expression of MCF-7 cells at every weeks of TAM exposure to investigate the genes involved in acquiring TAM resistance. The expression levels of FoxO signaling and autophagy related genes were elevated only before the cell growth rate were recovered, suggesting that these pathways may play key roles in the acquiring of drug resistance. Analysis of cell-to-cell variability of these molecules during the time-course implied the heterogeneity may contribute to the selection of resistant cell population.

J-1004

Annexin II plays diverse functions during pancreatic cancer progression

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We identified Annexin II (ANX2) as a gemcitabine-resistant factor in pancreatic ductal adenocarcinoma (PDAC), and demonstrated that high ANX2 expression in PDAC tissues was closely associated with rapid recurrence in patients with gemcitabine-based chemotherapy. In this study, we elucidated the diverse functions of ANX2 in PDAC progression. First, we compared the profiles of signaling phosphoproteins between gemcitabine resistant (GEM) MIA PaCa-2 and its wild type using proteomic analyses. The comprehensive analyses showed up-regulation of p-Akt in GEM-MIA PaCa-2 cells. The expression level of p-Akt was significantly correlated with that of p-mTOR in PDAC tissues. Inhibition of mTOR activation canceled gemcitabine resistance in GEM-MIA PaCa-2 cells. Next, we investigated the interaction between ANX2 and stromal tenascin C (TNC) in PDAC progression. Immunohistochemistry revealed the association between ANX2-TNC expression and metastasis. Furthermore, the interaction of ANX2 and stromal TNC regulated invasion in addition to stemness and anoikis resistance, which are crucial for metastasis. These results indicated AX2 as an attractive therapeutic target for PDAC progression.

▲TOP

J-1005

Targeting FSTL1-DIP2A axis for treating osteosarcoma

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Osteosarcoma (OS) frequently occurs regional invasion and distant metastasis, which are hard to be successfully cured by the conventional treatments. We previously demonstrated that FSTL1 molecule having both immune suppressive and inflammatory features is highly produced from mouse and human OS tumor cells. Blocking FSTL1 significantly suppresses tumor progression and metastasis through reducing the FSTL1-induced mesenchymal stem cells (MSCs) in mouse tumor models. However, clinical analysis shows a tendency that the positivity of its receptor DIP2A, but not FSTL1, in tumor tissues is correlated with overall survival of the OS patients. Indeed, siRNA studies reveal that DIP2A regulates the EMT-like metastatic and drug-resistant properties of OS tumors. In addition, the extrinsically expanded MSCs as well as OS tumors highly produced FSTL1. These suggest that DIP2A expression in OS tumors could be a key to amplify the vicious circulation for tumor progression and metastasis in the presence of a huge amount of FSTL1, regardless of the sources, in hosts with OS tumors. These suggest that FSTL1-DIP2A axis may be a promising target for effectively treating OS tumors.

J-1006

Antitumor activity by ADCC against oral squamous cell carcinomas by anti-podocalyxin antibody

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Podocalyxin (PODXL) is a highly glycosylated mucin-like type I transmembrane protein. PODXL is reported to be expressed in many cancers, such as lung, esophageal and oral cancers. In addition, its overexpression is associated with progression, metastasis, and poor outcome of those cancers. However, the function of PODXL in oral cancers have not been fully elucidated. We previously produced a novel anti-PODXL mAb, PcMab-47, which reacts with endogenous PODXL-expressing cancer cell lines. In this study, we engineered PcMab-47 into mouse IgG2a type (47-mG2a) to add antibody-dependent cellular cytotoxicity (ADCC) activity, and further developed core-fucose-deficient 47-mG2a (47-mG2a-f) to augment ADCC activity. In vitro analysis revealed that 47-mG2a-f showed much higher ADCC than 47-mG2a though those mAbs did not show complement-dependent cytotoxicity (CDC). Furthermore, 47-mG2a-f exerted antitumor activity against oral squamous cell carcinoma (OSCC) cell lines, SAS and HSC-2 xenograft models; in contrast, 47-mG2a did not. These results suggested that a core-fucose-deficient anti-PODXL mAb could be useful for antibody-based therapy against PODXL-expressing OSCCs by ADCC activities.

▲TOP

[J-1007] J6 [Japanese]
DNA replication / cell cycle / genomic instability (2)

2018 / 9 / 27 (Thu) 10:15-11:30 Room 8/10F 1008, Osaka International Convention Center Room 8

Masatoshi Fujita / Dept. Cell. Biochem., Grad. Sch. Pharm. Sci., Kyushu Univ.

J-1007

The mechanism of tumor cell fate decision by an antitumor nucleoside analogue, trifluridine

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Trifluridine (FTD) is a key component of the novel oral antitumor drug TAS-102 (also named TFTD), which consists of FTD and a thymidine phosphorylase inhibitor. FTD is efficiently incorporated into DNA and induces p53-dependent sustained G2 phase arrest. However, the underlying mechanism is still unclear. We show that FTD is a unique type of DNA replication stress inducer, which dictates tumor cell fate decision. DRS is caused by retarded DNA synthesis by the replicative DNA polymerases, Polδ and Polε, because of the inefficient incorporation of FTD triphosphate (FTD-TP) into the nascent strand as well as the inefficient DNA synthesis at FTD sequence in the template strand. At the cellular level, FTD severely elongated the duration of S/G2 phase and induced cellular senescence through a mitosis skip in p53-proficient human cancer cells. In the isogenic p53-deficient cells, FTD induced cell death via aberrant mitosis progression associated with unseparated sister chromatids interlinked along their chromosomal arms. We will discuss the possible mechanism how DNA replication stress by FTD triggers cellular senescence or aberrant mitotic chromosomes.

▲TOP

J-1008

Intrinsic DNA Replication Stress Confers Sensitivity to ATR inhibitor in Lung Adenocarcinoma Cell

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The ATR is a master regulator of DNA damage responses and is thought to act as a tumor suppressor through maintaining genome stability. However, the decreased ATR expression suppresses tumorigenesis induced by oncogenic signals suggesting ATR is required for transformed cell survival. In this study, we analyzed the effect of ATR inhibitor (ATRi) in lung adenocarcinoma cell line (LADC). Without exogeneous DNA damage, Rad17 Ser 645 phosphorylation, an ATR substrate, was observed in all cell line tested including normal and LADC cells indicating ATR is activated by endogenous stress. ATRi induced cell death with increasing number of pan-nuclear γ H2AX positive cells, which is an indicator of replication catastrophe. Combination with ATRi and cisplatin killed even ATRi-low-sensitive LADC in a synergistic manner. Interestingly, sensitivities to ATRi of LADC correlated with ATRi-induced ssDNA which is a plausible indicator of intrinsic replication stress (RS). These results suggest that ATR contributes cell survival resisting endogenous RS in cancer cells and could be efficiently targeted by ATRi. We will discuss current progress and a possible biomarker predicting ATRi efficiency.

J-1009

Telomere-binding proteins Taz1 and Rap1 suppress chromosomal rearrangements and promote DNA double-strand break repair

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Recent genome analyses have revealed that chromosomal rearrangements are much more common in tumors than we thought. Telomere protection is important for maintaining genome integrity through suppression of aberrant DNA double-strand break (DSB) repair at chromosome ends. However, it is largely unknown how dysfunctional telomeres contribute to the formation of chromosomal rearrangements. In this study, we constructed the system to quantitatively measure the occurrence rate of gross chromosomal rearrangements (GCRs) such as translocation and deletion in fission yeast. We found that evolutionally conserved telomere-binding proteins, Taz1 and Rap1 suppress GCRs at non-telomeric region. Further analysis indicated that they suppress GCRs via two pathways: the one related to telomerase regulation and the unknown one which involves previously uncharacterized BRCT domain of Rap1. Moreover, we found that Taz1 and Rap1 are required for efficient DSB repair at non-telomeric region in collaboration with Kurt Runge's group. These results suggest that telomere-binding proteins exploit multiple pathways to ensure chromosome stability.

J-1010

Microsatellite Instability in Triple Negative Breast Cancers

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Background: Microsatellite instability (MSI) is a phenotype resulting from defect in mismatch repair genes. The FDA approved anti-programmed death 1 (PD-1) immune checkpoint inhibitor for any solid tumor with MSI-high (MSI-H). Some tumors had good response to PD-1 blockade and it is a promising treatment for a part of refractory breast cancers. Our goal was to determine the frequency of MSI in triple negative breast cancer (TNBC), one of the most clinically aggressive subtypes. Patients and Methods: This study included 228 patients with primary TNBC underwent resection without neoadjuvant chemotherapy between January 2004 and December 2014. We classified the tumors as microsatellite stable, MSI-low or MSI-H by PCR at the following 5 microsatellite markers: NR-21, BAT-26, BAT-25, NR-24, MONO-27. Results: Six (2.6%) of the 228 tumors revealed MSI, of which 2 (0.9%) were MSI-H. Four tumors were stage Ⅱ and others were stage Ⅰ. Two MSI-H tumors showed nuclear grade 3 and high Ki-67 (>30%), and had common following instable markers: NR-21, BAT-26 and BAT-25. Conclusions: Our results demonstrated that the frequency of MSI-H might be remarkably rare in TNBC.

▲TOP

J-1011

Cell line selective function of Ebp1 for replication fork arrest

Shunji Izuta
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ErbB3 binding protein 1 (Ebp1) is a cell cycle protein that appears in the nuclei from late G1 to S phase and diminishes at G2. To understand the exact role of Ebp1 on cell cycle progression, we studied the behavior of Ebp1 in HeLa cells arrested at S phase. HeLa cells were synchronized at G1/S phase and treated with hydroxyurea. Immunofluorescence staining revealed that Ebp1 remained in nuclei of the cells treated with hydroxyurea, while it decreased from nuclei without hydroxyurea. Fractionation of HeLa cells indicated that Ebp1 was loaded onto chromatin with hydroxyurea treatment. Similar behavior of Ebp1 was seen in HeLa cells treated with cisplatin and mitomycin C but not with etoposide or UV-irradiation. Chromatin loading of Ebp1 with hydroxyurea was also seen in Cos7 cells but not in C2C12 or A549 cells. Furthermore, the knockdown of Ebp1 in HeLa cells increased sensitivity to hydroxyurea, while that in C2C12 cells had no effect on the sensitivity. These results indicate that the role of Ebp1 on replication fork arrest is different among several cell lines. This cell line selective function of Ebp1 may provide a novel strategy of cancer chemotherapy.

J-1012

A novel CRISPR-based assay can evaluate homologous recombination activity of BRCA1 mutants more accurately

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Homologous recombination (HR) contributes to the repair of DNA damages including DNA double-strand breaks and the interstrand crosslinks. Defects in HR are related to high sensitivity to ionizing radiation, poly (ADP-ribose) polymerase inhibitors, and chemotherapeutics like platinum compounds. Therefore, measurement of HR activity in cells is important for patient stratification and drug development. So far, DR-GFP assay has been used for measurement of HR activity. Although this assay effectively detects HR deficiency of various BRCA1 variants , some variants including I26A or N-terminus deletion variants were judged as HR proficient in spite that those confer no resistance to a PARP inhibitor or cisplatin. Here we report a novel assay to measure HR activity using CRISPR system. Using this assay, we evaluated HR activities of several BRCA1 variants. As a result, we found that our assay could detect HR deficiency of BRCA1 I26A or N-terminus deletion variants, which seemed to be proficient by DR-GFP assay. In conclusion, we established a novel assay for HR named “Assay for Site-specific HR Activity: ASHRA”, which can evaluate HR activity more accurately, sensitively, and easily.

▲TOP

[LS6] LS6 [Japanese]
Advanced in vivo imaging technology revolutionizing cancer diagnosis and therapies in clinic

2018 / 9 / 27 (Thu) 11:50-12:40 Room 8/10F 1008, Osaka International Convention Center Room 8
：NIKON CORPORATION

Koshi Mimori / Department of Surgery, Kyushu University Beppu Hospital

LS6

Advanced in vivo imaging technology revolutionizing cancer diagnosis and therapies in clinic

Masaru Ishii
Department of Immunology and Cell Biology, Graduate School of Medicine, Osaka University

No Abstract

▲TOP

[J-1061] J21 [Japanese]
Gene therapy and oncolytic virus therapy (2)

2018 / 9 / 27 (Thu) 13:00-14:15 Room 8/10F 1008, Osaka International Convention Center Room 8

Hiroshi Fukuhara / Dept. Urol., Kyorin Univ. Fac. Med.

J-1061

Applicability of a recombinant SLAM-blind measles virus to breast cancer treatment

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Oncolytic virotherapy is a new approach for cancer treatment. We previously reported that our recombinant measles virus (rMV-SLAMblind) infects and kills cancer cell lines expressing its receptor, nectin-4. Nectin-4 is a potential diagnostic marker and therapeutic target for breast, lung, and ovarian cancers. However, there are only a few reports on the proportion of nectin-4 positive cases in each cancer. To examine the proportion and association of its expression with poor prognosis for breast cancer patients, we performed immunohistochemical analysis of nectin-4 and compared clinical status of the patients in Japan. The proportion of the nectin-4-positive cases was higher than 70%. Overall survival rate was significantly lower in nectin-4-positive cases. We found that rMV-SLAMblind shows strong anti-tumor activity against nectin-4-positive triple negative breast cancer cell lines. These results indicate that nectin-4 expression is associated with malignancy of breast cancer, and rMV-SLAMblind is a promising novel therapeutic agent for breast cancer.

▲TOP

J-1062

Suppression of malignant rhabdoid tumors through novel drug based on Gene Switch Technology

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Malignant rhabdoid tumor (MRT) is a rare, highly aggressive malignancy primarily affecting infants and young children. Despite intensive multimodal therapies, the prognosis of patients with MRT is dismal and a novel therapy is needed. Here, we show that switching off RUNX1 utilizing our novel Chlorambucil-conjugated pyrrole-imidazole polyamides (Chb-M'), which specifically bound to the consensus RUNX-binding sequences, was highly effective against human MRT cells in vitro and in vivo. We demonstrated that silencing of RUNX1 with shRNA in MRT cell lines led to a marked inhibition of tumor growth. Besides, we found that Chb-M' was effective against MRT cells. MRT cells treated with Chb-M' showed elevated expression of p53 and cleaved PARP, suggesting that inhibition of RUNX-mediated apoptosis of MRT cells are possibly mediated by p53 pathway. Furthermore, in vivo experiments, a remarkable suppression of tumor growth with Chb-M' treatment was obtained in xenograft MRT cells mouse model, as well as mice harboring RUNX1-silencing MRT cells. Therefore, the suppression of RUNX would be a novel strategy of the therapy to MRT.

J-1063

The Use of Therapeutic Monoclonal Antibody Enhances Antitumor Immune Responses Induced by Oncolytic G47δ in Mouse Models

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Monoclonal antibodies (mAb) have been widely used for the treatment of various malignancies. Recent reports have highlighted their antitumor activities through immune modulations, especially in tumors with abundant infiltrating lymphocytes. G47δ, an oncolytic HSV-1 with triple genetic modifications, selectively replicates in and destroys tumor cells, causing infiltration of immune cells. Here, we examined whether therapeutic mAb could enhance the efficacy of G47δ. Subcutaneous tumors of SCC7 or Neuro2a cells stably transfected with human EGFR were generated in the flanks of syngeneic mice and treated with intratumoral injections of G47δ with or without systemic administration of anti-EGFR mAb. The combination therapy suppressed the tumor growth significantly better than either monotherapy. Tumors treated with the combination therapy showed markedly increased infiltration of immune cells of both innate and adaptive lineages. Depletion of CD8+ or NK cells eliminated the enhanced therapeutic effect of the combination therapy. These results suggest that the efficacy of G47δ can be augmented by therapeutic mAb via facilitating antitumor immune responses.

J-1064

Oncolytic activity of HF10 for head and neck squamous cell carcinomas

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Five-year survival of head and neck squamous cell carcinoma (HNSCC) is still 40%, necessitating new therapeutic agents. HF10 is a highly attenuated herpes simplex virus isolated in our laboratory. In this study, we investigated the therapeutic potential of HF10 for HNSCC in vitro and in vivo. Human and mouse SCC cell lines, and primary-cultured HNSCC cell lines from human or mouse tumors were prepared. HF10 replicated well, induced cytopathic effect, and killed all kinds of HNSCC cells. Next, we investigated oncolytic effect of HF10 using ear tumor models. Infected cells within the ear tumors were observed using injection of HF10-GFP, expressing green fluorescent protein (GFP). HF10 injection decreased ear tumor and prolonged survival. Pathologically, HF10 infection induced necrosis, where infiltrating CD8-positive cells were observed. Granulocytes and CD8+ T cells increased in the spleen, and several anti-tumor cytokines increased in the supernatant of splenic cells after stimulation with the tumor cells. The mice that had survived from tumor rejected re-challenge of the tumor cells. These result suggested that HF10 can be a promising agent for the treatment of HNSCC patients.

▲TOP

J-1065

Evaluation of oncolytic herpes simplex virus type 1 armed with an immunomodulatory function in murine tumor models

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Oncolytic HSV-1 has emerged as a promising treatment option for refractory cancers, including advanced stage malignant melanoma. In this study, we evaluated the therapeutic efficacy of G47Δ-based oncolytic HSV-1 expressing a soluble form of murine co-stimulatory molecule B7-1 (T-B7.1), and another expressing a molecule with an immunomodulatory function (T-IMF01). In vitro, cytopathic effect and replication capability of T-B7.1 and T-IMF01 were comparable to those of T-01, a control virus, in murine neuroblastoma cell line Neuro2a. In A/J mice with bilateral subcutaneous Neuro2a tumors, intratumoral injections with T-B7.1 or T-IMF01 (2×10^5 pfu) to one side suppressed the growth of tumors in non-injected contralateral side more effectively than T-01. Similarly, in DBA/2 mice with bilateral subcutaneous Clone M3 melanoma, T-B7.1 and T-IMF01 showed enhanced antitumor efficacy compared with T-01 in both injected and non-injected tumors. These results suggest the use of armed oncolytic HSV-1 with immunomodulatory functions would be a potent therapeutic tool for cancer including melanoma.

J-1066

The investigation of a recombinant coxsackievirus B3 manufacturing process for human clinical trial

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Despite advances in clinical therapy for cancers, malignant tumors are still the leading cause of death in Japan. As one of the new therapies, we found that Coxsackievirus B3 wild type (CVB3-WT) is a promising new oncolytic virus for the treatment of cancers including non-small cell lung cancer and breast cancer. However, CVB3-WT showed several side effects in mice. To overcome these pathogenicity, we engineered CVB3-WT genome for the development of microRNA-regulated oncolytic virus (CVB3-miRT).Here, we attempted to determine the methods of the large-scale culture and purification of CVB3-miRT for clinical trial. We performed serum-free and large-scale culture for CVB3-miRT production using 293 suspension cells. As a result, we succeeded in culturing high density cells sufficient to produce virus. Next, we investigated the method of concentration and purification. Harvested CVB3-miRT was purified by ion exchange chromatography. The results of oriole fluorescent gel staining and western blotting indicated the fraction of CVB3-miRT was highly purified. Collectively, we demonstrated that CVB3-miRT could be produced by large-scale production system for human clinical trial in future.

▲TOP

[J-1067] J1 [Japanese]
Process of carcinogenesis (2)

2018 / 9 / 27 (Thu) 14:15-15:30 Room 8/10F 1008, Osaka International Convention Center Room 8

Michihiro Mutoh / Ctr. for Public Health Sci., Natl. Cancer Ctr.

J-1067

Roles of Vasohibin-2 in pancreatic cancer

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Vasohibin-2 (VASH2) is isolated as a homologue of vasohibin-1 (VASH1), and is recently identified as tubulin carboxypeptidase. Our previous analyses revealed that VASH2 is expressed in various cancers, and promotes tumor angiogenesis and epithelial mesenchymal transition (EMT). In addition, pancreas cancer patients with higher VASH2 expression exhibited poorer prognosis. The aim of the present study is to characterize the role of VASH2 in pancreatic ductal adenocarcinoma (PDAC). We used PDAC cells derived from KPC mice. Knockdown of VASH2 did not affect proliferation, but decreased migration, invasion and detyrosinated tubulin. When PDAC cells were injected into the tail vein, lung metastasis was significantly decreased in the mice injected with VASH2-knockdown (KD) cells. We then analyzed the gene expression changes in VASH2-KD cells. According to KEGG databases, downregulated genes by the knockdown of VASH2 were associated with inflammation. We further confirmed the decreased expression of CXCLs in VASH2-KD cells using qPCR. These data suggest that VASH2 regulates metastatic potential of PDAC cells, and is also involved in tumor immune microenvironment.

▲TOP

J-1068

Functional interaction between SWI/SNF complex and a hematopoietic transcription factor in malignant rhabdoid tumor

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SNF5 is a subunit of the SWI/SNF chromatin-remodeling complex, and its inactivation is the sole abnormality for malignant rhabdoid tumor (MRT), a pediatric disastrous tumor. We previously showed that p21Cip1/Waf1 expression was impaired in the MRT cells and that exogenous expression of SNF5 re-activated p21 transcription. In this study, we examined the relationship between SNF5 and a hematopoietic transcription factor (the TF) known to regulate p21 transcription. We constructed a plasmid with human-p21-promoter-luc construct, containing the consensus sequences of the TF. When this reporter plasmid was co-transfected with the TF and/or SNF5 expression vectors into A204 MRT cells, SNF5 activated the p21 reporter. Interestingly, the TF functioned in p21 suppression only when SNF5 was provided. Thus the repression activity of this TF appeared to be dependent on the sound SWI/SNF complex. In addition, SNF5 physically binds to the TF so far as examined by immunoprecipitation. Thus the close relationship between chromatin remodeling and transcriptional control has been documented. We hope that these findings should serve as an initial clue to further elucidate MRT mechanism.

J-1069

Whole genome sequencing analysis elucidates the interaction between environmental factors and causes of human cancer

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Next generation sequencing analyses of human cancers have revealed characteristic mutational signatures (MSs) that reflect their etiology. Chemical substances also exhibit characteristic MSs when non-biased global mutations are analyzed by whole genome sequencing of tumors occurring in experimental animals or model organisms exposed to chemicals. Comparison of MSs derived from chemicals with those derived from human cancers can provide valuable insights into chemical-human cancer relationships. In the present study, we analyzed MSs derived from environmental factors that have been suggested to be responsible for human cancer development, such as N-nitroso (NO)-bile acid conjugates (BACs), using Salmonella strains. The C:G to T:A transition was predominant in the NO-glycine/taurine-BAC-treated groups. Prominent mutations in a tri-nucleotide context were observed in NO-taurine-BACs for ApCpC, and NO-glycine-BACs for GpCpC. Based on a similarity analysis, MSs related to NO-BACs are similar to those of human signatures 11, 15, and 23 from the COSMIC database. From these observations, it is suggested that NO-BACs might partly be involved in the development of human cancer.

J-1070

MicroRNAs profiling of cancer cells after Fe3O4 nanoparticles exposure (II)

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MicroRNAs (miRNAs) are short ribonucleic acid (RNA) molecules that play important roles in post-transcriptional regulation of gene expression. Because different types of cellular stress affect miRNA expression, nanomaterials may induce changes in miRNA expression. In the previous study, we showed miRNA profiling of cancer cells after Fe_3O_4 nanoparticles exposure, suggesting that cytotoxicity or effect of Fe_3O_4 nanoparticles may be dependent on cell vision. In the present study, we selected a few microRNAs with significant changes in expression after Fe_3O_4 exposure and analyzed their expressions in A549 and DU145 cells using quantitative RT-PCR. These cells were treated with 0. 100 or 200 μg/ml bare or carboxyl-coated Fe_3O_4 nanoparticles for 24 or 72 hours. miR-1260a and b in A549 cells were analyzed, and miR-494-3p and miR-5787 in DU145 cells. Expression of these miRNAs in each cell increased in a dose-dependent manner. Interestingly, miR-494-3p and miR-5787 have been reported to control eIF5 expression, showing the possibility of a target in prostate cancer. These results show the possibility to clarify the mechanisms of cytotoxicity or effect of Fe_3O_4 nanoparticles.

▲TOP

J-1071

DNA damage response facilitates aberrant expression of APOBEC3B via the ATM/ATR-Chk1 pathway in myeloma cells

Hiroyuki Yamazaki
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Co-author：Kotaro Shirakawa、Hiroyuki Matsui、Yasuhiro Kazuma、Tadahiko Matsumoto、Akifumi Takaori-Kondo
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APOBEC3B (A3B) is a DNA editing enzyme which induces genomic DNA mutations in myeloma. However, it is unclear how A3B contributes to acquisition of chemoresistance. To investigate A3B expression in myeloma cells favorably, we introduced 3xFLAG tag and IRES-EGFP at the 3' UTR of A3B in three myeloma cell lines, using the CRISPR/Cas9 system. PMA, a PKC activator which upregulates A3B expression via the NF-κB pathway, increased EGFP fluorescence in these cell lines as expected. During anti-cancer treatment testing, we found that antimetabolites and irradiation increased EGFP fluorescence, but bortezomib and lenalidomide did not. Chemical inhibition of ATM/ATR-Chk1, but not of DNA-PKcs, blocked the EGFP increase upon antimetabolites treatment, suggesting that A3B is upregulated by DNA damage response (DDR) via the ATM/ATR-Chk1 pathway. shRNA against A3B decreased the basal level of γH2AX foci in myeloma cell lines, indicating that A3B is involved in constitutive DNA double strand breaks, promoting DDR activation. Therefore DDR-inducible treatments trigger a positive feedback loop for A3B expression, high levels of which may drive chemoresistant clone expansion during chemotherapy.

J-1072

Assessment systems of cell competition between irradiated and non-irradiated rat mammary epithelial cells

Tatsuhiko Imaoka
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Because of the quantum nature of ionizing radiation, its exposure at low doses is characterized by physically inhomogeneous distribution in a tissue, where only a small fraction of cells are affected, with others intact and surrounding the affected cells. Competition between these cells, if there is any, can lead to exclusion of affected cells, and hence, smaller cancer risk than predicted from linear-no-threshold dose response. To investigate whether such novel biological phenomenon exists and, if ever, to what extent, we attempted to establish in vivo and in vitro experimental systems related with a rat model of radiation-induced mammary carcinogenesis. For an in vivo model, we developed a technique to generate a mosaic mammary gland, where cells exposed to radiation and those left unexposed are differentially fluorescent-tagged, mixed and transplanted. We also established a method to observe the mosaic tissue with optical tissue clearing and confocal imaging. For an in vitro model, we developed a method to culture fluorescent-tagged rat mammary cells and differentially irradiate them with an X-ray microbeam. We will further present preliminary results using these techniques.

▲TOP

[J-1073] J10-2 [Japanese]
Cell adhesion / invasion

2018 / 9 / 27 (Thu) 15:30-16:45 Room 8/10F 1008, Osaka International Convention Center Room 8

Kaoru Miyazaki / Mol. Pathol. Genetics Div., Kanagawa Cancer Ctr. Res. Inst.

J-1073

Coherent motion of the epithelial cells by the expression of the KRAS

Etsuko Kiyokawa
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Co-author：Eishu Hirata
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At the invasive front of the colorectal cancer, multi-cellular structures exists; the glands, and the cell clusters including tumor budding and poorly differentiated cluster (PDC) lacking a gland-like structure. It is technically difficult to evidence the movement of glands and clusters in vivo. We here utilized MDCK cells to understand the cell intrinsic characters for invasion. A single MDCK cell proliferates and forms a mature cyst in the matrix. We found that the expression of the active K-RAS induced rotation of a cyst. Treatment of Vorinostat, a HDAC inhibitor, increased the rotation velocity. We identified that *b*-catenin pathway was upregulated in the presence of active K-RAS and Vorinostat, and confirmed that *b*-catenin and the active K-RAS induced cyst rotation. Before forming a cyst, MDCK cells forms clusters, similar to budding or PDC. Quantitative analysis indicates that cells in a cluster moves faster than those in a mature cyst. Treatment of Vorinostat or the *b*-catenin expression did not alter the velocity of the cells in clusters. These results indicate that the signaling pathways of cell dynamics are different between the mature cyst and cell clusters.

▲TOP

J-1074

Acidic microenvironment induces epithelial mesenchymal transition in breast cancer cells

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Acidic extracellular pH (pHe) is known as an important feature of solid tumors. We investigate whether acidic pHe affects the morphology of cancer cells. MCF-7 cells cultured with acidic medium for 2 days showed loss of cell-cell adhesion and delocalization of E-cadherin and β-catenin from cell-cell contact. However, the absolute amounts of E-cadherin, β-catenin, vimentin and transcriptional factors that regulate EMT were unchanged, indicating that the change is not complete EMT. Phosphorylation of SRC at Y 416 and FAK at Y 925 was enhanced after culturing with acidic medium for 2 hours. SRC kinase inhibitor inhibited EMT-like change induced by acidosis. Phosphorylation of cortactin at Y421 and Y466, known as tyrosine residue by SRC, was also upregulated after culturing with acidic medium, and phosphorylated cortactins were also colocalized with phosphorylated FAK and SRC in focal adhesions. Futhermore, Integrin β6 was elevated after culturing with acidic medium, and integrin αV and β6 were colocalized with phosphorylated FAK, SRC and cortactin in focal adhesions. In conclusion, lowered pHe could induce EMT-like change of breast cancer cells via FAK/SRC/cortactin signaling.

J-1075

Cancer invasion geometry of giant cancer cells cooperating with stromal cells

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It is known that aberrant mitosis frequently creates giant cancer cells. Repeated mis-segregation of chromosomes in cancer cells accelerates genetic instability and produces giant cancer cells. It is not well understood whether giant cancer cells are involved in tumor progression. We noticed that SAS oral squamous cell carcinoma cells cause mitotic slippage and became large with giant nuclei after treatment with nocodazole. When these giant SAS cells were subcutaneously implanted in mice, they could proliferate. In co-culture assays in ECM gels, giant SAS cells co-cultured with cancer-associated fibroblasts (CAFs) further proliferated with aberrant mitosis and invaded into the gel led by co-cultured CAFs, indicating that they are not dormant. To explore an incidence of giant cancer cells in vivo, parent SAS cells were implanted in various tissues of nude mice. One week later, multinucleated giant SAS cells were most remarkably observed in the adipose tissues of abdominal cavity. Because giant cancer cells are sometimes detected in human specimens after chemotherapy, coordinated invasion by giant cancer cells and CAFs would be important in terms of relapse and progression of cancer.

J-1076

Macrophages transmit tumor-derived extracellular vesicles to stromal cells and create pro-tumor microenvironment

Masamitsu Tanaka
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It is not well understood how Tumor-derived extracellular vesicles (TEVs) are delivered and effect on stromal cells in a tumor microenvironment. We report a novel function of macrophages in delivery and transmission of TEVs. TEV incorporated macrophages (TEV-MΦ) increased invasion and widely disseminated. Upon contact with host stromal cells including mesothelial cells (MC), fibroblasts and endothelial cells, TEV-MΦs released membrane blebs containing TEVs. Then, scattered blebs were incorporated in these stromal cells, which transferred tumor-derived proteins and RNAs. Bleb formation and TEV-transfer partially depends on localized caspase-3 activation in macrophages. Macrophage mediated transfer of TEVs caused EMT and myofibroblastic change in the recipient cells. In TEV-MΦ injected mice stomach, TEVs were delivered by MΦs to MCs covering the stomach, and MCs entered the gastric wall to create a niche that favored invasion of gastric cancer cells. Deprivation of macrophages prevented TEV-transfer and MC niche formation. These results suggest a novel function of TAMs that transfer TEVs to surrounding stromal cells and increase CAF-like cells.

▲TOP

J-1077

The involvement of CADM1 in enhancement of malignant features of small cell lung cancer

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Small cell lung cancer (SCLC) is one of the representative intractable cancers with the 5-year survival of less than 10 %. CADM1, a member of immunoglobulin superfamily cell adhesion molecules, is highly expressed in SCLC and enhances aggregation activity of SCLC cells in liquid culture. To understand pathological significance of CADM1 overexpression in SCLC, we introduced full-length CADM1 and the deletion mutants in its intracellular domain into SCLC cells, SBC5, lacking endogenous CADM1 expression and assessed malignant features in comparison with control SBC5 cells. CADM1 expression significantly enhanced colony formation in soft agar and subcutaneous tumor formation in nude mice of SBC5, whereas its deletion mutants did not. Next, to identify molecules involved in anchorage-independent growth by CADM1, we screened library of 188 chemicals to inhibit growth of SBC5 cells in ultra-low attachment dish and found that a PI3K inhibitor suppressed cell growth of SBC5. These results suggest that CADM1 activates PI3K through binding proteins to its intracellular domain and promotes anchorage-independent growth of SCLC cells. CADM1 could provide a therapeutic target for SCLC.

J-1078

Mesothelial cells create invasion frontier in peritoneal metastasis of epithelial ovarian cancer

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This study illustrates how mesothelial cells create invasion frontier in peritoneal metastasis of epithelial ovarian cancer with detailed in vitro experimental models. In stage Ⅲ ovarian cancer, there were stromal cells positive for both calretinin and α SMA. We defined these cells as cancer-associated mesothelial cells (CAM) and found that this mesenchymal change is mainly caused by TGF-β 1 stimulation. Invasion was promoted in primary human mesothelial cells stimulated by TGF-β 1 and we detected that enriched invadepodia on the cell surface. We established 3D peritoneal dissemination model and revealed that both cancer cells and TGF-β 1 stimulated mesothelial cells invaded and did more collagen degradation compared with control ones. Fascin, the one of the essential factor to create invadepodia was upregulated in TGF-β 1 stimulated mesothelial cells. Using inhibitor of Fascin, the number of invadepodia and the amount of collagen degradation were decreased. Our results suggested that alteration of the tumor microenvironment by TGF-β 1 was enhanced invasion of CAM via Fascin upregulation and promote peritoneal dissemination of epithelial ovarian cancer.

▲TOP

[IS2-1] IS2 [English]
New antibody therapeutics in oncology

2018 / 9 / 27 (Thu) 9:00-11:30 Room 9/10F 1009, Osaka International Convention Center Room 9

Yuki Abe / Daiichi Sankyo Co., Ltd. Biologics & Immuno-Oncology Laboratorie、Maggie Lu / Targeted Drug & Delivery Tech. Div., Biomed. Tech. & Device Res. Laboratories, Industrial Tech. Res. Inst.

Monoclonal antibody therapy has been successfully applied to treat cancer patients in the clinic for these 20 years. Best examples are anti-HER2 monoclonal antibody trastuzumab and anti-CD20 monoclonal antibody rituximab to treat solid tumors and hematological malignancies, respectively. Second generation of antibody based therapy includes antibody drug conjugate (ADC), an emerging class of anti-cancer medicine. Identification of new target antigen, generation of good antibody with anti-cancer property, and selection of suitable modality (ie. monoclonal antibody or ADC) are major components of new drug development. In this session, we will highlight new targets, therapeutic antibodies, ADC technologies, and introduce recent achievements of ADCs in clinical development.

IS2-1

Novel Hydrophilic and Site-Specific Antibody-Drug Conjugates to Treat Tumors

Maggie Lu
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Targeted Drug & Delivery Tech. Div., BDL, ITRI

ADC is a promising therapeutic type of precision medicine. Our first approach is to introduce the sugar structures into the unnatural peptide to increase the hydrophilicity of the whole linker-toxins, this can eliminate organic solvent during ADC conjugation process and increase the stability of ADC property. The tumor disappeared completely in the head and neck cancer FaDu xenograft model treated with one dose E-MHT-71 ADCs of 5 mg/kg, and there was no tumor recurrence more than 160 days, which was significantly better than that of E-MC-Val-Cit-PAB-MMAE, and also superior to ABT-414. The therapeutic index of nearly 110-fold. The second approach is to improve the homogeneity of ADC by site-specific conjugation. The D4 ratio of H-SSCLT24 is over 70% without further separation. The general IC 50 of Herceptin-SSCLT24 is below 1nM in BT474 and JIMT-1 cell. H-SSCLT24 in mouse and human plasma are at great stability. H-SSCLT24 has similar PK as Kadcyla in a mouse model. The MTD is 100 mg/kg in a mouse model. Excellent efficacy in tumor inhibition after a single dose of 1 mg/kg in Herceptin-resistant JIMT-1 xenograft model. The efficacy of Herceptin-SSCLT24 is better than Kadcyla.

▲TOP

IS2-2

Globo series glycosphingolipids serves as promising targets for cancer therapy

Jiann-Shiun Lai
Res., OBI Pharma, Taiwan

Tumor-associated carbohydrate antigens (TACAs) are overexpressed in many epithelial tumors. Two hexasaccharide TACAs, Globo H and Stage-specific embryonic antigen 4 (SSEA4) of Globo series glycosphingolipids, were found on a variety of epithelial cell tumors such as colon, ovarian, gastric, pancreatic, endometrial, lung, prostate, and breast cancers. The marked difference in expression of Globo H and SSEA4 between cancer cells and normal tissues makes them promising targets for cancer immunotherapy. A multinational randomized phase Ⅱ clinical trial has been conducted to evaluate the efficacy of Globo H active immune therapy in patients with metastatic breast cancers. The study showed that patients with a humoral immune response to the Globo H antigen correlated highly with improved progression-free survival although it did not meet its primary end point. This result renders an important opportunity for monoclonal antibody target therapy in cancer patient with high expression of Globo H. Detailed approaches will be discussed.

IS2-3

Preclinical study for solid tumors using anti-tissue factor antibody drug conjugate

Yoshikatsu Koga
Div. Develop. Therap., EPOC, Natl. Cancer Ctr.

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Div. Develop. Therap., EPOC, Natl. Cancer Ctr.

ADCs are composed of the antibody, the linker, and the cytotoxic payload. For the succession of ADCs, the antibody should possess cancer specificity, the efficient internalization and long circulation in blood. The linkers are categorized into cleavable and non-cleavable linker and should be stable in blood. The payloads should have 100 to 1000 fold high potent cytotoxicity than the conventional anti-cancer agents because the number of drugs conjugated to each antibody is limited. When the ADCs reach to the solid tumors through blood stream, the cancer stroma interrupts the penetration of the ADCs. There are several issues to be considered in the solid tumors, which are the stability and the extravasation from tumor vessels, the cancer stroma barrier, the binding site barrier, the internalization into cancer cells, the drug releasing, and the bystander killing effect. The last 3 hurdles can be conquered by a linker technology and novel potent drugs. However, at the DDS point of view, antibody should be delivered to all of the cancer cells within the cancer tissue. In this presentation, we focus on the characteristics of antibody and various barriers for successful ADCs.

IS2-4

Anapocosis-inducing mAbs may be promising therapeutic device for hematological cancer

Tokuko Toyota
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A newly established mouse anti-pan HLA class Ⅱ mAb (4713) triggered cytoskeleton-dependent, but complement- and caspase-independent, cell death in Hodgkin Lymphoma (HD) cell lines, Burkitt lymphoma cell lines, and ATL cell lines. mab 4713 also has cytolytic effect on ex-vivo lymphoma cells of patient of diffuse large B-cell lymphoma. We develop this therapeutic mAb 4713, by immunizing a BALB/c mouse with live HD cell lines. mAb 4713 was revealed to be a mouse anti-human pan-HLA class Ⅱ mAb. Treatment with this mAb induced the formation of large pores on the surface of target lymphoma cells within 30 min. This finding suggests that the cell death process induced by this anti-pan HLA-class Ⅱ mAb may involve the same death signals stimulated by a cytolytic anti-pan mouse MHC class Ⅰ mAb that also induces large pores formation on the surface of mouse lymphoma cells. We named these not apoptotic not necrotic complement-independent cell death “anapocosis" because of large pore formation on the surface of lymphoma cells. Humanized mAb 4713 has also cytolytic effect on lymphoma cells.

▲TOP

IS2-5

DS-8201a, a next generation anti-HER2 antibody drug conjugate addressing across the wide range of HER2 expressing tumors

Takahiro Jikoh
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Antibody drug conjugate (ADC) is targeted agents that deliver cytotoxic payload to cancer cells via a linker attached to a monoclonal antibody that binds to a specific target expressed on cancer cells. Designed using Daiichi Sankyo's ADC technology, DS-8201a is a smart chemotherapy comprised of an anti-HER2 antibody attached to a novel topoisomerase I inhibitor payload by a tetrapeptide-based linker. It is designed to target and deliver chemotherapy inside cancer cells and reduce systemic exposure to the cytotoxic payload compared to common chemotherapies. The ongoing global phase 1 trial consists of dose escalation and multiple dose expansion cohorts including HER2 expressing breast cancer, gastric cancer and other solid tumors. As of Apr 18, 2018, 254 subjects received DS-8201a; N=241 at 5.4 or 6.4 mg/kg. Overall, confirmed overall response rate (ORR) in the evaluable subjects was 102/208 (49%) with the highest ORR in HER2 positive breast cancer (54.5%). Most frequent adverse events were gastrointestinal or hematologic with generally low grade. In conclusion, DS-8201a shows promising antitumor activity in HER2 expressing multiple tumor types in heavily pretreated subjects.

IS2-6

Novel antibody drug conjugates for high grade gliomas and EGFR-expressing tumours

Hui Gan
Med. Oncol., Austin Health, Olivia Newton-John Cancer Res. Inst., Austin Health, Heidelberg, Australia, Sch. of Cancer Med., Latrobe Univ., Heidelberg, Australia, Dept. Med., Melbourne Univ., Heidelberg, Australia

Antibody drug conjugates (ADCs) are drugs comprised of a targeting antibody linked to therapeutic payloads such as cytotoxic agents, immunotoxins or radioisotopes. Upon binding to their cell surface target, ADCs are internalised and degraded, with subsequent release of their payload and resultant cell death. Newer ADCs have shown impressive efficacy with minimal toxicity, as exemplified by the drug trastuzumab emtansine in breast cancer. This talk will focus on the use of novel ADCS in high grade gliomas, a tumour with poor prognosis and limited treatment options. Many of these have focused on EGFR or EGFRvIII, as EGFR is the most frequently dysregulated pathway in high grade gliomas such as glioblastoma. The biology of these agents and current status of clinical development will be discussed. The available data about resistance mechanisms and biomarkers will be reviewed. Lastly, the potential use of these agents in other EGFR expressing tumours will also be briefly presented.

▲TOP

[LS7] LS7 [Japanese]

Basic study of new molecular target therapy for ER-positive/HER2-negative advanced/metastatic breast cancer

2018 / 9 / 27 (Thu) 11:50-12:40 Room 9/10F 1009, Osaka International Convention Center Room 9
：Pfizer Japan Inc

Masahiko Watanabe / Kitasato University School of Medicine, Department of Surgery

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LS7

Basic study of new molecular target therapy for ER-positive/HER2-negative advanced/metastatic breast cancer

Shin-ichi Hayashi
Department of Molecular and Functional Dynamics, Tohoku University Graduate School of Medicine

No Abstract

▲TOP

[IS4-1] IS4 [English]

Application of artificial intelligence for cancer research; integrated analysis of cancer omics data using machine learning and deep learning

2018 / 9 / 27 (Thu) 13:00-15:30 Room 9/10F 1009, Osaka International Convention Center Room 9

Ryuji Hamamoto / Natl. Cancer Ctr. Res. Inst.、Jinhua Yu / Fudan Univ.

In the enlightened times of the postgenomic era, we could get a large quantity of omics data such as genome, epigenome, transcriptome, proteome, medical images with detailed clinical information. However, it was technically difficult to efficiently analyze enormous medical data in an integrated manner until recently. On the contrary, the current progress of the artificial intelligence (AI) technology, which is mainly based on the development of machine learning and computer performance, enables the integrated analysis of medical big data. In particular, deep learning, which is part of a broader family of machine learning methods based on learning data representations, is responsible for many of the resent breakthroughs in AI, and it has already been reported that deep learning outperformed humans in many tasks. In this session, researchers, who are all specialists of medical AI, introduce current progress of AI technologies in the field of cancer research, and we will discuss the possibility of the clinical application.

IS4-1

Development of the integrated cancer medical system using artificial intelligence

Ryuji Hamamoto
Div. Mol. Mod. Cancer Biol., Natl. Cancer Ctr. Res. Inst., Cancer Transl. Res. Team, RIKEN Ctr. for AIP project

The high quality basic medical study, clinical study and epidemiological study have been performed continuously in Japan, and enormous quantity of the medical data have already been accumulated. Although there was no means to analyze enormous medical data in an integrated manner before, the rapid progress of the recent artificial intelligence (AI) technology enables the big data analysis. In particular, by the rise of the deep learning technique, a trial to apply an AI technology to a diagnosis, the treatment of the disease and innovative drug development advances at world level. Importantly, the medical care optimized for an individual called Precision Medicine becomes the world trend now. It is certain that the advanced IT and AI technologies are essential to promote Precision Medicine. We started a project entitled "Development of the integrated cancer medical system using the artificial intelligence" as one of the research themes of CREST to develop the new cancer medical system using AI in National Cancer Center. On the basis of achievements obtained in the project, we introduce the usefulness of AI for the cancer medical care, and the possibility of its clinical application.

▲TOP

IS4-2

Deep Learning based Radiomics (DLR) and its usage in noninvasive IDH1 prediction for low grade glioma

Jinhua Yu
Electronic Engineering Dept., Fudan Univ.

Radiomics is an emerging technique explicitly designed to extract high-throughput features from medical images, convert the features into minable data, and subsequently make an effective clinical decision. Several groups of image features, including intensity, shape, texture and wavelets, were usually extracted in traditional radiomics. Although a lot of features can be calculated, it is not possible for all imaging characteristics to be included in the predesigned features. To overcome the shortcomings of traditional radiomics, we developed two sets of radiomics approaches, namely deep learning-based radiomics (DLR) and sparse representation-based radiomics (SRR). DLR obtains radiomics features by normalizing the information from a deep neural network designed for image segmentation. DLR could produce superior performance equipped with sufficient data. When dealing with rare disease with small amount of image data, DLR may easily suffers from overfitting. For modeling of rare disease with limited data, SRR was proposed. In SRR, dictionary learning- and sparse representation-based feature extraction, feature selection and classification were developed.

IS4-3

Artificial Intelligence for Cancer detection and Genetic Research

Jun Miyake
Global Ctr. for Med. Engineering & Informatics, Osaka Univ.

We have studied the whole-sequence-based classification of DNAs using Deep Learning (Stacked auto encoder). An important application is human leukocyte antigen (HLA) DNAs because they are deeply related to the resistance to cancer and immune checkpoint inhibitor. HLA Nucleotide sequence data corresponding to the length of 822 bp, were compressed to 2-dimensional representation and were plotted. Profiles of the two- dimensional plots indicate that the alleles can be classified as clusters are formed. The two-dimensional plot of HLA-A DNAs gives a clear outlook for characterizing the various alleles. We should like to propose the auto encoder based conceptual expression of the nature of HLA DNAs could give a tool for the research on the mechanism of immune system, giving solutions or indication of the medical analysis and drug design. For the next step of the research, collaborations with clinical teams are needed. A sufficient number of highly qualified clinical data are required for the correlation study. It is the first step of the interpretation of HLA DNA by artificial intelligence to give a conceptual view of alleles.

IS4-4

Computational inference of cancer-specific vulnerabilities in clinical samples

Jung Kyoon Choi
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Current methods of profiling cancer dependency are applicable only to in vitro cell culture. Here, we developed an in silico RNAi method and deep neural network model to predict cancer-specific vulnerabilities from tumor and matched normal transcriptomes of clinical samples. Acquired dependencies of tumors were inferred in cases in which one allele was disrupted by inactivating mutations or in association with oncogenic mutations. Nucleocytoplasmic transport by Ran GTPase was identified as a common vulnerability in triple-negative or Her2-positive breast cancers. Vulnerability to loss of Ku70/80 was predicted for tumors that are defective in homologous recombination and rely on nonhomologous end joining for DNA repair. We experimentally validated the potential of Ran, Ku70/80, and a proteasome subunit as precision therapeutic targets for particular tumors that are predicted to be dependent on these genes. This approach can be applied to facilitate the discovery of novel therapeutic targets for different types of cancers.

▲TOP

IS4-5

Molecular Diagnosis and Survival Predicition of Glioma Patients by Using Machine-Learning based Radiomics Methods

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Glioma is the most common and lethal malignant brain tumor with huge genetic heterogeneity. Nowadays, molecular diagnosis has become regular clinical practice in China served as a robust tool for neurosurgeons to predict sensitivity of chemo-radiotherapy and patients survival outcome. However, due to heterogeneous genetic alterations inside tumor, sampling for pathological and molecular diagnosis has been always challenging. Machine-learning based radiomics methods provide possibility to explore deep and microcosmic information of gliomas from conventional MRI data. Herein, we have developed three radiomics systems which was named as Classical Radiomics, Deep-Learning based Radiomics and Sparse Representation Radiomics to predict genetic alterations and perform molecular subgrouping noninvasively. These methods take the priority to analysis glioma as a whole that can makes survival prediciton more accurate than single biomarker did. In the future, along with the development of intelligent chips, machine-learning based radiomics methods will be commercialized and enjoy great potential in clinical practice.

IS4-6

Integrating Artificial Intelligent System with Clinical Workflow of Radiologist in the Hospital

Kazuma Kobayashi
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The potential of deep learning, as one of the state-of-the-art methods of machine learning, to influence the practice of medicine has been attracting attention, particularly in the field of radiology. Current application of deep learning in medical imaging has proven its capability of detection, diagnosis, and characterization of diseases. However, there are still several challenges for artificial intelligence-based diagnostic support system to be embedded in the hospital, such as lack of the clinical context of patients, necessity for the adaptation to individual clinical workflow, and potential burden of data labeling for the variety of diseases. Here, we introduce a proof-of-concept model of an integrated system of artificial intelligence in the hospital to overcome these difficulties and to improve clinical workflow based on the collaboration between radiologists and intelligent machines. This learning system works to augment the process of image-based diagnosis by its ability of detection, segmentation, and quantification of tumors while receiving feedbacks from radiologists at the same time, optimizing the judgement of both physicians and machines for better practice.

▲TOP

[E-1115] E25 [English]
Data science / AI

2018 / 9 / 27 (Thu) 15:30-16:45 Room 9/10F 1009, Osaka International Convention Center Room 9

Masaaki Matsuura / Teikyo Univ. Grad. Sch. of Public Health

E-1115

Combination of scRNA-seq platforms reveals the heterogenous transcript response to gefitinib

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scRNA-seq is a powerful tool for revealing heterogeneity. However, each of the current scRNA-seq platforms have pros and cons. We combined the data from two platforms to reveal variable transcriptome responses to gefitinib using five lung adenocarcinoma cell lines. We demonstrate that it is possible to estimate missing values for expression information. We further demonstrate that even in the cases where precise information for an individual gene cannot be inferred, it is possible to analyze the activity of given transcriptional modules. Interestingly, we found that two distinct transcriptional modules associated with the Aurora kinase and DUSP genes are aberrantly regulated in a minor population of cells, and thus, may contribute to the possible emergence of dormancy or eventual drug resistance within the population.Combining the different single-cell RNA-seq platforms can be one effective approach to obtaining complete information for both continuous expression varieties and a sufficient size of the cellular population to understand transcriptional heterogeneity in cancers.

▲TOP

E-1116

Convolutional neural network distinguishes cancer cell lines by microscopic images

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Recently, the technology of artificial intelligence (AI) trained with a convolutional neural network (CNN) has been developed, although it remains to be understood perfectly whether AI can distinguish cancerous cells that have different natures. The goal of the present research is to predict the effect of treatment such as radiation therapy and chemotherapy by microscopic images. We here tried to distinguish cancer cells and their radiation-resistant derivatives using phase contrast microscopic images. We prepared phase contrast microscopic images of radiation-resistant clones from two cell lines: NR-S1, mouse squamous cell carcinoma; and ME-180, human cervical carcinoma. We trained the CNN called VGG16 with these images and used the trained VGG16 to distinguish these cells and to extract image features. As the results, we obtained high accuracy in the model (>95%). Features extracted by VGG16 were clustered into 5 groups more easily than some apparently reasonable characteristics for classification were hardly extracted. Taken together, we demonstrated the potential of image recognition ability of AI, while the meaning of features extracted need further study.

E-1117

Comprehensive Search for Prognostic Biomarkers using PCAWG Data

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The PanCancer Analysis of Whole Genomes (PCAWG) project is a big international project organized by ICGC to understand whole genomes of the pan-cancer. It collected multi-omics data for the tumor and healthy tissues of 2,800 whole genomes across 39 distinct tumor types. There are 31 working groups; in the clinical correlation group, we search for molecular markers associated with overall survival. Carefully checking the qualification of the big data, we determined 12 tumor types with the usable variables of SNVs/indels, structural variations (SVs), CNAs, and transcriptome. We also analyzed mutation burden, mutational signatures, HLA class I, neo-antigens, gene pathways, and clinical factors such as age and genders.

We used our previous framework (Fujimoto et al, Nat. Genet., 2016): a combination of the survival Lasso regression and the Cox model. We found that, for example, SNVs/indels of ARID1A and TP53 were independently associated with about two times increase of hazard ratios in hepatocellular carcinoma. Prostate adenocarcinoma was the cancer type for which prognosis was most accurately (cindex > 0.9) predicted. We plan to validate our results using outside TCGA data.

E-1118

Application of logical exclusive OR gate and support vector machine to predict response to dCRT of ESCC

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Extensive multi-layer genome-wide screening with sufficient sample numbers may not yield high-quality biomarkers. This was true also to predict the response to definitive chemoradiotherapy (dCRT) in esophageal squamous cell cancers (ESCC), for which we previously isolated a DNA methylation marker with limited power [Takahashi, JCRCO, 141:453, 2015]. In this study, we aimed to isolate a novel biomarker with a combination of DNA methylation sites, which would show an effect following the rules of logical exclusive OR gate. We screened a combination that showed a positive outcome when two DNA methylation sites were hypermethylated or hypomethylated (++/- -) and that showed negative outcome when either of these sites were hypermethylated (+- /-+). We analyzed DNA methylation array data of 41 patients using a support vector machine (SVM). Twenty-nine candidates were isolated demonstrating 80% or more specificity and 50% or more sensitivity. Currently, a validation study is being conducted. Therefore, logical exclusive OR gate and SVM may lead to the identification of novel biomarkers, which cannot be identified by simple classification.

▲TOP

E-1119

Predicting neuroblastoma prognosis with deep learning model based on the genetics, epigenetics, and clinical status

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Neuroblastoma is the most frequent solid tumor in early childhood. Recent advances of molecular genetics have made it clear that several genetic or epigenetic abnormalities affects neuroblastoma prognosis. In addition, clinical disease status also correlates with prognosis. In the clinical setting, it remains uncertain how genetic or epigenetic factors, and clinical status should be integrated to predict the prognosis because of their complexity.To solve this problem, we hypothesized that deep neural-network based model is an appropriate method to estimate the neuroblastoma prognosis. We built a prognostic model based on the following factors; each factors are well known to correlate with prognosis.(I) genetic and epigenetic factor (DNA methylation, DNA copy number variation, MYCN status)(II) clinical disease status (INSS, Histology)(III) Patients' characteristics (age of onset, gender) After training with data set, the model could clearly classify validation cohort into a favorable subgroup, or an unfavorable one. We expect that in the clinical setting, our model is a useful tool to predict more accurate prognosis of neuroblastoma patients with conventional treatment.

E-1120

The Effects of Internet Self-Diagnosis on the Physician-Patient Power Dynamic

James A. Goddard
Kitasato Univ.

The phenomenon of patients doing self-diagnosis and treatment investigation using the Internet is well-documented in the literature. What is not as well-reported is the effect of this proactivity on patient empowerment, and how this affects the authority and power relationship with the consulting physician. From the patient and/or physician viewpoint, the perceived expertise and authority (expert power) of the physician can be diminished with increased patient information and empowerment. When there is a move to a more patient-centered treatment arising from Internet self-education, there can be effects on the comfort and confidence of the physician and/or patient, and patient satisfaction with outcomes. This session will present a brief literature review highlighting recent studies findings of surveys, interviews and case studies of physician experiences and reactions to patient education and empowerment through Internet self-education and diagnosis.

▲TOP

[J-1013] J16-1 [Japanese]
New molecular target (1)

2018 / 9 / 27 (Thu) 9:00-10:15 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Kazuo Shin-ya / BRD, AIST

J-1013

Crosstalk between somatic mutation and genetic variation modulates drug response in CRC

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Extensive efforts for predicting the response to chemotherapeutic agents have been made to identify the somatic mutations, which can be used as useful markers for cancer treatment. However, the limitations of genomic profiling as predicting makers still hampered the improvement of efficacy as well as the development of novel therapeutic agents. Recent studies have identified patient-derived organoids as robust preclinical models. We established 42 organoids from five familial adenomatous polyposis coli (FAP) patients. Sequence analysis revealed they have distinct genomic alterations including KRAS, which provide us the opportunity to explore the role of somatic mutations. In addition, comparison between the patients allowed us to investigate the contributions of genetic background. Using the FAP organoid sets, we explored the responses to chemotherapeutic agents targeting RAS signaling, and found that, in addition to activating mutations in KRAS, genetic variations strongly affect the response. We will discuss the potential applications to develop the novel strategy for cancer therapeutics and to obtain the biomarker to stratify the CRC patients harboring RAS mutations.

▲TOP

J-1014

Genomic analysis of predictive biomarker for pazopanib treatment in patients with advanced soft tissue sarcoma

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Pazopanib is an oral multi-tyrosine kinase inhibitor with activity against VEGFR, PDGFR and c-Kit, and has been shown to improve progression free survival (PFS) of patients with advanced soft tissue sarcoma (STS). However, there is no biomarker to predict the effect. In the current study, to discover the predictive biomarker, we collected 28 fresh frozen samples derived from non-small round cell STS patients treated with pazopanib, and performed whole exome sequencing (WES) and whole transcriptome sequencing (WTS) analyses. From WES data, we detected 33.3 base substitution and 2.9 insertion/deletion mutations per patient. No patient demonstrated a known functional mutation on genes directly targeted by pazopanib. Although 3 variants were detected in these genes, they were variants of unknown significance and were not associated with PFS. On the other hand, from Gene Set Enrichment Analysis using WTS data, we found that the patients with longer PFS (over 20 weeks) exhibit significantly higher expression of hypoxia signature than those with shorter PFS ($p<0.00001$). These results suggest that not genetic alterations but tumor microenvironment affect responses to pazopanib treatment.

J-1015

HSP90 inhibitor 17-AAG suppresses the proliferation of FLT3-ITD/D835 mutant AML cells

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Quizartinib is an effective drug for acute myeloid leukemia (AML) harboring internal tandem duplication (ITD) in Fms-like tyrosine kinase 3 (FLT3). However, its long-term administration conducts quizartinib resistance with gatekeeper (F691) and activation loop (D835, Y842) mutations in FLT3-ITD. In the last JCA meeting, we reported that HSP90 inhibitors, 17-AAG and 17-DMAG, overcame the resistance in Ba/F3 transfectants. Here, we established quizartinib-resistant AML cell lines, QR1 and QR2, based on FLT3-ITD-positive MV4-11 cells and evaluated the efficacy of 17-AAG. QR1 and QR2 cells harbor D835H and D835V mutations, respectively, in FLT3-ITD. These cells showed much higher resistance to quizartinib, but they were more sensitivity to 17-AAG than MV4-11 cells. In QR1 and QR2 cells, treatment with 17-AAG well downregulated STAT5, AKT, ERK, cyclins and phosphorylated RB, and induced caspase-dependent apoptosis. In addition, 17-AAG significantly enhanced the sensitivity of QR1 and QR2 cells to daunorubicin. In conclusion, 17-AAG suppresses the proliferation of quizartinib-resistant AML cells caused by FLT3-ITD/D835 mutations.

J-1016

CUDC-907, a new dual PI3K and HDAC inhibitor, as ATL therapeutics

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Active PI3K/Akt has a causal role in ATL caused by HTLV-1. PI3K inhibitors are evaluated in cancer clinical trials, but single agents display limited activity. Combination of PI3K inhibitor BKM120 and HDAC inhibitor LBH589 resulted in synergistic cytotoxic effect in HTLV-1-infected T-cell lines. CUDC-907, a dual PI3K/HDAC inhibitor was more efficacious compared with BKM120 and LBH589. In contrast, an uninfected T-cell line and PBMC were resistant to CUDC-907. It induced G1 arrest accompanied by downregulation of cyclin D1/D2, Cdk4/6, c-Myc and phosphorylated pRb. Apoptosis was also induced via caspase-3/8/9 activation concomitantly with downregulation of Bcl-xL, XIAP, survivin and c-IAP1/2, as well as upregulation of Bax and Bak. Furthermore, histone H3 acetylation, H2AX activation, and upregulation of HSP70 and phosphorylated HSP27 were observed after CUDC-907 treatment. It suppressed Akt, NF-κB and AP-1 through a reduction of phosphorylated and/or total Akt, S6K, 4E-BP1, RelA, IKKα/β, IκBα, JunB and JunD. The inhibitory effect of CUDC-907 against HTLV-1-infected T-cell lines supports the application of this dual PI3K/HDAC inhibition as a potential therapeutic strategy in ATL.

▲TOP

J-1017

BIG3-PHB2 complex as a novel target for overcoming trastuzumab-resistant HER2-overexpressing breast cancer

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Anti-HER2 monoclonal antibody, trastuzumab extends the overall survival of patients with HER2-overexpressing breast cancer, but resistance to trastuzumab remains a serious clinical problem. We previously demonstrated that Brefeldin A-Inhibited Guanine nucleotide-exchange protein 3 (BIG3) functions as a cancer specific A-kinase anchoring protein that binds PKA and PP1C α in breast cancer, thereby dephosphorylating and inactivating tumor suppressor, prohibitin2 (PHB2). We here report that BIG3/PKA/PP1C α regulates inactivation of PHB2 through its dephosphorylation in HER2-overexpressing breast cancer cells. Importantly, ERAP, a specific peptide inhibitor targeting the BIG3-PHB2 interaction, resulted in intrinsic PHB2 released from BIG3, followed by recovering its phosphorylation and activation, and eventually disrupted the HER2-HER3 interaction and NF-κB pathway, which are associated with trastuzumab-resistance. Our findings suggest that it is essential that dephosphorylation and inactivation of PHB2 is mediated by BIG3/PKA/PP1C α for oncogenic signalling in HER2-overexpressing breast cancer cells, and ERAP may be a promising anti-cancer drug for trastuzumab-resistant breast cancer.

J-1018

Imatinib mesylate induced antitumor effect by increased infiltration of effector T cells in tumor

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Imatinib mesylate inhibits the constitutively activated BCR-ABL tyrosine kinase protein, platelet-derived growth factor receptor and c-kit. It has also been reported that Imatinib showed off-target immunological effects in preclinical and clinical studies. However, the immunological mechanism is not fully understood. Here, we compared the effect of Imatinib in tumors of CT26 colon cancer and Lewis lung carcinoma (3LL) cell lines. Imatinib was no cytostatic effect for CT26 and 3LL cell lines in vitro, whereas Imatinib significantly suppressed the growth of CT26 subcutaneous tumor but not 3LL tumor in mice. The ratio of CD8 gene count (2.011) in CT26 tumor of Imatinib-treated mice compared to PBS-treated mice was significantly higher than that in 3LL tumor (0.963) ($p = 0.0027$) using a digital multiplexed measurement (nCounter). The expression of T cell recruitment-associated cytokines was significantly elevated in CT 26 tumors but not in 3LL tumors, suggesting that the Imatinib changed the cytokine expression profile in a CT26-specific manner, which enhanced the infiltration of effector T cells into tumor. We showed the novel mechanism of Imatinib-mediated suppression in tumor.

▲TOP

[J-1019] J16-2 [Japanese]
New molecular target (2)

2018 / 9 / 27 (Thu) 10:15-11:30 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Mikihiko Naito / Div. Mol. Target & Gene Ther. Products, Natl. Inst. Health Sci.

J-1019

Benzaldehyde inhibits the multiple signals and E2F transcription in cancer cells by suppression of overexpressed 14-3-3 ζ

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Benzaldehyde(BA) is the structurally simplest aromatic aldehyde constituent of many foods. In 1980's, multi institutional clinical trials by using derivatives of BA were performed, however only it's safety was confirmed. Given that we had multiple effective clinical cases including pancreatic cancers and chemo-resistant non-Hodgkin lymphoma, we attempted to elucidate the mechanism of anti-cancer effect of BA. We have previously reported that BA suppresses proliferation of cancer cell lines including pancreatic cancer cell BxPC3, but not in normal cells and that BA inhibits multiple cancer-related pathways such as PI3K/AKT/mTOR, STAT3, NFκB and ERK. Using biomedical analyses including pull-down assay and immunoprecipitation, we found that BA selectively inhibits the interaction of 14-3-3ζ with its phosphorylated client proteins. Transcriptome analysis revealed that BA suppresses E2F targeted genes and increases stress responsible genes, which was found to be regulated through the BA-mediated inhibition of 14-3-3ζ functions. Such cancer-related transcriptional regulation is a novel mechanism of BA-mediated anti-cancer effect.

▲TOP

J-1020

Target identification of bioactive small molecules using thermal shift assay based on 2-D electrophoresis

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Target identification of bioactive small molecules is an important step in drug discovery research. We have developed 2-D DIGE based protein profiling system to identify molecular targets of bioactive compounds, namely ChemProteoBase. Recently, cellular thermal shift assay (CETSA) has been developed as a label-free method for target identification which monitors thermal-stability changes of proteins by interactions with ligands. In this study, we applied 2-D DIGE to CETSA (2DE-CETSA) to widely screen thermal-shifted proteins in cells treated with target-unknown anti-cancer compounds. NPX84 is a newly identified small molecule exerting inhibitory effects on cell growth in colorectal cancer cell lines (HCT116, HT-29, LoVo). We performed 2DE-CETSA using HCT116 cell lysates treated with NPX84 and then heated at a rage of temperatures. In the 761 spots detected by quantitative analysis, several spots showed destabilization upon NPX84 treatment in the 55°C-heated sample, and LC-MS/MS identified PKM2 as a candidate target of NPX84. We revealed that NPX84 suppressed protein interactions between PKM2 and STAT3 or β -catenin and downstream signaling pathway.

J-1021

Targeting the oncogenic MUC1-C with a GO-203 nanoparticle overcomes MCL-1- and BCL2A1-mediated resistance

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Aberrant expression of MCL-1 and BCL2A1 is a major cause of drug resistance in human cancers. Mucin 1 (MUC1) is a heterodimeric oncoprotein that is abnormally expressed in most human carcinomas. There is no known relationship between the MUC1 C-terminal subunit (MUC1-C) and these proteins. MUC1-C was targeted by a tetracycline-inducible shRNA or a pharmacologic peptide GO-203. In vitro and in vivo studies were conducted using our polymeric nanoparticles (NPs) for intracellular delivery of peptide cargos. Cells were selected for resistance to ABT-drugs. Targeting MCL-1 with MS-1/NPs inhibited the survival of cancer cells in vitro and in vivo, and was associated with upregulation of BCL2A1. MS-1/NPs had limited effects on ABT-resistant cells due to increased BCL2A1 expression. Targeting MUC1-C is associated with suppression of both MCL-1 and BCL2A1. MUC1-C (i) stabilizes MCL-1 by activating the MEK→ERK and PI3K→AKT pathways, and (ii) activates BCL2A1 transcription. GO-203/NPs suppressed survival of parental and ABT-resistant cells. These findings demonstrate that targeting MUC1-C with GO-203/NPs is a potential strategy for abrogating MCL-1- and BCL2A1-mediated resistance.

J-1022

FGFR inhibitor BGJ398 and HDAC inhibitor OBP-801 synergistically induce apoptosis in bladder cancer cells

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Recently, in advanced bladder cancer, a number of clinical trials using molecular-targeted agents have been conducted, and new therapies are expected that could replace conventional cytotoxic chemotherapy. We herein report that concurrent treatment with fibroblast growth factor receptor (FGFR) inhibitor BGJ398 and the novel histone deacetylase (HDAC) inhibitor OBP-801/YM753/spiruchostatin A synergistically inhibited cell growth and markedly induced apoptosis in high-grade bladder cancer cells. This combination activated caspase-3, -8 and -9, and the pan-caspase inhibitor significantly reduced the apoptotic response to the combined treatment. The combination upregulated the expression of Bim, one of the pro-apoptotic molecules. In the present study, Bim siRNA efficiently reduced apoptosis induced by the co-treatment of BGJ398 and OBP-801. Therefore, the apoptosis induced by the combination was shown to be at least partially dependent on Bim. Taken together, these results suggest that the combination of BGJ398 and OBP-801 is a novel high potential therapeutic strategy for muscle-invasive bladder cancer. (Co-presenters: Toshiya Takamura, Shusuke Yasuda, Seijiro Toriyama, Yuichi Aono)

▲TOP

J-1023

Development of a new therapeutic agent for photoimmunotherapy with small peptides as a targeting ligand

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Photoimmunotherapy (PIT) is a novel phototherapy for cancer using near-infrared (NIR) light and antibody-photosensitizer (Ab-IR700) conjugates. If small peptides can be used in PIT instead of the antibody, the pharmacokinetics should be improved, and the therapeutic agents will be synthesized in large quantities at a lower cost. In this study, we have synthesized a new agent consisting of IR700 and a bivalent cyclic RGD peptide targeting for integrin $\alpha_v\beta_3$, which is widely expressed in metastatic tumors. In in vitro experiments, cell damage was observed after NIR-PIT to an integrin $\alpha_v\beta_3$ positive cell line U-87 MG. In in vivo experiments, tumor-to-background ratio reached the maximum much earlier than the case of Ab-IR700 due to improved pharmacokinetics. Thus, in PIT with small peptides, treatment could be performed 1 hr after the injection. This is much better than PIT with Ab-IR700 in which treatment is performed after 24 hr. Moreover, we found that the treated tumors were disappeared about 10 days after the treatment, which was more efficient than PIT with Ab-IR700. We suggested that PIT with small peptides should be useful and efficient therapy, and contribute to stable supply.

J-1024

Near-infrared photoimmunotherapy (NIR-PIT) using wireless light-emitting diode system to treat tumors in deep tissue

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Near-infrared photoimmunotherapy (NIR-PIT) is a new cancer phototherapy based on NIR light and antibody-IR700 conjugates. A phase III trial on patients with inoperable head and neck cancer is currently ongoing. However, it is difficult to treat deep regions because of light absorption by tissues. Thus, we have developed a small implantable light-emitting diode (LED), whose power is supplied through electromagnetic induction from outside of the body to avoid implantation of batteries. Our wireless LED system consisted of a LED capsule including two LED sources (680-700 nm) and a receiver coil coupled with an external coil and power source. The light density was strong enough for NIR-PIT (40 mW/cm^2, @5 cm), and it was able to emit NIR light up to a distance of 20 cm from the power supply coil. In in vitro experiments, severe cell damage such as bleb formation was induced after NIR light irradiation. The tumor growth in LED-implanted mice was significantly suppressed, suggesting that tumors were successfully treated by NIR-PIT with the LED system. In conclusion, the developed wireless LED system was encouraged as a possible solution for NIR-PIT to treat deep lesions.

▲TOP

[LS8] LS8 [Japanese]
What to read, How to read.

2018 / 9 / 27 (Thu) 11:50-12:40 Room 10/11F 1101+1102, Osaka International Convention Center Room 10
：Springer Nature

Takashi Joh / Gamagori City Hospital

LS8

What to read, How to read.

Keiko Nakayama
Graduate School of Medicine, Tohoku University

No Abstract

[J-1079] J14-2 [Japanese]
Colorectal cancer: prognostic factor

2018 / 9 / 27 (Thu) 13:00-14:15 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Takashi Yao / Dept. Human Path., Juntendo Univ., Grad. Sch. Med.

J-1079

Prognostic impact of POLE mutation in Colorectal Cancer

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Recent genetic studies clarified a complete registry of gene mutations in colorectal cancer (CRC), whose clinical impact has not been fully investigated. We performed a large-scale mutation analysis of CRC, in which mutations in a panel of 128 common CRC drivers were interrogated in 544 CRC patients using targeted-capture sequencing and correlated with clinical outcomes. Of interest, 55 (10%) patients showed high mutational burden. Among these, 44 were shown to have microsatellite instability (MSI). The remaining 11 cases harbored mutation in the proof reading domain of POLE, a gene encoding DNA polymerase ε. We further interrogated POLE mutations in an additional 815 CRC patients using amplicon-based deep sequencing of exons 9-14 of the proof-reading domain of POLE. In total, we found 17 (1.3%) cases with POLE mutations among 1359 CRC cases. Mutations showed clear hotspots, V411L (29.4%) and P286R (24.5%). With a median follow-up of 4 years, POLE-mutated patients showed a very low frequency of tumor recurrence (n=1, 5.9%) and a significantly better prognosis (P=0.074). Our findings highlight a role of POLE mutation in the pathogenesis of CRCs with a favorable prognosis.

▲TOP

J-1080

HVEM Expression Contributes to Tumor Progression and Prognosis in Human Colorectal Cancer

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Background: Herpesvirus entry mediator (HVEM) has been recently suggested to play some roles in cancer biology. We examined HVEM expression in human colorectal cancer (CRC) to reveal its clinical importance. Materials and Methods: Immunohistochemical staining was done in normal epithelium, benign and malignant lesions. Results: While intense HVEM expression was not observed in normal epithelium and hyperplastic polyp, 24% of adenoma and more than half of CRC had high HVEM expression. In 234 CRC, HVEM expression was significantly associated with tumor status and pathological stage. The patients with high HVEM expression had a significantly poorer prognosis than those with low expression. Importantly, HVEM status had an independent prognostic value in CRC. Furthermore, HVEM status was inversely corrected with tumor-infiltrating T cells. Conclusion: HVEM may play a critical role in tumor progression and immune evasion, and also can be a novel prognostic marker and potential therapeutic target in human CRC.

J-1081

High expression of microRNA-10b is associated with poor prognosis and chemo-resistance to 5-FU in Colorectal Cancer

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Background: MiR-10b has been shown to be involved in progression of various kinds of cancers. We investigated the clinical significance of miR-10b and its involvement in chemo-resistance in colorectal cancer. Methods: We evaluated the clinicopathologic significance of miR-10b expression using 88 colorectal cancer tissue samples by quantitative RT-PCR. We also investigated the chemotherapeutic sensitivity to 5-fluorouracil (5-FU) in miR-10b-overexpressing colorectal cancer cells. Results: Clinicopathological analysis revealed that High miR-10b expression is significantly associated with higher incidence of lymphatic invasion (P=0.026) and poor prognosis (P=0.0057). Multivariate analysis indicated that high miR-10b expression is an independent prognostic factor for survival (P=0.0025). In vitro studies revealed that miR-10b directly inhibits the pro-apoptotic BH3-only BCL-2 family member BIM, and the overexpression of miR-10b confers chemo-resistance to 5-FU in colorectal cancer cells. Conclusions: MiR-10b is a meaningful prognostic biomarker and a potential indicator of chemo-resistance in colorectal cancer.

J-1082

Overexpression of GTF2IRD1 on chromosome 7 promotes cell cycle progression and poor prognosis in colorectal cancer

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Background: Chromosome 7q is clonally amplified in CRC, and novel driver genes are　located on the arm. Our aim is to disclose the clinical and biological significance of a candidate driver gene, general transcription factor 2I repeat domain-containing protein 1 (GTF2IRD1) on Ch.7q in CRC.Methods: We measured the expression of GTF2IRD1 in 98 CRC patients using immunohistochemistry and RT-qPCR, and assessed the clinicopathological and prognostic significance. We performed in vitro cell proliferation and cell cycle assay using siGTF2IRD1-transfected CRC cells. We further investigated the oncogenic mechanism how GTF2IRD1 promoted CRC progression.Results: GTF2IRD1 was overexpressed in tumor cells and its expression positively correlated with liver metastasis ($p<0.05$). On multivariate analysis, high GTF2IRD1 expression was an independent poor prognostic factor ($p<0.05$). GTF2IRD1 knockdown inhibited cell proliferation and induced cell cycle arrest. GTF2IRD1 downregulated TGF β R2 expression, a tumor suppressor gene in CRC.Conclusion: GTF2IRD1 promoted tumor aggressiveness and could be a novel prognostic biomarker in CRC.

▲TOP

J-1083

Tenascin C in colorectal cancer stroma is a predictive marker for liver metastasis and is a potent target of miR-198

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Tumor stroma plays important roles in CRC development. We sought to clarify the roles of miRNAs and their target genes in CRC stroma during liver metastasis (LM). Tumor stroma was isolated from FFPE tissues of primary CRC with or without LM, and miRNA expression was analyzed by miRNA array. Hierarchical clustering classified 16 CRCs into two groups according to the existence of synchronous LM. Tenascin C (TNC) was identified as a predicted target of miR-198, one of the top 10 miRNAs downregulated in tumor stroma of CRC with synchronous LM. Immunohistochemical analysis revealed that a high staining intensity (SI) correlated with synchronous LM (P <0.001). Multivariate analyses identified TNC-SI as an independent prognostic factor to predict postoperative overall survival (P=0.005; n=139) and liver metastasis-free survival (P=0.001; n=128). The relative protein expression of TNC was suppressed by treatment with miR-198 mimic and increased by miR-198 inhibitor in SW620 and CCD-18Co cells. Alterations of miRNAs in CRC stroma form a metastasis-permissive environment that can elevate TNC to promote LM. TNC in primary CRC stroma is a novel biomarker to predict postoperative prognosis.

J-1084

Budding-related gene expressions at the invasive front and tumor surface in colorectal cancer

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Background High-grade tumor budding has been reported as a poor prognostic factor in colorectal cancer(CRC). We previously identified 7 budding-related genes based on DNA microarray data obtained from surgically resected CRC specimens.Objective This study aimed to clarify intratumor heterogeneity of the expression of these 7 budding-related genes and its impact on predictive value.Methods In 121 CRC, total RNAs were prepared from frozen tissues at the invasive front and those at the tumor surface, respectively. We first performed RNA quality control test and 25 cases were excluded from this study. Based on the RNA expression levels of 7 budding-related genes, budding score was calculated using the sum of logarithm of each gene expression.Results The two budding scores were significantly correlated (P<0.001). Higher budding score at the invasive front of tumor was strongly associated with high-grade tumor budding (P<0.001). Similarly, budding score at the surface of tumor was associated with tumor budding status with a marginal significance (P=0.054).Conclusions Calculated score based on 7 budding-related genes has a correlation with tumor budding status regardless of sampling site.

▲TOP

[J-1085] J14-3 [Japanese]
Colorectal cancer

2018 / 9 / 27 (Thu) 14:15-15:30 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Ichiro Takemasa / Dept. Surg., Surg. Oncol. & Sci., Sapporo Med. Univ.

J-1085

Conversion surgery and mutational analysis for advanced colorectal cancer after systemic chemotherapy

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Multidisciplinary treatment is needed for advanced colorectal cancer (CRC). We herein investigated the gene mutational tracking for locally advanced CRC during serial treatment. Surgical resections were performed in 10 cases of advanced CRC following preoperative systemic chemotherapy. Tissues from the primary tumor, distant metastatic tumors, and blood plasma were obtained during serial treatment, and genome DNA was extracted. Comprehensive mutation analysis of 47 cancer-associated genes was performed using a pre-designed gene panel and next generation sequencing. All cases showed a partial response to systemic chemotherapy containing oxaliplatin, and pathologically radical resection was accomplished. In primary tumors, non-synonymous mutations were detected at between 1 and 8 sites before chemotherapy and at between 1 and 3 sites after. Driver mutations were precisely detected in blood plasma and metastatic tumors during serial treatment. Serial mutational analysis indicated that subclonal selection occurs during chemotherapy and that plasma can substitute for tumorous tissue in mutational analysis. These mutational analyses may useful for treatment decision in advanced CRC.

▲TOP

J-1086

Can ABCC11 protein expression in colon cancer predict the effect of chemotherapy?

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Aim: ABCC11 was involved in 5-FU resistance. We examined the relation ABCC11 expression with chemotherapy.Patients and method: One hundred thirty nine patients with colorectal cancer (CRC) were enrolled. The relation with ABCC11 expression and clinicopathological factors were analyzed.Results: The positive expression of ABCC11 was observed in 31 patients (22.3%). The cases with node positive and with venous invasion were significantly fewer in the cases with ABCC11 positive (p=0.0246, p=0.0285). In Stage 3 cases with adjuvant chemotherapy, DFS in cases with ABCC11 negative/positive were 80.0% and 76.0% (p=0.9364). OS in cases with ABCC11 negative/positive were 80% and 94.7% (p=0.2148). No relation between ABCC11 expression and the effect of adjuvant chemotherapy. In Stage 4, all three positive cases were obtained with CurB resection (p=0.0406), however, there were no significant differences in DFS and OS. In all the cases, OS in negative cases was 81.1% and significant poorer than in positive cases with 96.2% in OS (p=0.0248), but it was not significant by multivariate analysis.Conclusion: It is not expected to predict the effect of chemotherapy on CRC by the expression of ABCC11.

J-1087

Predictive markers of downstaging to ≤ypT1in rectal cancer patients with preoperative chemoradiotherapy

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Aim: Local excision for non-metastatic rectal cancer is reportedly feasible for selected patients who achieve an favorable response (depth≤ypT1) to neoadjuvant chemoradiotherapy (CRT). We previously reported that the density of CD8+ tumor-infiltrating lymphocytes (TILs), expression levels of CD133 and COX-2 could be useful predictive markers of tumor response to CRT. We aimed to evaluate the predictive power of these markers in terms of downstaging of the depth.Method: We prospectively recruited 49 patients who underwent CRT (45 Gy) followed by curative resection. Immunohistochemical staining for CD8, CD133, and COX-2 was performed on pretreatment biopsy specimens.Results: Increased number of TILs, negative expressions of CD133 and COX-2 were all demonstrated to be favorable predictive factors of CRT response associated with a higher incidence of ≤ypT1 (p=0.01, 0.23, 0.16). The incidence of ≤ypT1 was 48% in patients with 2 or 3 favorable factors; it was only 11% in patients with 0 or 1 factor (p=0.004).Conclusion: Comprehensive evaluation of the three markers could be a useful tool to predict successful downstaging in rectal cancer patients treated with neoadjuvant CRT.

J-1088

Area-specific prognostic values of E-cadherin and β-catenin in Stage Ⅱ colorectal cancer: a tissue-microarray approach

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BACKGROUND: Histological changes associated with epithelial-mesenchymal transition (EMT) at the tumor front are reportedly linked with the aggressiveness in colorectal cancer (CRC). We aimed to reveal the prognostic values of the status of EMT-related proteins, E-cadherin (Ecad) and β-catenin (Bcat). METHODS: We prepared area-specific tissue microarray (TMA) samples of tumor in 301 Stage Ⅱ CRC. Specifically, four core specimens prepared from paraffin-embedded sections at the submucosal invasive front (SM), the subserosal invasive front (SS), the central area (Ce), and the rolled edge (Ro) were constructed into a TMA block. Using these blocks, Ecad and Bcat expressions were evaluated immunohistochemically. RESULTS: Bcat nuclear expression in SM (Hazard ratio (HR) 3.4, P=0.029), SS (HR 4.5, P=0.019), decreased membranous expression of Ecad in SM (HR 2.3, P=0.032), SS (HR 3.0, P=0.0034), and Ro (HR 2.2, P=0.027) were associated with unfavorable relapse-free survival. Multivariate analysis revealed that Ecad expression status in SS (HR 2.7, P=0.008) was an independent factor. CONCLUSIONS: Area-specific evaluation of Ecad expression could heighten its prognostic value in Stage Ⅱ CRC.

▲TOP

J-1089

Sequential expression of epithelial-mesenchymal transition related genes in cancer epithelium and stroma

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Cancer tissues contain abundant amount of stromal tissues, which can dramatically alter characteristics of adjacent epithelial cells and may lead to cancer progression and metastasis. Although transcriptome profiles derived from bulk samples demonstrate a relative abundance of each gene, these gene expressions are originated from mixed populations consisting of epithelial and stromal cells. In the current study, using own developed independent gene expression profiles of cancer epithelium and stroma, we assessed the contribution of mesenchymal gene expressions to transcriptome of bulk samples. In this analysis process, we identified putative EMT regulators, which is highly expressed in cancer cells and even play important role when epithelial cells acquire mesenchymal properties. This analytical approach may help identifying novel EMT inducers, which are key regulators of caner progression.

▲TOP

[J-1090] J14-4 [Japanese]
Colorectal cancer: clinical

2018 / 9 / 27 (Thu) 15:30-16:45 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Masataka Ikeda / Div. Lower GI, Hyogo College of Med.

J-1090

Treatment strategy for intra-pelvic local recurrence of rectal cancer - Is it feasible?

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Once the intra-pelvic local recurrence (IPLR) occurs after rectal cancer surgery, its optimal treatment is always problematic. To clarify the significant prognostic parameters, retrospective chart review was performed. Localized IPLR was diagnosed in 33 patients. Primary tumors were located in the recto-sigmoid in 8, the upper rectum in 9, and the lower rectum in 16 patients. Surgical treatment for IPLR was performed in 24 patients (73%). External beam radiotherapy was given in 17 patients (52%), intra-operative electron beam radiotherapy in 3 patients, and chemotherapy in 26 patients (79%). The number of fixation sites (F-number) was significantly correlated with the grade of resection margin status (R-number, p=0.002). The median OS of the entire group was 41 months, with a 3 year-OS of 62.3%. Multivariate analysis revealed that patient gender and the type of initial operation were significantly and independently associated with post-IPLR median survival. These results suggested that patients' outcome was not determined by our treatment modalities, but by the factors at the initial presentation. Our current strategy should be reconsidered, and new modality should be introduced.

▲TOP

J-1091

Organ preservation of active surveillance with chemoradiotherapy for rectal cancer

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(Introduction)Organ preservation therapy for rectal cancer has been proposed in Nederland or Brazil to reduce morbidity or mortality for radical surgery. (Methods)Of 288 cases of rectal cancer were identified in our institution from 2010 to 2016. Of 47 cases with locally advanced rectal cancers were referred for neoadjuvant chemoradiotherapy (CRT)(52Gy + 5FU). All cases were reassessed by colonoscopy and diagnostic imaging with CT or MRI. An active surveillance program (watch and wait) was offered to patients of identification complete endoluminal response. Some cases with residual tumor or lymph node metastasis in reassessment were performed radical surgery. (Results)5 cases (12.8%) were achieved complete endoluminal response. 1 case with residual tumor was performed additional radiotherapy with request by patient. These 6 cases have been to watch and wait, after a median follow-up 26 months (range 6-48 months). 41 cases (87.2%) were performed radical surgery, 6 cases were identified with complete pathological response. (conclusion) Organ preservation of active surveillance with chemoradiotherapy for rectal cancer is feasible with achievement of endoliminal complete response.

J-1092

REVERCE: A Randomized Phase II trial of Regorafenib - Cetuximab for mCRC previously treated with chemotherapy

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Background: The study objective was to evaluate the efficacy and safety of the therapeutic sequence of regorafenib (R) followed by cetuximab (C), compared with C followed by R, as the current standard sequence for metastatic colorectal cancer (mCRC). Methods: Patients with KRAS exon 2 wild-type mCRC after failure of chemotherapy were randomized to R-C or C-R arm. The primary endpoint was overall survival (OS). Secondary endpoints included progression-free survival (PFS), safety, and quality of life (QOL). Serial biomarker analyses were exploratory analyzed. Results: One-hundred one patients were randomized to the study. Median OS for R-C and C-R was 17.4 and 11.6 months, respectively (p=0.0293), with a hazard ratio (HR) of 0.61 (95% CI, 0.39-0.96). Among the all wild-type patients for RAS, BRAF V600E, HER2 or MET at baseline, 3 patients in the R-C arm and 12 in the C-R arm showed at least 1 of these emerging genomic alterations, which were associated with shorter OS vs. patients without these alterations. Conclusions: The therapeutic sequence of R-C showed longer OS than the current standard sequence. Emerging oncogenic alterations are more frequently observed after C than R.

J-1093

Phase II study on starting with reduced dose of regorafenib for metastatic colorectal cancer after standard chemotherapy

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Background: Regorafenib is an oral multikinase inhibitor with a proven survival benefit for metastatic colorectal cancer patients. The standard dose of REG is 160mg/body/d, however, some cases require dose reduction to 120mg/d or less due to adverse events (AE). Dose modification due to AE was observed frequently in Japanese compared with non-Japanese (84.6% and 51.3%).Methods: This single arm, multicenter phase II study evaluated lower initial dose of REG (120mg/d, for 21 days, followed by 7-day break). Patients underwent radiographic evaluation every 8 wks. The primary endpoint was disease control rate (DCR: CR+PR+SD > 6 wks).Results: Total 60 patients were enrolled into the study. Median age was 68.5 (range: 30-84), and ECOG PS 0/1 were 70%/30%. DCR was 36.7% (22/60); 7% (4/60) have had SD for 6 months or longer. Median PFS was 2.5 months (95% CI: 1.9 - 3.7). 40% (24/60) had dose reduction to 80mg due to AE, and that dose reduction was needed in 10% (6/60) at the first cycle. Grade 3-4 AEs were observed in 52% (31/60).Conclusions: REG 120mg appears to have comparable efficacy to 160mg. AEs were generally consistent with the known safety profile of REG in this setting.

▲TOP

[J-1025] J8-1 [Japanese]
Cell death / immortalization / cell cycle

2018 / 9 / 27 (Thu) 9:00-10:15 Room 11/12F Conference Hall, Osaka International Convention Center Room 11

Hiroyuki Kugoh / Dept. Biomed. Sci., Ins. Regenerative Med., Tottori Univ.

J-1025

The identification of molecular mechanism underlying gastric cancer progression by senescent fibroblasts

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Secreted factors from cancer associated fibroblast (CAFs) is likely to be implicated in cancer progression, but the precise mechanism is not fully understood. It is reported that senescent cells survive and secrete some inflammatory cytokines, so called senescence-associated secretory phenotype (SASP). The purpose of current study is to identify the molecular mechanism underlying gastric cancer (GC) progression by CAFs showing SASP. Here we show that senescent state of CAFs, which was activated NFκB signaling by inflammatory cytokines, had abundant SASP factors. GC cell lines treated with SASP-CAFs derived condition medium (CM) increased growth ability. Moreover, we transplanted GC cell lines with intraperitoneal administration to generate peritoneal metastasis mouse model and evaluated the influence of SASP factors on cancer progression. SASP-CAFs derived CM remarkably enhanced intraperitoneal tumors. These findings suggest that SASP factors from senescent CAFs can promote peritoneal dissemination of GC cells.

▲TOP

J-1026

Development of a novel anticancer therapeutic strategies targeting maintenance of telomere

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There is a close relationship between telomere length and immortalization of cancer cells. During cell division, both ends of the telomere are not completely replicated and gradually shorten, resulting in DNA damage induction and cellular senescence. However, in many immortalized cancer cells, activated telomerase reverse transcriptase (hTERT) restores telomerase activity and stabilizes telomeres. Therefore, the maintenance of telomeres is an important therapeutic target for cancer treatments. Here, we have synthesized a variety of alkylating Pyrrole-Imidazole (PI) polyamides targeting telomere maintenance. We designed and synthesized PI polyamides conjugated with CBI targeting hTERT and PI polyamides conjugated with Chlorambucil targeting telomere repeat sequences. We will report recent progress of the antitumor effect targeting maintenance of the telomere by those synthetic drug candidates of the alkylating PI polyamide.

J-1027

HNRNPLL promotes cell cycle progression in colon cancer cells by stabilizing mRNAs for regulators of DNA replication

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We have reported in the previous annual meetings that HNRNPLL (heterogeneous nuclear ribonucleoprotein L-like) suppresses colorectal cancer metastasis by regulating the pre-mRNA alternative splicing. Here we demonstrate a functional role of HNRNPLL in colorectal cancer cell proliferation. Overexpression of HNRNPLL in colorectal cancer cells increased the percentages of the cells in S/G2/M phase. RNA sequencing analysis for colon cancer cells overexpressing or knocked down for HNRNPL indicated that 13 DNA replication-related genes were upregulated by HNRNPLL, among which PCNA, RFC3, and FEN1 showed an increase at protein levels. Knockdown of any of these genes alone suppressed the proliferation-promoting effect of HNRNPLL. RNA-immunoprecipitation assay presented a binding of FLAG-tagged HNRNPLL to mRNA of these genes, and HNRNPLL overexpression significantly suppressed the downregulation of these genes during 12 hours of treatment with the transcription inhibitor actinomycin D, suggesting a role of HNRNPLL in the mRNA stability. The link between HNRNPLL and colorectal cancer cell proliferation was further supported by immunohistochemical analysis of clinical samples.

J-1028

Autophagy controls centrosome number by degrading Cep63

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Centrosome number abnormalities are associated with chromosome mis-segregation and genomic instability. It can contribute to tumor development.The ubiquitin-proteasome system is considered to be the main regulator of centrosome number. However, here we show that autophagy also regulates the number of centrosomes. Autophagy-deficient cells carry extra centrosomes. The autophagic regulation of centrosome number is dependent on a centrosomal protein of 63 (Cep63) given that cells lacking autophagy contain multiple Cep63 dots that are engulfed and digested by autophagy in wild-type cells, and that the upregulation of Cep63 increases centrosome number. Cep63 is recruited to autophagosomes via interaction with p62, a molecule crucial for selective autophagy. In vivo, hematopoietic cells from autophagy-deficient and p62-/- mice also contained multiple centrosomes. These results indicate that autophagy controls centrosome number by degrading Cep63. Our results may provide new insight into a novel mechanism for tumorigenesis caused by a defect of autophagy.

▲TOP

J-1029

Functional characterization of EPN3 as a senescens inducer

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Cellular senescence is defined as permanent cell cycle arrest induced by various stresses, such as DNA damage. We have compared gene expression profiles of senescent cells by using microarray analysis and identified EPS-15-Interacting Protein3 (EPN3) to be upregulated in senescence. EPN3 is a member of EPN family that functions as an adaptor protein in the clathrin-mediated endocytosis of membrane receptors, yet its relationship to senescence has not been described. Therefore, we explored the EPN3 role in senescence. We observed that ectopic EPN3 expression induced senescence-associated β-galactosidase activation and p21 expression, well-known senescence markers, and reduced the proliferation capacity, indicating that EPN3 is a has a potential to induce senescence. Next, we investigated whether EPN3 enhances DNA damage, by immunostaining with 53BP1, a DNA damage marker. As a result, the extent of DNA damage was increased not only in EPN3-overexpressing cells but intriguingly also in the surrounding cells that did not express ectopic EPN3. These results suggest that EPN3 induces senescence and affects neighboring cells through the enhancement of DNA damage.

J-1030

Modest static pressure can suppress the growth of columnar adenocarcinoma cells

Man Hagiyama
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Dept. Pathol., Fac. Med., Kindai Univ.

Intraluminal pressure elevation can be pathogenic to mucosal epithelia even at a few tens cmH_2O, but its effect on cancer cell growth has not been examined intensively. A two-chamber culture system was modified to make cells receive water pressure of up to 60 cmH_2O. Adenocarcinoma cells with columnar epithelial morphology (NCI-H441 and Caco2) were growth-suppressed in a manner dependent on water pressure ranging from 2-50 cmH_2O without cell cycle arrest, whereas spherical adenocarcinoma cells were not growth-suppressed even at 50 cmH_2O. Phalloidin staining of the former cells revealed that a pressure load of 50 cmH_2O vertically flattened and laterally widened columnar epithelial cells and made actin fiber distribution sparse, without affecting total phalloidin intensity per cell. In addition, 50 cmH_2O pressure load enhanced serine-127 phosphorylation and cytoplasmic retention of YAP, the major constituent of the Hippo signaling pathway, suggesting that Hippo pathway was involved in the pressure-induced cell growth suppression. These results suggest that the method to elevate the intra-glandular pressure could be a new therapeutic strategy for well-differentiated adenocarcinoma.

▲TOP

[J-1031] J10-1 [Japanese]
Angiogenesis

2018 / 9 / 27 (Thu) 10:15-11:30 Room 11/12F Conference Hall, Osaka International Convention Center Room 11

Hiroyuki Konno / Hamamatsu Univ. Sch. of Med.

J-1031

Trastuzumab resistance accompanies vasculogenic mimicry in HER2-positive breast cancer cells

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Co-author：Shinzaburo Noguchi
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Resistance to trastuzumab (Tzm) is a major obstacle in controlling the progression of HER2+ breast cancer (BC). We hypothesized that Tzm load might induce a phenotypic change in HER2+ BC cells, enabling them to escape and survive Tzm activity. We conducted comprehensive immunophenotyping to detect the phenotypic changes in HER2+ BC cells loaded with Tzm, and compared the immunophenotype of Tzm-loaded cells with that of control cells. Out of 242 cell surface antigens, 9 antigens were significantly upregulated and 3 were significantly downregulated. Surprisingly, all the antigens were related to endothelial and stem cell phenotypes, suggesting that Tzm load induced vasculogenic mimicry (VM). Then, using three Tzm-resistant (R) cell lines, we showed that all Tzm-R cell lines exhibited tube formation on Matrigel. Eribulin inhibited tube formation in Tzm-R cells at a clinically relevant concentration. In conclusion, Tzm load induces an incomplete vasculogenic phenotype in HER2+ BC cells. The cells exhibit VM after eventually acquiring Tzm resistance. Since VM drives metastasis, its regulation in Tzm-R HER2+ BC appears to be a promising approach for this devastating disease.

▲TOP

J-1032

Down Syndrome Critical Region (DSCR)-1 in endothelial cells controls tumor angiogenesis and pulmonary tumor metastasis

Masashi Muramatsu
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Co-author：Takashi Minami
Div. Mol. Vas Biol, IRDA, Kumamoto Univ.

VEGF signaling plays a crucial role in the regulation of vascular integrity, which performed an ingenious manner under the physiological and pathological conditions including cancer. We previously reported that Down syndrome critical region (Dscr)-1 is the most highly induced gene in VEGF-treated activated endothelial cells (ECs), and plays as a feedback regulator for calcineurin-NFAT signaling axis contribute to suppress endothelial cell activation and tumor growth. Here, to further elucidate the vessel regulation by the VEGF-NFAT/DSCR-1 pathways in vivo, we performed phenotypic analysis of newly generated EC-specific DSCR-1 transgenic (DSCR-1ECTg) mice. DSCR-1ECTg mice exhibited embryonic lethality before day E9.5 due to an aberrant vessel formation and dysfunctional branch malformation. To examine whether DSCR-1 in endothelium affects tumor growth, tumor angiogenesis and metastasis, we used a tumor-bearing model with DSCR-1ECTg or DSCR-1 null (DSCR-1-/-) mice. Tumor volume and angiogenesis were dramatically reduced in DSCR-1-/- while increased in DSCR-1-/- mice. Collectively, NFAT-DSCR-1 axis plays a crucial role for tumor angiogenesis and pulmonary metastasis.

J-1033

Establishment of an in vivo model to observe patient-derived tumor blood vessels with intravital microscopy

Yohei Tsukada
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[1]Dept. Signal Transduction, RIMD., Osaka Univ.、[2]Dept. Signal Transduction, Res. Int. Microbial Disease, Osaka Univ.

BACKGROUND: Drugs that target tumor blood vessels have been developed for cancer therapeutics. However, the anti-tumor effects in the clinic are lower than that of those expected in animal tumor models. So far, in vivo effects have been studied in animal tumor blood vessels; then there is no standardized in vivo model that can evaluate the effect on the human tumor blood vessels. In this study, we aim to establish a patient-derived xenograft (PDX) model to observe human tumor blood vessels. METHODS: Primary colon tumor was dissected into pieces and then subsequently implanted into NOD/scid mice. To observe human tumor blood vessels with intravital microscopy, Alexa Fluor 488-conjugated anti-human CD31 antibody was administered intravenously. RESULTS: Human tumor blood vessels could be visualized at multiple time points. By the observation in the same field, the length of perfused human tumor blood vessels increased. CONCLUSIONS: We have shown that patient-derived human tumor blood vessels can be observed at multiple time points in vivo. These results showed that the current model could be a useful tool to evaluate the effect of drugs that target human tumor blood vessels in vivo.

J-1034

NDRG1 promotes tumor angiogenesis and metastasis by activation of VEGFR2/PLC γ /ERK signaling in vascular endothelial cell

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[Purpose] N-myc downstream regulated gene 1 (NDRG1) has been known to play pleiotropic roles in embryogenesis and cell differentiation. Our previous studies demonstrated that NDRG1 in cancer cells could modulate tumor angiogenesis. Recently we have reported that NDRG1 promoted tumor angiogenesis through induction of differentiation of macrophages to angiogenic macrophages. In our present study, we asked whether and how NDRG1 in vascular endothelial cells (ECs) could play any pivotal role in tumor angiogenesis, especially VEGF-induced angiogenesis.
[Results] In NDRG1 deficient mice as compared to their wild type counterparts, [1] tumor growth and angiogenesis in syngeneic tumors were suppressed; [2] exogenous administration of VEGF could not induce angiogenesis whereas FGF-2 could induce angiogenesis by both corneal micropocket and aorta ring assays; [3] VEGF could not induce ERK and PLC γ 1 phosphorylation without affecting expression and function of VEGFR2 by ECs.
[Conclusion] We first present a novel finding that NDRG1 in ECs contributes to tumor angiogenesis in response to VEGF. [Collaborator: Ai Shinoda, Shigeo Yoshida, Takahito Nakama, Kinuko Sasada (Kyushu Univ.)]

▲TOP

J-1035

Function of PLOD2 signaling to modulate invasion and metastasis of oral cancer

Ken Saito
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The family of 2-OG (2-oxoglutarate)-dependent dioxygenase enzymes catalyze the hydroxylation of targets including the transcription factor, histone and nucleotide etc. Its activities participate in the regulation of cell-cycle, epigenetics and migration in cancer cells. Here we report the critical role of PLOD2, a member of procollagen lysyl hydroxylase family molecules, in invasion of oropharyngeal squamous cell carcinomas (SCCs). PLOD2 was found to be highly expressed at endoplasmic reticulum of oropharyngeal SCC cells, in contrast to non-neoplastic keratinocytes. Besides, PLOD2 facilitated SCC cell migration, while its blockage with RNAi decreased cell mobility. Diminution of PLOD2 expression caused downregulation of integrin beta-1 and attenuation of cellular response to IL-6. Analysis of oral SCC tissues from the patients revealed PLOD2 concordant expression with integrin beta-1 at the invasive front of tumor nests. Thus, our results would provide a first insight into the molecular basis of cellular migration via PLOD2 in oral cancer.

J-1036

Concomitant deletion of PAR-2 abrogated the increased tumor formation in HAI-1 deficient ApcMin+ mice

Makiko Kawaguchi
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Co-author：Koji Yamamoto、Tsuyoshi Fukushima、Hiroaki Kataoka
Dept. Pathol., Facul. or Med., Univ. of Miyazaki

Hepatocyte growth factor activator inhibitor type 1 (HAI-1) is a membrane-bound serine protease inhibitor expressed on epithelial and carcinoma cell surface. We have previously reported that Hai-1-deficient ApcMin/+ mice showed significantly accelerated tumor formation in the intestine. We also found that NF-κB signaling was activated in Hai-1-deficient ApcMin/+ mice tumors and inhibition of NF-κB significantly reduced tumor formation of Hai-1-deficient ApcMin/+ mice. Recent studies have revealed that protease-activated receptor (PAR)-2 activation induces NF-κB activity. This study aimed to examine whether excess activation of Par-2 is involved in the Hai-1 loss-induced increased tumor formation in Hai-1-deficient ApcMin+ mice. We observed that knockout of Par-2 alleviated the activation of NF-κB and enhanced formation of intestinal tumors caused by Hai-1 deletion. These results suggested that HAI-1 loss-induced tumor susceptibility may be mediated by activation of NF-kB signaling through activation of Par-2.

▲TOP

[SP02-Keynote1] SP2 [Japanese]
What is the optimal social insurance system for sustainable best practice in cancer care?

2018 / 9 / 27 (Thu) 15:00-18:00 Room 11/12F Conference Hall, Osaka International Convention Center Room 11

Hitoshi Nakagama / Natl. Cancer Ctr.、Masaki Mori / Dept. Gastroenterological Surg., Osaka Univ.

日本の人口は減少傾向にあるが，人口に占める高齢者の割合は増加を続けており，当面がん患者の減少の兆しは見られない。ヒトゲノムの 解明，新薬，新規治療法の開発により，がんの治療成績は着実に向上している。しかし，その一方でこれらの実用化に伴い，がん医療のための医療費も着実に増 加している。
今後 持続可能ながん医療を支えていくためには，最善のがん医療と医療費制度について様々な立場から意見を交換し，合意を形成していく必要がある。
本 特別企画では，近年 代表的ながんの医療費がどの様に変わってきたかをみながら，アカデミア，医療提供者，行政，企業，患者など様々な立場からこれからがん医療の方向性について 検討する。行われた議論の結果，今後の国民的な合意形成のための礎となることを期待したい。

SP02-Keynote1
[Keynote]

Hitoshi Nakagama
Natl. Cancer Ctr.

No Abstract

▲TOP

SP02-Keynote2

[Keynote]

Yuko Kitagawa
Dept. Surg. Keio Univ. Hosp.

No Abstract

SP02-Keynote3

[Keynote]

Hironobu Minami
Med. Oncol.

No Abstract

SP02-Debater1

Debater

Hitoshi Nakagama
Natl. Cancer Ctr.

No Abstract

▲TOP

SP02-Debater2

Debater

Yuko Kitagawa
Dept. Surg. Keio Univ. Hosp.

No Abstract

SP02-Debater3

Debater

Hironobu Minami
Med. Oncol.

No Abstract

SP02-Debater4

Debater

Kenji Matsubara
Japan Med. Association

No Abstract

▲TOP

SP02-Debater5

Debater

Morito Monden
The Japan Med. Sci. Federation

No Abstract

SP02-Debater6

Debater

Motomichi Kamohara
Ministry of Health, Labour & Welfare

No Abstract

SP02-Debater7

Debater

Joji Nakayama
Japan Pharm. Manufactures Association

No Abstract

SP02-Debater8

Debater

Shinsuke Amano
Japan Federation of Cancer Patient Groups

No Abstract

SP02-Debater9

Debater

Keizo Sugimachi
Onga Hosp.

No Abstract

SP02-Debater10

Debater

Nobuaki Suzuki
Med. News Dept., Editorial Bureau, The Yomiuri Shinbun

No Abstract

▲TOP

SP02-Debater11

Debater

Haruno Horike
News Commentators Bureau

No Abstract

SP02-Debater12

Debater

Toshiharu Yamaguchi
Cancer Inst. Hosp., JFCR

No Abstract

SP02-Debater13

Debater

Tetsuo Noda
Cancer Inst. of JFCR

No Abstract

▲TOP

SP02-Special_Remarks

Special Remarks

Toshiharu Yamaguchi
Cancer Inst. Hosp., JFCR

No Abstract

▲TOP

[J-1037] J17-1 [Japanese]
Anticancer drug and cell death

2018 / 9 / 27 (Thu) 9:00-10:15 Room 12/12F 1202, Osaka International Convention Center Room 12

Masaya Imoto / Fac. Sci. Tech. Keio Univ.

J-1037

Anti-cancer effects of staurosporine against human malignant pleural mesothelioma cells

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Malignant pleural mesothelioma (MPM) is a highly refractory cancer arising from the pleural cavity. It is difficult to detect MPM during the early stages, because there are no effective diagnostic markers or radiological methods such as chest X-rays or computed tomography scan. Furthermore, MPM is highly resistance to conventional therapies, so that the prognosis is very poor. The combination of cisplatin and pemetrexed is considered the first-line chemotherapy for MPM. However, there are no effective anti-cancer drugs when MPM becomes resistant to these chemotherapy. Recently, it was reported that staurosporine (STS) is effective against cancer stem cells of MPM cells.
In this study, we examined the effects of STS against some MPM cell lines and found that the sensitivity for STS is different depending on cell lines. In addition, expression levels of some apoptosis-related molecules including activated caspase-3 differed among STS-treated MPM cell lines. Collectively, our findings suggest that staurosporine is effective for MPM cells but resistant cell lines are present.

▲TOP

J-1038

SIRT2 is involved in mitotic cell death by blocking P/CAF-MDM2-p53-p21 axis through interacting with P/CAF

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Sch. of Life Sci., Fac. of Med. Tottori Univ.

SIRT2 is a deacetylase that regulate mitosis and maintain genome integrity as a tumor suppressor. We previously reported that SIRT2 suppression restrained cell death during prolonged mitotic arrest provoked by nocodazole, a microtubule inhibitor in HCT116(p53-/-) cells, whereas stronger resistance was observed in HCT116(p53+/+) cells with increase of p53 and p21 without significant G1 or G2 arrest. These insights indicated that p53 might mediate the depression of mitotic cell death in cells with SIRT2 suppression. Herein we investigated how SIRT2 regulates p53 level in mitotic cell death. We found that SIRT2 suppression leads to the increase of acetylation and accumulation of P/CAF, a histone acetyltransferase, which also acts as an ubiquitin ligase against MDM2, and the decrease of MDM2. We found that the physiological interaction between P/CAF and SIRT2 rather than deacetylase activity of SIRT2 regulates these alterations. Depression of mitotic cell death with SIRT2 suppression was released by suppression of P/CAF or p21. Collectively, the present study indicated that P/CAF-MDM2-p53-p21 axis leads to depression of mitotic cell death through interaction of SIRT2 and P/CAF.

J-1039

Dual inhibition of Mcl-1 and Bcl-2 could be a safe and effective way to induce apoptosis in some cancer cells

Ryuji Yamaguchi
Kansai Med. Univ. Anesthesiology

HeLa cells express Mcl-1, Bcl-2, Bcl-xL and Bcl-w. S63845 inhibits Mcl-1, while ABT-263 inhibits all Bcl-2 relatives. Thus, the combination of S63845 with ABT-263 could free Bak from all its inhibitory associations, activating Bak, and inducing apoptosis. However, the loss of Bcl-xL from platelets and lymphocytes causes thrombopenia and lymphopenia, limiting the clinical use of ABT-263. We tested S63845 and Bcl-2 specific inhibitor, ABT-199 on Hela cells. Under single agent treatments (both at 100 nM), cells grew better than untreated, while the combination treatment killed over 90% of the cells overnight in a caspase-dependent manner, showing that the dual inhibition of Mcl-1 and Bcl-2 is enough to induce apoptosis in HeLa cells. However, quiescent, untransformed HUVEC were sensitive to S63845, suggesting limited clinical use for the agent. Because in our previous work, 2-Deoxyglucose (2DG) and b-Cyclodextrin (bCD) combination caused dissociation of Mcl-1 from Bak only in cells with elevated glucose uptake, we replaced S63845 with 2DG-bCD. The triple combination induced apoptosis in HeLa cells and not in quiescent HUVEC cells, suggesting the combination is better for clinical use.

J-1040

Auranofin exhibits preferential cytotoxicity under nutrient-deprived conditions in human pancreatic cancer cells

Takefumi Onodera
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Cancer tissues are frequently exposed to hypoxia and nutrient-deprived conditions. We screened small molecular cytotoxic agents which preferentially decreased the survival of cancer cells under nutrient-deprived conditions. Auranofin (AF), an organic gold compound used in clinical treatment of rheumatoid arthritis, is known to inhibit thioredoxin reductase (TrxR) in the thioredoxin (Trx) system. Our previous studies in nutrient-deprived conditions showed that AF significantly suppressed TrxR activity, increased the level of intracellular reactive oxygen species (ROS) and promoted apoptosis. We have analyzed whether the suppression of TrxRs is involved in cell survival. The shTrxRs knockdown of PANC-1 cells reduced tolerance to nutrient-deprived conditions. It suggests that TrxRs play a crucial role in the survival of cancer cells. Based on the accumulation of ROS by dysfunction of Trx system, we predicted that targeting the related redox pathway in combination with AF and cisplatin could be a promising strategy. As a result, this combination has displayed a highly effectiveness. Our findings suggest that the Trx system in cancer cells is an important target for anticancer drugs.

▲TOP

J-1041

Mechanism of action of novel anticancer agents derived from naturally occurring fatty acid

Saeko Ando
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Co-author：Katsumi Fukamachi、Harutoshi Matsumoto、Masumi Suzui
Dept. Mol. Toxicol., Nagoya City Univ. Grad. Sch. Med.

We earlier discovered one candidate compound, palmitoyl piperidinopiperidine, named PPI (patented in 2014). We then found that PPI exerts growth inhibition of human colon carcinoma cells by inhibiting transcriptional activity of STAT3. In a recent study, PPI's growth inhibition of carcinoma cells was canceled by treatment of these cells with PPI plus STAT3 specific inhibitor. QSAR analysis demonstrated that nucleophilicity of nitrogen atom of the piperidine structure plays an important role in tumor specificity of the drug. Therefore, we designed and synthesized a new next generation compound, named DPH (patent pending in 2015). In silico docking calculation with DPH the by Discovery Studio 2017R2 computer program resulted in the higher docking score than that of PPI and exhibited that DPH can bind to SH2 domain of STAT3. DPH also inhibited the growth of HT29 human colon carcinoma cells with IC_{50} values of 0.08 to 0.1 μM, and caused a dose dependent decrease in expression levels of pSTAT3 in these cells, indicating the DPH's anticancer effects similar to those of PPI.

J-1042

Aurora kinase blockade enhances sensitivity of cancer cells to EGFR inhibitors via de novo addiction to oncogene

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The major obstacles in molecular target drug-based cancer chemotherapy are attributed to acquired resistance (i.e. secondary mutations and activation of alternative signal pathways) and to limited patients who harbor target gene alterations. To overcome these obstacles of the treatment, we present the novel concept based on de novo addiction to oncogene (Dead-On). In this study, we found the combination of aurora A/B- and EGFR-inhibitor synergistically induced cell death in cervical, esophageal, and head and neck cancer, but not normal cells. Both aurora A/B inhibitor and the gene knockdown activated EGFR phosphorylation and its downstream pathways. This type of Dead-On was triggered by the accumulation of EGFR in the cell membrane via promoting endocytic recycling. In a mouse xenograft model, the combination treatment of aurora A/B- and EGFR-inhibitor more effectively induced apoptosis and suppressed tumor growth than the single treatment. Taken together, dual inhibition of aurora A and B can induce addiction of cancer cells to the oncogenic EGFR pathway, thereby increase the sensitivity to EGFR inhibitors. This concept may be new sword to combat cancer in future.

▲TOP

[J-1043] J17-2 [Japanese]
Anticancer drug resistance

2018 / 9 / 27 (Thu) 10:15-11:30 Room 12/12F 1202, Osaka International Convention Center Room 12

Toshiyuki Sakai / Dept. Mol. -Target. Cancer Prev., Kyoto Pref. Univ. Med.

J-1043

Examination of association between mitochondrial copy number and resistance to chemotherapy in esophageal cancer

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Co-author：Makoto Yamasaki、Tomoki Makino、Yasunori Masuike、Yasuhiro Miyazaki、Tsuyoshi Takahashi、Yukinori Kurokawa、Kiyokazu Nakajima、Masaki Mori、Yuichiro Doki
Dept. Gastroenterological Surg., Osaka Univ.

Introduction: We have reported that mitochondrial DNA (mtDNA) copy number was decreased in esophageal squamous cell carcinoma (ESCC). In this study, we investigated the relationship between mtDNA copy number and the effect of chemotherapy. Methods: mtDNA copy number of surgically resected primary tumors from ESCC patients was measured by qPCR for Cytochrome Oxidase I. Human ESCC cell (TE8 and TE11) with decreased mtDNA copy number were established by knockdown of mitochondrial transcription factor A. The response of chemotherapy was accessed by cell viability assay and apoptosis assay. Results: In human sample, lower mtDNA copy number was correlated with pathological response of chemotherapy. Viability assay and apoptosis assay showed that mtDNA depleted TE8 and TE11 were more resistant to chemotherapy. Invasion assay and wound healing migration assay showed that mtDNA depleted TE8 and TE11 were more invaded and migrated than the control-sh cellsConclusions: These results suggest that low mitochondrial copy number is associated with resistance to chemotherapy in esophageal cancer.

▲TOP

J-1044

The association of chemoresistance and p22phox/HIF-1α pathway in EGFR-TKI resistant lung adenocarcinoma

Masayuki Kobayashi
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Co-author：Ryoko Saito[1]、Yasuhiro Miki[2]、Hironobu Sasano[3]
[1]Dept. Pathol., Tohoku Univ., Grad. Sch. Med.、[2]Dept. Pathol., Tohoku Univ., Grad. Sch. Med., Disaster Ob

Chemotherapy is gold standard for the patients with epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) resistant lung adenocarcinoma (LADC). Therefore, administration of effective chemotherapy is an enormous challenge toward the successful therapeutic outcome of these patients. This study aimed to explore the mechanisms underlying chemosensitivity and EGFR-TKI resistance. Studies of microarray analysis in erlotinib resistant PC9 cell line (PC9-ER) which we established revealed that $p22^{phox}$/hypoxia inducible factor-1α (HIF-1α) pathway resulted in chemoresistance and EGFR-TKI resistance via an induction of epithelial-mesenchymal transition (EMT). Results of our study also showed that $p22^{phox}$ and HIF-1α expressions were elevated in PC9-ER harboring EMT and chemoresistance. Further, the knockdown of $p22^{phox}$ and HIF-1α by siRNAs enhanced chemosensitivity while HIF-1α induced EMT and chemoresistance in PC9-ER. We finally evaluated the correlation of clinicopathological factors and the status of $p22^{phox}$. We provided the first evidence that targeting $p22^{phox}$/HIF-1α should represent new therapeutic strategy to enhance the chemosensitivity in EGFR-TKIs resistant LADC.

J-1045

Suppression of lysosomal enzyme improves chemoresistance in pancreatic cancer cells

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[Background] Autophagy plays an important role in homeostasis, chemoresistance and tumor malignancy. Suppression of autophagy is expected to be a new strategy for cancer. Autophagy depends on hydrolysis by lysosome enzymes, so down-regulation of lysosomal enzyme suppressed autophagy. Therefore, we knock down the gene of acid-α glucosidase (GAA), one of the lysosome enzyme to analyze the changes in anti-tumor effect of gemcitabine. [Methods] The autophagy levels and the enzyme activity of GAA were assessed using gemcitabine-resistance pancreatic cancer cell lines (PANC-1, MIA PaCa-2). The apoptosis signals and cell viabilities were also assessed in condition of GAA knock down by siRNA method.[Result] The expression of autophagy-related protein levels (LC3, LAMP2) and enzyme activity of GAA were elevated by gemcitabine ($p<0.05$). In addition, knockdown of GAA enhanced the induction of apoptosis and expression levels of apoptosis signals. Consequently, the cell viabilities were decreased by siGAA (control vs. siGAA+gemcitabine, 88.15 ± 7.65 % of control, $p<0.01$).[Conclusion] Down-regulation of GAA expression enhances the anti-tumor effect of gemcitabine in pancreatic cancer.

J-1046

Anti-proliferative effect of fatty-acid derivative AIC-47 in Ph-positive leukemia with imatinib-resistant mutation

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Therapy based on targeted inhibition of BCR-ABL tyrosine kinase has greatly improved the prognosis for Ph-positive leukemia and tyrosine kinase inhibitors (TKIs) have become the standard therapy. However, some patients acquire resistance to TKIs which is frequently associated with point mutations of BCR-ABL. We previous reported that a medium-chain fatty-acid derivative AIC-47 induced transcriptional suppression of BCR-ABL, leading to autophagic cell death in CML cells. In this study, we investigated the anti-leukemic effects of AIC-47 in BCR-ABL-mutated (M351T, Y253F and T315I) cells. AIC-47 exhibited growth inhibition in either wild type (WT)- or mutated-BCR-ABL-harboring cells. AIC-47 also suppressed transcription of BCR-ABL with mutation. Moreover, AIC-47 induced switching the expression profile of pyruvate kinase muscle (PKM) isoforms from PKM2 to PKM1, suggesting that AIC-47 perturbed the Warburg effect. In leukemic mice model, AIC-47 extremely suppressed the increase in BCR-ABL mRNA and hepatosplenomegaly regardless of the BCR-ABL mutation. These findings suggest that AIC-47 is promising agent for overcoming resistance of Ph-positive leukemia.

▲TOP

J-1047

A role of PTBP1 in cancer specific energy metabolism and the chemoresistance

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The Warburg effect is the cancer specific energy metabolism system that use glycolysis mainly even in aerobic conditions. Pyruvate kinase muscle (PKM) is a rate-limiting enzyme in glycolysis. PKM has two splicing variants; PKM1 and PKM2. These expression is regulated via alternative splicing by PKM splicers, PTBP1 and SRSF3. We found that the PKM1/2 ratio was lower in tumor cells than normal cells and commonly higher in chemoresistant (CMR) cancer cells than parent cells. Metabolome analysis that showed CMR cells were increased the oxidative phosphorylation (OXPHOS) with TCA cycle compared with parent cells. These data indicated that CMR cells are stable even in partially shifted from glycolysis to OXPHOS. Furthermore, we found that CMR cells had more active type pf PKM1 tetramers than parent cells. To clarify the functions of PKM1 in CMR cancer cells, we established PKM1 over-expression cells by silencing PTBP1 for a long term. Such cells showed CMR at least in part through the induction of ABCB1 and Bcl-2 over-expression. These findings suggested that PTBP1 plays a central role in cancer specific energy metabolism and also contribute to CMR probably through modulating OXPHOS.

J-1048

Extracellular vesicles derived from cancer associated fibroblasts induce drug resistance of gastric cancer cells

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ackground: Cancer associated fibroblasts (CAFs) have been reported to enhance drug resistance, but the crucial mechanism is not determined. The aim of this study is to identify the molecular mechanism underlying anticancer drug resistance mediated by CAFs in gastric cancer (GC). Methods: We have isolated CAFs from surgically resected GC tissues and collected conditioned medium (CM) of CAFs. GC cell lines cultured in normal medium or CAF-CM were examined the drug sensitivity with extracellular matrix (ECM). We isolated extracellular vesicles (EVs) from CAF-CM by ultracentrifugal separation. We performed mass analysis of GC-EVs and CAF-EVs.Results: We found that GC cells with CAF-CM on ECM coat plates showed unique network formation and much higher drug resistance than those without CAF-CM. We next focused on CAF-EVs, and mass analysis revealed that CAF-EVs contained high amount of AnnexinA6, which did not exist in GC-EVs. Further functional analysis demonstrated that AnnexinA6 has an important role on network formation and drug resistance.Conclusion: The findings in current study suggest that AnnexinA6 from CAF-EVs enhance network formation and drug resistance of GC cells.

▲TOP

[LS9] LS9 [Japanese]
Surgical nutrition invasion study aimed at improving prognosis

2018 / 9 / 27 (Thu) 11:50-12:40 Room 12/12F 1202, Osaka International Convention Center Room 12
：Otsuka Pharmaceutical Factory , Inc.

Eigo Otsuji / University Hospital Kyoto Prefectual University of Medicine , Department of Surgery , Division of Digestive Surgery

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LS9
Surgical nutrition invasion study aimed at improving prognosis

Yuichiro Doki
Osaka University , Graduate School of Medicine , Department of Gastroenterological Surgery

No Abstract

[J-1094] J11-2 [Japanese]
Metabolism / metabolome (2)

2018 / 9 / 27 (Thu) 13:00-14:15 Room 12/12F 1202, Osaka International Convention Center Room 12

Tetsuo Morita / Dept. Biochem. Grad. Sch., Fukuyama Univ.

J-1094

PDK2 inhibition has synergic effect with cisplatin targeting mitochondrial metabolism in ovarian clear cell carcinoma

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<Objective > Cancer metabolism is an attractive strategy to overcome chemo-resistance in cancers. The aim of this study is to identify metabolic targets induced by genomic alterations which render chemo-resistance in ovarian clear cell carcinoma(OCCC).<Methods> 39 clinical samples and 13 cell lines of OCCC were analyzed by whole exome sequencing and gene expression microarray. IHC, cell viability assay, Mito stress test and in-vivo experiments were conducted.<Results > Chromosome (Chr) 17q21-24 was significantly amplified in recurrent cases. Cell viability assay revealed chr17q21-24 amplification is correlated with resistance to cisplatin. Inhibitors targeting PI3K, MDM2, or MYC reduced PDK2 expression, which is located in chr17q23. IHC showed high PDK2 expression was associated with poor prognosis. Knock down of PDK2 increased cisplatin sensitivity by activation of oxidative phosphorylation and mitochondrial ROS production. Suppression of PDK2 synergically inhibited tumor growth with cisplatin in vivo.<Conclusion > High PDK2 expression rendered platinum-resistance in OCCC. Targeting metabolism such as PDK2 is a new therapeutic strategy against OCCC.

▲TOP

J-1095

Profiling of metabolic changes in EVs from breast cancer cells stimulated by interferon- γ

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Cancer cell-derived extracellular vesicles (EVs) suppress functions of immune cells by transferring EV components. Recently some reports have shown that EVs contain metabolites. However the functions of metabolites in EVs remain largely unknown. Indoleamine-2,3-dioxygenase1 (IDO1) is a tryptophan (Trp) catabolic enzyme which is induced by cytokines such as interferon (IFN)- γ and permits cancer cells to escape the immune system. Therefore, we aim to identify IDO1-induced metabolites that are associated with immunosuppressive functions in breast cancer cell-derived EVs. The breast cancer cell line, MDA-MB-231-luc-D3H2LN (D3H2LN) was cultured in the presence or absence of IFN- γ to regulate IDO1 expression. Metabolome analysis of cell and EVs were performed on D3H2LN treated with or without IFN- γ , using CE-TOFMS and IC/LC-QE. The results showed IFN- γ treated cell-derived EVs contain higher amount of uracil, uridine, adenosine, and guanosine than IFN- γ non-treated cell. We investigated the metabolome profile of EV from IDO1 knockout cells generated by CRISPR-Cas9 and their immunoregulatory roles by functional analysis.

J-1096

Pterostilbene sensitizes osteosarcoma cells to killing by cMYC inhibitors

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[Objective]It has been reported that cancer stem cells(CSC) metabolize predominantly oxidative metabolism (OXPHOS). We report anti osteosarcoma stem cell action of Pterostilbene (PTE) through inhibition of mitochondrial enzyme F0 -F1 ATP synthase(comp.5). [Materials & Methods]Using SaOS2, U2OS and MG63 cells, cell survival was assessed with or without PTE , and the expression of stem cell markers of Oct3, NS, CD44 was examined. Next, the amount of ATP and comp.5activity was measured. We detected the synergistic effect between JQ1 and Honokiol, a cMYC inhibitors. [Results]PTE treatment reduced viabilities of all cells in dose dependent manner . Expression of stem cell marker was also decrease . The activity of comp.5was reduced, and ATP synthesis was also decreased in spheroid cultured condition. Suggesting that PTE inhibits ATP synthesis of sarcoma stem cells via comp.5 inhibition. The anticancer activity of JQ1 and Honokiol is strongly potentiated by PTE.[Discussion]PTE inhibits OXPHOS and caused energy Crisis of osteosarcoma stem cells . cMYC leads to glycolytic dominant metabolism. cMYC inhibitors lead metabolic flux to OXPOS, and it exerted a great synergistic effect with PTE.

J-1097

Serine racemase is a potential new therapeutic target for colon cancer

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Serine racemase (SRR) was cloned in 1999 and its enzymatic function is catalyzing the racemization and elimination reaction of D-and L-serine. The only known physiological function of SRR is producing D-serine which is a co-activator of N-Methyl-D-aspartate receptor in human brain. How SRR work in cancer metabolism has not been studied. Here, we present a novel role of SRR in colon cancer. We found that the SRR expression levels were significantly elevated in colon adenoma and adenocarcinoma compared to normal colon mucosa in the datasets of ONCOMINE and immunohistochemistry of the clinical samples. SRR expression levels correlated to colon cancer cell proliferation rates and SRR knockout by CRIPR/CAS9 system resulted in reduced cell proliferation. SRR knockout cells had reduced mitochondria mass and potential. Moreover, phenazine methosulfate, which was previously reported as a serine racemase inhibitor, suppressed colon cancer cell proliferation in vitro. These results indicated that SRR contributed to homeostasis of mitochondria and enhanced cell proliferation in colon cancer, and expected to be a potential new target for the development of novel therapies for colon cancer.

▲TOP

J-1098

Ovarian cancer therapeutic potential of glutamine depletion based on GS expression

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Amino acids (AAs) are biologically important nutrient compounds necessary for the survival of any cell. Of the 20 AAs, cancer cells depend on the uptake of several extracellular AAs for survival. However, which extracellular AA is indispensable for the survival of cancer cells and the molecular mechanism involved have not been fully defined. In this study, we found that the reduction of cell survival caused by glutamine (Gln) depletion is inversely correlated with the expression level of glutamine synthetase (GS) in ovarian cancer (OVC) cells. GS expression was downregulated in 45 of 316 OVC cases (14.2%). The depletion of extracellular Gln by treatment with l-asparaginase, in addition to inhibiting Gln uptake via the knockdown of a Gln transporter, led to the inhibition of cell growth in OVC cells with low expression of GS (GS^{low}-OVC cells). Furthermore, the re-expression of GS in GS^{low}-OVC cells induced the inhibition of tumor growth in vitro and in vivo. Thus, these findings provide novel insight into the development of an OVC therapy based on the requirement of Gln.

J-1099

Combinatorial inhibition of xCT and ALDH3A1 induces synthetic lethality in xCT inhibitor-resistant cancer cells

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The cystine-glutamate antiporter xCT has an important role for redox homeostasis in tumor cells and its expression enhances chemotherapy resistance and distant metastasis of tumor cells. Therefore, xCT is considered as a promising target for tumor therapy. However, differences of response to drugs including xCT inhibitors in tumor cells caused by intratumor heterogeneity are often observed and this heterogeneity causes therapy resistance in malignant tumor. Thus, we performed drug screening for sensitizer of xCT inhibitor with the use of xCT inhibitor-resistant cancer cell line HSC-4 cells, and we found that ALDH inhibitor drastically sensitizes cancer cells to xCT inhibitor sulfasalazine. Furthermore, we found that ALDH3A1 gene is overexpressed in sulfasalazine-resistant cancer cells and knockdown of ALDH3A1 also sensitizes sulfasalazine-resistant tumor cells to xCT inhibitors. Combination of sulfasalazine and ALDH inhibitor inhibited growth of HSC-2 xenograft tumors, which is insensitive to monotherapy of sulfasalazine in vivo. Our results suggest that ALDH inhibitor is promising drug for combinatorial use of sulfasalazine or other xCT inhibitors for cancer therapy.

▲TOP

[J-1100] J18-1 [Japanese]
Molecular target therapy

2018 / 9 / 27 (Thu) 14:15-15:30 Room 12/12F 1202, Osaka International Convention Center Room 12

Daisuke Okuzaki / Res. Inst. for Microbial Diseases, Osaka Univ.

J-1100

FSTL1 creates refractoriness of colorectal cancer

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“Hot” tumors with a number of mutational neoantigens have been believed to be relatively curable by immunotherapy with immune checkpoint inhibitors (ICIs). However, accumulating are evidences that the infiltrating T cells might be sometimes dysfunctional to fight against cancer. We recently found that, in the host with unbalanced immunity including both suppressive and inflammatory statuses associated with cancer metastasis and aging, blocking immune inhibitory pathways weights down the inflammatory side, and rather facilitates tumor progression and metastasis through T-cell immune exhaustion and dysfunction, and further identified FSTL1 as a key molecule involved in the mechanisms. Indeed, transduction with fstl1 into murine colon cancer MC38 cells with microsatellite instability disabled ICI therapy in the tumor-implanted mouse models, suggesting that FSTL1 could create the refractoriness of colon cancer. In clinical settings, FSTL1 positivity is reversely correlated with overall survival of colon cancer patients, suggesting a poor prognostic factor. Thus, targeting FSTL1 may be a promising strategy for treating colon cancer through rightly balancing immunity against cancer.

▲TOP

J-1101

Novel anticarcinoembryonic antigen antibody-drug conjugate has antitumor activity in the existence of soluble antigen

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Carcinoembryonic antigen (CEA) is a classic tumor-specific antigen that is overexpressed in several cancers, including colorectal cancer. Although some anti-CEA antibodies have been tested, to our knowledge, there are currently no clinically approved anti-CEA antibody therapies.In this study, we generated a novel anti-CEA antibody, 15-1-32, which was capable of stronger binding to membrane-bound CEA on cancer cells than existing anti-CEA antibodies. In addition, 15-1-32 showed poor affinity for soluble CEA, thus the binding activity of 15-1-32 to membrane-bound CEA was unaffected by soluble CEA. Moreover, we constructed a 15-1-32-monomethyl auristatin E conjugate (15-1-32-vcMMAE) to improve the therapeutic efficacy of 15-1-32. 15-1-32-vcMMAE showed enhanced anti-tumor activity against gastric cancer cell lines. Unlike existing anti-CEA antibody therapies, 15-1-32-vcMMAE retained anti-tumor activity in the presence of high concentrations of soluble CEA.Our findings indicate that novel anti-CEA antibody 15-1-32 can affect CEA expressing tumor cells without interference with soluble form. Thus, 15-1-32 would be one of the promising candidate for anti-CEA cancer therapeutics.

J-1102

Immunogenetic Profiling Identifies Sulfated-Glycosaminoglycans as Major Functional B Cell Antigens in Human Malignancies

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Diffuse-type gastric cancer (DGC) exhibit "genomically stable" phenotype; therefore, the efficacy of immune-checkpoint blockades may not be sufficient for DGCs with few Neo-antigens. To develop effective immunotherapies against DGCs, it is important to clarify their tumor immunity profiles. Here, we aimed at immunogenetically profiling tumor-infiltrating T and B cell repertoires for 30 DGCs. As a result, mature B cell immunity was revealed to play an important role especially in DGCs. Focusing on the B cell repertoires, we identified varieties of tumor-specifically dominant immunoglobulins. Analysis of reconstructed IgGs showed that some of them exhibited auto-reactivities to abundant cellular proteins, however, it was of note that multiple of the rest of IgGs commonly recognized sulfated-glycosaminoglycans (GAGs). More than 30% of the tumor-specific dominant IgGs found in DGCs showed anti-sulfated-GAG nature. Importantly, those antibodies exhibited robust growth suppression against various cancers. Thus, sulfated-GAGs were revealed to be major functional B cell antigens in tumor microenvironments, and could be suitable for the targets of therapeutic antibodies against cancers.

J-1103

Pharmacological Characterization of Anti-Glypican 3/CD3 Bispecific T Cell-Redirecting Antibody ERY974

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The bispecific T cell redirecting antibody (TRAB) is a new form of promising immunotherapy. We generated a novel TRAB, ERY974, targeting tumor specific antigen Glypican-3 (GPC3). Using a mouse model reconstituted with human immune cells, we revealed that ERY974 was highly effective in killing cells of various tumor types that have GPC3 expression levels comparable to those in clinical tumors. Interestingly, ERY974 also induced robust antitumor efficacy even against tumors with non-immunogenic features, which are thought to be difficult to treat by inhibiting immune checkpoints like PD-1 and CTLA-4. Combination effect of ERY974 with other anti-cancer agents also demonstrated in several mouse models. Toxicology studies in non-human primates showed a transient cytokine elevation, but it was manageable and reversible, and importantly, no organ toxicity was evident. These data provide a rationale for the clinical testing of ERY974 for the treatment of patients with various GPC3-positive solid tumors. A Phase 1 clinical trial of ERY974 targeting GPC3-positive solid tumors is ongoing (NCT02748837).

▲TOP

J-1104

CD98 is a novel target for antibody therapy against multiple myeloma

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Although mAb drugs targeting CD38 or CS1are effective, more target antigens may be needed to cure MM. In this study, we screened more than 10,000 anti-MM mAb clones, and identified R8H283 as a mAb that bound to MM cells but not to CD45+ normal leukocytes. R8H283 specifically recognized CD98. Although CD98 protein is expressed broadly in all hematopoietic cells, R8H283 did not reacted with normal hematopoietic cells, in which N-glycosylation of CD98 was different from that in MM cells. Expression of the R8H283 epitope were induced by treatment with endoplasmic reticulum (ER) stress-inducers such as bortezomib, suggesting synergistic effect of R8H283 and proteasome inhibitors. Proliferation and survival of CD98-deficient MM cells were severely impaired, implying that MM cells are unlikely to lose CD98 expression. R8H283 exhibited significant anti-MM effects without damaging normal hematopoietic cells in vivo. Collectively, CD98 is a promising therapeutic target against MM and can be efficiently targeted using a new CD98 mAb R8H283.

J-1105

Contribution of Fc γ RIIB to creating suppressive tumor microenvironment

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It is well established that cellular immune responses, such as cytotoxic or suppressive activities play an important role in regulating tumor growth and metastasis. However, contribution of humoral immune responses against tumor is incompletely understood. The Fc receptors constitute critical elements for activating or down-regulating immune responses through immunoglobulin immune complex. Here, we examined the potential role of inhibitory Fc γ receptor, Fc γ RIIb in tumor immunity using a mouse model. Deletion of Fc γ RIIb receptor significantly improves both cellular and humoral anti-tumor immunity, delays tumor growth. Deletion of Fc γ RIIb receptor also strongly enhance the expression of inflammatory cytokines at tumor site. Results indicate that tumor associated macrophages in Fc γ RIIb KO mice up-regulate inflammatory cytokine expressions. Mechanistically, immune complexes significantly enhance the activation of antigen presenting cells in Fc γ RIIb KO mice. These findings indicate that spontaneous anti-tumor antibody create the suppressive tumor microenvironment through Fc γ RIIb signaling, and suggests attractive therapeutic target for cancer immunotherapy.

▲TOP

[J-1106] J12-2 [Japanese]
Innate immunity (2)

2018 / 9 / 27 (Thu) 15:30-16:45 Room 12/12F 1202, Osaka International Convention Center Room 12

Tsukasa Seya / Dept. Pathol. Hokkaido Univ. Sch. Med.

J-1106

PEDF promotes tumor dissemination of ovarian cancer cells through an interaction with peritoneal immune system

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Peritoneal dissemination leads to poor prognosis in ovarian cancer. In this study, we aimed to elucidate a mechanism how ovarian cancer cells escape immune system in peritoneal cavity.First, highly disseminative mouse ovarian cancer cell line (GO2) was established by in vivo passage of ID8-GFP (ID8G) cells in B6J mice. Microarray analysis revealed PEDF as one of the up-regulated genes in GO2 cells. Second, we found that PEDF-overexpressed ID8G cells (ID8G-PEDF) possess higher ability of peritoneal dissemination compared to ID8G cells. PEDF expression levels correlated with cell survival ability in the peritoneal cavity whereas anoikis resistance was not affected by PEDF. *IL10* and *CD206* expression in peritoneal macrophages, PD-L1 expression in B lymphocytes, and IL10 concentration in ascites were higher in mice inoculated with ID8G-PEDF cells than in mice inoculated with parental ID8G cells. Furthermore, high PEDF expression in tumor correlated with unfavorable overall survival in patients.Correctively, these data suggest that PEDF plays an important role in the peritoneal dissemination of ovarian cancer cells potentially by constructing immunosuppressive microenvironment.

▲TOP

J-1107

Chimeric antigen receptor T (CAR-T) cell therapy with intrinsic PD-1 blocking for ovarian cancer

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Chimeric antigen receptor T (CAR-T) cell therapy has shown significant clinical effect for hematological malignancies, while insufficient effect has been obtained for solid tumors due to PD-1 signal-related immunosuppression and/or exhaustion of CAR T cells. Therefore, we established PD-1 dominant negative receptor (DN) co-transduced CAR-T cells, which blocked PD-1/PD-L1 pathway and explored the antitumor effect on the mouse ovarian cancer in vitro. We selected mesothelin(MSLN) as a target antigen, and generated anti-MSLN-CAR-T cells (M-CAR-T) and PD-1 DN-M-CAR-T cells. For target cells, MSLN- and/or PD-L1- expressed mouse ovarian cancer cell lines were established. When co-cultured with ID8-MSLN or HM-1-MSLN, M-CAR-T showed high cytotoxicity, while cytotoxicity lessened when co-cultured with HM-1-MSLN-PDL1. However, PD-1 DN-M-CAR-T showed enhanced cytotoxicity compared with M-CAR-T. CAR-T cells targeting MSLN exhibited potent activity against MSLN-expressing mouse ovarian cancer cell lines. Blockading PD-1 pathway reinforced the cytotoxic activity of the CAR-T cells. In conclusion, PD-1 DN-CAR-T could exert antitumor effect in the tumor microenvironment of ovarian cancer.

J-1108

Enhancement of NK Sensitivity against ICAM-1 Over-expressing Cancer Cell by Inactivated Sendai Virus Particles

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This study revealed a novel cancer therapy which is employing virus particles to induce anti-cancer NK cell immunity. Inactivated Sendai virus (hemagglutinating virus of Japan) envelope (HVJ-E) has multiple anti-cancer effects, including induction of cancer-selective cell death and activation of anti-cancer immunities. First, we found HVJ-E induced intercellular adhesion molecule-1 (ICAM-1) production in several cancer cell lines, a breast cancer cell line (MDA-MB-231) and a prostate cancer cell line (PC3). The cancer cells were treated with HVJ-E or its viral RNA and the expression was analyzed, and cancer cell specific increase of ICAM-1 expression was observed. In vivo, treatment of HVJ-E significantly suppressed MDA-MB-231 tumor growth in SCID mice while the tumor suppression effect of HVJ-E was impaired when NK cells were depleted. These findings suggest that HVJ-E enhances NK cell sensitivity of cancer cells by increasing ICAM-1 expression via RIG-I/MAVS signal pathway resulting in promotion of NK cell anti-cancer cytotoxicity. This is the first report demonstrating that the viral therapy can enhance NK cell sensitivity in cancer cells.

J-1109

Role of Mint3 in tumor-associated macrophages

Takeharu Sakamoto
Div. Mol. Pathol., Inst. Med. Sci., Univ. Tokyo.

Co-author：Tetsuro Hayashi[1]、Motoharu Seiki[2]、Yoshinori Murakami[1]
[1]Div. Mol. Pathol., Inst. Med. Sci., Univ. Tokyo.、[2]Faculty Med., Kanazawa Univ.

Tumor-associated macrophages (TAM) can be classified into the tumor-suppressive M1-like and the tumor-promotive M2-like subtypes. Most TAMs are polarized to the M2-like subtype and promote tumor malignancy. Previously, we revealed that the activator of hypoxia-inducible factor, Mint3, promotes metastasis by controlling metastatic niche formation via inflammatory monocytes. However, whether Mint3 contributes to the function and polarization of TAMs remains unclear. To address this, we inoculated Lewis lung carcinoma (LLC) cells in control and myeloid cell-specific Mint3 conditional knockout (Mint3 cKO) mice and analyzed tumor growth and TAM polarization. Mint3 cKO mice showed decreased tumor growth of LLC cells compared with control mice. The ratio of total TAMs in tumors was not different between control and Mint3 cKO mice. However, more TAMs were polarized into the M1-like subtype in Mint3 cKO mice compared with control mice. These results suggest that Mint3 in myeloid cells promotes tumor growth and M2 polarization of TAMs.

▲TOP

J-1110

Regulation of myeloid cell differentiation and regression of cancer by saturated fatty acids

Hiroshi Goda
Lab. Biochem. Mol. Biol., Grad. Sch. Pharm., Osaka Univ.

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[1]Lab. Vaccine Immune Reg. (BIKEN), Grad. Sch. Pharm., Osaka Univ., MEIC, Osaka Univ.、[2]Lab. Biochem. Mol. Biol., Grad. Sch. Pharm., Osaka Univ.、[3]Lab. Biochem. Mol. Biol., Grad. Sch. Pharm., Osaka Univ., NIBIOHN, MEIC, Osaka Univ.

Myeloid-derived suppressor cells (MDSCs) accumulate in cancer patients and suppress various immune responses such as T cell proliferation. In this study, to reveal the effects of fatty acids on the differentiation and/or immunosuppressive function of MDSCs, we added each fatty acid to bone marrow cells during in vitro MDSC differentiation. We found that palmitic acid (PA) and stearic acid (SA) inhibited the differentiation to MDSCs and promoted the differentiation into dendritic cells (DCs). Inhibition of fatty acid uptake via long-chain fatty acid transporter, CD36, suppressed the promotion of differentiation into DCs by PA or SA. Furthermore, we observed significant suppression of tumor progression by feeding tumor-bearing mice on PA-rich diet. These results suggest that PA inhibits the MDSC differentiation and promotes the differentiation into DCs, leading to activation of anticancer immunity. These results lead to elucidation of the mechanisms of MDSC differentiation and development of novel cancer therapies targeting the metabolic pathways of PA and SA.

J-1111

Development of TCR gene therapy with allogeneic Stealth T cells deficient in endogenous TCR and MHC class I molecules

Satomi Okada
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Introduction: In adoptive immunotherapy, the usage of patients' lymphocytes genetically engineered to express tumor-reactive TCR limits the quality and quantity. We demonstrate the TCR gene therapy using allogeneic “Stealth T cells” deficient in endogenous TCR and MHC class I (MHC-I). Methods: We transduced human lymphocytes with TCR specific to a cancer/testis antigen, NY-ESO-1, by a retrovirus vector with siRNA specific to the endogenous TCR (siTCR vector). In addition, utilizing CRISPR/Cas9 system, we generated TCR gene-engineered T cells deficient in MHC-I.Results: Human lymphocytes genetically edited with siTCR vector showed reduced expression of endogenous TCR associated with the diminished reactivity to allogeneic cells. When administrated into NOG mice, these TCR gene-transduced T cells induced tumor regression without development of GVHD. In addition, the CRISPR/Cas9 gene-edited T cells showed reduced expression of MHC-I. Allogeneic T cells showed reduced reactivity against MHC-I deficient “Stealth T cells”. Conclusions: Allogeneic “Stealth T cells” is a promising strategy to achieve an off-the-shelf therapy for wide application of adoptive immunotherapy.

▲TOP

[S2-1] S2 [English]
Characteristics of cancer revealed by bio-imaging technology

2018 / 9 / 27 (Thu) 9:00-11:30 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13

Masaru Ishii / Dept. Immunol. Cell Biol., Osaka Univ. Grad. Sch. Med.、Etsuko Kiyokawa / Dept. Oncol. Pathol, Kanazawa Med. Univ.

Fluorescent imaging is a powerful tool to investigate the cancer cell behavior. There are two kinds of fluorescent probes; one is genetically encoded proteins, such as GFP (green fluorescent protein), and the other is the chemical dyes. Fluorescent proteins can be a nice reporter for the expression of proteins-of-interest. Therefore the generation of various transgenic mice has been bringing us lots of knowledge of tumor heterogeneity, micro-environments with various types of cells. In contrast, chemical dyes already have been used in the clinical field. It is still challenging to label specifically the cancer cells.
In this symposium, we have speakers covering a variety imaging methods both in basic and clinical research. Not only for obtaining the images, but the fluorescent light can be used also for manipulation of the cellular events in vitro and in vivo. The people in the hospital might be surprised to know that the fly (Drosophila melanogaster) forms cancers like mammals. We will learn what we can and cannot see by the specific methods, and how to integrate these techniques or tools for your own research.

S2-1
Optogenetics technology applicable to cancer research

Moritoshi Sato
Grad. Sch. Arts & Sci., Univ. Tokyo

The genome consists of more than 20,000 genes and is essential for most of biological phenomena. To understand these biological phenomena, including diseases, and to utilize or modify them, approaches that enable the genome to be regulated at will are required. We developed a light-inducible, RNA-guided programmable system for endogenous gene activation based on the CRISPR-Cas9 system (CPTS). We demonstrated that this optogenetic system allows rapid and reversible targeted gene activation by light. Using this system, we exemplified photoactivation of multiple user-defined genes in mammalian cells. Additionally, we also developed another optogenetic system, named photoactivatable Cas9 (PA-Cas9). We divided the Cas9 nuclease into two fragments and connected photo-inducible dimerization proteins to the fragments, leading to the development of PA-Cas9 of which nuclease activity is switchable with light. PA-Cas9 allows direct editing of DNA sequence of the genome by light stimulation. Genome editing technology and optogenetics technology have emerged as different technologies from each other so far. Our studies described above merge these emerging research fields together.

▲TOP

S2-2

Tumor Hotspots, a newly discovered epithelial tissue-intrinsic oncogenic niche

Yoichiro Tamori
Natl. Inst. Genet.

Pro-tumor mutant cells can evolve through a multistep process in which they become malignant. Many genes that are involved in the different steps towards cancer development have been identified; however, how certain mutant cells destroy normal tissue organization and undergo uncontrolled proliferation during the initial stages of this process remains largely unclear. I show through analysis of conserved neoplastic tumor-suppressor genes in Drosophila wing imaginal disc epithelia that tumor initiation depends on tissue-intrinsic local cytoarchitectures, causing tumors to consistently originate in a specific region of the tissue. In this "tumor hotspot," the filopodial protrusions observed on the basal side of epithelia showed directional, flow-like patterns. A combination of confocal microscopy and computational imaging analysis suggests that the confluence of flow patterns corresponds to the tumor hotspots, where pro-tumor cells preferentially induce tissue disorganization. These suggest that an encounter of oppositely-directed mechanical stress forms mechanically unstable spots that might be vulnerable to a tissue-disorganizing event such as the emergence of pro-tumor cells.

S2-3

Identification of cancer stem cells by multicolor lineage tracing method

Hiroo Ueno
Dept. Stem Cell Path., Kansai Med. Univ.

Although the existence of cancer stem cells in various tumors has been suggested, it is still controversial. Because stem cells are defined as the cells that can both self-renew and differentiate, it is crucial to show that the cells of interest have both potentials to prove that there exist stem cells within tumors. To this end, the multicolor lineage tracing method should be one of the useful tools. In this presentation, we examined two mouse cancer models. First, by using three different models of intestinal adenocarcinoma and adenoma, we found that Bmi1 or Lgr5 positive cells within tumors expand to form single cell derived single color areas, suggesting that they behave as cancer stem cells. Second, using a mouse model of chemically (4-nitroquinoline-1-oxide: 4-NQO) induced tongue cancer, we found that although many Bmi1-positive cells within the tongue cancer specimens failed to proliferate, some proliferated continuously and supplied tumor cells to the surrounding area, suggesting that such cells could serve as cancer stem cells. We will discuss about the existence of cancer stem cells from our results and recent experimental results from other groups.

S2-4

Intravital multiphoton imaging revealing cellular dynamics in inflammation and cancer in vivo

Masaru Ishii
Dept. Immunol., Cell Biol, Osaka Univ. Grad. Sch. Med.

Multiphoton fluorescent microscopy has launched a new era in the field of biological imaging. The near-infrared laser for multi-photon microscopy can penetrate thicker specimens, enabling the visualization of living cell behaviors deep within tissues and organs without thin sectioning. Bone has been an organ where various kinds of cells are resident and undergo differentiation into specific lineages, processes that occur in specialized locations called niches, and proper localization and migration of cells is the first critical step for effective processes. However, by means of conventional methodology, the information regarding cellular movements was completely missing. To analyze their movements in vivo, we must definitely see them in live bone tissues. By means of intravital microscopy, we succeeded in visualizing the various dynamic phenomena in bone marrow cavity. Especially we have focused on the behavior of osteoclast, a cell type contributing bone destruction in inflammation and cancer. In this presentation, I will present the latest data on this new concept in the field of inflammatory biology, as well as show our recent trials for visualizing human cancer specimens.

▲TOP

S2-5

Finding out new enzymatic activities as biomarkers for various cancers by a novel chemical probe library-based approach

Yasuteru Urano
Grad. Sch. Pharm. Sci., Univ. Tokyo, Grad. Sch. Med., Univ. Tokyo, AMED-CREST, AMED

Recently, we have succeeded to develop novel fluorogenic probes for aminopeptidases including that for γ-glutamyltranspeptidase (GGT), and tumor regions in real clinical specimen of breast cancer patients were proved to be easily discriminated from normal mammary gland tissues within 1-15 min after GGT-activatable probe application.
Encouraged by these promising results, we prepared a library of fluorogenic probes composed of more than 400 probes for various aminopeptidases. By applying these probes to fresh biopsy samples of esophageal cancer patients, we found the enzymatic activity of dipeptidyl peptidase-4 (DPPIV) was upregulated in esophageal cancer, and cancer region in the resected human fresh specimens was clearly visualized by topically spraying DPPIV-activatable fluorescence probes within 10 min. These results clearly demonstrate that our library-based approach is quite versatile to find out novel biomarkers in terms of enzymatic activity for vast range of clinical purposes including fluorescence-guided surgery and therapies

S2-6

Multiple antitumor mechanisms of Zn-protoporphyrin nanoparticle (ZPPN) utilizing EPR-effect for PDT

Hiroshi Maeda
BioDynamics Res. Foundation., Dept. Microbiol., Grad. Sch. Med. Sci., Kumamoto Univ., Osaka Univ. Med. Sch.

Co-author：Waliul Islam[1]、Jun Fang[2]
[1]BioDynamics Res. Foundation., Dept. Microbiol., Grad. Sch. Med. Sci., Kumamoto Univ.、[2]Dept. Micorbiol. & Oncol., Faculty of Pharm. Sci., Sojo Univ.

We showed Zn-protoporphyrin (ZnPP) inhibited hemeoxygenase (HO-1), and a tumor survival factor that is also called HSP32. ZPPN induces tumor cell death by losing defensive potential against oxystress and involving in oncogenes and cancer growth. We also found it exhibits fluoresce when irradiated by blue light and simultaneously generates singlet oxygen and kills tumor cells. Advantages of ZPPN are: selective accumulation in solid-tumor by EPR-effect; fluorescent tumor-imaging observed in autochthonous cancers; endoscopic light irradiation resulting tumor regression; ZPPN in plasma results in neither fluorescence nor ROS (‘O2) generation by π-π interaction of ZnPP. When ZPPN nanoparticle-micelles were disrupted with detergents or lecithin (component of lipid-bilayer) it resumes fluorescence and generates singlet-oxygen. Existing methods of PDT using tetrapyrrole (Laserphyrin etc) shows no tumor selective effect, and it distributes indiscriminately in entire body. Emission of 633nm by HeNe-laser has no effect on tetrapyrroles thus no ROS generation and little antitumor effect. All together there is a good prospect for development of ZPPN for PDT.

S2-Special_Remarks

Special Remarks

Yasuyuki Seto
Dept. Gl. Surg., The Univ. Tokyo, Grad. Sch. Med.

No Abstract

▲TOP

[LS10] LS10 [Japanese]
Clinical impact of cancer stemness and immune microenvironment

2018 / 9 / 27 (Thu) 11:50-12:40 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13
：TAIHO PHARMACEUTICAL CO., LTD

Hideshi Ishii / Osaka University, Graduate School of Medicine, Department of Medical Data Science

LS10

Clinical impact of cancer stemness and immune microenvironment

Shinji Tanaka
Tokyo Medical and Dental University, Graduate School of Medical and Dental Sciences, Department of Molecular Oncology

No Abstract

[S5-1] S5 [English]
New developments in cancer research revealed by genome instability

2018 / 9 / 27 (Thu) 13:00-15:30 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13

Kozo Tanaka / Dept. Mol. Oncol., Inst. Dev. Aging Cancer, Tohoku Univ.、Hiroyuki Seimiya / Div. Mol. Biother., JFCR Cancer Chemother. Ctr.

Most cancer cells exhibit genome instability at nucleotide or chromosome level, which is known as a hallmark of cancer. Rapidly growing cancer genome sequencing data have been unraveling the prevalence and detailed picture of genome instability. Genome instability leads to increased genetic alterations and heterogeneity, facilitating tumor formation and progression as well as drug resistance. Investigating the underlying mechanisms of genome instability in cancer contributes to the identification of biomarkers for cancer diagnosis and treatment. Genome instability is also considered as a target for cancer therapy, an Achilles' heel that provides an opportunity to pinpoint cancer cells and effectively eradicate the disease. In this symposium, the speakers will highlight recent topics in cancer research focusing on genome instability, both at nucleotide and chromosome levels, and discuss their implications in tumorigenesis and cancer therapy.

S5-1
A robust transition from metaphase to anaphase prevents chromosome missegregation

Toru Hirota
Div. Exp. Path., Cancer Inst., JFCR

Aneuploidy is a widespread feature of malignancies, which is primarily caused by defective cell division. To understand molecular grounds underlying these defects, we have placed our research focus to the cellular functions ensuring chromosome segregation in mitosis. We yet know very little about if and how their regulation is impaired in cancer cells, however. In my presentation, I will share the latest findings on the control of the spindle-assembly checkpoint and subsequent activation of cohesin-severing protease separase, the mechanisms to commence anaphase at the correct time. Our data suggest that a wide range of cancer cells consistently have an impaired regulation for the 'brake' and 'engine', culminating in chromosome missegregation, which allows us to propose previously unanticipated etiologies for chromosome instability.

▲TOP

S5-2

Tetraploidy in cancer and aging

Hidemasa Goto
Dept. Neural Regen. Cell Commun., Mie Univ. Sch. Med., Dept. Physiol., Mie Univ. Sch. Med.

Co-author：Akira Mizoguchi[1]、Masaki Inagaki[2]
[1]Dept. Neural Regen. Cell Commun., Mie Univ. Sch. Med.、[2]Dept. Physiol., Mie Univ. Sch. Med.

About 20-30% of solid tumors show tetraploid or near-tetraploid karyotypes. Tetraploidy is likely a transient intermediate to develop CIN and intra-tumor heterogeneity, phenomena linked to drug resistance and poor prognosis. However, the biological impact of unscheduled tetraploidy remains largely unknown. We first produced genetically modified mice in which unscheduled tetraploidy occurred in a tissue-specific manner. In these mice, unscheduled tetraploidization was observed in lens epithelial cells and subcutaneous fibroblastic/adipose cells. The protein level of p53 was elevated in these cells, but the tetraploid cells continued to divide and then developed into aneuploid ones, a similar phenomenon observed in cancer. Such aneuploid cells accumulated DNA damages and in turn became senescent. Our tetraploidy-prone mice exhibited several tissue-specific aging phenotypes such as lens cataract, impaired wound hearing, subcutaneous fat loss, and lordokyphosis. In addition, when fibrosarcoma was induced by chemical carcinogen, its growth was partially suppressed in our tetraploidy-prone mice. In this symposium, we will discuss the effects of tetraploidy on carcinogenesis.

S5-3

ASXL1 regulates cellular differentiation and initiates tumorigenesis in colon

Taichi Isobe
Inst. for Stem Cell Res. & Regenerative Med., Stanford Univ.

Co-author：Mark A Zarneger[1]、Junichi Matsubara[1]、Omar Abdel-Wahab[2]、Michael F Clarke[1]
[1]Inst. for Stem Cell Res. & Regenerative Med., Stanford Univ.、[2]Human Oncol. & Pathogenesis Program, Memorial Sloan-Kettering Cancer Ctr.

It is known that genetic instabilities fuel tumorigenesis in the large intestine. Colorectal cancers (CRCs) are divided into two types of genetic instabilities: microsatellite instability (MSI) and chromosomal instability (CIN). The latter is the most common in CRCs, especially in left-sided CRCs. TCGA database revealed that the amplification of Chr20q11.21 is most frequent chromosomal alteration in left-sided CRCs. The expression of ASXL1, a gene residing on Chr20q11.21 and coding an epigenetic modifier, is upregulated in CRCs with CIN. Since epigenomes regulate cellular differentiation, impaired cellular differentiation by disordered epigenome can be a cause of oncogenesis. We showed that the knockout of Asxl1 perturbed lineage commitment to absorptive or secretory fate in vivo. Tumor cells highly expressed Asxl1 and the knockout of Asxl1 decreased the spontaneous tumor formation in Apc^{Min} mice. In patient-derived xenografts of CRCs, the knockdown of ASXL1 impaired the tumorigenicity of cancer stem cells and changed the pathological type of engrafted tissues. These results suggest that amplified ASXL1 locus plays a central role in the initiation and maintenance of CRCs with CIN.

S5-4

Dysfunction of DNA damage response and effect of a PARP inhibitor in neuroblastoma

Junko Takita
Dept. Ped., The Univ. of Tokyo.

Co-author：Masatoshi Takagi
Dept. Pediatrics, Tokyo Med. & Dent. Univ.

Loss of 11q was frequently observed in neuroblastoma (NB) with poor prognosis, and DNA damage response (DDR) genes, such as ATM and H2AFX are located within the commonly deleted 11q region. To assess the oncogenic role of these DDR genes in the pathogenesis of NB, genomic alterations of DDR-related genes including ATM and CHEK1 were investigated in 45 NB-derived cell lines and 237 fresh tumor samples. In addition, PARP inhibitor sensitivity of NB was investigated in in vitro and in vivo xenograft models. Among the fresh tumor samples, ATM, MRE11A, H2AFX, and/or CHEK1 loss or imbalance of 11q was detected in 20.7% of NBs, 89.8% of which were stage 3/4. Rare SNVs in DDR-associated genes other than ATM were detected in 26.4% and were mutually exclusive. Overall, samples with SNVs and/or copy number alterations in these genes accounted for 48.4%. Intriguingly, 83.3% NB-derived cell lines exhibited sensitivity to PARP inhibition, and NB growth was markedly attenuated in the xenograft group receiving PARP inhibitors. Genomic alterations of DDR-associated genes including ATM, were observed in almost half of NBs, suggesting that PARP inhibitors represent candidate NB therapeutics.

▲TOP

S5-5

Replication stress as a trigger for microsatellite destabilization and hypermutation

Ken-ichi Yoshioka
Div. Carcino. & Can. Prev., Natl. Can. Ctr. Res. Inst.

Mismatch repair (MMR)-deficient cancers are characterized by microsatellite instability (MSI) and hypermutation. However, it remains unclear how MSI and hypermutation arise and contribute to cancer development. Here, we show that replication stress simultaneously triggers MSI and hypermutation, which leads to clonal expansion of cells with ARF/p53-module mutations and resistance to the anti-cancer drug camptothecin. Mutations accumulated in MMR-deficient cells exposed to replication stress, under which low-fidelity translesion synthesis polymerases operated in the absence of proofreading and MMR. Unlike replication stress-associated DNA double-strand breaks in MMR-proficient cells, those in MMR-deficient cells were repaired by a Pol θ -mediated pathway that suppressed chromosomal instability, but caused MSI. Thus, replication stress-triggered MSI and hypermutation lead to the efficient generation of cells with abrogated defense systems and enable clonal evolution.

S5-6

G-quadruplex nucleic acids as a molecular target for cancer therapy

Hiroyuki Seimiya
Div. Mol. Biother., Cancer Chemother. Ctr., JFCR

Co-author：Kazuo Shin-ya[1]、Kazuo Nagasawa[2]
[1]Natl. Inst. Adv. Indust. Sci. Tech.、[2]Fac. Eng., Tokyo Univ. Agr. Tech.

G-quadruplex (G4) is a four-stranded nucleic acid structure formed at guanine-rich sequences, such as the telomeric repeats. G4s are widely distributed throughout the genome and their dynamics affects genomic stability and functions. We have previously demonstrated that G4 stabilization by small compounds called G4 ligands preferentially targets glioma stem cells. However, precise mechanisms for the efficacy remain elusive. Here we demonstrate that G4 ligands induce the replication stress and DNA damage response more potently in glioma stem cells than in non-stem glioma cells. By screening a cancer cell line panel, we further identified cell lines that were hypersensitive to G4 ligands, such as telomestatin and Phen-DC3. Intriguingly, these cells did not give DNA damage foci upon treatment with G4 ligands, suggesting additional mode-of-action. In fact, G4 ligands inhibited in vitro transcription and translation of mRNAs that contained G4-forming sequences. As a potential pharmacodynamic biomarker, immunofluorescence intensities of intracellular G4 foci were enhanced by G4 ligands. These observations suggest that G4s both in DNA and RNA are promising targets for cancer therapy.

S05-Special_Remarks

Special Remarks

Hiroyuki Yamamoto
Div. Gastroenterol Hepatol, St. Marianna Univ. Sch. Med.

No Abstract

▲TOP

[S6-1] S6 [English]
Epigenome abnormalities and translational research

2018 / 9 / 27 (Thu) 15:30-18:00 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13

Toshikazu Ushijima / Div. Epigenomics, Natl. Cancer Ctr. Res. Inst.、Yutaka Kondo / Div. Cancer Biol., Nagoya Univ., Grad. Sch. Med.

Epigenomic abnormalities are now used in practice for diagnosis and therapy. At the same time, targets of epigenomic abnormalities are expanding from cancer cells to cancer microenvironments, offering new opportunities for translational research. In this symposium, we assembled speakers who are actively involved in the identification of novel targets and development of cutting-edge applications. Novel targets include immunoepigenomics, namely epigenomic abnormalities that induce altered cancer immune reactions, and non-coding RNAs that regulate cancer cell growth and immune cell development. Applications include cancer diagnosis that takes maximum advantage of epigenomics, development of epigenetic drugs, and a search for an efficient combination of epigenetic and other drugs. The chairpersons hope that this Symposium will provide insight into the expanding nature on epigenetic translational research.

S6-1
Precision Cancer Risk Diagnosis by Assessing the Epigenetic Field

Toshikazu Ushijima
Div. Epigenomics, Natl. Cancer Ctr. Res. Inst.

Precision cancer risk diagnosis by measuring aberrant DNA methylation accumulated in a normal tissue, mainly due to chronic inflammation, is now approaching to clinical use. Our multicenter prospective cohort study involving 826 gastric cancer patients showed that, even among cancer patients, the risk of metachronous gastric cancer can be predicted [Maeda, Gut, 66:1721, 2017]. Such precision is achieved first by the use of appropriate markers that reflect the overall epigenome damage, as supported by the high correlation among the markers and irrelevance to gene expression [Maeda, Gastric Cancer, online]. Second, the deeper involvement of aberrant DNA methylation than of mutations in gastric cancer risk is important. To prove this issue, we evaluated both point mutations and aberrant methylation in mucosae with different risk levels, using a novel method to quantify rare point mutations [Yamashita, Cancer Lett, 403:152, 2017]. Cancer risk was heavily influenced by aberrant DNA methylation in the stomach while it was by both in the esophagus [Yamashita, PNAS, 115:1328, 2018]. Epigenomic precision cancer risk diagnosis will also be effective for other inflammation-associated cancers.

▲TOP

S6-2

Combination treatments with histone deacetylase inhibitors

Yoshihiro Sowa
Dept. Molecular-Targeting Cancer Prevention

Co-author：Toshiyuki Sakai
Dept. Molecular-Targeting Cancer Prevention

Not only genetic abnormalities but also epigenetic abnormalities are involved in cancer onset and progression. Histone deacetylases (HDAC) remove acetyl groups from histone, resulting in the transcriptional repression of a variety of genes, e.g. cell cycle- and apoptosis-control genes. Therefore, HDAC inhibitors inhibit the cell cycle and induce apoptosis through the induction of various genes in cancer cells. Although several HDAC inhibitors have been approved as anticancer drugs, the benefit of HDAC inhibitors as a monotherapy is limited to a part of hematologic malignancies. Moreover, there is no approved companion diagnostic indicator for the usage of HDAC inhibitors so far. Since HDAC inhibitors have pleiotropic effects, combination treatments of HDAC inhibitors with cytotoxic agents, molecular-targeting agents or radiation might be potential approaches by enhancing their efficiency and decreasing each adverse effect. We have found a variety of promising combination treatments with HDAC inhibitors and other agents, e.g. eribulin, FGFR inhibitor, PI3K inhibitor, and so on. These findings suggest that the combination of HDAC inhibitors with other agents might be promising.

S6-3

Cancer stem cell-targeted therapy for hematological and sold cancers by inhibition of EZH1/EZH2 and mutant IDH1

Issay Kitabayashi
Div. Hematological Malignancy, Natl. Cancer Ctr. Res. Inst.

EZH1/EZH2 are catalytic components of Polycomb repressive complexes 2 (PRC2), which trimethylates histone H3 at lysine 27 (H3K27) to repress transcription of target genes. We found that EZH1/2 are essential for maintenance of cancer stem cells (CSCs) in acute myeloid leukemia and multiple myeloma. The EZH1/2 dual inhibitor selectively reduced the number of CSCs and prevented tumor progression in xenograft models for acute myeloid leukemia, multiple myeloma, lymphomas and colon cancers. Phase1 clinical trials of the dual inhibitor have been initiated in malignant lymphoma and acute leukemia. Mutations in isocitrate dehydrogenase (IDH) 1 and 2 are frequently observed in various cancers. We have found that mutant IDH is essential for progression and maintenance of cancers. And we have shown that mutant IDH1 inhibitor prevented growth of the tumors with IDH1 mutation in acute myeloid leukemia, glioma and condrosarcoma. Based on these results, Phase I clinical trial of the inhibitor has been initiated for patients of glioma with IDH1 mutation.

S6-4

Enhancing the efficacy of liver cancer immunotherapy by specific inhibition of histone deacetylase 8 (HDAC8)

Alfred S. Cheng
Sch. of Biomed. Sci., The Chinese Univ. of Hong Kong

Accumulating evidence is underscoring the fundamental importance of epigenetic regulation in tumor immune evasion. We have previously elucidated a critical role of histone deacetylase 8 (HDAC8) in hepatic carcinogenesis. Here, we aim to investigate the therapeutic potential of a HDAC8-specific inhibitor PCI-34051 in preclinical hepatocellular carcinoma (HCC) models. PCI-34051 significantly reduced HCC tumorigenicity in immunocompetent but not immunodeficient mice. Immune profiling revealed specific reduction in tumor-infiltrating regulatory T cells, which was associated with significant increase in CD8+ T cells. Notably, combined PCI-34051 and anti-PD-L1 treatment resulted in complete tumor eradication in all of the co-treated mice. Moreover, the combination therapy promoted long-term survival, which was associated with elevated CD8+ T effector and central memory cells. Our data suggest that selective chromatin modifications by HDAC8 alter the tumor immune surveillance program and demonstrate the potential of rational combinatorial epigenetic immunotherapy to fully unleash T-cell responses, leading to long-term remission of HCC.This work is supported by the RGC CRF (C4017-14G).

▲TOP

S6-5

Non-coding RNA transcription modulates nuclear architecture to specify T-cell fate and blocks T-cell malignancies

Takeshi Isoda
Dept. Pediatrics, Developmental Biol., Tokyo Med. & Dent. Univ.

Co-author：Masatoshi Takagi
Dept. Pediatrics, Developmental Biol., Tokyo Med. & Dent. Univ.

It is now well established that the transcriptional regulator Bcl11b specifies T cell fate. During T cell commitment, the super-enhancer is repositioned from the lamina to the nuclear interior. In the search for candidate factors that repositioned the Bcl11b control region, we identified long non-coding RNA ThymoD. ThymoD expression pattern in T cell progenitors immediately precedes that of Bcl11b. ThymoD-deficient mice displayed a block in T cell development and rapidly developed T-cell malignancies. We found that ThymoD transcription acted in cis to promote demethylation at CTCF bound sites and activated cohesin-dependent looping to reposition the Bcl11b enhancer from the lamina to the nuclear interior and to juxtapose the Bcl11b enhancer and promoter into a single-loop domain. These large-scale changes in nuclear architecture were associated with the deposition of activating epigenetic marks across the loop domain, plausibly facilitating phase separation. These data indicate how, during developmental progression and tumor suppression, non-coding transcription orchestrates chromatin folding and compartmentalization to direct with high precision enhancer-promoter communication.

S6-6

Targeting long non-coding RNA as a novel treatment option in human cancers

Yutaka Kondo
Div. Cancer Biol., Nagoya Univ., Grad. Sch. Med.

Accumulating studies revealed that in addition to the DNA/histone modification-based chromatin regulation in the DNA regulatory elements (i.e. enhancers and promoters), non-coding transcripts are also involved in regulation of gene function. Among such non-coding transcripts, dysregulations of long non-coding RNAs (lncRNAs) have been recognized as important functional molecules in tumorigenesis via acting in a concerted fashion to regulate chromatin states at the tumor-associated genes. We recently identified the molecular functions of TUG1 lncRNA in human cancers including gliomas and pancreas cancers. Targeting TUG1 using antisense oligonucleotides (ASO) coupled with a drug delivery system (DDS) effectively reduced the tumor size of both gliomas and pancreas cancers in xenograft models. Although the mechanistic details for targeting lncRNAs is still under investigating, combination with new technologies, such as DDS, may improve the efficacy and specificity of oligonucleotide-based therapies in cancer treatments.

S6-Special_Remarks

Special Remarks

Kazuhiro Yoshida
Dept. Surg. Oncol., Gifu Univ., Sch. Med.

No Abstract

[S3-1] S3 [English]
The role of intestinal microbiota in cancer progression

2018 / 9 / 27 (Thu) 9:00-11:30 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Hiroshi Kiyono / Int'l Res. & Development Ctr. for Mucosal Vaccines, The Inst. of Med. Sci., The Univ. of Tokyo、Naoko Ohtani / Grad. Sch. of Med., Osaka City Univ.

Emerging evidences have shown that the activity of the gut microbiota and their composition could be associated not only with the intestinal inflammation but also with the onset and progression of cancer. In this symposium, the selected speakers will show you the links between the gut microbiota and gut barrier function, inflammation and immune-checkpoint activities in the development of cancer. Topical themes on gut microbiota that can strengthen the anticancer immune responses will be announced. In addition, the bioinformatics approaches towards the understandings of the functions of gut microbiota on diseases will be shown. This symposium will provide us with the understandings on a diverse microbial ecosystem which favors organismal homeostasis, particularly in cancer prevention.

S3-1
Role of microbiome in immune suppressive tumor microenvironment

Hiroyoshi Nishikawa
Div. Can. Immunol., Res. Inst.

FOXP3+ T cells are composed of three functionally distinct subpopulations in humans:naive Tregs, effector Tregs (eTreg) and non-Tregs. A fraction of CRCs contained higher non-Tregs associated with a favorable prognosis. Thus, CRCs were classified into two types: type A with increased infiltration of eTreg, and type B with increased infiltration of both eTreg and non-Treg. This distinct T-cell infiltration pattern was due to inflammatory and tumorigenic intestinal bacteria, especially Fusobacterium nucleatum. All MMR-deficient CRCs were classified into type B with higher amounts of F. nucleatum, suggesting the possible relationship between MSI status and gut microbiome. Additionally, CRCs were further clustered into two groups with immunological gene signatures. In a sub-population of MMR-deficient CRCs, for which immune checkpoint blockade (ICB) are generally effective, low-immunogenic cluster was identified. Of note, these low-immunogenic MMR-deficient CRCs contained higher infiltration of eTreg. Together, MMR-deficient CRCs in type B can be further classified into high- and low-immunogenic tumors, and Tregls could be a therapeutic target in combination with ICB in the latter.

▲TOP

S3-2

Interplay of microbiota and the host in the intestine

Kiyoshi Takeda
Dept. Microbiol. Immunol., Grad. Sch. Med., Osaka Univ., IFReC, Osaka Univ.

Intestine is a unique tissue, where many commensal bacteria, called microbiota, inhabit. Therefore, intestinal mucosa is protected from microbiota as well as pathogenic bacteria by several types of barriers. One of these barriers is constructed by mucus layers, composed of inner and outer mucus layers in the colon. Microbiota is present in the outer mucus layer, whereas there is no microbiota in the inner mucus layer. Separation of microbiota from the intestinal epithelial cells contributes to prevention of intestinal inflammation. However, the precise mechanisms by which the inner mucus layer is free of microbiota in the colon remained unknown. We identified that Lypd8, which is secreted from the colonic epithelial cells, mediates the separation of intestinal microbiota and the host by binding to highly motile flagellated bacteria. In the intestine, there exist other types of microorganisms, such as viruses and fungi. Fungi inhabit in the intestinal lumen, like intestinal bacteria. We analyzed how intestinal bacteria and fungi interact with each other for their efficient colonization. We will discuss the transkingdom interaction of bacteria and fungi in the intestine.

S3-3

Virome analysis in intestine

Satoshi Uematsu
Dept, Genome Immunol., Med., Osaka City Univ., Lab. Innate Immune Regulation, IMS, Univ. of Tokyo

Our intestinal tract carries a lot of bacteria in the lumen as the resident microorganisms. In addition to resident bacteria, viruses are present in our intestinal tract, most of which are bacteriophages. However, it is still unclear what kind of bacteriophages exist in our intestine and what kind of bacteria they infect with. As one of the reasons, isolation of viral nucleic acids and preparation of libraries have not been established yet. Since conserved sequences such as 16S rRNA gene do not exist in viruses, whole genome shotgun analysis is necessary. Even if comprehensive whole genome analysis of intestinal viruses were performed, most of the sequence fragments couldn't be classified by homology search due to the insufficient public databases. Thus, virome analysis is relatively difficult compared with bacteriome analysis and this situation is expressed by the word, viral dark matter. Here, we will outline the virome analysis pipeline constructed at our center. We will also explain the intestinal bacteriophages analyzed by this pipeline in details and host-parasite association identified based on the shotgun sequencing data of the bacterial flora and viral plexus.

S3-4

Commensal bacteria that can induce CD8 T cells and cancer immunity

Takeshi Tanoue
MicroBiol. & Immunol., Keio Univ., Sch. Med., Gut Homeostasis, RIKEN., IMS.

Co-author：Koji Atarashi、Kenya Honda
MicroBiol. & Immunol., Keio Univ., Sch. Med., Gut Homeostasis, RIKEN., IMS.

There are mounting evidences to support that the gut microbiota is essential role for various physiologies in host. The control of the microbiota may be a promising approach to regulate disease and health condition. From fecal samples of a healthy human volunteer, we identified and isolated a defined consortium of commensal bacterial strains which can strongly induce CD8 T cells. In particular, oral administrations of the bacterial cocktail to germ-free mice resulted in the accumulation of interferon-gamma-producing CD8 T cells and enhanced the therapeutic effect by immune-checkpoint inhibitor in subcutaneous tumor model of mice. These bacterial strains may be applicable for regulating immunological condition of human host.

▲TOP

S3-5

The role of gastric microbiome in gastric carcinogenesis

Yoku Hayakawa
The Univ. of Tokyo, Dept. Gastroenterology

Co-author：Ryota Niikura[1]、Kazuhiko Koike[2]
[1]The Univ. of Tokyo, Dept. Gastroenterology、[2]Dept. Gastroenterology, The Univ. of Tokyo

Gastric cancers arise from inflamed stomach that is infected with Helicobacter pylori. Since H. pylori eradication therapy was established, gastric cancer incidence appears to be decreased, but not to be eliminated, probably because long-term H. pylori infection causes irreversible genetic and histopathological alterations in the stomach. One of the major risk factor for gastric cancer after H. pylori eradication is the high degree of gastric atrophy and intestinal metaplasia. We and others recently reported that sustained use of proton pump inhibitors may increase gastric cancer risk after H. pylori eradication. Several potential mechanisms have been proposed; long-term PPI use leads to strong acid suppression, resulting in sustained hypergastrinemia and bacterial overgrowth in gastric mucosa. We investigate serum gastrin levels and the profile of gastric microbiome in patients with chronic gastritis, and explore their effects on gastric cancer. We also use mouse gastritis models and analyze the impact of gastric microbiome on gastric carcinogenesis, particularly from the aspect of nerve-immune interaction.

S3-6

Development of meta-transcriptome analysis method and its application to meta-transcriptome map of common marmoset

Yasubumi Sakakibara
Dept. BioSci. Info., Keio Univ.

To understand the bacterial flora and its relationship with cancer disease in the intestine, the aim of our study is to construct a meta-transcriptome analysis method based on the metagenome assembly and create a common marmoset meta-transcriptome map. First, we accomplish a highly accurate metagenome assembly that can deeply detect bacteria with high sensitivity by developing deep-learning based metagenome assembler MetaVelvet-DL for high throughput sequencing. Next, we sequence the genomic DNA and simultaneously mRNAs using next-generation sequencer, determine the metagenome sequence with the optimized metagenome assembler MetaVelvet-DL, and mapped the RNA-seq read data onto it. The gene expression profile of the bacterial flora is thus obtained. The second objective is to focus on the gastrointestinal tract including colon that forms a resident microbial flora using common marmoset, which is a preclinical research animal belonging to the same primate as humans and closer to humans than mouse, and create a meta-transcriptome map.

▲TOP

[LS11] LS11 [English]
Immunomodulatory drugs for multiple myeloma: from bench to bedside

2018 / 9 / 27 (Thu) 11:50-12:40 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14
：Celgene KK

Junya Kuroda / Division of Hematology and Oncology, Department of Medicine, Kyoto Prefectural University of Medicine

LS11
Immunomodulatory drugs for multiple myeloma: from bench to bedside

Leif Bergsagel
Division of Hematology

No Abstract

[S7-1] S7 [English]
Progress in cancer research through RNA biology

2018 / 9 / 27 (Thu) 13:00-15:30 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Fuyuki Ishikawa / Kyoto Univ., Grad. Sch. of Biostudies、Hideshi Ishii / Med. Data Sci., Osaka Univ. Grad. Sch. Med.

RNA of higher organisms is not genetic material, as defined by a central dogma. For example, mRNA is responsible for expressing information coded on DNA. tRNA fulfills the translation, while rRNA constitutes ribosomes. ncRNA has attracted attention as functional nucleic acids. Thus, these RNA molecules are basically not reproducible beyond cell division, unless reverse transcription or retrotransposon is involved, which is reminiscent of passive functions of RNA. However, recent studies have demonstrated that RNA, through its regulation of chromatins, plays a critical role in the development of cancer, such as metastasis. Given RNA has the property of being secreted from cells in vesicles, genetic information can be transmissible between cells. Furthermore, RNA epigenome is reportedly playing a primary role in cancer, rather than follows from DNA. We here have formed a symposium centered around young researchers who are resolutely attempting to elucidate the essence of cancer in views of RNA. While we will receive the special remark, we would like to discuss the future outlook based on the interdisciplinary technologies to overcome cancer.

S7-1
The role of extracellular RNAs in ovarian cancer

Akira Yokoi
Dept. Gyne. Onco. & Repro. Med., MD anderson Cancer Ctr., Dept. Mol. Cell. Med., Natl. Cancer Ctr. Res. Inst., Dept. Obst. & Gyne. Univ. Nagoya, Sch. Med.

Co-author：Yusuke Yoshioka[1]、Yusuke Yamamoto[1]、Juntaro Matsuzaki[1]、Tomoyasu Kato[2]、Ken Kato[3]、Hiroaki Kajiyama[2]、Fumitaka Kikkawa[2]、Takahiro Ochiya[1]
[1]Dept. Mol. Cell. Med., Natl. Cancer Ctr. Res. Inst.、[2]Dept. Obst. & Gyne. Univ. Nagoya, Sch. Med.、[3]Dept. Gastrointest. Med. Oncol., Natl. Cancer Ctr. Hosp.

Ovarian cancer has been the leading cause of gynecologic cancer mortality and its dismal outcomes are mainly due to high metastatic capability and late-stage diagnosis. We have investigated to identify the underlying molecular mechanisms of peritoneal dissemination, and to detect novel biomarkers, focusing on extracellular RNAs, related to extracellular vesicles (EVs) including exosomes. Recent evidence has demonstrated that cancer cells secrete EVs to both proximal surrounding cells and distal sites, thereby enabling the development of cancer microenvironment that in turn promotes cancer metastasis. Ovarian cancer cells aggressively migrate into the peritoneal cavity, and ascites provide a favorable environment for wide dissemination. Recently we reported new functions of EVs carrying MMP1 mRNAs derived from ovarian cancer cells remarkably inducing metastatic behavior, and these findings could provide a new therapeutic strategy to prevent the metastatic process. Furthermore, we also obtained the evidence that extracellular miRNAs in serum can serve as new diagnostic biomarkers. Investigations of extracellular RNAs can shed the light on novel mechanism of ovarian cancer malignancy.

▲TOP

S7-2

Development of anticancer agents targeting long non-coding RNA

Keisuke Katsushima
Div. Cancer Biol., Nagoya Univ. Grad. Sch. Med.

Co-author：Atsushi Natsume[1]、Fumiharu Ohka[1]、Keiko Shinjo[2]、Tatsuhiro Shibata[3]、Kanjiro Miyata[4]、Kazunori Kataoka[5]、Yutaka Kondo[2]
[1]Dept. NeuroSurg., Nagoya Univ. Sch. Med.、[2]Div. Cancer Biol., Nagoya Univ. Grad. Sch. Med.、[3]Div. Cancer Genomics, Natl. Cancer Ctr. Res. Inst., Hum. Genome Ctr., Inst. of Med. Sci., Tokyo Univ.、[4]Dept. Mater. Eng., Grad. Sch. Eng., Univ. Tokyo、[5]iCONM

Accumulating studies have proven that long non-coding RNAs (lncRNAs) are essential epigenetic regulators with critical roles in tumorigenesis. However, the accurate mechanisms of many lncRNAs are still under investigation. We recently showed that a lncRNA, taurine upregulated gene 1 (TUG1) , which was highly expressed in glioblastoma. TUG1 plays an oncogenic role by antagonizing miRNA in the cytoplasm and recruiting polycomb to repress neuronal differentiation-associated genes in the nucleus. Inhibition of TUG1 induces glioma cell differentiation and efficiently represses glioma cell growth both in vitro and in vivo. Furthermore, intravenous treatment with antisense oligonucleotides targeting TUG1 coupled with drug delivery system (TUG1-DDS) efficiently impaired tumorigenesis. Our results highlight the importance of TUG1 in regulating glioma cell proliferation and provide a novel strategy for the prevention of glioblastoma.

S7-3

Significance and application of epitranscriptome in cancer

Masamitsu Konno
CoMIT, Osaka Univ. Grad. Sch. Med.

Co-author：Taroh Satoh[1]、Yuichiro Doki[2]、Masaki Mori[2]、Hideshi Ishii[1]
[1]CoMIT, Osaka Univ. Grad. Sch. Med.、[2]Dept. Gastro. Surg., Osaka Univ. Grad. Sch. Med.

Cancer is a genetic disease, while the significance of epitranscriptome (ETR) modification of RNA in cancer has been emerged with unexpected level. Recent studies have indicated abundant ETR modifications occur and that ETR plays a role in RNA function such as translation of proteins; nevertheless, the significance in cancer remains to be understood perfectly, i.e., how ETR is involved in cancer development, which alterations in ETR pathway associate with cancer malignancy, and whether ETR information may be useful for cancer profiling and detection. Here we studied pancreatic cancer model of transgenic animals with RNA methyltransferase, and found that it functioned as a writer in collaboration with other erasers and readers, demonstrating its critical role in cancer development and metastasis. We then developed the technology of MS based measurement for ETR methylation, and confirmed the results. In addition, we studied the RNA methylation in patients with gastrointestinal cancer and found methylation of small RNAs was reduced after surgical resections of tumors. The present study indicates that information of ETR may be useful for precise detection and diagnosis of cancer.

S7-4

Bioinformatics for Single-Cell Transcriptomic Analysis of Tumor Heterogeneity

Teppei Shimamura
Div. Systems Biol., Nagoya Univ. Grad. Sch. Med.

The recent development in high-throughput single-cell RNA sequencing enables us to isolate or trap a single cell of interest from a cell population and permits genome-wide gene expression measurement at the single-cell level. It makes it possible to analyze the diversity of tumors (tumor heterogeneity) based on individual cells that make up a population from the conventional analysis of the diversity of tumors based on the average profile of a population. In this presentation, we introduce a bioinformatics approach to understand a tumor ecosystem from enormous and heterologous single cell transcriptome data generated by single-cell RNA sequencing.

▲TOP

S7-5

Size-regulated siRNA carriers from small complex loading single siRNA molecule for systemic delivery to tumor tissue

Hiroyasu Takemoto
Inst. Innov. Res., Tokyo Tech.

Co-author：Kanjiro Miyata[1]、Nobuhiro Nishiyama[2]、Kazunori Kataoka[3]
[1]Dept. Eng., Univ. Tokyo、[2]Inst. Innov. Res., Tokyo Tech.、[3]Innov. Ctr. NanoMed.

Small RNA molecules, including siRNA, have attracted much interest as a potential drug for cancer. For systemic delivery of siRNA (~5 nm) to solid tumor tissues, the carrier with regulated size is necessary for circumvention of renal clearance (>10 nm) and for tumor-selective accumulation (<50 nm). To this end, we constructed a nanoarchitecture from siRNA-loaded unimer polyion complex (uPIC, ~6 nm) and a template nanoparticle, for enhanced tumor accumulation of siRNA and associated gene silencing within the tissue.Firstly, block copolymer of PEG and polylysine (PEG-PLys) having thiol moiety at the PLys terminus was synthesized. Herein, the cationic charges in PLys segment was tuned at 40 for charge-matched uPIC formation with siRNA (40 anionic charges). uPIC with thiol moiety was decorated onto gold nanoparticle (AuN), in order to obtain the nanoarchitecture (uPIC-AuN). uPIC-AuN had the hydrodynamic diameter of ~40 nm with narrow size distribution. Finally, uPIC-AuN exhibited 10-fold higher tumor accumulation of siRNA, relative to liberated siRNA, after intravenously administration for tumor-bearing mouse, leading to effective gene silencing activity in the tumor cells.

S7-6

Acceleration of RNA biology-targeting drug discovery in cancer using cell-free technology

Hiroyuki Takeda
PROS, Ehime Univ.

Co-author：Tatsuya Sawasaki
PROS, Ehime Univ.

The control of oncoprotein expression is one of the promising strategies in anticancer therapeutics. RNA is a key intermediate between DNA and protein in central dogma. Therefore, RNA biology in cancer cells can provide a breakthrough in therapeutics and drug discovery of cancer. Transcription and surrounding physiological phenomena in cancer are attracting attention, such as transcriptome, post-transcriptional RNA modifications, and RNA editing enzymes.
To promote target-based therapeutics and drug discovery that targets cancer and RNA, in-vitro translation system can be a promising biochemical approach. We have developed wheat cell-free protein synthesis system and applied it to various drug discovery. We adopted technologies such as cDNA libraries, high-throughput assay, chemical libraries, and fused them with cell-free system. In this talk, we will introduce our recent challenges such as 24,000-human protein array and protein-protein or protein-nucleotide interaction-based chemical screening, and their applications to RNA biology-targeting researches, aiming at diagnostics and therapeutics in cancer.

S7-Special_Remarks

Special Remarks

Satoshi Inoue
Tokyo Metropolitan Inst. of Gerontology

No Abstract

▲TOP

[S8-1] S8 [English]
Medical achievements of nucleotide analogs in cancer treatment

2018 / 9 / 27 (Thu) 15:30-18:00 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Satoshi Obika / Grd. Sch. Pharm. Sci., Osaka Univ.、Nobuhiro Nishiyama / Lab. Chem. Life Sci., Tokyo. Tech.

Recently the epoch-making nucleic acid drugs, Eteplirsen for Duchenne muscular dystrophy and Nusinersen for spinal muscular atrophy have emerged on the market and the nucleic acid medicine is becoming the center of attraction. The drugs are vigorously being developed in the world, and more than one hundred international clinical trials are currently in progress. A third studies treat cancers and great expectation rises for creation of innovative drugs. In development of nucleic acid drugs, there are several key points including molecular targets, proper design of antisense oligo (ASO), siRNA or miRNA, the chemical modification, and the delivery techniques that enable cancer site-specific delivery of nucleic acid drugs. In this session, the leading presenters will introduce the individual state-of-the-art technology or its combination to realize the efficient cancer therapy. Cross-cutting collaboration is indispensable beyond the different scientific fields of medicine, pharmacy and engineering. We hope that many collaborations start here through discussion and a new encounter and expect that they leads to creation of nucleic acid drugs for revolutionary cancer therapy.

S8-1
Development of a DNA/RNA vaccine platform based on the intracellular environment-responsive lipid-like material

Hidetaka Akita
Grad. Sch. Pharm. Sci., Chiba Univ.

For the successful DNA/RNA vaccine development, promising carrier to stabilize the antigen-encoding DNA/RNA in vivo, and release them to the cytoplasm is necessary. As one is the idea to accomplish it, we recently reported on the intracellular environment-responsive material (SS-cleavable and pH-activated lipid like materials: ssPalm). In this material, dual sensing motifs: positively charging unit (tertiary amines) in response to an acidic compartment (endosome/lysosome) for membrane destabilization, and cleavable unit (disulfide bonding) that can trigger the collapse in response to the reductive environment (cytosol). Subcutaneous administration of DNA-encapsulating lipid nanoparticle (LNP) (approximately 1 μg DNA) prepared with vitamin E-scaffold (ssPalmE) conferred the strong activation of cytotoxic immune response. The migration of the LNP to the lymph node was also confirmed. Currently, we are challenging to apply this material for the development of RNA vaccine. Also, chemical modification of the ssPalm for the acceleration of the cytoplasmic delivery of RNA is ongoing. These process will be also presented.

▲TOP

S8-2

Distinct usage of sCA as EPR enhancer

Hirofumi Yamamoto
Dept. Mol. Pathol., Health & Sci., Grad. Sch. Med., Osaka Univ., Dept. Gastroenterological Surg., Grad. Sch. Med., Osaka Univ.

Co-author：Masaki Mori
Dept. Gastroenterol. Surg. Osaka Univ. Grad. Sch. Med.

Super carbonate apatite (sCA) nanoparticle is an efficient in vivo DDS for miRNA and siRNA. We reported that sCA delivered efficiently siRNA for survivin and PTBP as well as miR-340 (against liver metastasis), miR-4689 (KRAS mutated colon cancer), miR-302 and miR-369 (cancer reprograming), and miR-29b-1-5p (pancreatic cancer) and the RNA function was fully realized in every case. Moreover, sCA exhibited the superb efficacy in augmentation of influenza vaccination with CpG nucleic acid and in inhibiting inflammatory bowel disease with miR-29s. These innovative findings are based on the unique ability of sCA in enhancement of EPR effect and reduction of tumor stromal pressure, which help various substances in blood circulation to be accumulated into the tumor. In other words, sCA alone can collect the anti-tumor drugs to the tumors without incorporating them. We here show the distinct usage of sCA, which serves to collect chemo-agents (doxorubicin, oxaliplatin), polymer conjugate chemo-agent (P-THP), liposome-microRNA complex into the tumors, and provides application to minimally invasive treatment of photodynamic and radio wave therapy.

S8-3

Novel strategy of pancreatic cancer treatment: gapmer antisense oligonucleotides against nucleolar noncoding SNORA23 RNA

Kenji Nakano
Japanese Red Cross Society, Fukuoka Red Cross Blood Ctr.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive solid malignancies. Small nucleolar noncoding RNAs (snoRNAs) play a housekeeping role in ribosome biogenesis, and their aberrant expressions were recently reported in some cancers. Here, we demonstrate SNORA23, one of snoRNAs, is overexpressed in PDAC, which correlates with the pathological invasion grade and inversely correlates with survival of PDAC patients. In vitro gain- and loss-of-function experiments indicated that SNORA23 contributes to the invasion and anchor-independent survival of PDAC cells partly via spectrin repeat-containing nuclear envelope 2 signalling. To examine whether SNORA23 is a potential therapeutic target, we generated a SNORA23-targeted antisense oligonucleotide (ASO) flanked with amido-bridged nucleic acids that exhibit high nuclease resistance. Administration of the SNORA23-targeted ASO suppressed the dissemination and liver metastasis of highly metastatic PDAC tumours in mice. These results indicate that SNORA23 is a promising target to control the PDAC's aggressiveness and gapmer-type ASOs targeting aberrant snoRNAs may open the way for intractable cancer treatment.

S8-4

Development of therapeutic antisense oligonucleotide against small cell lung cancer

Masahito Shimojo
Bioorganic Chem., Grad. Sch. Pharm. Sci., Osaka Univ.

Small cell lung cancer (SCLC) is one of the deadliest human lung cancers. SCLC is a high-grade neuroendocrine carcinoma, which associates with tobacco smoking and air pollution. SCLC spreads quickly and widely, the risk of death is tremendously high. Effective treatment and early diagnosis are needed to combat this aggressive cancer. Ser/Arg repetitive matrix 4 (SRRM4)(also known as nSR100), a splicing activator, is abnormally expressed at high levels in SCLC and thus is a potential therapeutic target of SCLC. SRRM4 activates splicing of a tumor suppressor, the repressor element 1-silencing transcription factor (REST) to a truncated form of known as sREST. The neuroendocrine to non-neuroendocrine fate mediated by REST is reversed by sREST. Here we report that targeting of SRRM4 mRNA using novel antisense oligonucleotides (ASO) causes repression of SRRM4 synthesis leading to SCLC apoptosis. In addition, the specific miR-4516 was shown to be incorporated into exosomes in the blood of SCLC patients and serves as a novel prognostic for SCLC. These data thus establish that a gapmer ASO against SRRM4 represents a novel therapeutic medicine for SCLC.

▲TOP

S8-5

Development of therapeutic approaches based on the tumor suppressor microRNA-27b for breast cancer patients

Ryou-u Takahashi
Dept. Cell. Mol. Biol., Grad. Sch. Biomed. Health, Hiroshima Univ., Div. Mol. Cell. Med., Natl. Cancer Ctr. Res. Inst.

Co-author：Takahiro Ochiya[1]、Hiroyasu Takemoto[2]、Nobuhiro Nishiyama[2]
[1]Div. Mol. Cell. Med., Natl. Cancer Ctr. Res. Inst.、[2]Lab. for Chem. Life. Sci., Tokyo Tech.

Drug resistance is one of the major clinical obstacles to successful treatment for cancer patients. Accumulating lines of evidence demonstrated the involvement of microRNA in the various aspects of tumor malignancies including the resistance to chemotherapy. In the current study, we identified microRNA-27b (miR-27b) as a key regulator of cancer stem cell (CSC) properties such as tumorigenicity and drug resistance. In luminal type breast cancer, miR-27b suppressed the acquisition of chemo-resistance via inhibiting the generation of side-population that shows the CSC properties. miR-27b reduced the side-population through the direct target of ectonucleotide pyrophosphatase 1 which is associated with the development of type II diabetes. After these findings, we have started the development of therapeutic approaches using miR-27b mimic and observed that miR-27b mimic with the docetaxel treatment significantly reduced the tumor growth in the mouse model of luminal type breast cancer. Therefore, our study suggests that conventional chemotherapy with modulating miR-27b expression by RNA-based treatments might improve the therapeutic outcomes of breast cancer patients.

S8-6

Enhanced anti-cancer activity of microRNA by chemical modification for clinical use

Yukihiro Akao
Uni. Grad. Sch., Drug., Med. Info. Sci., Gifu Univ.

Co-author：Kazuki Heishima
United Grad. Sch., Drug Dis. Med. Inf., Gifu Univ.

Much evidence indicates that miRNAs are involved in human carcinogenesis as tumor suppressors or oncogenes. Previously, we demonstrated that the expression of miR-143 and -145 was severely down-regulated in the majority of human cancer cell lines and particularly in colon cancers and gastric cancers. Moreover, the growth of human colon or gastric cancer cells expressing miR-143 and -145 at low levels was significantly inhibited by their ectopic expression (replacement treatment of anti-oncomir). For clinical application, we added aromatic benzene-pyridine (BP-type) analogs to the 3'overhang region of the RNA-strand and changed the sequences of the passenger strand in the miRNA duplex, leading to greater activity and increased resistance to nuclease (Mol. Cancer Ther. 2010). Recently, we have developed a novel chemically-modified miR-143 (Syn-miR-143#12) that acquired nuclease-resistance and enabled us to better understand the K-Ras signaling networks. The Syn-miR-143#12 impaired the K-Ras signaling networks efficiently through silencing not only K-Ras but also RAS-effector signaling molecules Erk and Akt, and Ras-GDP exchange factor Sos1 (Cancer Sci. 2018).

S08-Special_Remarks

Special Remarks

Hiroyuki Konno
Hamamatsu Med. Univ., Sch. Med.

No Abstract

▲TOP

[S4-1] S4 [English]
Cancer invasion and metastasis - A summary and the future

2018 / 9 / 27 (Thu) 9:00-11:30 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15

Nariaki Matsuura / Osaka InterNatl. Cancer Inst.、Yoshinori Murakami / Div. Mol. Pathol., Inst. Med. Sci., the Univ. of Tokyo

Since the essential characteristic of cancer is its metastatic potential, one of the most important challenges for cancer research has been to explore the mechanisms of cancer metastasis and to control them. In 1990s, thanks to advances and prevalence of experimental procedures in molecular biology, many researchers started to investigate cancer metastasis and its closely related pathological phenomenon, cancer invasion, thus making great contributions in this research field. However, we have a long way to go to translate the outcome of basic research on metastasis to clinical application because it is essentially difficult to design clinical trials for the assessment and verification of metastasis suppression in human patients suffering from early- or advanced-stages of cancer. Recently, the main field of cancer research has been gradually shifted to drug development research, including molecular targeting drugs, and many anticancer agents are being discovered which lead to the prognostic progress of cancer patients. Since most successful targets developed so far are molecules involved in direct cell growth and its oncogenic signaling, research on cancer metastasis itself has resulted in fewer innovations in these past 10 years. However, there still remain a number of subjects to be elucidated upon concerning the mechanisms of cancer metastasis and invasion. Now that a variety of innovative technologies and concepts on molecular and cellular biology and genetics have been generated, we will discuss incoming cancer metastatic research from the new perspective of cancer biology.

S4-1
Involvement of a cell adhesion molecule, CADM1, in cancer invasion and metastasis

Yoshinori Murakami
Div. Mol. Pathol, Inst. of Med. Sci., Univ. Tokyo

Co-author：Toko Funaki[1]、Yumi Tsuboi[1]、Zenichi Tanei[2]、Daisuke Matsubara[3]、Motoi Ohba[4]、Takeshi Ito[5]
[1]Div., Mol. Pathol., Inst., Med. Sci., Univ. Tokyo、[2]Dept. Pathol., Tokyo Metropol. Geriatric Med. Ctr.、[3]Div., Mol. Pathol., Inst., Med. Sci., Univ. Tokyo, Diept. Integ. Path., Jichii Med. Univ.、[4]Inst. Mol. Oncol., Showa Univ.、[5]Div. Mol. Pathol, Inst. of Med. Sci., Univ. Tokyo

Disruption of cell adhesion is a crucial step in invasion and metastasis of human cancer. A cell adhesion molecule, CADM1, is originally identified as a tumor suppressor. CADM1 is involved in formation and maintenance of an epithelial cell structure, whereas it is frequently inactivated in various cancers in their advanced stages. Here, we demonstrate that CADM1 inhibits HGF-MET-induced epithelial mesenchymal transition of MDCK cells by associating with MET on the cell membrane. CADM1 also suppresses SRC signaling by associating with c-Src binding protein (CBP) on the membrane. Intervention of growth factor signaling by cell adhesion molecules, such as CADM1, on the cell membrane could provide a novel approach to suppress oncogenic signals in cancer cells. By contrast, CADM1 is overexpressed in small cell lung cancer (SCLC) and enhances malignant features of SCLC cells by associating with distinct cytoplasmic proteins involved in cell motility. Moreover, expression of CADM1 variant on the membrane and its cleavage by proteases could provide a promising candidate for diagnosis of SCLC. Context-dependent functions of cell adhesion molecules in tumor progression will be discussed.

▲TOP

S4-2

The role of interaction between growth factor and integrin in cancer progression

Seiji Mori
Facul. Health. Sci. Morinomiya Univ. Med. Sci., Dept. Mol. Pathol., Health & Sci., Grad. Sch. Med., Osaka Univ.

Co-author：Hirofumi Yamamoto[1]、Nariaki Matsuura[2]、Yoshikazu Takada[3]
[1]Dept. Mol. Pathol., Health & Sci., Grad. Sch. Med., Osaka Univ.、[2]Osaka InterNatl. Cancer Ctr.、[3]Dept. Derm. UCDavis Med. ctr.

Integrins play a key role in cell invasion and metastasis, not only for tethering cells to the matrix, but also for receiving and transducing signals. Epithelial-to-mesenchymal transition (EMT) is critical for cancer progression, and is regulated by growth factors such as transforming growth factor β (TGF-β) and fibroblast growth factors (FGFs). We have proven that FGF1and 2 directly bind to integrin αvβ3 and the interaction was required for cell proliferation and migration. However, the relation between FGFs and integrin in EMT has not been cleared. We studied the role of integrin αv β3 on TGF-β-induced EMT. We demonstrated that FGFs augmented EMT induced by TGF-β1 in mammary epithelial cells. Hence, we studied if the enhancing effect of FGFs on TGF-β1-induced EMT requires enhanced levels of integrin αvβ3 expression. Knockdown of β3 suppressed the enhancement by FGFs of TGF-β1-induced EMT. Integrin-binding defective FGF mutants did not augment TGF-β1-induced EMT. These findings imply that integrin αv β3 expression is a critical component for FGFs to regulate TGF-β1-induced EMT in mammary epithelial cells. This represents a new model of growth factor-integrin crosstalk in EMT.

S4-3

Phosphorylation of kindlin-2 regulates invadopodia formation and cancer cell invasion

Ge Liu
Dept. Biochem. & Mol. Biol., Sch. Med., SZU

Ge Liu, Yantao Bao, Xiaopeng Lu, Qian Zhu, Wei-Guo Zhu

Metastasis is the major cause of cancer morbidity and mortality, and responsible for about 90% of cancer deaths. Invadopodia are critical structures utilized by cancer cells to degrade extracellular matrix during metastatic process. Hence, blocking invadopodia formation can effectively inhibit cancer metastasis. However, the mechanisms underlying regulation of invadopodia formation are not fully understood. We found that the cell adhesion protein kindlin-2 localizes to invadopodia along with Tks5. IKKε-mediated kindlin-2 phosphorylation at S159 affects its stability on the plasma membrane and is involved in inadopodia formation. Overexpression of kindlin-2 phosphorylation mimic mutant (S159D) results in increased invasion ability of cancer cells and transcription of matrix metalloprotein (MMP). Our study defines a previously unknown function for IKKε in formation of invadopodia, and reveals the role of kindlin-2 phosphorylation at S159 in regulation of invadopodia formation and cancer cells metastasis.

S4-4

EphA2 proteolysis converts it from a tumor suppressor to an oncoprotein

Naohiko Koshikawa
Kanagawa Cancer Ctr. Res. Inst.

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EphA2 is considered a candidate therapeutic target in cancer but it can exert opposing effects on cell growth. In the presence of its ligands, EphA2 suppresses ErbB signaling, whereas EphA2 ligand-independent activation augments ErbB signaling. To deploy EphA2 targeting drugs effectively in tumors, the anti-oncogenic ligand-dependent activation state of EphA2 must be discriminated from its oncogenic ligand-independent state. Since the molecular basis for the latter is little understood, we embarked how the activation state of EphA2 can be switched in tumors. We found the EphA2 ligand-binding domain is cleaved frequently by MT1-MMP. EphA2 immunostaining revealed a significant loss of the N-terminal portion in tumor areas that expressed MT1-MMP. Moreover, EphA2 ligand-independent activation were observed specifically in these tumor areas. Mechanistic experiments revealed that EphA2 cleavage promoted tumor cell growth and migration by ErbB. Conversely, expression of a proteolysis-resistant EphA2 mutant prevented tumorigenesis and metastasis. Overall, we showed how the EphA2 proteolysis in tumors determines their effector function and could serve as a biomarker for targeted therapy.

▲TOP

S4-5

Alveolar macrophages drive lung metastasis in cooperation with interstitial macrophages by generating leukotriene B4

Takuto Nosaka
2nd Dept. Int., Univ. of Fukui., Cancer Res. Inst., Kanazawa Univ.

Co-author：Tomohisa Baba[1]、Yamato Tanabe[1]、Soichiro Sasaki[1]、Makoto Arita[2]、Yasunari Nakamoto[3]、Naofumi Mukaida[1]
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Macrophages can be classified into alveolar macrophages (AMs) and interstitial macrophages (IMs) in lungs. AMs can generate leukotrienes (LTs) from arachidonic acid (AA), but the roles in lung metastasis remain elusive. An i.v. injection of a mouse hepatocellular carcinoma (HCC) cell line, BNL, caused lung metastasis and comprehensive determination of AA metabolites revealed increases in LTs and prostaglandins in metastatic lungs. A 5-LOX but not a COX-2 inhibitor reduced the numbers of metastatic foci ($P<0.01$). A number of macrophages infiltrated in metastatic foci and AMs expressed a higher level of the 5-LOX, consistent with HCC patients ($P<0.05$). Intratracheal clodronate liposomes injection selectively depleted AMs, together with reduced LTB_4 content ($P=0.03$) and metastatic foci ($P<0.01$). Moreover, IMs in metastatic foci produced CCL2, thereby recruiting blood-borne, CCR2-expressing AMs into lungs; that is, AMs can be recruited under the guidance of IM-derived CCL2. Two distinct subsets of macrophages, AMs and IMs, cooperatively support the progression of metastasis. AM-derived 5-LOX and its metabolites, LTB_4, may be a novel target to treat and/or prevent metastasis to lungs.

S4-6

The contribution of tumor endothelial cells in tumor metastasis

Kyoko Hida
Vascular Biol. Mol. Pathol., Hokkaido Univ. Grad. Sch. Dent. Med.

Tumor metastasis is the primary cause of cancer-related death. It is now well known that bidirectional interaction between tumor cells and their microenvironment is essential for tumor progression and metastasis. Tumor angiogenesis is a requirement for tumor progression.Tumor endothelial cells (TECs), which line tumor blood vessels, exhibit several altered phenotypes compared with those of their normal counterparts. During intravasation, tumor cells physically contact tumor endothelial cells and interact with them by juxtacrine and paracrine signaling. We have previously reported that TEC derived biglycan enhanced tumor metastasis via increasing tumor intravasation. Biglycan expression was induced in endothelial cells by several tumor-derived factors. Furthermore, we recently found that TECs acquire drug resistance via upregulation of drug transporter, suggesting that TECs can provide the route for metastasis for cancer cells during chemotherapy. TEC phenotypic alteration was induced by tumor secreting factors. These results suggested that TECs contribute to tumor invasion and metastasis. Targeting TEC - tumor cell interaction may be beneficial in antimetastasis approaches.

▲TOP

[LS12] LS12 [Japanese]
Toward development of combination cancer immunotherapy

2018 / 9 / 27 (Thu) 11:50-12:40 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15
：ONO PHARMACEUTICAL CO., LTD. / Bristol-Myers Squibb K.K.

Eishi Baba / Department of Comprehensive Clinical Oncology Faculty of Medical Sciences,Kyushu University

LS12

Toward development of combination cancer immunotherapy

Yutaka Kawakami
Division of Cellular Signaling, Institute for Advanced Medical Research, Keio University School of Medicine

No Abstract

▲TOP

[YIA-1] YIA [Japanese]
The Young Investigator Awards Lecture

2018 / 9 / 27 (Thu) 13:00-15:30 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15

Wataru Yasui / Dept. Mol. Pathol., Hiroshima Univ. Gradatesch. Biomed. Sci.

YIA-1

Anti-tumor effect of RUNX cluster regulation by genetic switch

Ken Morita
DFCI, HMS

Runt-related transcription factor 1 (RUNX1) has been generally considered as a tumor suppressor, but a growing body of evidence strongly suggests its pro-oncogenic property in acute myeloid leukemia (AML). We demonstrate that anti-tumor effect elicited by RUNX1 silencing is compensated by the other RUNX family members such as RUNX2 and RUNX3, and a simultaneous attenuation of whole RUNX family members as a cluster displays superior anti-tumor effect to the individual suppression of family members in AML cells. Notably, switching off RUNX cluster using the novel alkylating agent-conjugated pyrrole-imidazole (PI) polyamides, which specifically binds to the consensus RUNX-binding sequences, is highly effective against leukemia as well as several dismal-prognostic solid tumors in vivo without any significant adverse events. These results collectively indicate the crucial role of RUNX cluster in the maintenance and the progression of cancer cells, and the indicated genetic switch technology-dependent its modulation would be a novel strategy to control malignancies in general.

▲TOP

YIA-2

Identification of Drug Targets and Molecular Mechanisms to Prevent Drug Resistance of Cancer Cells

Reiko Satow
Lab. of Genome, Tokyo Univ. of Pharm. & Life Sci., AMED-CREST

Co-author：Kiyoko Fukami
Lab. of Genome, Tokyo Univ. of Pharm. & Life Sci., AMED-CREST

To elucidate mechanisms of drug resistance could extend the efficacy of cancer therapy. Our recent screening of neural crest genes identified ZIC5 (Zic family member 5), which enhances malignant phenotypes of melanoma via PDGFD (platelet derived growth factor D) expression. In melanoma and other types of cancer cells, we revealed that knockdown of ZIC5 or PDGFD downregulated the proliferation with induction of cell death. Furthermore, ZIC5- or PDGFD-suppression diminished the amount of phosphorylated FAK (focal adhesion kinase) and STAT3 (signal transducer and activator of transcription 3), which is known to promote several cancer aggressiveness as well as drug resistance. When ZIC5 or PDGFD was suppressed, molecular targeted drugs or anti-cancer agents, such as oxaliplatin, docetaxel, or BRAF inhibitors, induce tumor cell death effectively. Because ZIC5 expression in normal human tissues is limited, ZIC5 could be a molecular target to prevent drug resistance of cancer cells. Drug library screening to identify ZIC5 inhibitors is in progress, and two candidate compounds have been obtained.

YIA-3

Emerging roles of ubiquitin-proteasome system in primary cilia dynamics

Kousuke Kasahara
Dept. Physiol., Mie Univ. Grad. Sch. Med.

Co-author：Masaki Inagaki
Dept. Physiol., Mie Univ. Grad. Sch. Med.

Primary cilia are microtubule-based sensory organelles that organize numerous key signals during developments and tissue homeostasis. Ciliogenesis is generally inhibited in dividing cells and induced upon exposure to cell cycle exit signals, such as serum starvation, however, it has been unclear which signaling cascades regulate the phenomenon. In this study, we reveal that EGF receptor (EGFR) kinase suppresses ciliogenesis by directly phosphorylating USP8 deubiquitinase. This phosphorylation activates USP8, which protects trichoplein, a negative regulator of ciliogenesis, from ubiquitin-proteasome-mediated proteolysis. Thus, serum starvation inactivates the EGFR-USP8-trichoplein pathway and results in ciliogenesis. These data provide first evidences that EGFR signal and ubiquitin-proteasome machinery control ciliogenesis. We further find that ciliogenesis itself functions to block cell proliferation. Down-regulation of the inhibitory systems for ciliogenesis, including EGFR-USP8-trichoplein pathway, induces forced ciliogenesis even in growing condition, thereby leading to cell cycle arrest, suggesting therapeutic potential for targeting the primary cilia dynamics in cancer.

YIA-4

Genetic alterations in adult T cell leukemia/lymphoma

Yasunobu Nagata
Cleveland Clinic

Adult T-cell leukemia/lymphoma (ATLL) is a distinct form of peripheral T-cell lymphoma (PTCL) with poor prognosis which is caused by human T-lymphotropic virus type 1 (HTLV-1). The role of acquired mutations in HTLV-1 infected T-cells has not been fully elucidated. We identified unique RHOA mutations in ATLL, which showed distinct distribution and function from those found in other cancers. Involving 15% (30/203) of ATLL cases, RHOA mutations were almost invariably located at the GTP-binding pocket, with Cys16Arg being most frequently observed. Unexpectedly, depending on mutation types and positions, these RHOA mutants showed different or even opposite functional consequences in terms of GTP/GDP-binding kinetics, regulation of actin fibers, and transcriptional activation. The Gly17Val mutant did not bind GTP and act as a dominant negative manner, whereas Cys16Arg and Ala161Pro showed fast GTP cycling with enhanced transcriptional activation. Thus, both loss- and gain-of-RHOA functions could be involved in ATLL leukemogenesis. Our study highlights not only the molecular pathogenesis of ATLL but also a unique role of variegation of heterologous RHOA mutations in human cancers.

▲TOP

YIA-5

Identification and functional analysis of novel Non-Small Cell Lung Cancer related genes

Yasuyuki Hosono
Aichi Cancer Ctr. Res. Inst., Div. Mol. Therap.

Lung cancer is the leading cause of cancer death in most of the developed countries. Non-Small Cell Lung Cancer (NSCLC) that accounts for about 80% of all lung cancers, is one of the refractory cancers that exhibit resistance to both chemo and radiation therapies. My studies thus far are collectively aimed at understanding the oncogenic mechanism that operates in NSCLC, to expand therapeutic options. For instance, I characterized several downstream targets of TTF1, lineage-survival oncogene in NSCLC. Here I identified MYBPH as a downstream transcriptional target of TTF, which was also subjected to epigenetic regulation. In addition I showed that MYBPH exerts metastasis suppressive function by directly binding to ROCK1 and NMHC IIA. In other projects, I have also explored the noncoding portion of the genome, where I characterized the role of several miRNAs and long noncoding RNAs in cancer. For example, in my recent studies, we identified a novel highly conserved cancer testis lncRNA by analyzing a large compendium of RNA-sequencing data set and named it THOR. Importantly, we showed that THOR regulates cancer development and progression using cell lines, zebrafish and murine models.

YIA-6

A method of high-throughput functional evaluation of EGFR gene variants of unknown significance in cancer

Shinji Kohsaka
Div. Cell. Signaling, Natl. Cancer Ctr. Res. Inst.

Numerous variants of unknown significance (VUS) have been identified through large-scale cancer genome projects, although their functional relevance remains uninvestigated. We developed a mixed-all-nominated-mutants-in-one (MANO) method to evaluate the transforming potential and drug sensitivity of oncogene VUS in a high-throughput manner and applied this method to 101 nonsynonymous epidermal growth factor receptor (EGFR) mutants. We discovered a number of mutations conferring resistance to EGFR tyrosine kinase inhibitors (TKIs), including gefitinib- and erlotinib-insensitive missense mutations within exon 19 and other gefitinib-resistant mutations, such as L833V, A839T, V851I, A871T, and G873E. L858R-positive tumors (12.8%) harbored compound mutations primarily in the cis allele, which decreased the gefitinib sensitivity of these tumors. Thus, these data support the importance of examining the clinical relevance of uncommon mutations within EGFR and of evaluating the functions of such mutations in combination. This method may become a foundation for the in vitro and in vivo assessment of variants of cancer-related genes and help customize cancer therapy for individual patients.

YIA-7

Identification of druggable oncogenic gene fusions and a novel mechanism of drug resistance in lung cancer

Takashi Nakaoku
Div. Genome Biol., Natl. Cancer Ctr. Res

Co-author：Takashi Kohno
Div. Genome Biol., Natl. Cancer Ctr. Res, Div. Translational Genomics, EPOC, Natl. Cancer Ctr.

Precision oncology based on genetic alterations is about to be implemented in clinic. For the progress of the genome-based oncology, identification of more genetic alterations and their annotations; such as drug sensitivity and resistance, would be helpful. To achieve that purpose, we performed genomic analysis on invasive mucinous lung carcinoma, and identified novel oncogenic fusion of the NRG1 gene encoding neuregulin as a novel therapeutic target (Nakaoku et al., Clin Cancer Res, 2014). We also showed the NRG1 gene fusion increases cancer stem-like property through enhancing the signaling circuit of the NF-kB-IGF2 pathway (Murakami, Nakaoku et al, Cancer Res, 2015). In addition, we identified a secondary mutation on RET kinase which were detected in a patient harboring CCDC6-RET fusion who showed resistance to RET tyrosine kinase inhibitor, vandetanib. We showed the allosteric mechanism of its resistance by biological, biochemical studies and molecular dynamics simulation analysis (Nakaoku et al., Nat Commun, 2018). Our studies provide novel druggable target and genetic annotation and advance precision oncology.

▲TOP

YIA-8

Elucidation of a proteomic contexture of exosomes for development of cancer early detection diagnostics

Koji Ueda
Personalized Can. Med., CPM Ctr., JFCR

Exosome has come to be recognized as an attractive resource of diagnostic molecular targets since it delivers molecular cargoes from original cancer cells into body fluids. To figure out specific molecular signatures of cancer cell-derived exosomes, we have recently developed high-purity exosome isolation devices, such as anti-CD9 MSIA tips (Sci Rep, 2014), Exo-Trap spin columns (Cosmo Bio), and EVSecond columns (GL Sciences) (Sci Rep, 2016). In combination with leading-edge mass spectrometry technologies, we have gained comprehensive and quantitative proteome dataset of exosomes. Especially, a proteome-wide profiling of viable renal cell carcinoma (RCC) tissue-derived exosomes identified AZU1 as a specific cargo on RCC exosomes. Importantly, exosomal AZU1 was also detectable in RCC patients' sera even from early clinical stages. Furthermore it induced intercellular tight junction collapse of vascular endothelial cells, indicating involvement in hematogenous metastasis of RCC (Int J Cancer, 2018). These findings provide novel insights regarding relationship between behaviors of exosomes and cancer biology, leading to development of novel diagnostic and therapeutic strategies.

YIA-9

Physiological Srsf2 P95H expression causes impaired hematopoietic stem cell functions and aberrant RNA splicing in mice

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Co-author：Satoshi Yamazaki[1]、Yasuhito Nannya[2]、Keisuke Kataoka[2]、Yasunori Ota[3]、Masahiro Nakagawa[2]、Kenichi Yoshida[2]、Tetsuichi Yoshizato[2]、Masashi Sanada[4]、Manabu Nakayama[5]、Haruhiko Koseki[6]、Hiromitsu Nakauchi[7]、Seishi Ogawa[2]
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Splicing factor mutations are characteristic of myelodysplastic syndromes (MDS), but their roles in the MDS pathogenesis have not fully been elucidated. We investigated the consequence of mutant Srsf2 expression using newly generated Vav1-Cre-mediated conditional knock-in mice. Srsf2 P95H mutant mice showed reduced numbers of hematopoietic stem cells (HSCs), differentiation defects and impaired long-term reconstitution of HSCs. Although the Srsf2 mutant mice did not develop MDS under the steady-state condition, when their HSCs were transplanted into lethally irradiated mice, the recipients developed anemia, leukopenia and erythroid dysplasia, suggesting the role of replicative stress in the development of an MDS-like phenotype. RNA-seq of the Srsf2 mutant HSCs revealed a number of abnormal splicing events and differentially expressed genes, including several potential targets implicated in the MDS pathogenesis. Our findings suggest that the mutant Srsf2 leads to a compromised HSC function by causing abnormal RNA splicing, contributing to the deregulated hematopoiesis that recapitulates the MDS phenotypes, possibly as a result of additional genetic and/or environmental insults.

YIA-10

Identification of genetic biomarkers to predict efficacy and adverse effect of anti-cancer drugs

Kazuma Kiyotani
Cancer Precision Med. Ctr., JFCR

Genetic factors are one of the causes of individual variability in the efficacy or adverse reactions observed in cancer patients who received anti-cancer drug therapy. To identify genetic biomarkers determining the efficacy or risk of adverse reactions, we performed gene-wide and gnome-wide association studies, identified significant associations of ABCC2 and SLCO1B3 with docetaxel-induced leukopenia/neutropenia in 39 cases and 74 controls, and 4 SNP markers with gemcitabine-induced leukopenia/neutropenia in 54 cases and 120 controls. We found that CYP2D6 as well as ABCC2, C10orf11 were significantly associated with clinical outcomes of patients with tamoxifen treatment, and that tamoxifen dose adjustment based on CYP2D6 genotype is useful to maintain the effective level of active metabolite, endoxifen. We also investigated an immunogenomic biomarker, and found the clonal T-cell expansion by evaluating TCR sequencing to predict responses to immune checkpoint inhibitors. These results suggested that these genetic/immunogenomic biomarkers would be useful for personalized therapy, which could optimize therapeutic outcome and minimize adverse effect for individual patients.

▲TOP

YIA-11

Translational research for overcoming resistance to apoptosis induced by targeted drugs in lung cancer

Shinji Takeuchi
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Co-author：Seiji Yano
Cancer Ctr., Kanazawa Univ. Hosp., Div. Med. Oncol., Cancer Res. Inst., Kanazawa Univ.

The BIM deletion polymorphism is associated with apoptosis resistance to EGFR-TKIs in NSCLC harboring EGFR mutations. We have shown that a histone deacetylase inhibitor, vorinostat, can epigenetically restore BIM function and apoptosis sensitivity to EGFR-TKIs in EGFR mutant NSCLC cells with BIM deletion polymorphisms. Based on our preclinical findings, we designed the phase 1 study named VICTORY-J to evaluate the safety of combined therapy with vorinostat and gefitinib, and to determine the maximum tolerated dose (MTD) of vorinostat combined with a fixed dose of gefitinib based on the conventional 3 + 3 design for Japanese patients with EGFR mutant NSCLC with a BIM deletion polymorphism. No DLTs were observed in 12 patients, and we determined 400 mg of vorinostat as the recommended dose for phase 2. Disease control (stable disease assessed at least 6 weeks) was achieved in 10 out of 12 (83.3 %) patients with a history of progressive disease during EGFR-TKI treatment. Combined treatment with vorinostat and gefitinib may improve outcomes in patients with EGFR mutant NSCLC with a BIM deletion polymorphism.

▲TOP

[J-1112] J10-3 [Japanese]
Invasion and metastasis (2)

2018 / 9 / 27 (Thu) 15:30-16:45 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15

Kazuki Nabeshima / Dept. Pathol., Fukuoka Univ. Sch. Med. & Hosp.

J-1112

Role of epigenetic remodeling in neutrophil-dependent metastatic dissemination of renal cancer cells

Jun Nishida
Dept. Mol. Path., Grad. Sch. Med., Univ. Tokyo

Co-author：Yusuke Tamura、Kei Takahashi、Daizo Koinuma、Shogo Ehata、Kohei Miyazono
Dept. Mol. Path., Grad. Sch. Med., Univ. Tokyo

Cancer cells in the primary tissue metastasize by motivating pro-metastatic role of myeloid cells or extracellular vesicles at the pre-metastatic site in some contexts. However, it is unclear how cancer cells acquire this phenotype through the interaction with tumor microenvironment. To clarify this, highly malignant renal cell carcinoma cell lines were established in renal microenvironment. These cancer cells exhibited the metastatic ability with neutrophil priming, which was not observed in parental cancer cells. Transcriptome analysis revealed that highly malignant cancer cells expressed increased levels of multiple transcripts related to neutrophil dynamics. ChIP-sequence analysis of acetylated histone showed that super enhancer was present near the coding regions of these transcripts in highly malignant cancer cells. DNA demethylation at the promoter region was also implicated in the regulation of another transcript. These findings suggest that interaction with tumor microenvironment causes epigenetic remodeling in cancer cells, which enables the neutrophil-dependent metastasis.

▲TOP

J-1113

Osteocyte-driven downregulation of Snail restrains effects of Drd2 inhibitors on mammary tumor cells

Kazumasa Minami
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Co-author：Masahiko Koizumi[1]、Yuhki Yokoyama[2]、Hirofumi Yamamoto[2]、Kazuhiko Ogawa[1]
[1]Dept. Radonc., Osaka Univ., Grad. Sch. Med.、[2]Dept. Mol. Path., Osaka Univ., Grad. Sch. Med.

While bone is a frequent target of breast cancer-associated metastasis, little is known about the effects of tumor-bone interactions on the efficacy of tumor-suppressing agents. Here we examined the effect of Fluphenazine (FP) and Trifluoperazine (TFP), on mammary tumor cells, osteoclasts, osteoblasts, and osteocytes. These agents suppressed proliferation and migration of mammary tumor cells chiefly by antagonizing dopamine receptor D2 and reduced bone resorption by downregulating nuclear factor of activated T-cells, cytoplasmic 1. Three-dimensional spheroid formation assays revealed that tumor cells have high affinity to osteocytes and type I collagen, and interactions with osteocytes as well as administration of FP and TFP downregulated Snail and suppressed migratory behaviors. In the bone microenvironment, osteocytes downregulated Snail and acted as an attractant as well as a stimulant to mammary tumor cells. These results demonstrate that tumor-osteocyte interactions strengthen dopamine receptor-mediated suppression of tumor migration but weaken its inhibition of tumor proliferation in the osteocyte-rich bone microenvironment.

J-1114

Identification of genes highly expressed in metastases of an orthotopic transplantation model of SCLC

Shuichi Sakamoto
Inst. Microbial Chemistry, Numazu, Microbial Chemistry Res. Foundation

Co-author：Manabu Kawada
Inst. Microbial Chemistry, Numazu, Microbial Chemistry Res. Foundation, Inst. Microbial Chemistry, Microbial Chemistry Res. Foundation

Small cell lung cancer (SCLC) is one of the most severe malignancy with early and widespread metastasis. For further studies of SCLC, we have developed a new orthotopic transplantation model with human SCLC cell line DMS273. GFP-labeled DMS273 cells formed metastatic foci in distant tissues such as bone and brain, as observed in SCLC patients (Sakamoto et al. Cancer Sci. 2015).
In this study, we evaluated the differentially expressed genes between orthotopic and metastatic tumors in the model. We isolated the tumor cells from orthotopic and metastatic sites, and then total RNAs were isolated from the cells. A mRNA microarray analysis revealed that 19 genes in the metastatic tumors showed a >4.0-fold increase ($p<0.01$) compared with the orthotopic tumors. One of these genes encodes a transmembrane protein IFITM1, and an immunohistochemical analysis confirmed the higher expression of the protein in metastatic sites than orthotopic sites. Overexpression of IFITM1 in the GFP-labeled DMS273 cells increased their metastatic formation in the orthotopic model and in an experimental metastatic model. These findings suggest that IFITM1 may promote metastatic formation in human SCLC.

J-1115

Dimethyl fumarate suppresses the tumor growth and metastasis through suppression of NF-kappaB

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<Purpose> Dimethyl fumarate (DMF) is a fumaric acid ester that is used to treat psoriasis and multiple sclerosis. Recently, DMF was found to exhibit anti-tumor effects. However, the effect of DMF on metastasis and tumor growth of metastatic melanoma remains unclear in vivo. In this study, we evaluated the effect of DMF on metastasis and tumor growth in a mouse metastatic melanoma model. <Methods> B16BL6 cells were subcutaneously injected in C57BL/6 mice. Effects of statins on signal molecules were determined by western blots. <Results> We found that DMF inhibited spontaneous metastasis and tumor growth. Furthermore, DMF suppressed the nuclear translocation of NF-kappaB in vivo. In addition, we found that DMF inhibited the expression of MMPs and VLAs. DMF treatment also decreased the expression of Bcl-xL and Survivin in vivo. <Discussion> These results indicate that DMF selectivity suppresses the NF-kappaB activation, thereby inhibiting metastasis and tumor growth. Taken together, our data suggest that DMF may be a potential therapeutic agent with the treatment of metastatic melanoma.

▲TOP

J-1116

Elucidation of the pathophysiological roles of AM-RAMP system in inter-organ metastasis

Kun Dai
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Adrenomedullin (AM) is a multifunctional peptide, which is regulated by receptor activity-modifying proteins (RAMPs). We reported AM-RAMP2 system regulates vascular development, whereas AM-RAMP3 system regulates lymphatic function. In this study, we investigated the roles of AM-RAMP system in inter-organ metastasis. PAN02 cancer cells were injected into the spleen, which enabled liver metastasis. Compared with control, the metastasis was enhanced in endothelial cell-specific RAMP2 knockout mice (E-RAMP2-/-). We found compensative upregulation of RAMP3 and its downstream target podoplanin (PDPN) in E-RAMP2-/-. Moreover, larger number of α SMA and FSP1-positive CAF was present among PDPN-positive cells in the peripheral region of metastasis. Contrary, the metastasis was suppressed in RAMP3 knockout mice (RAMP3-/-), which showed downregulation of PDPN. From these results, we suggest that distribution of PDPN-positive CAF may determine the degree of malignancy in our model. PDPN expression and the degree of malignancy were reduced in RAMP3-/-, whereas compensative upregulation of RAMP3 in E-RAMP2-/- enhanced metastasis. AM-RAMP system is thus expected as a new therapeutic target.

J-1117

The mechanistic insight of bone marrow-metastasized breast cancer cell survival in nutrient-limited conditions

Akiko Kogure
Div. Mol. Cell. Med., Natl. Cancer Ctr. Res. Inst.

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Late-recurrences in breast cancer (BCa) are well explained by their metastasis to the distant region such as bone marrow (BM). However, the mechanism underlying the survival and recurrence of BCa cells in the metastatic site remains unknown. Especially, it is not clear how cancer cell manages to survive in the BM even in the limited nutrient conditions. To understand this, BM2 cells, which are isolated from mouse BM after the metastasis of human malignant BCa cells MDA-MB-231-D3H2LN, were employed. As a result, the survival rate of BM2 cells was higher than parental BCa cells in the glucose-deprived medium. Furthermore, the addition of 2-Deoxy-D-glucose, which interfere with the glucose metabolism, to BM2 cells showed little effect on its cell survival, although parental cells could not survive in this condition. Moreover, BM2 cells showed the tolerance to the anti-cancer drug, such as doxorubicin or docetaxel. Taken together, these results suggest that BCa cells metastasized into BM have a different glucose metabolism rate, which may mediate cell survival and therapeutic resistance in the metastatic site, leading to the recurrence of BCa cells.

▲TOP

[SST1-1] SST1 [Japanese]
Developments in gastrointestinal cancer treatments

2018 / 9 / 27 (Thu) 9:00-11:30 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16

Atsushi Ochiai / Exp. Oncol. Res. Clin. Trial Ctr.、Yoshihiro Kakeji / Div. of Gastrointest Surg, Dept. of Surg, Grad Sch Med, Kobe Univ

With the development of next generation sequencing technology, analysis of the whole genome in cancer are progressing and genetic abnormalities of each carcinoma have been clarified. Epigenomic changes are causally involved in cancer development and progression by inducing silencing of tumor-suppressor genes and genomic instability. The development and combination of new anticancer drugs including molecular targeting agents have brought patients significant benefits of long survival. Major advances with the development of immune check-point inhibitors have ushered in a new era in cancer therapy. Surgical resection, which is a local treatment, has been increasing its role in multimodal therapies. In this symposium, six experts in the gastrointestinal cancer field will highlight advanced research regarding the molecular mechanisms in the development and progression of cancers which leading to establish the next generation therapy.

SST1-1

Gastric Cancer is Heavily Influenced by Aberrant DNA Methylation and Shows Sensitivity to DNA Demethylating Therapy

Toshikazu Ushijima
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Co-author：Hiroshi Moro、Yoshiaki Nakamura、Naoko Hattori
Div. Epigenomics, Natl. Cancer Ctr. Res. Inst.

Helicobacter pylori infection potently induces aberrant DNA methylation. The accumulation level of aberrant DNA methylation in gastric mucosae is correlated with gastric cancer risk, and has a stronger impact on cancer risk than point mutations [Yamashita, PNAS, 115:1328, 2018]. The utility of DNA methylation in cancer risk diagnosis has been shown even by a multicenter prospective study [Maeda, Gut, 66:1721, 2017]. In gastric cancer cells, whole-genome and exome sequencing revealed only a limited number of novel driver genes. Instead, aberrant DNA methylation frequently affected driver pathways [Yoda, Gastric Cancer, 18:65, 2015]. All this suggests that DNA demethylating therapy is effective against gastric cancer. Experimentally, treatment of 21 gastric cancer cell lines with a DNA demethylating agent, 5-aza-2'-deoxycitidine (5-aza-dC), demonstrated that a substantial fraction of them are sensitive to 5-aza-dC with IC_{50} < 50 nM. Treatment of irinotecan-resistant gastric cancer cells with 5-aza-dC sensitized them to irinotecan. Gastric cancer is heavily influenced by aberrant DNA methylation, and epigenetic therapy is a promising strategy for gastric cancer.

▲TOP

SST1-2

Endoscopic treatment for early gastrointestinal neoplasia in upper GI tract

Hiroyuki Ono
Div. Endoscopy, Shizuoka Cancer Ctr.

Endoscopic treatment of gastrointestinal cancer in Japan began with gastric cancer. In the background, the incidence of gastric cancer in Japan is high, and many efforts have been paid for diagnosis and treatment up to today.Since we introduced endoscopic submucosal dissection (ESD) using insulation tip electrosurgical knife for early gastric cancer in the late 1990's, it has been widely accepted not only in Japan, but also Asian countries. ESD has dramatically spread.Considering the patient's quality of life (QOL) after operation, ESD is much better than surgical gastrectomy. Therefore, we, endoscopists pursue the expansion of the indication of ESD. From the era of surgical radical resection for cure, the treatment of cancer is to make the patient's QOL as much as possible.Recently, treatment methods using robotic endoscopy, laser endoscopy and the like are being developed. In this trend, endoscopic therapy is pregressing as a treatment aiming at curing while maintaining function.

SST1-3

Association between gastrointestinal tract cancer and genetic alterations: from the pathological viewpoint

Tomio Arai
Dept. Pathol., Tokyo Metro. Geriatric Hosp.

This talk focuses on association between gastrointestinal cancer, especially gastric and colorectal cancers (GCs and CRCs, respectively), and genetic alterations from the pathological viewpoint. Recent advances in molecular biology demonstrated novel classifications of GCs and CRCs. GCs are classified into 4 groups: Epstein-Barr virus (EBV)-related, microsatellite-unstable, chromosomal-unstable and genomically stable cancers. EBV-related and microsatellite-unstable GCs show peculiar clinicopathological features. On the other hand, CRCs are composed of microsatellite instability (MSI) immune, canonical, metabolic and mesenchymal subtypes as the consensus molecular classification. Medullary carcinoma is a representative tumor which shows prominent MSI, CpG island methylator phenotype (CIMP) and BRAF mutation. Approximately a half of mucinous carcinoma also demonstrates MSI. Recent advances in molecular targeting agents demonstrated an efficacy of PD-1 blockade in MSI-positive cancers. In order to apply combined pathological and genetic information in a daily practice, the development of histopathological screening and test to confirm genetic alterations is required.

SST1-4

Activity on Nationwide Genome Screening Project for Advanced Gastrointestinal Cancer in Japan; SCRUM-Japan GI-SCREEN

Takayuki Yoshino
Dept. Gastrointestinal Oncol., Natl. Cancer Ctr. Hosp. East

Cancer genome alterations have been identified as targets for gastrointestinal (GI) cancer treatment, which include BRAF mutation, HER-2 alteration, MSI-High, cMET and etc., for which corresponding agents showed promising activity in clinical trials. In addition, emerging concept in clinical development targeting MSS mCRC comes into question which compound adding on an immune checkpoint inhibitor evokes immunity. Successful combination strategy evoking immunity will break out of the paradigm.Current clinical development of corresponding agents in molecularly characterized cohorts of cancer patients as well as in acquired targets by means of NGS- and blood-based molecular testing may represent more substantial progress toward precision medicine. On the basis of cancer genome alterations by NGS- and blood-based molecular testing, umbrella & basket-type investigator-initiated clinical trials with the National Patient Registry are anticipated to contribute to the establishment of the appropriate infrastructure in terms of the promotion of clinical development in Japan for orphan-fractionated cancer subtypes, which are regarded as having the low priority in pharmaceutical companies.

▲TOP

SST1-5

What is the checkpoint of the immunotherapy against GI cancer?

Kiyoshi Yoshimura
Dept. Clin. Immuno Oncol., CRI, Showa Univ., Div. Med. Oncol., Med., Showa Univ.

Co-author：Satoshi Wada[1]、Takuya Tsunoda[2]
[1]Dept. Clin. Diagnostic Oncol., CRI, Showa Univ.、[2]Div. Med. Oncol., Med., Showa Univ.

Due to the rise of immune checkpoint inhibitors, the strategy of cancer treatment has been dramatically changed. Gastrointestinal cancer is no exception, and there are no doubt that immune checkpoint inhibitors will be increasingly used for colon cancer accompanying genetic mutation following gastric cancer and esophageal cancer. Although not only immune checkpoint inhibitors but also the cancer immunotherapy itself as a cancer treatment option is greatly recognized again, problems in cancer immunotherapy regarding checkpoint inhibitors and genetically modified T cell therapy have been highlighted as well. One of the serious problems is that infiltration of T cells and NK cells into solid cancers has a great influence on the therapeutic effect. Factors on the tumor side and factors on the immune cell side are greatly involved, and for this reason, we need to develop strategies based on the both. We are approaching the tumor side and the immune cell side respectively, and developing the idea that will be a major step toward a remarkable effect of immunotherapy on gastrointestinal cancer.

SST1-6

Dual-targeting Photoimmunotherapy for esophageal cancer and cancer-associated fibroblasts in tumor microenvironment

Hiroaki Sato
Dept. Gastroenterological Surg., Okayama Univ. Grad. Sch.

Co-author：Kazuhiro Noma、Toru Narusaka、Satoshi Komoto、Toshiaki Ohara、Hiroshi Tazawa、Toshiyoshi Fujiwara
Dept. Gastroenterological Surg., Okayama Univ. Grad. Sch.

The prognosis of esophageal cancer remains poor, despite improvement of multi-modalities. Cancer associated fibroblasts (CAFs) have been reported to be associated with therapeutic resistance. Thus, we hypothesized to target both cancer and CAFs, using novel cellular targeting “Near-infrared Photoimmunotherapy (PIT)”. Firstly, we evaluated the correlation between the expression of EGFR/HER2 and FAP (fibroblast activation protein: CAFs) in 89 patients. EGFR/FAP positive group demonstrated the poor prognosis in esophageal cancer. From this result, targeting therapy against EGFR and FAP positive cells may improve prognosis in esophageal cancer. We analyzed the effect of PIT for esophageal cancer cells using Panitumumab- and Trastuzumab-IR700, successfully showing rapid cell death respectively. FAP-targeting PIT also demonstrated specific cell death even in co-culture condition with cancer cells. “Dual-targeting PIT” for both cells demonstrated rapid cell death simultaneously. In conclusion, targeting cancer and tumor stroma by PIT is a new concept. It might have stronger anti-tumor effect by cutting off stromal supply, and furthermore by inhibiting stromal immune-suppression.

SST1-Special_Remarks

Special Remarks

Masato Kusunoki
Dept. Gastrointestinal & Pediatric Surg., Mie Univ.

No Abstract

▲TOP

[LS13] LS13 [Japanese]
Single cell multi-parameter analysis of tumor infiltrating lymphocyte (TIL)

2018 / 9 / 27 (Thu) 11:50-12:40 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16
：Nippon Becton Dickinson Company,Ltd.

Hitoshi Kiyoi / Department of Hematology and Oncology, Nagoya University Graduate school of Medicine

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LS13

Single cell multi-parameter analysis of tumor infiltrating lymphocyte (TIL)

Hiroyoshi Nishikawa
Division of Cancer Immunology, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center

No Abstract

▲TOP

[SST2-1] SST2 [Japanese]
Current status and the future of hepatobiliary and pancreatic cancer research and treatment

2018 / 9 / 27 (Thu) 13:00-15:30 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16

Masatoshi Kudo / Dept. Gastroenterol & Hepatol, Kindai Univ.、Mitsuo Shimada / Dept. Surg., Tokushima Univ.

In this symposium, current status and the future of hepatobiliary and pancreatic cancer research and treatment will be discussed. After the emergence of sorafenib, the dark age of molecular-targeted drugs continued in the field of hepatocellular carcinoma treatment; however, newer molecular-targeted drugs including immune checkpoint inhibitors have recently appeared, which may open the next stage. Professor Kudo, also a moderator, will talk about the past reviews and future prospects as a global expert in this field. Professor Okusaka will talk about the reviews and future prospects on genomic diagnosis, combination therapies of anticancer drugs and combined immunotherapies for advanced biliary tract cancer and pancreatic cancer. Professor Sakurai will talk about the advancement and future possibilities of radiation therapy including heavy-ion radiotherapy for hepatobiliary and pancreatic cancer. Professor Mizugami will give us a lecture on the diversity of pancreatic cancer precursor lesions from the standpoint of genetic abnormalities and biological behaviors in IPMN, and will give us suggestions on the mechanism of carcinogenesis. Professor Sekiguchi will give us a lecture on the identification by comprehensive epigenomic analysis and future usefulness of NQ01, which has shown as a new therapeutic target in high-risk hepatoblastoma
Through this symposium, new findings on carcinogenesis, cancer development, diagnosis and treatment in hepatobiliary pancreatic cancer will be shown, and hopefully presented knowledge will be beneficial for members of the Japanese Cancer Association.

SST2-1

Systemic Therapy for Hepatocellular Carcinoma: Current Status and Future Perspective

Masatoshi Kudo
Dept. Gastroenterology & Hepatology, Kindai Univ.

Current status and future perspective of systemic therapy for hepatocellular carcinoma will be presented.

▲TOP

SST2-2

Current and Future Perspectives of Medical Oncology for Pancreaticobiliary Cancer

Takuji Okusaka
Dept. Hepatobiliary & Pancreatic Oncol., Natl. Cancer Ctr. Hosp.

The prognosis of patients with pancreaticobiliary cancer has remained fairly dismal, although many trials have being conducted to establish more effective therapies. Recent advances in the treatment are characterized by novel combinations of conventional cytotoxic chemotherapy. Unfortunately, with the exception of erlotinib for pancreatic cancer, no recently completed and published later phase trials have resulted in approved targeted strategies. However, following the recent increase in the interest in drug development, attempts at clarifying the molecular mechanisms underlying the onset and proliferation have been made proactively, accompanied by clinical studies on promising new agents with specific mechanisms for the treatment of pancreatic cancer. Additional targeted and immuno-oncology agents are also currently under development and results from these trials have the potential to impact the future of treatment, especially for patients with biliary cancer. Integrative approaches that combine various new strategies will hopefully lead to the development of novel therapies that may produce a dramatic improvement in outcomes for patients with this dismal disease.

SST2-3

Radiation Therapy for hepatobiliary and pancreatic cancer

Hideyuki Sakurai
Dept. Radiat Oncol, Univ. of Tsukuba

Co-author：Toshiyuki Okumura、Hitoshi Ishikawa、Tetsuo Nonaka、Haruko Numajiri、Masashi Mizumoto、Kayoko Ohnishi、Keiko Murofushi、Yuta Sekino、Nobutaka Mizoguchi、Takashi Iizumi、Daigo Miyauchi
Dept. Radiat Oncol, Univ. of Tsukuba

For hepatobiliary and pancreatic cancer, conventional radiation therapy (RT) was considered to be insufficient to give enough radiation dose to the target with tolerable safety for the normal organ, therefore, many physicians recognized RT had an palliative role for these types of cancer. In recent years, however, many technological progress has been made in radiation oncology, RTs are used as an important curative and more effective treatment option for these cancers. Stereotactic body radiotherapy is a technique to accurately concentrate doses three-dimensionally on small liver target, and particle beam therapy can accumulate more dose to the large target with lessor dose to the surrounding normal tissue. Not only X-ray intensity modulated radiation therapy but also particle beam therapy can give higher dose to the pancreatic cancers without increasing surrounding normal tissue dose, as a result, these therapy combined with chemotherapy have been potentially extending median survival time for the patients. In this presentation, the optimum choice of new radiation therapies for several condition of the hepatobiliary and pancreatic cancer were summarized.

SST2-4

Comprehensive epigenetic analysis identifies NQO1 as a potential therapeutic target of high-risk hepatoblastoma

Masahiro Sekiguchi
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Co-author：Masafumi Seki[1]、Kenichi Yoshida[2]、Misa Yoshida[1]、Ryota Shirai[3]、Ryota Souzaki[4]、Yuichi Shiraishi[5]、Tomoaki Taguchi[4]、Motohiro Kato[3]、Yukichi Tanaka[6]、Satoru Miyano[7]、Seishi Ogawa[2]、Junko Takita[1]
[1]Dept. Pedatr., Univ. Tokyo、[2]Dept. Path. & Tumor Biol., Kyoto Univ.、[3]Childen's Cancer Ctr., NCCHD、[4]Dept. Pedatr. Surg., Kyushu Univ.、[5]Div. Cell. Signaling, Natl. Cancer Ctr. Res. Inst.、[6]Dept. Pathol., Kanagawa Children's Med. Ctr.、[7]Hum. Genom. Ctr., IMS, Univ. Tokyo

Although hepatoblastoma (HBL) is the most common pediatric liver tumor, molecular mechanisms that define clinical variety of HBL are yet to be clarified. To investigate the genetic diversity of HBL and identify therapeutic targets, we performed targeted capture sequencing, RNA sequencing, and DNA methylation array analysis on 42 samples of HBL. Consensus clustering of methylation data revealed the presence of three clusters correlated with distinct clinical and biological features. By comparing these subgroups, we identified high expression of NQO1 with promoter hypomethylation as a key marker in high-risk HBL groups. To confirm the role of NQO1 in HBL, we performed inhibition experiments using NQO1 siRNA and dicoumarol (an NQO1 inhibitor) on HBL cell lines, HepG2 and HuH-6. In experiments using HepG2 (high NQO1 expression), both siRNA and dicoumarol induced not only significant growth inhibition, but also sensitization to anti-cancer drugs. These effects were not observed in HuH-6 with low expression of NQO1. In conclusion, comprehensive epigenetic analysis defined three distinct subgroups of HBL, and identified NQO1 as a promising target in high-risk groups.

▲TOP

SST2-5

Diversity of precursor lesions for pancreatic cancer: Genetics and biology of intraductal papillary mucinous neoplasm

Yusuke Mizukami
3rd Dept. lnt. Med., Asahikawa Med. Univ., lnst. Biomed. Res., Sapporo Higashi Tokushukai Hosp.

Co-author：Yusuke Ono[1]、Hidenori Karasaki[2]、Yuko Omori[3]、Jun Ueda[4]、Toshikatsu Okumura[5]
[1]3rd Dept. lnt. Med., Asahikawa Med. Univ., lnst. Biomed. Res., Sapporo Higashi Tokushukai Hosp.、[2]lnst. Biomed. Res., Sapporo Higashi Tokushukai Hosp.、[3]Dept. Histopathol., Tohoku Univ., Sch. Med.、[4]Ctr. Adv. Res. Edu., Asahikawa Med. Univ.、[5]3rd Dept. lnt. Med., Asahikawa Med. Univ.

Pancreatic ductal adenocarcinoma (PDA) is associated with two main types of morphologically distinct precursors, pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasm (IPMN). The fundamental mechanisms differentiating PanIN and IPMN remain largely obscure; however, recent studies have identified distinct genetic profiles between the precursors. Whereas activating KRAS mutations are common to both, gain-of-function mutations in GNAS are specifically associated with IPMN. Our studies uncover GNAS-driven oncogenic mechanisms, identify SIK kinases as potent tumor suppressors, and demonstrate unanticipated metabolic heterogeneity among KRAS-mutant pancreatic neoplasms. Also, we propose a revised progression model for IPMN by classifying its carcinogenic pathways by multiregion sequencing of IPMN-related PDAs, serving as a platform to design efficient surveillance for detecting IPMN with potential progression to PDA. Together, the combination of genetic lesions with associated changes in transcriptional and metabolic circuitry may help define novel biomarkers for the surveillance of pancreatic cancer in high-risk individuals.

SST2-Special_Remarks

Special Remarks

Mitsukazu Gotoh
Osaka General Med. Ctr.

No Abstract

▲TOP

[E-1121] E14-4 [English]
Hepatocellular carcinoma (2)

2018 / 9 / 27 (Thu) 15:30-16:45 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16

Michiie Sakamoto / Dept. Pathol., Keio Univ. Sch Med

E-1121

Functional analysis of WFA+ Mac-2 Binding Protein (M2BPGi) in hepatocellular carcinoma in vitro

Dolgormaa Gantumur
Dept. HBP Surg, Gunma Univ., Sch. Med.

Co-author：Kenichiro Araki、Kei Hagiwara、Takahiro Yamanaka、Norihiro Ishii、Mariko Tsukagoshi、Takamichi Igarashi、Akira Watanabe、Norio Kubo、Norifumi Harimoto、Ken Shirabe
Dept. HBP Surg, Gunma Univ., Sch. Med.

Background: Recent clinical studies has revealed that novel fibrosis marker of M2BPGi (WFA^{+}-M2BP) may correlated with hepatocellular carcinoma(HCC) development and progression (Yamasaki K. Hepatology. 2014 Nov;60(5):1563-70.). However, no basic research has been explained role of M2BPGi in live cancer yet. Methods: The effects of M2BPGi on the proliferation and invasion ability of human HCC cell lines were examined. The effects of silencing of galectin-3(gal-3), possible ligands of M2BPGi, using siRNA was done. Possible signaling pathway was evaluated with Western Blotting(WB). To clarify the localization of M2BPGi and gal-3, multiple staining was performed for human HCC tissue sample. Results: M2BPGi enhanced proliferation and invasion rate of the HCC cells. However, gal-3 silencing reduced proliferating effect of M2BPGi. Gal-3 expression increased in the HCC cell's conditioned medium after M2BPGi treatment. Furthermore, M2BPGi may activate Erk and Akt in HCC cell lines by WB. Multiple staining showed that M2BPGi located in Gal-3 high expressing HCC cells.Conlusions: M2BPGi may related to HCC progression involving activation of Erk and Akt via gal-3.

▲TOP

E-1122

IDH1 mutation affects the sensitivity against BET inhibitor in human intrahepatic cholangiocarcinoma

Hiroaki Fujiwara
Dept. Gastroenterol., Univ. Tokyo

Co-author：Keisuke Tateishi、Takuma Nakatsuka、Kazuhiko Koike
Dept. Gastroenterol., Univ. Tokyo

Intrahepatic cholangiocarcinoma (ICC) is a type of bile duct cancers arising from intrahepatic biliary epithelial cells. The prognosis is poor; its five-year survival rate is less than 10%. Dysregulation of chromatin remodeling genes has been identified in nearly half of ICCs and so targeting aberrant epigenetic status is expected to make a breakthrough of therapeutic strategy against ICC. Recently, BET inhibitors including JQ1 have demonstrated anti-cancer efficacy in many types of tumors. Here we found that the sensitivity of JQ1 is affected by IDH1 mutation in human ICC cells. JQ1 impaired the growth of IDH1- mutant RBE cells but not of HuCCT1 or HuH28 cells harboring wild-type IDH1. JQ1 induced cell cycle arrest both in RBE and HuCCT1 cells, followed with the upregulation of CDK inhibitor p21. By contrast, JQ1 induced apoptosis only in RBE cells, but not in HuCCT1 cells. The selective inhibitor of mutant IDH1 attenuated JQ1-induced apoptosis in RBE cells. These results suggested that IDH1 mutation is involved in the growth inhibitory effect of JQ1 in ICC cells. Our study provides a new insight into the therapeutic compatibility between BET inhibitors and IDH1 mutation.

E-1123

Prevalence of TERT Promoter Mutations in Immunohistochemistry-based Subgroups of Hepatocellular Carcinoma

Wit Thun Kwa
Dept. Path., Keio Univ. Sch. Med.

Co-author：Kathryn Effendi、Naoto Kubota、Mami Hatano、Hanako Tsujikawa、Yutaka Kurebayashi、Yohei Masugi、Michiie Sakamoto
Dept. Path., Keio Univ. Sch. Med.

We aimed to investigate the presence of telomerase reverse transcriptase (TERT) promoter mutation in hepatocellular carcinoma (HCC), which has been classified into subgroups based on immunohistochemistry markers. DNA was extracted from cell culture, formalin-fixed paraffin-embedded (FFPE) tissues and surgically resected fresh liver tissues as well. Sanger sequencing was performed to identify sequence alteration. Mutation was observed in HepG2, KIM1 and KYN2, while no mutation was found in Alex and Li7. High frequency of mutation was seen in the adjacent non-tumor region from FFPE-derived DNA, raising a concern of false positive results. Meanwhile, no mutation was observed in the non-tumor region of fresh tissues-derived DNA. Currently, TERT promoter mutation was observed in 25/44 (57%) of HCC fresh tissues. In biliary/stem cell marker-positive group, Wnt/ β -catenin signaling-related group, and -/- group, mutation was seen in 2/5 (40%), 8/12 (67%), and 15/27 (56%) respectively. Less frequent of TERT promoter mutation was seen in HBV-related HCC (33%). Our findings propose a careful analysis using FFPE-derived DNA and suggest implications of TERT mutation in HCC subgroups.

E-1124

Regulation of cell proliferation and apoptosis by PDIA3 through STAT3 signaling pathway in hepatocellular carcinoma

Ryota Kondo
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Dept. Integr. Diag. Path., Nippon Med. Sch.

Protein disulfide-isomerase A3 (PDIA3) catalyzes folding of newly synthesized glycoproteins. Increased expression of PDIA3 in hepatocellular carcinoma (HCC) correlates with poor prognosis, but the mechanism is poorly understood. The aim of this study is to reveal the role of PDIA3 in HCC. PDIA3 expression in HCC tissues was higher than that in adjacent non-cancerous tissues. HCC tissues with high expression of PDIA3 were higher Ki-67 index and less apoptotic cells than those with low expression of PDIA3. In HCC cell lines, PDIA3 knockdown inhibited cell proliferation and induced apoptosis. To elucidate the mechanism of cell death induced by PDIA3 siRNA, we investigated the relationship between PDIA3 and STAT3 pathway. We found that expression of PDIA3 and phosphorylated STAT3 (Tyr705) in HCC tissues had a positive correlation. In cells, immunofluorescent staining and co-immunoprecipitation showed co-localization and direct binding of PDIA3 to STAT3, respectively. In addition, PDIA3 knockdown decreased p-STAT3 levels and their downstream targets. Taken together, our data suggest that PDIA3 has the HCC promoting potential through STAT3 signaling pathway.

▲TOP

E-1125

Researches on molecular mechanisms of TAZ/miR-31-3p/CA2 in metastasis and invasion of hepatocellular carcinoma

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The recurrence and metastasis of HCC are the most important factors which influence on the prognosis of HCC patients. We have proved that TAZ could regulate the recurrence and metastasis of HCC, however, the mechanism is basically unknown. Our studies found that miR-31-3p expression was decreased after TAZ knock-down. Furthermore, miR-31-3p is over-expressed in clinic HCC tissue, and miR-31-3p expression was positively correlated to the TAZ expression. In vivo, MiR-31-3p knockdown resulted in significant decrease of tumor size, decreased average size of pulmonary metastatic lesions. We also found that altered expression of TAZ have an effect on the expression of miR-31-3p in vitro and vivo. Meanwhile, we found miR-31-3p induced invasion and metastasis by regulating its downstream target gene carbonic anhydrase 2. This study will attempt to illuminate the molecular mechanism of TAZ in HCC invasion and metastasis and to establish the TAZ/miR-31-3p/CA2 signaling pathway which should supply some new therapeutic strategies in prevention of HCC invasion and metastasis.

E-1126

A third generation oncolytic HSV-1 G47Δ enhances the efficacy of radiofrequency ablation therapy

Tomoharu Yamada
Div. Innovative Cancer Therapy. Inst. Med. Sci., Univ. Tokyo

Co-author：Yasushi Ino[1]、Miwako Iwai[1]、Kazuhiko Koike[2]、Tomoki Todo[1]
[1]Div. Innovative Cancer Therapy. Inst. Med. Sci., Univ. Tokyo、[2]Dept. Gastro. Med., Univ. Tokyo

Radiofrequency ablation (RFA) is a standard local treatment for hepatocellular carcinoma (HCC). Here, we examined the efficacy of an oncolytic herpes simplex virus type 1 (HSV-1), G47Δ, in combination with RFA therapy. In A/J mice harboring bilateral Neuro2a subcutaneous tumors, tumors on one side only were treated with intratumoral injections with G47Δ (2×10^6 pfu) on days 0, 2 and 4 followed by complete ablation by RFA on day 6. The G47Δ+RFA treatment caused smaller volumes of contralateral tumors, accompanied by increased $CD8^+$ /$CD45^+$ T cells, compared with G47Δ monotherapy. When cured mice were rechallenged with Neuro2a implantation, those treated with G47Δ+RFA rejected the Neuro2a rechallenge more frequently than those treated with G47Δ alone. ELISpot assay on day 20 revealed that the number of Neuro2a reactive, IFN-γ secreting splenocytes was significantly greater in the G47Δ+RFA group than the RFA group. These results indicate that intratumoral administration of G47Δ prior to RFA may augment elicitation of systemic antitumor immunity. The combination of G47Δ and RFA could be an effective regimen for the treatment of HCC.

▲TOP

[P-1001] P2-1 [English/Japanese]
Animal models for cancer (1)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Kazumi Nakano / Grad.Sch. Frontier Sci, The Univ. Tokyo

P-1001

Infliximab inhibits colon carcinogenesis in AOM/DSS-induced colitic cancer mouse model

Dang Yang Wang
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Co-author：Shinobu Ohnuma[1]、Kentaro Ishii[1]、Megumi Murakami[1]、Norihiko Sugisawa[1]、Hideaki Karasawa[1]、Hiroaki Musha[1]、Fuyuhiko Motoi[2]、Michiaki Unno[3]
[1]Dept. Surg., Tohoku Univ., Sch. Med.、[2]Dept. Surg. Tohoku Univ.、[3]Dept. Gastroenterological Surg. Grad. Sch. Med. Tohoku Univ.

Aim: To reveal whether anti TNF-α antibody (Ab) induces colon carcinogenesis or not in colitic cancer models. Methods: The effects of TNF-α and anti TNF-α Ab were analyzed with cell-proliferation, migration, and invasion assays in human colon cancer cell lines, in vitro. Then, the effects of anti TNF-α Ab, infliximab (10 mg/kg, i.p.) were investigated with drug-induced colitis-associated colon cancer model by azoxymethane (AOM: 10 mg/kg, i.p.)/dextran sodium sulfate (DSS: 2.5%, oral) in vivo. Results: 50 ng/ml of TNF-α inhibited cell proliferation, migration, and invasion of HCT8 and COLO205, however, anti TNF-α Ab offset the TNF-α mediated-inhibition in vitro. Infliximab significantly attenuated the development of colon cancers in AOM/DSS treated mice. The microarray analyses revealed that mast cell related genes were down-regulated in cancer tissues of the infliximab-treated AOM/DSS mice. The number of mast cells were also reduced in those mice. Conclusions: Mast cell may have a pivotal role in the development of colitic cancer. Anti TNF-α Ab may prevent colitic cancer in the patients with inflammatory bowel diseases.

▲TOP

P-1002

A method of producing genetically manipulated mouse mammary gland for breast cancer gene analysis

Kosuke Ishikawa
Japan Biological Informatics Consortium (JBiC)

Co-author：Hiroaki Tagaya[1]、Shinya Watanabe[2]、Kentaro Semba[1]
[1]Grad. Sch. of Advanced Sci. & Engineering, Waseda Univ.、[2]Translational Res. Ctr., Fukushima Med. Univ.

To obtain a deep understanding of the mechanism of breast cancer, it is essential to analyze the genes involved in tumorigenesis in vivo. Mouse mammary gland has the capacity to regenerate completely from a mammary stem cell (MaSC), which enables us to analyze the effect of gene expression on tumorigenesis in mammary gland regenerated from genetically manipulated MaSCs. Although viral vectors had been applied for gene delivery into MaSCs, they had difficulties in introducing long or transcriptional termination sequences. To overcome these difficulties, we had developed a vector system using PiggyBac transposon vectors and electroporation and achieved fluorescent gene expression by Tet-On inducible system. Here we demonstrate that even a long DNA fragment from BAC (>100kb) including multiple expression units including polyA signals can be delivered with this system. Also, we will report that tumorigenic phenotypes were seen by introducing polyoma middle T antigen (PyMT), similar to MMTV-PyMT transgenic mouse model. Thus, this system is useful for analyzing genes involved in breast cancer. (Collaborator: Yoshito Hosokawa, Shun Kobayashi, Yukino Ueoka, Mayuna Shimada)

P-1003

Roles of Dio2 (deiodinase, iodothyronine, type II) in colorectal tumorigenesis

Yasushi Kojima
Div. Pathophysiol. Aichi Cancer Ctr. Res. Inst.

Co-author：Teruaki Fujishita[1]、Emi Mishiro[1]、Rie Kajino[1]、Makoto M. Taketo[2]、Masahiro Aoki[3]
[1]Div. Pathophysiol., Aichi Cancer Ctr. Res.、[2]Dept. Pharmacology, Kyoto Univ. Grad. Sch. Med.、[3]Div. Pathophysiol. Aichi Cancer Ctr. Res. Inst.

The prohormone thyroxine (T4) in circulation is converted to bioactive T3 in peripheral organs by the enzyme DIO2 (deiodinase, iodothyronine, type II), a major regulator of local thyroid hormone levels. Although recent epidemiologic studies suggest the significance of the thyroid hormone signaling in CRC formation, the precise role of DIO2 in CRC remains poorly understood. Here we demonstrate that expression of DIO2 is upregulated in the colon tumor microenvironment in $Apc^{+/\Delta 716}$ mice, a genetically engineered model of CRC. Treatment with iopanoic acid, a potent inhibitor of deiodinases, suppressed tumor formation and conferred a survival advantage in $Apc^{+/\Delta 716}$ mice. The Cancer Genome Atlas (TCGA) data of CRC also indicated significant up-regulation of DIO2 gene expression in colorectal cancer and a close association of its expression pattern with the stromal component of CRC. We have also found a link between DIO2 and cyclooxygenase-2 (COX-2), a well-known stromal player in colorectal carcinogenesis. Collectively, these data highlight biological roles of DIO2 in the CRC tumor microenvironment.

P-1004

Connexin 32 prevents the development of insulin resistance and hepatocarcinogenesis in non-alcoholic steatohepatitis

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Co-author：Hiroyuki Kato、Shugo Suzuki、Yoriko Yamashita、Satoru Takahashi
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We previously reported that Cx32 has protective roles in non-alcoholic steatohepatitis (NASH) by analyzing methionine-choline deficient diet received Cx32 dominant negative transgenic (Tg) rat. However, this model did not reflect metabolic syndrome in terms of biochemical data. To clarify the roles of Cx32 in NASH associated with obesity and insulin resistance, Tg and wild-type (Wt) rats were received high-fat diet (HFD) with dimethylnitrosamine. HFD gained body, liver and visceral fat weights in both genotypes. Serum insulin, glucose and HOMA-IR in Tg were significantly higher than those in Wt rats. Elevation of AST, ALT, inflammatory cytokine expressions (Tnf α , Il6, Tgf β , Il1 β , Timp2, Col1a1), NF- κ B activity, and progression of steatohepatitis, fibrosis were severe in Tg as compared with Wt rats. Concerning carcinogenesis, the number, area of GST-P positive foci and expression of brain expressed, X-linked 1 (Bex1), which was established as a maker for hepatocarcinogenesis with NASH, were significantly increased in Tg versus Wt rats. These results suggest that Cx32 dysfunction promoted the development of steatohepatitis and carcinogenesis in NASH accompanied by metabolic syndrome.

▲TOP

P-1005

Context-dependent induction of distinct liver tumors in mice with Kras activation and Pten inactivation

Tsuneo Ikenoue
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MAPK and PI3K pathways are frequently involved in the development of human liver cancer. To study the effect of co-activation of these pathways in hepatocarcinogenesis, we first analyzed mice carrying $Kras^{G12D}$ expression and Pten deletion in hepatoblasts, the embryonic bipotential liver progenitor cells using the Albumin-Cre (Alb-Cre) strain. In this model, $Kras^{G12D}$ expression cooperated homozygous Pten deletion to induce intrahepatic cholangiocarcinoma (ICC) but not hepatocellular carcinoma (HCC). In contrast, $Kras^{G12D}$ expression with heterozygous Pten deletion caused both ICC and HCC, while $Kras^{G12D}$ alone induced only HCC. We next introduced liver-specific $Kras^{G12D}$ expression and Pten deletion using the tamoxifen-inducible Alb-CreERT2 strain. Interestingly, the induction of $Kras^{G12D}$ expression and homozygous Pten deletion at 8 weeks and 10 days after birth resulted in the formation of HCC and ICC, respectively, while the induction of them at 14 days after birth resulted in the formation of both HCC and ICC. These results suggest that the activation of MAPK and PI3K pathways cooperates to induce distinct type of murine liver tumors in a context-dependent manner.

P-1006

Identification of responsible genes for Stmm1a locus conferring resistance to early-stage chemically induced skin tumors

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In our previously study, we have identified strong genetic loci Skin tumor modifier of MSM 1a (Stmm1a) on chromosome 7 for early stage papillomas using forward genetics approach. In order to identify responsible genes for Stmm1a, we performed RNA-seq analysis using normal skin tissues of MSM (resistant strain) as well as FVB (susceptibility strain) mice. As a result, Pak1 was identified as the most differentially expressed gene between MSM and FVB in Stmm1a genetic region. Pak1 is a member of serine/threonine kinases that are implicated in regulating skin carcinogenesis via Ras signaling pathway. Therefore, we generated MSM-Pak1 knockout mice by CRISPR/Cas9 system. Interestingly, we found that $Pak1^{-/-}$ mice shows fewer residential langerhans cells (LC) in the epidermis, which effects on cutaneous carcinogenesis. Next, we performed skin carcinogenesis experiments with F_1-konockout heterozygous mice ($Pak1^{FVB/-}$) by DMBA/TPA protocol. As a result, lower expression of Pak1 in LC conferred stronger resistance to skin tumors. Taken together, our data suggest that Pak1 might be a good candidate gene for Stmm1a.

P-1007

MOB1-YAP1 is the most potent oncogenic driver pathway for the onset of head and neck squamous cell carcinoma

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Head and neck squamous cell carcinoma (HNSCC) is a cancer with a poor prognosis. Survival rate has not been improved even by the intensive platinum based treatment in recent decades. Large-scale whole exome sequence analysis by The Cancer Genome Atlas project in 2015 revealed only TP53 mutation is observed as an universal genetic mutation in HNSCC, but any other significant oncogenic driver is not yet identified.Here, we generated a HNSCC model by tongue-specific Mob1 (cancer suppressor gene) deletion in mice. This model is the first HNSCC mouse model by single pathway alteration; and showed the most rapid onset of HNSCC in the world. In addition, we identified Yap1 (oncogene, downstream of Mob1) was significantly activated from the precancerous dysplasia stage in human tongue cancer patients. Thus, we found MOB1-YAP1 is the most potent oncogenic driver pathway for the onset of HNSCC. The pathway is one of the most attractive molecular targets for HNSCC treatment, and the mutant mice is the suitable HNSCC model to explore the new drugs in vivo.

▲TOP

[P-1015] P2-3 [English/Japanese]
Animal models for cancer (3)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Shunsuke Noguchi / Vet. Radiol. Osaka Pref. Univ.

P-1015

Human PBMC transferred NOG mouse model to evaluate human T cell activity by immune checkpoint inhibitors

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Therapeutic treatment of anti-PD-1 antibodies clearly suppresses tumor growth by blockade of the PD-1/PD-L1 pathway, but over activation of T cells exhibits immune-related adverse effects with exacerbation of autoimmune reactions. The human T cells are spontaneously activated in a human peripheral blood mononuclear cells (hPBMC) transferred NOG mouse because of xenogenic-reaction and they frequently die due to graft-versus-host disease (GVHD). In the present study, we evaluate the potential adverse effects of 2 therapeutic antibodies, Keytruda and OPDIVO by using NOG mice transferred with hPBMC. These mice showed a weight loss, wasting, rough fur, and skin thickening suggesting of GVHD symptoms. These changes exacerbated in either antibody treated hPBMC-NOG mice with increase of activation marker for T cells, and they died earlier than saline-treated group. By immune-cytoloical analysis, human T cells was significantly decreased in number in hPBMC-NOG mice even though the antibodies were treated. These results demonstrated the hPBMC-NOG mice might become powerful preclinical tools to analyze human T cells activated by therapeutic immune checkpoint inhibitors.

▲TOP

P-1016

Comparison analyses of PDX and organoids from colorectal cancer for optimized application to non-clinical studies

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PDX (Patient-Derived Xenograft) and organoid culture systems are useful as models reproducing the in vivo environment. However, molecular pathological evaluation has not yet been substantially conducted for confirmation whether PDX or organoids can faithfully reproduce the microenvironment and solid cancer epithelial cells.
Here, we established PDX and organoids from colorectal cancer cases and comparison analyses between them were conducted. As a result, gene expression profiles which distinguish between cancer and normal tissues are surely maintained in a site specific manner. In addition, mutations were mostly conserved in PDX and organoids, however, there are some variations in spite of the same solid cancer origins, suggesting that solid cancers are not a single population each with one genotype but an aggregate with various mutations. As PDX has more mutations than organoids, PDX maintains more diversity than organoids. On the other hands, organoids are uniform and increase (easily) relatively fast. These results suggested that considering of the advantages of each resource is required, when we apply patient-derived avatar models for non-clinical studies.

P-1017

Passage-related phenotypic changes of patient-derived xenografts from surgical specimens of endometrial cancer

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Patient-derived xenograft (PDX) model is considered to reproduce in vivo niche and maintain primary phenotypes. On the other hand, phenotypes of PDX are recognized to gradually change by passages, and degrees of the changes are suspected to be in an origin and/or a histological type of tumors-dependent manner. Here, we established PDX from endometrial cancers and comparison analyses between the corresponding passaged PDX and primary tumors were conducted.Twelve cases of endometrioid adenocarcinomas (8 cases), carcinosarcomas (3), and mixed cell adenocarcinoma (1) were subcutaneously implanted into immunodeficient NOG mice. Then, totally 7 of 12 cases engrafted. PDX of endometrioid carcinomas maintained their phenotypes of original tumors (histology and AE1/AE3, PAX8 and ER-positivities), whereas those of carcinosarcomas showed a tendency to proliferate with predominant sarcomatous tumor cells and minor epithelial cancer cells along with their passages. One mixed cell carcinoma case was represented as a lymphoma.These results suggested that considering of the passage number of each PDX particularly from carcinosarcoma case is required, when we apply them for non-clinical studies.

P-1018

Expression of adenosine generating ecto-enzymes, CD39 and CD73, in lymphocytes in tumor bearing mouse

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【Background and Purpose】Extracellular adenosine exerts strong immunosuppressive activity. Adenosine is generated by the dephosphorylation of extracellular ATP by CD39 and CD73, which has been reported to be enhanced in tumor tissues. We analyzed the expression of CD39, CD73 in tumor tissues and regional lymph nodes in murine model.【Materials and Methods】LuM1, a subtype of colon26 was subcutaneously injected at the right flank of 4-8 week BALB/c mouse, tumor tissue and lymph node were excised on day 14 or day 21, and the expression of CD39 and CD73 in immune cells was analyzed by FACS.【Results】CD39 was expressed mostly on CD19(+) B cells but not in T cells. However, the ratio of CD39(+) in T cells was elevated in tumor tissue(CD4(+):Mean=94.3%,Min77.0-Max97.3%, CD8(+):71.3%,64.0-92.4%). The ratios of CD73 in CD4(+) and CD19 (+) cells were increased in tumor draining than in contralateral lymph nodes(CD4(+):22.6%,12.0-27.4% vs 14.6%,11.3-26.2%, $p < 0.05$, CD19(+):11.0%,1.60-25.3% vs 2.43%,0.46-10.6%, $p < 0.05$).【Conclusion】The upregulation of CD39 and CD73 in the tumor associated lymphocytes may be involved in enhanced adenosine production in tumor microenvironment.

▲TOP

P-1019

Metallo-balance index: tumor detection based on serum trace elements in dogs

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Introduction: Since regulation of trace elements is intimately associated with homeostasis of organisms, their imbalance reflects systemic condition of the body. “Metallo-balance” index has been developed to detect early cancer in human by measuring serum trace elements, which showed promising results. With substantial extension of lifespan in companion animals, more pet dogs are suffering from cancer. In this study, feasibility of Metallo-balance index for diagnosis of cancer in dogs is investigated. Materials and methods: Serum was obtained from 4 healthy control dogs and 19 tumor bearing dogs. Seventeen serum elements were measured by ICP-MS. Analysis was performed by discriminant analysis with forward selection of variables. Results: With six selected variables (Li, Na, Zn, Co, Rb, Mo), serum trace elements index could discriminate cancer patients from healthy control with 100% accuracy. Discussion:“Metallo-balance” index for tumor diagnosis may be feasible in pet dogs. Comparative aspect with human cancer and biological significance of each trace element should be further determined.

P-1020

The differential expression of micro RNAs responding to irradiation in canine melanoma cells

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Canine melanoma (CM) is the most common cancer occurred in the oral cavity. It is often treated with radiotherapy, and most melanomas achieve complete response or partial response. However, relapse is a serious problem and a part of CMs show resistance to irradiation (IR). To improve a therapeutic effect on radiotherapy, elucidation of the mechanisms of radioresistance in CM is needed. In late years, the correlation between radioresistance and microRNA (miRNA) in various human tumors has been clarified. The aim of this study is to validate the mechanism of radioresistance involved in miRNA-associated signaling cascade in CM. First, we performed microRNA microarray using a human melanoma cells with or without IR. As a result, we detected 30 miRNAs showing a differential expression pattern. Of these miRNAs, miR-454 was significantly upregulated in the IR-resistant CM cells, which were established by daily IR. We hypothesized that miR-454 is associated with radioresistance of CM. Now, the analysis of the role of miR-454 in response to IR is undergoing.

P-1021

Biological characterization of cancer stem cells in canine mammary gland tumor

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Identifications of cancer stem cells (CSCs) have been reported in mammary gland tumor (MGT) in the humans and the dogs. The dogs are one of the most common pets, and they have a high incidence of MGTs. However, few therapeutic options are available for advanced unresectable metastatic diseases. CSCs are reported to involve therapy resistance in MGTs in the humans and the dogs although the detailed mechanism are not well-understood. To characterize the therapy resistance mechanism of canine MGT (cMGT)s, we performed comprehensive screening of surface markers using cell lines of cMGTs. Among the markers tested, the expression of CD44, CD24, CD98 and xCT were measured each in adherent and sphere culture conditions by using flowcytometry. Although more that 90% of cells were positive in the bulk populations of CTBp, and CTBm, CD44 expression was decreased in sphere culture, as opposed to CD24, CD98 and xCT. The exposure to doxorubicin to CTBp, and CTBm cell lines induced the decrease of CD44 expressions in small subpopulation of the cell lines. Further characterization of CSCs in cMGTs might lead to elucidation of a new aspect of therapy resistance mechanisms.

▲TOP

[P-1029] P2-5 [English/Japanese]
Animal models for cancer (5)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Rieko Ohki / Natl. Cancer Ctr. Res. Inst.

P-1029

Identification of the therapeutic targets for brain tumor-related fusion genes using an animal model

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Fusion genes provide therapeutic insight as the breakpoints highlight cancer-relevant genes. In contrast to druggable kinase fusions, it is not easy to identify the therapeutic targets for ones composed of an unpredictable domain such as a transcription factor, thus likely necessary to examine the molecular function in detail. The genetically engineered mouse model is an essential tool for current cancer research but has the critical drawback which cannot perfectly reproduce the complex molecular heterogeneity of human cancers because a limited number of genes is enough to induce tumors in mice. However, given the potential driver function of the fusions, the model appears to fit for their functional analysis. So far, we have presented that the C11orf95-RELA fusion, recently identified in ependymomas (EPNs) was a potent oncogene capable of inducing human EPN-like tumors in an RCAS/tv-a system. In this talk, we would demonstrate to explore for the therapeutic targets of the RELA fusion through the functional analyses and drug screening with our EPN model, and then the application of our experimental approach to other brain tumor-related fusions to identify the therapeutic targets.

▲TOP

P-1030

Establishment and characterization of novel syngeneic oral squamous cell carcinoma mouse cell lines

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M1-2 and M2-3 cells were established from mouse tongue tumor specimens induced by 4-NQO and arecoline. M1-2 and M2-3 cells partially developed tumors after subcutaneous injection in immune-compromised mice. In contrast, M1-2 and M2-3 cells failed to develop any tumor by orthotopic inoculation of syngeneic mice. By in vitro selection, M1-2sph and M2-3sph cells were established and only M1-2sph cells successfully developed tumors in syngeneic mice. M1-2sph and M2-3sph cells showed the higher activities of cell growth and sphere formation than their parental cells. The higher Klf4 and Nanog expression was detected only in M1-2sph cells. M1-2sph and M2-3sph cells showed the higher capabilities of migration and invasion than their parental cells. The epithelial mesenchymal transition (EMT) was occurred only in M1-2sph cells. The higher Twist and Slug expression was detected only in M1-2sph cells. The profiling of p ERK1/2 activation was changed between M1-2 and M1-2sph cells but not between M2-3 and M2-3sph cells. The relationship between ERK1/2 activation and occurrence of EMT and sphere formation was under investigation.

P-1031

Interrogating genetically engineered mouse models of prostate cancer to aid in immunotherapy development

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Androgen deprivation therapy (ADT) remains the main treatment for advanced prostate cancer. Treatments targeting androgen receptor (AR) signaling by androgen withdrawal or AR antagonists have been implemented in the clinic and clinical trials evaluating combinations with immunotherapy are ongoing. However, AR expression is not limited to prostate epithelial cells and is expressed in several cells types including stromal and immune cells. Studies have suggested that targeting AR is a double-edge sword with regards to immune suppression and tumor immunity. To gain insights into the influence of ADT on tumor immunity, we performed robust histopathological, flow cytometric and gene expression analyses to profile tumor immunity in a well-established genetically engineered mouse model of prostate cancer driven by the conditional inactivation of Pten. We also used this model to investigate the effects of androgen deprivation based on surgical castration as well pharmacological ADT using Ar silencing, non-steroidal anti-androgens, steroidal CYP17A1 inhibition and AR silent agonists, and provide data that will be useful in developing treatments in combinations with immunomodulatory agents.

P-1032

A novel gastric cancer mouse model by using organoid technique and genetic manipulation

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【Background】 It has been said that a part of differentiated gastric cancer occurred by gene mutation related to chronic inflammation by H. pyloly infection. However, there are no serviceable mouse models which proved this theory. 【Methods】 We used K19-Wnt1/C2mE mice (GAN mice), which is a model of inflammation associated gastric cancer. We created organoids derived by adenoma cells of GAN mice (WT organoid). We knocked out p53 of WT organoid by CRISPR/Cas9 system . Farther, we transfected KRAS mutation viral vector to p53KO Organoids. We transplanted 3types organoids to subcutaneous and intraperitoneal of nude mice.【Results】 We observed uniform cyst when we transplanted WT organoid to subcutaneous. p53KO Organoids created solid type subcutaneous tumor. Moreover, p53KO-KRAS Organoids created more solid tumor. It involved high nuclear grade, high irregularity of ducts. Either, there were no tumor when transplanted WT Organoid and p53KO Organoid to intraperitoneal. On the other hand, p53KO-KRAS Organoid created intraperitoneal tumor.【Conclusion】 In this model, the tumor growth quickly and easy to observe, accordingly this model is useful for analyze gastric carcinogenesis.

▲TOP

P-1033

Establishment and analysis of a novel mouse line carrying a conditional knockin allele of cancer-specific FBXW7 mutation

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F-box and WD40 domain protein 7 (FBXW7) is a component of the SKP1-CUL1-F-box protein complex that mediates the ubiquitination of various oncogenic target proteins. Three mutation hotspots have been identified at conserved arginine residues (Arg^{465}, Arg^{479}, and Arg^{505}) in the WD40 domain of the FBXW7 gene in human cancer. It has been shown that these arginine residues are critical for substrate recognition. To study the function of $FBXW7^{R465C}$, the most frequent mutation in human malignancies, in carcinogenesis, we generated a novel conditional knockin mouse carrying murine $Fbxw7^{R468C}$ corresponding to human $FBXW7^{R465C}$. Systemic heterozygous knockin of the $Fbxw7^{R468C}$ mutation resulted in perinatal lethality due to defects in lung development. Although liver-specific knockin of $Fbxw7^{R468C}$ alone did not induce any liver tumor, the combination of oncogenic Kras mutation and homozygous $Fbxw7^{R468C}$ induced cholangiocarcinoma. The substrates affected by the mutant Fbxw7 differed between the embryos, embryonic fibroblasts, and adult liver. This novel conditional knockin $Fbxw7^{R468C}$ line should be useful for investigating the mechanisms of carcinogenesis associated with FBXW7 mutations.

P-1034

Modeling organelle-specific O-glycosylation in driving liver tumor growth, invasion and metastasis

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Cancer progression is driven by the ability of tumor cells to invade surrounding tissues. Invasiveness correlates with perturbation of a covalent modification of cell surface proteins: O-glycosylation. However, the molecular mechanisms are still unclear. Here, we show that O-glycosylation initiating enzymes are activated during liver tumor growth by intracellular relocation from Golgi to ER. In a Nras/shp53 mouse liver cancer model, co-expressing an ER-targeted glycosyl-transferase GALNT1 (ER-G1) massively increased tissue growth, invasion and metastases. ER-G1, but not its Golgi-localized counterpart, strongly stimulates glycosylation of the key cancer matrix metalloprotease MT1-MMP, this modification being essential for collagenase activity. Conversely, expression of an ER-targeted inhibitor of GALNTs inhibits liver tumor progression in Nras/shp53 injected mice. In summary, our model comprehensively demonstrates the generation and analyses of the GALNTs Activation pathway driving liver tumor invasiveness; thus further characterization of glycosylation substrates will unveil mechanisms of tumor growth and help identify potential therapeutic targets in human liver cancer.

P-1035

FEAT downregulates primary cilia formation and enhances INSL3 expression intesticular Leydig cells

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FEAT, the protein encoded by METTL13, is aberrantly upregulated in most human cancers. FEAT overexpression potently drives tumorigenesis in vivo. It is weakly expressed in normal human tissues, including the testis. However, its role in normal tissues remains elusive. Here, we found that FEAT is expressed in fetal and adult Leydig cells in the testis. Depletion of FEAT in MA-10 Leydig tumor cells using siRNA enhanced primary cilium formation and AMPK activation. Immunofluorescence analyses of FEAT-silenced MA-10 cells showed diminished INSL3 expression. A male $Mettl13^{+/-}$ mouse developed bilateral intraabdominal cryptorchidism, suggesting defects in INSL3 production by fetal Leydig cells. Immunohistochemistry revealed markedly decreased INSL3 in Leydig cells from the mouse. Together, these results suggest that FEAT suppresses primary cilia formation, downregulates AMPK signaling, and facilitates the INSL3 production that is essential for transabdominal testis migration.

▲TOP

[P-1041] P4-2 [English/Japanese]
Expression / functional analysis of novel oncogenes / tumor-suppressor genes

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Tohru Ishitani / IMCR, Gunma Univ

P-1041

Monoclonal antibodies against SLC7A1: assessment of gene expression and cytotoxicity in colorectal cancer

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SLC7A1 is a cationic amino acid transporter of a gene that we determined to exhibit a considerably higher transcription level in colorectal cancer (CRC). We attained monoclonal antibodies against SLC7A1 and assessed several characteristics of these antibodies to elucidate the functional role of SLC7A1 on CRC cells. In this study, the expression kinetics of SLC7A1 was investigated with immunohistochemical staining in CRC tissues of clinical samples using the monoclonal antibodies obtained. We detected hyperexpression of the SLC7A1 protein in 89% of CRC cases, especially in the cancerous part of the tissue slices. Next, we evaluated the antibody-dependent cellular cytotoxicity (ADCC) of the attained antibodies. Then, the in vitro antitumor activity of anti-SLC7A1 antibodies was validated by their ADCC activities on a colon cancer cell line HT29 in the presence of human peripheral blood mononuclear cells as effector cells. This study indicates that anti-SLC7A1 antibodies could be a potentially novel therapeutic agent for CRC.

▲TOP

P-1042

Over-expression of BRCA1-interacting protein BIP2 causes centrosome amplification by activating PLK1 and Aurora A.

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Introduction
BRCA1 interacts with various proteins to contribute DNA repair and centrosome regulation, yet the precise mechanisms are still unknown. We previously identified BIP2 (BRCA1-interacting protein 2) as a protein that interacts with BRCA1-interacting protein, OLA1. It has been reported that the higher expression of BIP2 is related to a malignancy in breast cancer. We found that over-expression of BIP2 causes centrosome amplification (CA) only in cells derived from mammary tissue. In this study, we analyzed how over-expression of BIP2 induces CA.

Results
Over-expression of BIP2 potentiated phosphorylated polo-like kinase 1 (PLK1) localization in centrosomes and increased C-Nap1 spots in the late G1/S phase in MCF-7, which suggested that premature activation of PLK1 induced centriole disengagement. Inhibition of Aurora A and PLK1 suppressed CA and premature centriole disengagement induced by over-expression of BIP2. Furthermore, over-expression of BIP2 enhanced the interaction between Aurora A and PLK1.

Conclusion
Over-expression of BIP2 enhanced activation of PLK1 and Aurora A resulting in premature centriole disengagement and CA.

P-1043

Flt-1 is a possible cell-type specific tumor suppressor gene in human choriocarcinoma

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Soluble Fms-like tyrosine kinase-1 (sFlt-1) is an anti-angiogenic factor trapping VEGF, and abundantly expressed in placental trophoblasts. Choriocarcinoma, malignant tumor derived from trophoblasts, is known to have high pro-angiogenic and metastatic activities. We hypothesized that sFlt-1 production is suppressed in choriocarcinoma cells. In this study, we examined Flt-1 gene expression and DNA methylation status of the Flt-1 gene promoter region in normal trophoblasts and choriocaricinoma cells. In choriocarinoma cells, the expression of Flt-1 gene was strongly suppressed compared with primary trophoblasts, and the CpG sites at its prompter region were hypermethylated. Choriocarcinoma cells treated with 5'-aza-2'-deoxycytidine, a DNA methyltransferase inhibitor, showed an increase in the expression of Flt-1 gene at mRNA level and the secretion of sFlt-1 proteins. Moreover, sFlt-1-expressing choriocarcinoma cells implanted into nude mice indicated significantly slower tumor growth in comparison with GFP-expressing control choriocarcinoma cells. There results strongly suggest that the Flt-1 gene is a cell-type specific tumor suppressor.

P-1044

Frequent detection of structural variations in TP53 gene of malignant mesothelioma by digital MLPA

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Genomic deletions occurs frequently in tumor, but around 30 bp to 3 Kb deletions are unlikely to be detected because NGS sequencing are sensitive to detect minute deletions (generally <30 bp only) and commercial array CGH can detect larger ones (>3Kb). We previously reported multiple minute simultaneous homozygous deletions in chromosome 3p21 of malignant mesothelioma (MM) using high-density custom made array CGH (probes average interval: 254 bp). These may be consistent with chromothripsis. And genomic loss is more frequent than sequence-level mutations detected by target NGS. We developed the digital MLPA, combined technology with MLPA (Multiplex Ligation dependent Probe Amplification) and NGS, in order to detect exon level copy number (CN) changes for 234 genes with frequent mutations in MM. This system could detect the CN change of 0.2 (log2 ratio) reproductively. Among 35 MM samples, there were 8 MMs with exon level copy number (CN) changes (22.9%) and 9 ones with 1 allele deletion (25.7%) in the gene TP53. Overlooking of minute CN changes would lead misunderstanding for MMs that are thought to have low level of mutations in TP53.

▲TOP

[P-1052] P5-1 [English]
MicroRNAs (1) [English]

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-1052

Regulatory function of MSC-derived EV-mediating delivery of miRNAs to T cells

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Mesenchymal stem cells (MSCs) hold an immunoregulatory capacity and induce immunosuppressive effects. As an extension, MSC-derived extracellular vesicles (EVs) exerted an inhibitory effect in the differentiation and activation of T cells. The therapeutic effects of MSC-EVs were partly mediated by the packaged miRNAs, which were suggested to be associated with proliferation and immunomodulation. The PD-1/PD-L1 pathway downregulates T-cell proliferation and cytokine production. Based on the excellent characteristics of EVs as a gene vector, the regulation of miRNAs on PD-1/PD-L1 pathway and the important role of T cells in cancer immunotherapy, we propose the hypothesis that EV-mediated delivery of miRNAs has immunomodulatory effects on T cells. To prove this, we firstly predicted the specific target miRNAs of PD-L1 using TargetScan and miRBase. MSC-EVs were used as a gene vector to deliver the predicted miRNAs to T cells and then check the immunomodulatory effects on T cells. The purpose of our study is the application of MSC exosomes for miRNAs delivery and its potential to block the PD-1/PD-L1 pathway to reverse the immunosuppressive effects on T cells.

▲TOP

P-1053

MicroRNA-1258/CKS1B axis plays an important role in mediating colorectal cancer progression

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Colorectal cancer (CRC) is second common cancer and remains a major challenge due to its progression mechanisms. MicroRNAs (miRNAs) are small non-coding RNAs that are involved in several diseases by regulating target genes expression. However, the role of miRNAs and their target genes in CRC remain to be elucidated. In this study, we investigate cellular functions of miR-1258 and its target gene, CKS1B, to generate novel insights into colorectal carcinogenesis. TCGA data results showed that CKS1B was upregulated in CRC. In silico and in vitro study revealed that miR-1258 negatively regulated CKS1B through 3'-UTR. In particular, ectopic expression of miR-1258 resulted in suppression of cell growth and migration. Furthermore, expression of miR-1258 in KM12SM cells significantly decreased the tumor size in xenograft model. In addition, knockdown of CKS1B by siRNA treatment revealed inhibition of cell growth and migration. Collectively, these findings suggest that miR-1258 may functions as a tumor suppressor by targeting the CKS1B, and re-expression of miR-1258 might prove beneficial to prevent tumor formation and provide a potential therapeutics strategy to suppress CRC progression.

P-1054

NNK induces miR-944 expression and modulates CISH/STAT3 signaling pathway in oral squamous cell carcinoma

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The cytokine-inducible Src homology 2-containing protein (CISH) is an endogenous suppressors of signal transduction and activator of transcription (STAT) and acts as a key negative regulator of inflammatory cytokine responses. Downregulation of CISH has been reported to associate with increased activation of STAT and enhanced inflammatory pathways. However, the underlying mechanisms of dysregulation of CISH/STAT pathway in oral squamous cell carcinoma (OSCC) remains unknown. Here, we report that CISH protein is significantly downregulated in OSCC patients and its levels are inversely correlated with miR-944 expression. We identified the CISH protein, which modulates STAT3 activity, as a direct target of miR-944. The miR-944-mediated CISH functions are crucial in regulating STAT3 activity, pro-inflammation molecules secretion (such as CCL3, CCL5, IL-1Ra and IL-1β), migration and invasive potential in OSCC cells. Furthermore, the expression of miR-944 was significantly induced by the 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and co-expressed with its host gene TP63. Taken together, miR-944/CISH/STAT3 may have therapeutic potential for the treatment of OSCC.

P-1055

Down-regulation of plasma miR-133b is related to sarcopenia and contributes to cancer progression in gastric cancer

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BACKGROUND: Recent studies have identified that sarcopenia is associated with poor outcomes in gastric cancer (GC). We tried to identify a novel microRNA reflecting sarcopenia and causing poor outcomes in GC patients with sarcopenia.
METHODS: We tested 4 myomiRs (miR-1, miR-133a, miR-133b and miR-206) as candidates of novel plasma biomarkers for sarcopenia in GC. We measured skeletal muscle mass index (SMI) in 55 GC patients as the indicator of total muscle mass volume.
RESULTS: (1) Among four candidate miRNAs, plasma miR-133b levels had a strong correlation with SMI values. (2) Low plasma miR-133b level was significantly related to poor prognosis. (3) Over-expression of miR-133b significantly suppressed cell proliferation through the production of p21 with G1/S arrest in GC cell lines. (4) Co-cultured with human skeletal muscle cell, GC cell proliferation was significantly suppressed through the up-regulation of miR-133b in medium and GC cells.
CONCLUSION: Plasma miR-133b might be a novel biomarker reflecting sarcopenia. The down-regulation of plasma miR-133b level caused by loss of skeletal muscle might be one of the mechanisms of poor prognosis in GC patients with sarcopenia.

▲TOP

P-1056

Serum miR-1290 is correlated with high grade serous epithelial ovarian cancer and can be a new potential biomarker

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Objective: New diagnostic markers detecting ovarian high grade serous ovarian cancer (HGSOC) are urgently needed.The aim of this study is to analyze whether serum miRNA can discriminate HGSOC patients from healthy controls. Methods: Exosomes from ovarian cancer cell lines were collected and exosomal miRNAs were extracted. miRNA microarray analysis showed several elevated miRNAs were specific to HGSOCs. Among them, we focused on miR-1290. Serum from 70 ovarian cancer patients and 13 healthy controls were collected and its expression levels were detected by quantitative Real Time PCR.Results: In HGSOC patients, miR-1290 emerged overexpressed compared to healthy controls unlike other types of epithelial cell ovarian cancer patients. Atadvanced stage of HGSOC, its expression was higher than that in early stage (P=0.23). Its expression significantly decreased after operation(P<0.01), indicating that this miRNA reflects tumor burden. ROC analysis showed at the cut-off of 1.20, the sensitivity and specificity were 63 % and 85 % respectively for detecting HGSOCs (AUC = 0.71). Conclusions: Serum miR-1290 can be a new potential diagnostic biomarker for HGSOC.

P-1057

Low miR-522 expression is related to paclitaxel resistance in ovarian cancer cells

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Purpose: The aim of this study is to identify key miRNAs which regulate paclitaxel resistance and to pursue those potential as therapeutic targets.Methods: Two paclitaxel resistant ovarian cancer cell lines were established by a continuous exposure. MiRNA PCR array was performed and miR-522 was found to be one of down-regulated miRNAs. RNA was extracted from recurrent tumor and miR-522 expression was compared with that of the corresponding primary tumor. The effect of miR-522 was assessed by transducing the precursor miRNAs. Especially, the relation between miR-522 and cell cycle-related proteins was extensively analyzed.Results: In resistant ovarian cancer cells, miR-522 was down-regulated. In three cases of recurrent tumors, miR-522 was down-regulated compared with primary tumor. Upregulation of miR-522 sensitized resistant cells to paclitaxel and its downregulation desensitized parental cells. MiR-522 attenuated paclitaxel resistance by down-regulating E2F and causes G1/S arrest in resistant cells.Conclusion: MiR-522 modulates paclitaxel resistance and can be considered a therapeutic target for recurrent ovarian cancer.

P-1058

miR-1285-5p functions as a tumor suppressor in breast cancer progression

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Accumulating evidences suggest that microRNA (miR)-1285-5p plays indispensable roles in various cancer. Previously, we found that miR-1285-5p expression was down-regulated in young breast cancer (BC) patients with poor prognosis, however, its precise roles in BC progression have not been clarified yet. The aim of this study was to investigate the functional significance through identification of molecular traits of miR-1285-5p. We confirmed that miR-1285-5p expression in breast tumor tissues was suppressed compared with that in adjuscent normal tissues. Additionally, low levels of miR-1285-5p were found in multiple BC cell lines compared with that in MCF10A. Transient transfection significantly inhibited cell proliferation in BC cell lines, suggesting miR-1285-5p as a tumor suppressor. Comprehensive microarray analysis revealed that various pathways such as c-Myc targets, DNA repair, and E2F targets were globaly attenuated in the cells with miR-1285-5p overexpression. Futhermore, we found candidate target genes of miR-1285-5p. In conclusion, we provided new insights into the mechanisms of BC oncogenesis through the molecular traits of miR-1285-5p.

▲TOP

[P-1059] P5-2 [Japanese]
MicroRNAs (2)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-1059

Stress-activated p38 and JNK pathways downregulate an anti-apoptotic miRNA

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Stress-activated MAPK (SAPK: p38 and JNK) pathways are activated by various environmental stresses, including ultraviolet (UV) irradiation, and play a central role in the regulation of cellular stress responses such as apoptosis by controlling gene expression. Here we report that the SAPK pathways down-regulate the expression of a certain miRNA, thereby potentiating stress-induced apoptosis. In order to identify miRNA whose expression is controlled by SAPK signaling, we first established mutant cells that are defective in SAPK activation (δ3/6/4/7 cells). These cells were then stimulated with UV irradiation and their miRNA expression profiles were monitored by microarray analysis. We found that the expression level of mir-X was significantly increased in δ3/6/4/7 cells, as compared with wild-type cells. Following stress stimuli such as UV irradiation, its expression was gradually decreased. We also found that mir-X downregulated the expression of several pro-apoptotic molecules, and its overexpression inhibited UV-induced apoptosis. Thus, the SAPK pathways promote apoptosis at least in part through downregulating mir-X expression.

▲TOP

P-1060

Serum miRNA as a predictive marker of recurrence and prognosis for biliary tract cancer after surgery

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[Background] Biliary tract cancer (BTC) is one of the aggressive malignant tumors. Even if the radical resection has been could performed at early stage, the risk of recurrence still largely remains and has a poor prognosis. We investigated the usefulness of serum miRNA as predictive markers of recurrence and prognosis after radical surgery. [Methods] We obtained a total of 66 samples from 22 BTC patients who performed radical surgery at three time points; before surgery, after surgery, recurrence or last observation period (in case of non-relapse). By microarray analysis, we compared serum miRNA expression profiles in BTC patients with or without recurrence. [Result] We successfully identified 6 specific miRNAs which associated with recurrence and prognosis of BTC after radical surgery. In addition, by the combination of these miRNAs, we could predict the recurrence and prognosis with significantly high accuracy (sensitivity, specificity, and accuracy of 84.6%, 100.0%, and 90.9%). [Conclusion] Our results provide compelling evidence for the potential usefulness of specific serum miRNAs as an effective predictive tool of recurrence and prognostic in postoperative BTC patients.

P-1061

Prediction of pathological complete response by microRNA in breast cancer patients treated with neoadjuvant chemotherapy

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[Background] miRNAs are potentially useful to evaluate treatment of cancer.[Materials and Methods] Serum sample of patients who received neoadjuvant chemotherapy (NAC) before treatment between 2008 and 2014 were collected. A comprehensive quantitative expression analysis of miRNA was performed using the by DNA chip “3D-Gene (Toray Industries Inc.)” Pathological complete response (pCR) was defined as the absence of residual cancer of the resected breast specimen and all sampled lymph nodes.[Results] 90 patients received NAC. Median age was 49 years (range 28-77). 42 patients were hormone receptor(HR)-positive (HR+) and HER2-negative (HER2-), 24 were HR+ and HER2-positive (HER2+), 11 were HR-negative (HR-) and HER2+ and 13 were HR- and HER2-. pCR was observed in 19 (21%) of NAC patients. pCR in each subtype were 3 (7.7%) in HR+/HER2-, 6 (33.3%) in HR+/HER2+, 4 (36.4%) in HR-/HER2+ and 3 (23.1%) in HR-/HER2-. Serum after NAC were obtained from 19 pCR patients and 71 non-pCR patients. miR-X/ and miR-Y/ were differentially expressed in the patients with pCR. [Conclusion]miR-X and miR-Y are the potential predictive marker of pCR in patients with BC treated with NAC.

P-1062

Detecting oral squamous cell carcinoma by novel serum microRNA panel

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Background: In the field of oral cancer diagnosis, reliable tumor biomarker is urgently needed. Serum microRNA (miRNA) is now known to be useful for cancer detection. The purpose of this study was to identify specific serum miRNA for oral cancer detection. Methods: Microarray and RT-PCR were used for evaluation of expression level of miRNAs in serum samples from 42 oral squamous cell carcinoma (OSCC) patients and 20 healthy controls. The accuracy of oral cancer detection by candidate miRNA was evaluated statistically. Results: Microarray analysis demonstrated 44 upregulated and 24 downregulated specific miRNAs in OSCC patients in comparing with healthy control individuals. Especially, expression level of anonymous miR-X was significantly decreased in patients group, whereas that of miR-Y was increased ($p=0.0053$ and $p=0.0009$). The sensitivity and specificity for OSCC detection is 92.9% and 66.7% by using miR-X, and 50% and 80% by miR-Y, respectively. The diagnostic accuracy was increased by combination of two miRNAs (double-positive vs. double-negative, $p<0.0001$). Conclusion: Panel of specific microRNAs might be novel candidate biomarker for oral cancer detection.

▲TOP

P-1063

The prognostic value of pre-miR-488 expression in peripheral blood of gastric cancer patients

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Background: Circulating microRNAs (miRs) in various cancers have been highlighted as diagnostic and prognostic biomarker. However, the significance of precursor miR (pre-miR) expression remains unclear. A recent study reported that miR-488 might act as a tumor suppressor gene in gastric cancer (GC). Our aim of this study is to determine the clinical significance of pre-miR-488 in GC patients.Methods: We measured the expression of pre-miR-488 by RT-qPCR, and assessed the clinicopathological and prognostic significance of pre-miR-488 expression in peripheral blood (PB) and tissue of GC patients.Result: The low expression group of pre-miR-488 in PB had poorer overall survival (OS) than the high expression group (p=0.005) and was an independent poor prognostic factor for OS (HR:2.59, p=0.03). The expression of pre-miR-488 in PB of GC was higher than that of healthy volunteers (p=0.001). The expression of pre-miR-488 in tumor tissue was lower than that of normal tissue (p=0.0003), but was not correlated with prognosis.Conclusion: Pre-miR-488 expression in PB was novel prognostic biomarker in GC patients. Furthermore, pre-miR as well as mature miR could be a clinical biomarker.

P-1064

The exploration of miRNAs that induce cell death in p53-inactive cancer cells using functional-miRNA screening

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p53 is most mutated tumor suppressor, which controls cell proliferation, survival, and death by the regulation of target genes. miRNAs, endogenous small non-coding RNAs, regulate target gene expressions posttranscriptionally via partial complementation. Because a single miRNA can alter the several signaling pathways by repressing several target genes simultaneously, miRNA mimics targeting vulnerable signaling pathways in cancers may be useful as a therapeutic agent for cancer therapy. In this study, we performed function-based screening with 2565 human miRNAs in p53-wild or p53-inactive cancer cells, and miR-X (lab name) was identified as a miRNA that induced cell death specifically in p53-inactive cancer cells. Gene expression analysis and database analysis revealed that 237 genes were candidate targets of miR-X. GSEA analysis showed that miR-X downregulated several signaling pathways including WNT- β catenin pathway and DNA repair pathway. We also validated several genes, including BRD4, were direct targets of miR-X by luciferase reporter assay. miR-X may be a promising candidate for the development of a miRNA-based therapeutics against p53-inactive cancer.

P-1065

Eribulin suppresses EMT through the changing of microRNA expression in breast cancer cells

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<Background>Eribulin mesylate is a microtubule-depolymerizing drug used in the treatment of metastatic breast cancer (BC). Eribulin has been reported to suppress the epithelial mesenchymal transition (EMT). However, the detailed mechanism is still largely unknown. <Purpose>In this study, suppression effects of EMT induced by Eribulin were investigated from the view of the microRNA. <Materials and methods>Three BC cell lines (BJMC3879, MDA-MB-231, MDA-MB-468) were used. Evaluations of the EMT were performed by Western blotting analysis and Wound healing assay. Also, the expression levels of interested microRNA expression were examined by qRT-PCR. <Result>The treatment with Eribulin suppressed the expression levels of N-cadherin, ZEB1, and SNAI2 and increased the expression levels of E-cadherin by WB analysis and wound healing in BC cells tested. Also, Eribulin increased the expression levels of miR-X in BC cells tested. Moreover, suppression of EMT related genes' expression and wound healing were observed in miR-X-treatment BC cells. <Conclusion>Our data suggested that Eribulin suppresses EMT and migration through the increment of miR-X in BC cells.

▲TOP

[P-1073] P5-4 [Japanese]
Signal transduction (1)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Masayuki Hiraki / Dept. Surg. Itami City Hosp.

P-1073

Heterogeneity of cellular responses in breast cancer cells

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The binding of growth factors to their corresponding receptors induce cellular events that ultimately change its behavior. Generally, cell responses differ according to the type of receptor, and the same chemical signal can trigger different responses in different types of cells. In this research, we investigated the time-dependent signal transduction pathways in 4 subtypes of breast cancer cells. MCF-7, BT-474, SKBR-3, MDA-MB-231 cells were stimulated with Epidermal Growth Factor (EGF), Heregulin (HRG) or Estradiol (E2), and the cellular responses within 3 hours were quantitatively analyzed. While MCF-7 cells were responded to all stimuli, other 3 types of cells display entirely different pattern of responses or even no response. We also found that signal transductions in EGF-treated BT-474 cells were not accompanied by the activation of corresponding EGF receptors and ErbB2 receptor phosphorylation was not occurred in HRG-treated SKBR-3 cells even though they have abundant Her2 receptors. These results indicate that signal pathways were not always associated with the corresponding receptor activation which can further illustrate existence of other functional molecular targets.

▲TOP

P-1074

Molecular mechanism of Survivin expression in anaplastic large cell lymphoma

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NPM-ALK fusion gene, which encodes a constitutively active kinase, is detected in most anaplastic large cell lymphoma (ALCL) cases. NPM-ALK is thought to be causative of ALCL by activating downstream signaling pathways. Although the most of ALCL cells highly express Survivin, which is assumed to be important for their immortalization, how NPM-ALK regulates Survivin expression is not fully elucidated. With regard to this point, it has been reported that Survivin expression is dependent on YAP-TEAD and that c-Src plays important roles in NPM-ALK signaling. Since Src family kinases are known to phosphorylate and activate YAP, we attemted to reveal relationship among NPM-ALK, c-Src and YAP in ALCL cells. We confirmed that NPM-ALK$^+$ SU-DHL-1 was much more sensitive to crizotinib, an ALK-specific inhibitor, than NPM-ALK$^-$ HL-60. Accordingly, the mRNA levels of Survivin was significantly decreased in SU-DHL-1 by crizotinib treatment. In HEK293T cells, transient expression of c-Src seemed to increase Survivin expression but the synergistic effects with NPM-ALK were not detected. Detailed analysis of relationship among NPM-ALK, c-Src and YAP in a hematopoietic cell line will be presented.

P-1075

Molecular mechanism underlying Survivin expression in FLT3-ITD+ AML cells

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FLT3 internal tandem duplication (FLT3-ITD) is present in one third of AML cases. FLT3-ITD has been reported to cause expression of Survivin, an anti-apoptosis factor, which is considered to be associated with resistance to chemotherapy. It is known that activated YAP, a transcription coactivator, together with TEAD, a transcription factor, induces Survivin expression. However, the signaling pathway from FLT3-ITD to Survivin remains largely unclear. In this study, we attempted to analyze the molecular mechanism underlying Survivin expression in FLT3-ITD$^+$AML cells. At first, FLT3-ITD$^+$ (MOLM-13) and FLT3-ITD$^-$ (HL-60) cell lines were treated with FLT3-specific inhibitor quizartinib. We confirmed that treatment with quizartinib decreased viable cell number as well as Survivin expression in MOLM-13 but not in HL-60, suggesting that Survivin expression is dependent on FLT3-ITD. In contrast, exogenous expression of FLT3-ITD in HEK293T did not increase Survivin expression. These results suggest that some hematopoietic cell-specific factor is needed for FLT3-ITD-induced Survivin expression. We are now preparing TF-1-based systems to further analyze these issues.

P-1076

Optimization of combination transfection with signal suppressive nucleic acid and reporter plasmid DNA

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We previously reported that miR-4689 showed a strong anti-tumor effect by suppressing the RAS/MAPK and PI3K/AKT signaling pathways. Luciferase reporter assay is an efficient tool for evaluation of signal activity; however, we are not certain that 'concurrent' transfection of reporter plasmid and miRNA reflects the real cellular signal activity. In this study, we searched for ideal protocol to show the effect of miR-4689 on EGFR signaling in colon cancer cells.The following three protocols were tested. (1) Reporter plasmid (SRE reporter or AP-1 reporter assay) and miRNA were transfected simultaneously. (2) MiRNA was transfected 8 or 24 hr prior to reporter plasmid transfection. (3) Reporter plasmid was transfected 2 or 4 hr before transfection of miRNA. During various time course studies, we found that the following protocol was the most rational for the evaluation of miRNA effect; miRNA is transfected 24 hr prior to transfection of reporter plasmid and then luciferase activity is measured 24 hr later after transfection of reporter plasmid. These experiments indicate that we should consider interference of plasmid DNA with miRNA and appropriate timing in the procedure.

▲TOP

P-1077

The analysis of CRISPR/Cas9-mediated GLI1 knockout lung adenocarcinoma cells

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The GLI1 gene was originally identified as an amplified gene in glioblastoma. Subsequently, several studies have demonstrated that the Gli1 protein is a transcriptional activator that functions as an effector of the hedgehog signaling pathway.
We have found hedgehog signaling independent novel Gli1 activation pathway. This pathway is involved in cancer stem cells (CSCs) maintenance in lung adenocarcinoma cells. To understand the role of the Gli1 activation pathway in lung CSCs properties, we generated Gli1 knockout lung adenocarcinoma cells by CRISPR/Cas9 engineering. The growth of these cells was partially suppressed, and the tumorigenicity in nude mice was markedly suppressed. Indeed, the numbers of injected Gli1-knockout HCC4006 cells gradually decreased and became undetectable after 16 weeks. Tumor initiating cells frequency was also suppressed in these cell. These results suggest that Gli1 is a critical factor for induction of lung adenocarcinoma and lung CSCs properties.

P-1078

Identification of a novel Wnt pathway activation mechanism mediated by TIM-3/Gal-9/HCK axis in human AML-LSCs

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Aberrant β-catenin pathway activation confers the enhanced self-renewalcapacity to AML stem cells (AML-LSCs). We have reported that in most AML cases, LSCs express T-cell immunoglobulin mucin-3 (TIM-3) on their surface, and LSCs secrete Galectin-9 (Gal-9), a TIM-3 ligand, in an autocrine manner to induce the accumulation of β-catenin in LSCs. In this study, we sought to identify the molecular mechanism of aberrant βcatenin pathway activation induced by TIM-3 signaling. We found that Gal-9 stimulation induced phosphorylation of LRP6, a key component of canonical Wnt pathway, and the subsequent β-catenin accumulation occurred in primary TIM-3+ AML cells. Next, we found that Gal-9 ligation to TIM-3 immediately recruited hematopoietic cell kinase (HCK). Furthermore, specific HCK inhibitors blocked phosphorylation of p120-catenin, a indispensable molecule for canonical Wnt pathway, and subsequent accumulation of β-catenin. These results suggested that TIM-3/Gal-9 autocrine loop constitutively activate HCKs and it mimics the canonical Wnt signaling independent of Wnt ligands. This novel machinery should contribute to the maintenance of self-renewing LSCs.

P-1079

Mathematical approach to the nuclear and cytoplasmic oscillation in the non-canonical NF-κB pathway

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NF-κB is transcription factor that regulates variety of functions such as inflammation, cell growth, cell division and apoptosis. The following two signaling pathways have been implicated in NF-κB activation: the canonical pathway and the non-canonical pathway.In the canonical pathway, it is known that NF-κB shuttles between nuclear and cytoplasm with a period of about 100 minutes by TNFα stimulation.Similar N-C oscillation is also reported in the non-canonical pathway.However, since the behavior of oscillation varies from cell to cell, it is difficult to experimentally analyze the behavior in a single cell. Therefore, we mathematically analyzed the hetero phenomena in non-canonical pathway by constructing a mathematical model.As a result, it was suggested that the different oscillation dynamics may be controlled depending on several parameters involved in transcription.

▲TOP

[P-1087] P5-6 [Japanese]
Signal transduction (3)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yosuke Hirotsu / Genome Analysis Center, Yamanashi Central Hospital

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P-1087

Canonical NOTCH2 signaling promotes bladder cancer progression through cell cycle progression, dedifferentiation and EMT

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Introduction: NOTCH is a family of cell surface receptors to regulate differentiation, proliferation and metastasis. Here we report the NOTCH signaling in bladder cancer (BC). Results: Genetic aberrations of NOTCH in BC showed that NOTCH2 demonstrated genomic gain, while NOTCH1/3 were frequently deleted, suggesting NOTCH2 oncogenic function. In TCGA dataset of BC, patient tumors with high NOTCH2 expression showed basal subtype and worse prognosis. To verify NOTCH2 signaling, we established NOTCH2 overexpressing BC cells. NOTCH2 overexpression increased cell growth and enhanced invasion compared to control. This was associated with cell cycle progression by down-regulation of p21 and p27, and increased expression of EMT and stem cell markers. Silencing CSL, a NOTCH transcriptional activator, inhibited NOTCH2-induced cell growth and invasion by up-regulation of p21 and p27, MET and decreased expression of stem cell markers. Orthotopic xenografts of NOTCH2 overexpression demonstrated increased BC growth and metastasis. Conclusion: Canonical NOTCH2 signaling plays a crucial role in growth and metastasis in BC.

▲TOP

P-1088

GEP oncogene induces epithelial-mesenchymal transition through LATS1 proteolysis in ovarian cancer

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$G\alpha_{12/13}$, heterotrimeric G-proteins, encoded by GEP oncogene, is implicated in the progression of several human cancers. However, the underlying mechanisms by which GEP oncogene promote cancer progression has not been fully elucidated. We herein evaluated downstream targets of GEP oncogene that are implicated in ovarian cancer progression. To examine the effect of $G\alpha_{13}$ activation on ovarian cancer cells, we employed constitutively active mutant $G\alpha_{13}$ ($G\alpha_{13}$QL) or synthetic biology approach using a GPCR activated solely by artificial ligands (RASSLs). Morphological change, protein expression profiles and intracellular signaling pathways were analyzed. Regarding both in cell morphology and protein expression profile, $G\alpha_{13}$ activation induced epithelial-mesenchymal transition (EMT)-like phenotypes. Cycloheximide chase assay revealed that LATS1 degradation mediated by $G\alpha_{13}$ was involved in EMT-like phenotype of ovarian cancer cells. These results suggest that $G\alpha_{13}$ activation may contribute to EMT-like phenotypical changes through LATS1 proteolysis in ovarian cancer.

P-1089

Synergistic anti-cancer effects of PP2A methyl-esterase PME-1 inhibition and p53 activation

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Protein phosphatase 2A (PP2A) is an essential holoenzyme that is implicated as an important tumor suppressor based on its central role in phosphorylation-dependent signaling pathways. Reversible methylation on C-terminal Leu309 of PP2A catalytic subunit (PP2Ac) is essential for proper biogenesis of the PP2A holoenzyme, and protein phosphatase methyl-esterase (PME-1) catalyzes specifically the demethylation of PP2Ac. PME-1 also inhibits PP2A activity by directly binding to its phosphatase active site; the role as PP2A inhibitory protein. Accumulating evidence indicates that PME-1 promotes oncogenic pathway activities in various cancer types. We revealed that PP2A inhibitory function, but not methyl-esterase activity, is important for tumor-promoting function of PME-1. We also found that PME-1 inhibition and p53 activation synergistically exert anti-cancer effects on human lung cancer cell line A549. In this presentation, detailed molecular mechanisms will be discussed.

P-1090

Molecular mechanisms of PP2A/PME-1 interaction in cancer cells: Implications from PPI screening assay

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Protein phosphatase 2A (PP2A) is an important tumor suppressor based on its central role in phosphorylation-dependent signaling pathways. PP2A forms heterotrimers, each comprised of a catalytic subunit (C, or PP2Ac), a scaffolding subunit (A, or PP2A-A) and one regulatory B subunit from 4 different families of genes. Regulatory B subunits control PP2A specificity by targeting PP2Ac to substrates. Reversible methylation on C-terminal Leu309 of PP2Ac is essential for proper biogenesis of the PP2A holoenzyme, and methylation of PP2Ac has been shown to enhance the formation of a subset of PP2A heterotrimers. Protein phosphatase methyl-esterase (PME-1) catalyzes specifically the demethylation of PP2Ac. PME-1 also controls the PP2A activity by directly binding to its phosphatase active site. Accumulating evidence indicates that PME-1 promotes oncogenic pathway activities in various cancer types such as glioma. However, the molecular mechanisms that control PP2A holoenzymes and PME-1 interaction remain largely unknown. We utilized NanoBiT system to analyze the effects of ~400 compounds on PP2A/PME-1 interaction. In this presentation, recent progress will be discussed.

▲TOP

P-1091

Analysis of MEK mutants derived from cancers and congenital Ras/MAPK syndromes

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The ERK pathway (Raf-MEK-ERK) plays crucial roles in various physiological and pathological processes. Recent genetic analyses in sporadic cancers and congenital Ras/MAPK syndromes identified multiple gain-of-function mutations in the MEK genes, and also revealed that sites of the MEK mutations vary depending on the diseases. However, the molecular mechanism by which these MEK mutations induce either cancers or congenital diseases remains unknown.
We found that these MEK mutations induced constitutive but distinct kinase activities: The Ras/MAPK syndrome-derived mutants showed moderate kinase activity, while cancer-derived mutants induced strong kinase activity and continuous ERK nuclear-localization. This spatiotemporal alteration of ERK signaling severely affects the downstream gene-expression, causing the characteristic manifestations of each disease. In addition, we demonstrated that trametinib, a MEK-targeting anticancer-drug, suppressed specific ERK-inducible genes which promote apoptosis, thereby inducing resistance of cancer cells to trametinib. These data suggest that the expression of these genes is crucial for a recurrence of cancer.

P-1092

SRC is involved in the ROR1-sustained ASK1 inhibition

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We previously reported that the receptor tyrosine kinase-like orphan receptor 1 (ROR1) is transcriptionally activated by TTF-1/NKX2-1 lineage-survival oncogene in lung adenocarcinoma and maintains a favorable balance between pro-survival PI3K-AKT and pro-apoptotic ASK1-p38 signaling. However, it remains elusive how ROR1 inhibits pro-apoptotic signaling. In this study, we investigated whether SRC plays a role in the ROR1-sustained ASK1 inhibition. Introduction of constitutively active SRC significantly reduced siROR1-induced ASK1activation in PC-9 cells. Co-transfection of SRC and ASK1 resulted in tyrosine phosphorylation of ASK1 in both 293T and COS-7 cells, in association with diminished ASK1 phosphorylation at threonine 845. Finally, the interaction between SRC and ASK1 was found to be enhanced by the presence of ROR1 by an in vitro pull-down assay. Taken together, the present findings support the notion that ASK1 is negatively regulated by SRC with the aid of ROR1 for their interaction, which constitutively suppresses disadvantageous pro-apoptotic signaling and contributes to survival of lung cancer cells.

P-1093

Identification of a novel protein that is induced by hyper-activation of the ERK pathway

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Hyper-activation of the ERK pathway eventually induces carcinogenesis. Its molecular mechanism is, however, still incompletely understood. Recently, we have identified genes whose expression is up-regulated only when the ERK pathway is hyper-activated by various oncogenes (e.g., Ras, Raf, or MEK active mutants). Of these, we focused on a previously uncharacterized molecule, which we named EIG-1 (ERK inducible gene 1). In this study, to understand the pathological role of EIG-1 in cancer, we investigated its molecular and physiological functions.We elucidated that EIG-1 was induced by activation of the ERK pathway via ELK1 and EGR1. We also discovered that EIG-1 interacted with PP2A-B56 complex and ArpC1B, and acted as an adapter between these proteins. Therefore, it is thought that EIG-1 recruits PP2A-B56 to dephosphorylate ArpC1B. ArpC1B is activated by phosphorylation, thereby enhancing cell migration. In fact, expression of EIG-1 suppressed cell migration and invasion in cancer cells. These results suggest that EIG-1 is a negative regulator of cancer invasion and metastasis. Consistently, in some cancer cells, EIG-1 was suppressed by promoter hypermethylation.

▲TOP

[P-1101] P5-8 [English]
Proliferation (1) [English]

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-1101

The role of CTGF in cancer cells

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CTGF (Connective Tissue Growth Factor) is a 36-38-kD cysteine-rich protein originally identified as a growth factor secreted by Human endothelial cells. In the context of oncogenic properties, CTGF expression was observed in the stroma of tumors and affects vascularization, migration, and epithelial-mesenchymal transition (EMT). Recent studies indicated that tumor cell-derived CTGF plays an important role in the proliferation of many types of cancer cells, although the mechanism is not still unclear. Our previous report have shown that We demonstrated a crosstalk mechanism between the TGFβ and Hippo signaling pathways, in which both were strong regulators of MM (malignant mesothelioma) cell growth, suggesting that CTGF is an important regulator of MM tumor growth. In our experiment, CTGF expression was predominantly overexpressed in sarcomatoid and biphasic tumor cells in tissues samples, although little was observed in epithelioid tissues. We have constructed the expression vector which lacks signal peptide and checked the different activation of gene expression compared with that of wild type.

▲TOP

P-1102

Regulation of small intestinal homeostasis by Tsc2-mTORC1 signaling

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Intestinal tumorigenesis driven by mutations in adenomatous polyposis coli gene requires the mammalian target of rapamycin complex 1 (mTORC1) activity, whereas the physiological roles of mTORC1 in the homeostasis of intestinal epithelial cells (IECs) remain virtually unknown. We here generated mice, in which tuberous sclerosis complex 2 (Tsc2), a negative regulator of mTORC1, was specifically ablated in IECs (Tsc2 CKO mice). Tsc2 CKO mice manifested the enhanced proliferative activity of IECs in intestinal crypts as well as the promoted migration of these cells along the crypt-villus axis. The apoptotic rate of IECs was increased in Tsc2 CKO mice. The LC3-II/I ratio (a marker for autophagosome formation) in IECs and the number of Paneth cells (secretory IECs) were decreased in Tsc2 CKO mice. In addition, ablation of Tsc2 promoted development of intestinal organoids. We also found that Tsc2 CKO mice exhibited increased sensitivity to irradiation injury. These results thus suggest that Tsc2-mTORC1 signaling regulates the proliferation, turnover, and differentiation of IECs. On the other hand, excessive activity of mTORC1 in IECs increases the susceptibility to irradiation injury.

P-1103

Early growth response protein 1 (EGR1) is involved in the 14-3-3 ε -regulated tumor progression of HCC

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We have previously reported that overexpression of 14-3-3 ε is correlated with high risk of extrahepatic metastasis and worse survival in hepatocellular carcinoma (HCC). Early growth response protein 1 (EGR1) is a transcriptional regulator which contributes to modulating cell proliferation and differentiation. However, whether EGR1 modulates HCC progression has not yet been elucidated. In this study, we found that EGR1 is a potential downstream regulator of 14-3-3 ε . Both of transient and stable overexpression of 14-3-3 ε significantly induced EGR1 expression which was determined by Western blotting and real-time PCR analysis. Furthermore, we found that EGR1 was involved in 14-3-3 ε -promoted HCC progression through activation of MEK/ERK pathway. Moreover, we found that sorafenib attenuated 14-3-3 ε -induced EGR1 expression. These results suggest that EGR1 plays as a crucial factor that contributes in 14-3-3 ε -promoted HCC tumor progression.

P-1104

AXL regulates IRS-associated metabolism in pancreatic cancer cells via a novel target TNS2

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AXL is known to be involved in the later stages of tumor progression such as migration/invasion, metastasis and/or drug resistance. Studies show that TNS2 is a binding partner of many proteins, including the tyrosine kinase Axl, but the details of their interactions have not been elucidated. Tensin 2 is a member of the Tensin family. Here, we demonstrate that TNS2 is a phosphorylation substrate of Axl. We further confirmed the correlationship of TNS2 expression and the expression of Axl, insulin receptor substrat-1 (IRS-1), pyruvate dehydrogenase kinase 1 (PDK1), pyruvate kinase M2 (PKM2) and glucose transporter type 4 (GLUT4) in pancreatic cancer patients. Based on these results, we suggest that Axl may modulate glucose metabolism potentially through TNS2 and IRS-1. We hypothesize that there exists a novel mechanism whereby Axl binds and phosphorylate TNS2 at Y483, releasing TNS2 from interacting with IRS-1 and resulting in increased stability of IRS-1. The three key enzymes of aerobic glycolysis, GLUT4, PDK1 and PKM2, were found to be up-regulated by the Axl/TNS2/IRS-1 cross-talk and may potentially play a critical role in glucose metabolism of cancer cells.

▲TOP

P-1105

Inhibition of cell cycle progression in megakaryoblastic cell line MEG-01 under simulated microgravity

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Platelets played a major role as first responders in cancer progression and metastasis. Megakaryocytes are specialized cells responsible for the production of platelets. Using a Desktop random positioning machine (RPM) we studied the cell cycle distribution of megakaryoblastic cell line MEG-01 under simulated microgravity. Flow cytometry analysis showed a significant increase in the percentage of cells in G0/G1 phase after 1-week of RPM-simulated microgravity as compared to the static group (1g control). Under RPM-simulated microgravity the change of expression of the CD13, CD19 and CD33 was not significant as compared to the control group. However, after 1-week of microgravity culture the expression of CD33 on cells was decreased by 15% to compare with the control cells. Thus, we concluded that simulated microgravity inhibits cell cycle progression of MEG-01 cells from G0/G1 into S phase, decrease cell proliferation and change the expression of surface markers. Understanding what inhibits cell cycle progression in the simulated microgravity could help researchers to develop a novel strategy for selective therapy.

P-1106

Lung squamous cell carcinoma exclusively depends on CD271 for cell proliferation

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Background: Lung squamous cell carcinoma (LSCC) has high degree of genome complexity, resulting in few common therapeutic targets for LSCC. We recently reported that cell proliferation in head and neck squamous cell carcinoma (HNSCC) is completely inhibited by CD271 depletion. Screening of the Tissue Atlas revealed that CD271 is also expressed in the SCC of other organs including lung, but only minimally expressed in lung adenocarcinoma, suggesting that CD271 has a critical role exclusively in LSCC. Aim of study: To elucidate the role of CD271 in LSCC. Results and discussion: Immunohistochemistry of 70 lung cancer specimens showed that CD271-positive specimens accounted for approximately 56% in LSCC, but 0% of the lung adenocarcinoma cells. Using LSCC cell lines originally established from patient-derived xenograft, CD271-knock down induced cell growth arrest through ERK-related pathway and G_0/G_1 arrest, while Trk receptor-knock down, known to bind CD271 and activate ERK signaling, induced only partial inhibition of cell proliferation. These results indicated that CD271 may be a good therapeutic target for LSCC.

▲TOP

[P-1114] P5-10 [Japanese]
Translation / non-coding RNAs

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Masahisa Ohtsuka / Osaka Police Hosp., Dept. Surg.

P-1114

RNA-helicase DDX6 regulates the expression of HER2 and FGFR2 at the translational step in gastric cancer cells

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Previously, we reported that the oncogenic RNA helicase DDX6 was overexpressed in most of malignant cell lines and clinical colorectal tumor samples through enhancement of c-Myc mRNA translational process.In the current study, we examined Gastric Cancer (GC) clinical samples by western blot analysis. As a result, DDX6/HER2/FGFR2 protein was overexpressed in about 60%/35%/30% of clinical samples. (both n=20) Interestingly, DDX6 protein was overexpressed in all HER2 positive samples(n=7), and in 83%(5 of 6) FGFR2 positive samples.In GC cell lines MKN-7 which has HER2 amplification, KATO-III and HSC39 which have FGFR2 amplification, knockdown of DDX6 by siR-DDX6 induced the decreased expression of HER2 and FGFR2 protein. On the other hand, knockdown of HER2 and FGFR2 did not influence DDX6 expression any more.RIP（RNP Immunoprecipitation）-assay in the GC cells indicate that DDX6 protein acts as RBP for HER2 and FGFR2 mRNAs and positively regulates the post-transcription process.These findings suggested that DDX6 may contribute to the pathogenesis of HER2 and FGFE2-positive GC through the promotion of HER2 and FGFR2 expression.

▲TOP

P-1115

PERK prevents accumulation of unfolded LGR5 protein during ER stress

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Previously, we reported that the expression of LGR5 protein, a representative stem cell marker, is depleted during ER stress, which is specifically regulated by PERK, one of the regulators of the unfolded protein response. In this study, we investigated precise mechanisms of PERK-dependent depletion of LGR5. Inhibition of PERK either by knockdown or a small compound led to dramatic increase in the amount of immature, underglycosylated forms of LGR5 during ER stress. On the other hand, PERK inhibition had no effect on the pre-existing mature form of LGR5, which was constitutively degraded under both normal and ER stress conditions. Mechanistically, the generation of underglycosylated LGR5 increased in concordance with the recovery of global translation repression by PERK inhibition. In that case, the underglycosylated proteins were not delivered to the cell surface but retained in the ER, and exhibited unexpectedly increased stability. Collectively, PERK is responsible for LGR5 depletion under ER stress conditions, through suppression of biosynthesis of LGR5 protein. This PERK-mediated regulation can prevent augmentation of stress to the ER by accumulation of unfolded LGR5.

P-1116

Extracellular vesicle-mediated miRNA transfer enhances growth and survival of multiple myeloma

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Recent evidence indicated that extracellular vesicles (EVs)-mediated multiple myeloma (MM) cell-bone marrow stromal cell (BMSC) communication plays an important role in the MM microenvironment. In this study, we investigated the biological property of EVs derived from BMSCs, aiming to establish the emerging strategies to target MM microenvironment to prevent tumor growth and spread.
BM samples were obtained from MM patients (MM-BMSCs), and assessed EV-miRNA profiling using a TaqMan PCR array. For functional analysis of candidate miRNAs, the miRNA mimics were transfected into MM cells using HiPerFect. Cell viability were determined using WST-8.

We found that the MM-BMSC-EVs enhanced the cell proliferation of MM cell line RPMI8226, and some miRNAs, including miR-10a, were significantly up-regulated in the MM-BMSC-EVs. We then visualized the uptake of Cy3-miR-10a into RPMI8226 via EVs. To identify the function of miR-10a in MM cells, miR-10a mimic was transfected into RPMI8226. Of note is that the overexpression of miR-10a enhanced MM cell growth and survival.
The EV-miR-10a derived from MM-BMSCs might therefore be one of promising target for controlling tumor proliferation in MM.

P-1117

Oncogenic activation of the ERK pathway alters miRNA expression profiles in exosomes

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The ERK pathway is a key signaling pathway involved in the regulation of cell proliferation. Hyper-activation of this pathway by oncogenes, such as Ras, Raf and MEK, eventually induces carcinogenesis. An emerging evidence indicates that exosomes, 30-100nm cell-derived vesicles, are also involved in cancer development and progression. Exosomes play biological roles in intracellular communication through their cargo molecules, such as microRNAs.Although both abnormal activation of the ERK pathway and exosomal miRNAs are deeply involved in cancer progression, the functional relationship between ERK signaling and exosomal miRNAs is still unknown. To understand this, we analyzed the transcriptome of exosomes using next generation sequencing to detect ERK-inducible, exosomal microRNAs.We found that hyper-activated ERK greatly affected the miRNA profile in exosomes, as compared with that in cell-body fractions. Interestingly, several ERK-inducible miRNAs were detected only in exosomes. Understanding the molecular function of these miRNAs may unravel the mechanism of ERK-mediated carcinogenesis. We are currently investigating if these miRNAs can serve as new cancer biomarkers.

▲TOP

P-1118

Establishment of DICER1 syndrome model cells

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DICER1 syndrome is an inherited disorder that increases the risk of various tumors. DICER1 mutations have been frequently found in Pleuropulmonary blastoma (PPB), and this finding has revealed that this mutation is the cause of the disease. Furthermore, in many diseases, a characteristic mutation in this gene has been found. Thus, it has been called DICER1 syndrome.DICER1 is member of RNase3 and essential for miRNA maturation. RNase3a and RNase3b domains of DICER1 process miRNA-3p and miRNA-5p arms of pre-miRNA, respectively. DICER1 syndrome has frequently detected missense mutations to RNase3b domain. It is thought that this disease develops by impairment of microRNA processing. However, the pathogenic mechanism has yet to be elucidated. To understand the role of DICER1 mutant, we established DICER1G1809R expressing cells. miRNA assay confirmed that the expression of miRNA-5p significantly reduced in the DICER1G1809R expressing cells. This cell is expected to be an available as tools for clarify mechanisms of DICER1 syndrome.

P-1119

Hypoxia downregulates tumor-suppressive sST2 in CRC cells in an IL-33/HIF-dependent manner

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[PURPOSE] We have previously demonstrated that sST2, a decoy receptor of IL-33, exerts tumor-suppressive effects on colorectal cancer (CRC) in the tumor microenvironment. IL-33, a member of “alarmin” family, is reported to act on sST2 expression as a nuclear repressor. Because hypoxia is known to enhance malignant properties of tumor cells, we investigated whether hypoxia downregulates sST2 expression in CRC cells through IL-33-HIF pathway. [METHODS] CRC-derived mouse NM11 cells and human SW480 cells were cultured in 20% or 1% O_2. Expression levels and transcription of the sST2 gene were measured by qRT-PCR and luciferase reporter assay, respectively. Subcellular localization of IL-33 was determined by Western blotting and immunofluorescence analysis. IL-33 binding to the sST2 promoters was assessed by ChIP assays. [RESULTS] Hypoxia stimulated IL-33 nuclear translocation followed by binding to the sST2 promoter and subsequently caused downregulation of sST2. Moreover, hypoxia-induced sST2 downregulation was ameliorated by siRNA-mediated knockdown of HIF-1α and HIF-2α. [CONCLUSION] Hypoxia may downregulate sST2 expression in an IL-33/HIF-depndent manner in CRC cells.

P-1120

Identification of hub-long non-coding RNAs (lncRNAs) by the network analysis of lncRNA expression in colorectal cancers

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In previous study, we revealed that the lncRNA NEAT1 is a direct transcriptional target of p53 and the suppression of NEAT1 induction by p53 attenuates the inhibitory effect of p53 on cancer cell growth and also modulates gene transactivation, including that of many lncRNAs. Furthermore, low expression of NEAT1 is related to poor prognosis in several cancers including colorectal cancers. Although other lncRNAs may have important functional roles in cancers, the analysis of every lncRNAs have difficulties because a myriad of lncRNAs are expressed in cancers. Therefore, the candidate lncRNAs need to be narrowed down in some way. We performed a gene network analysis of cancer transcriptome including lncRNAs using public RNA-seq data of cancer tissues. As a result, we identified several hub-lncRNAs which regulate the transcription of many downstream genes. The knockdown of several hub-lncRNAs significantly suppressed cancer cell growth. Our results suggest that lncRNAs comprise a complex transcriptional network for various biological functions and tumor suppression in colorectal cancers.

▲TOP

[P-1127] P8-2 [English/Japanese]
Cell death

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yuichi Takiguchi / Dept. Med. Oncol., Grad. Sch. Med., Chiba Univ.

P-1127

Involvement of Reactive Oxygen Species in Apoptosis Induced by Combination of UVA and Enoxacin

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Enoxacin (ENX) is a widely used fluoroquinolone antimicrobial agent that plays an important role in the treatment of human and animal infections; however, it has been reported to cause phototoxicity. In this study, we investigated the induction of apoptosis due to ultraviolet A (UVA) light in the presence or absence of ENX as the mechanism of phototoxicity in human promyelocytic leukemia (HL-60) cells. HL-60 cells were exposed to UVA for up to 20 min in the presence and absence of ENX, and the induction of apoptosis was examined by analyzing cell morphology, DNA fragmentation, and caspase-3 activity. The proportion of apoptotic cells was significantly higher in cells treated with both UVA and ENX than in those treated with UVA or ENX alone. Reactive oxygen species were measured by detecting nitroxide formation from 2,2,6,6-tetramethyl-4-piperidone by electron spin resonance (ESR) spectroscopy. NaN_3, the quencher of singlet oxygen significantly reduced the induction of apoptosis and nitroxide formation. These results suggested that singlet oxygen is most likely to be involved in the photodynamically induced apoptosis enhanced with ENX.

▲TOP

P-1128

Efficacy of MCL1 inhibitor S63845 in small cell lung cancer

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Small cell lung cancer (SCLC) is a histologic subtype of lung cancer and the proportion of SCLC is approximately 15%. The treatment of SCLC has not been improved over recent decades. It is important to explore new treatment strategies of SCLC. MCL1 is a member of the BCL-2 family, which regulates apoptosis. Targeting MCL1 represents a potential breakthrough of cancer treatment. We used four SCLC cell lines (DMS114, DMS53, SW1271, and NCI-H69) and one patient derived SCLC cell (KTOR201). The expression of MCL1 was higher in DMS114, DMS53, SW1271, and KTOR201 than NCI-H69. S63845, a MCL1 inhibitor, had greater efficacy in DMS114 and KTOR201. Knockdown of MCL1 resulted in lower cell viability in DMS114 and KTOR201 than DMS53 and SW1271. DMS114 and KTOR201 had lower expression of BCL-X_L, which is another member of the BCL-2 family, than DMS53 and SW1271. Knockdown of BCL-X_L increased sensitivity of S63845, suggesting that BCL-X_L is important to resistance of S63845 in SCLC. Immunohistochemistry of specimens from 37 patients with SCLC showed high expression of MCL1 at 27 cases.These data suggested that S63845 might be a powerful treatment of SCLC as a novel therapeutic strategy.

P-1129

Therapeutic effects and antitumor mechanism of trehalose liposomes against lung carcinoma mice model

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Inhibitory effects of trehalose liposomes (DMTre) composed of L- α -dimyristoylphosphatidylcholine and α -D-glycopyranosyl- α -D-glucopiranoside monomyristate (TreC14) on the proliferation of human non-small cell lung carcinoma (A549) cells were examined in vitro and in vivo. DMTre with a hydrodynamic diameter less than 100 nm, were preserved for 4 weeks. The inhibitory effects of DMTre on the proliferation of A549 cells accompanied with apoptosis were obtained after the enhancement of accumulation of DMTre into A549 membranes. An increase in cellular membrane fluidity of A549 cells treated with DMTre was observed on the basis of fluorescence depolarization method. DMTre caused apoptosis for A549 cells through the activation of caspases and mitochondrial pathway. Lung weights on the orthotopic graft bearing mice of lung carcinoma intravenously administrated with DMTre were markedly decreased as compared with those of the control group. Remarkable decrease in dimensions of tumor area of lung on the orthotopic graft bearing mice of lung carcinoma intravenously administrated with DMTre was obtained in histological analysis using the hematoxylin and eosin staining.

P-1130

Chemotherapy with cationic liposome that strategically targets pancreatic cancer cell membrane with negative charge

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Remarkably inhibitory effects of cationic liposomes (CL) on the growth of human renal and colon carcinoma cells along with apoptosis have been obtained in vitro and in vivo. In this study, we examined the therapeutic effects of CL against pancreatic carcinoma (BxPC-3) cells. Negatively charged phosphatidylserine (PS) and sialic acid-containing glycosphingolipids (GM1) were over represented on the cell membranes of BxPC-3 cells but not on normal pancreatic cells. CL selectively fused in the negatively charged cell membranes of BxPC-3 cells inhibiting the growth of BxPC-3 cells and inducing apoptosis, but had no effect on the viability of normal pancreatic cells. Apoptotic signal induced by CL for BxPC-3 cells passed through the mitochondrial events and the activation of caspase-8, -9 and -3. CL also inhibited tumor enlargement of xenograft model mice of pancreatic cancer by inducing apoptosis. It is noteworthy that remarkably high therapeutic effects of CL against pancreatic cancer cells with negative charge were obtained using model mice of pancreatic cancer.

▲TOP

P-1131

SphK1 inhibitor PF-543 induces autophagy regulated by ROS generation in oral squamous cell carcinoma cells

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Sphingosine kinase 1 (SphK1) overexpressed in head and neck squamous cell carcinoma (SCC) regulates tumor growth. In the present study, we examined PF-543-induced autophagy in oral SCC cells, and the role in several cell death mechanisms using autophagy inhibitors. The proportion of viable cells after PF-543 treatment decreased in a time- and dose- dependent manner, and cell death occurred in SphK1-expressing SCC cells. Flow cytometry analysis revealed that PF-543 induced both necrosis and apoptosis. In PF-543-treated cells, light chain 3 (LC3)-I was changed to LC3-II, indicating the occurrence of autophagy. Treatment of oral SCC cells with autophagy inhibitors and PF-543 increased the proportion of cells with necrosis and apoptosis, indicating that autophagy acts to promote cell survival. Reactive oxygen species (ROS) scavenger reduced the cytotoxicity of PF-543. These results demonstrated that PF-543 induces apoptosis, necrosis, and autophagy in oral SCC cells, and that autophagy antagonizes either necrosis or apoptosis.

P-1132

Switching mechanisms of two types of cancer cell death, necrosis and apoptosis

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Two major forms of cancer cell-death have been identified: necrosis and apoptosis. We have been studying the mechanisms that regulate the cell death observed during treatment of mouse tumor FM3A with antitumor 5-fluoro-2'-deoxyuridine (FUdR): it induces in the original F28-7 to necrosis, but for its variant F28-7-A, it induces apoptosis. We previously identified the cell-death regulators: molecular chaperone Hsp90, nuclear scaffold protein lamin-B1, cytoplasmic scaffold protein cytokeratin-19, transcription factor ATF3, and microRNAs (miR-351 and miR-743a) by using proteomic and transcriptomic analyses and siRNA/miRNA screening. Here, we investigated the gene mutations in these sister cells, using whole exome sequencing. In this data analysis, we revealed the critical gene mutations related to NAD^+-poly(ADP-ribose) metabolism and epigenetic regulation in necrosis-fated F28-7 cells. Interestingly, inhibition of NAD^+-poly(ADP-ribose) metabolism related gene blocked the FUdR-induced necrosis in F28-7 but not apoptosis in F28-7-A. These findings suggest that the gene mutation may have key roles in regulating the necrosis-apoptosis cell death mode.

P-1133

Impact of glutathione peroxidase enzyme 4 (GPX4) in human oral cancer

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GPX family, a selenoprotein, was first described as an enzyme that protects hemoglobin from oxidative degradation. Selenium-containing enzyme GPx4 antagonizes this damage by reducing lipid hydroperoxides to respective hydroxides. However, the role of GPX4 in human oral cancer remains unclear. We examined the impact of GPX4 against oral cancer. Five human oral squamous cell carcinoma (HOSCC) cell lines were used in this study. As a result of real-time quantitative RT-PCR, GPX4 expression levels were the highest in SAS cells. GPX4 knockdown with GPX4 siRNA in SAS cells revealed to decrease the cell number. It was suggested that GPX4 knockdown in SAS cells did not lead to apoptosis via the activation of caspases, but to lead non-apoptotic cell death such as ferroptosis. In addition, the positive reaction for MAb GPX4 was observed on the membrane of tumor cells in HOSCC tissues. Meanwhile, p53 immunoreactivity was clearly observed, especially consistent with GPX4 positive cells in HOSCC tissue. These findings suggest that GPX4 plays a significant role in the growth and proliferation of oral cancer.

▲TOP

[P-1139] P12-1 [English/Japanese]
Molecular target therapy (1)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Takashi Ishida / Div. Hematology & Oncology, Iwate Med. Univ.

P-1139

Functional analysis of high-affinity antibody mimetics with structurally constrained CDR peptides for tumor imaging

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Small proteins that have high affinity for cancer cell surface markers can be promising cheap alternatives for antibody (antibody mimetics). Therefore, various types of antibody mimetics have been extensively developed. We recently designed 130 species of small antibody mimetics (SAM) composed of a biocompatible small scaffold protein and a structurally constrained complementarity–determining region peptide derived from trastuzumab, a monoclonal antibody against HER2, and identified the highest–affinity mimetics (SAM–1) with a low dissociation constant (K_D = 24 nM).

In this study, we evaluated the applicability of SAM–1 for tumor imaging. We firstly confirmed that SAM–1 had same binding epitope with trastuzumab by competitive ELISA. Then we found that SAM–1 was highly thermostable as the heat treatment (95°C for 5 min) did not reduce the target–binding activity. Moreover, HER2 expression in cultured cancer cells and tumor sections were clearly detected with SAM–1. The applicability of SAM–1 as an in vivo imaging probe is under investigation using HER2–expressing tumors. Our approach may open a new avenue to develop practical molecular–targeting imaging probes for clinical use.

▲TOP

P-1140

Interdependent reactivity of anti-HER family antibodies against HER-family gene knock-outed cells

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We have recently succeeded in the production of a set of monoclonal antibodies (mAb) against surface epitopes of cancer-associated molecules containing amino-acid transporters (CD98hc, LAT1, xCT, ASCT2, CAT1), oncogene products (HER1, HER2, HER3, HER4, MET) and adhesion molecules (CD44, EpCAM) expressed on various human cancers. Since CRISPR-Cas9 system can efficiently abolish the protein expression by knockout (KO) of target gene, in this study, we investigated the effect of KO on the reactivity of anti-HER family mAb by flow cytometry. Although KO of HER1 or HER2 specifically abolish the expression of each HER protein, KO also affected the reactivity of anti-HER3 and anti-HER4 mAb on HER1-KO or HER2-KO human lung cancer cells (NCI-H1838), and HER3-KO affected the reactivity of anti-HER4 mAb in human colorectal cancer cells (SW1116). These results suggest that selective heterodimerizations in HER family proteins interdependently influence the affinity of mAb against a given component in heterodimeric proteins. We have started on the analysis of dissociation constants of anti-HER family mAb against KO cells of individual HER family genes. collaborators: Kazue Masuko, Kinji Yoshida

P-1141

Functional evaluation of HER2-binding small proteins harboring a structurally constrained peptide

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Peptides with high affinity for tumor markers such as HER2 are attractive molecules for targeted therapy. However, target–binding peptides constructed so far showed low binding affinity for target molecules. Our previous study found that structurally constrained target–binding peptides showed higher affinity compared to fluctuating ones. This finding prompted us to develop a clinically applicable HER2–binding small protein (F–body Ⅰ) using a human fibronectin type Ⅲ (Fn3) as a scaffold. By screening a hexapeptide library, F–body Ⅰ was isolated with high binding affinity for HER2 (K_d = 282 nM), but for practical applications a higher binding affinity is required.

In this study, we identified three amino acids of the HER2–binding hexapeptide of the F–body Ⅰ, which are important for binding to HER2. Then, we optimized the remaining three amino acids for HER2 binding by screening a library of T7 phage–displayed peptides. The purified F–body Ⅰ with optimized hexapeptide, designated F–body Ⅱ, exhibited HER2 binding activity comparable to antibodies (K_d = 57.7 nM). We present results demonstrating that F–body Ⅱ has the potential to be clinically used as a practical tool.

P-1142

Enhancing efficacy of anti-PD-1 antibody by combination with an HDAC/PI3K dual inhibitor in a mouse model of melanoma

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Purpose: Immune checkpoint inhibitors targeting PD-1 have brought possible clinical benefit to patients with advanced cancers. However, the significant limitation is their insufficient response rates. It is needed to develop a novel strategy aimed to increase the response efficiently. Some agents including HDAC inhibitors and PI3K inhibitors were reported to enhance anti-PD-1 antibody by modulating conditions of immune response. We have developed an HDAC/PI3K dual inhibitor, FK-A11. In this study, we examined the efficacy of the combination of the anti-PD-1 antibody and FK-A11 in a melanoma mouse model. Method: 5 x 10^5 B16F10 melanoma cells were injected to C57BL/C mouse subcutaneously. After confirming tumor formation, anti-PD-1 antibody was administrated with or without FK-A11 intraperitoneally. Result: In spite of the non-significant efficacy of single-agent anti-PD-1 therapy, the combination of anti-PD-1 antibody and FK-A11 significantly inhibited tumor growth. Furthermore, the combination therapy increased CD8 lymphocytes infiltrated into tumor tissues. Conclusion: FK-A11 efficiently enhanced anti-PD-1 antibody in a mouse model of melanoma.

▲TOP

P-1143

T-cell-engaging B7-H4/CD3 bispecific Fab-scFv antibody targeting human breast cancer

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We found an upregulation of B7-H4 in breast cancer using comprehensive whole exome sequencing and gene expression profiling (project HOPE) launched by the Shizuoka Cancer Center based on tumor tissues. We designed and manufactured anti-B7-H4/CD3 bispecific antibodies (BsAb) based on the Fab and scFv structure. We found that the BsAb had potent cytotoxic activity against B7-H4-positive cell lines in vitro, and in vivo using a humanized mouse model. hPBMCs with the BsAb lysed the human breast cancer cell line MDA-MB-468 (EC50: 0.2 ng/ml) and others in vitro. hPBMC transplanted MHC-dKO NOG mouse had an immediate anti-tumor activity after the BsAb injection, and with no adverse effect after long term observation. With the BsAb, both CD8+-T cells and CD4+ T cells killed the target cells in vitro, and mainly CD8+ T cells contributed to in vivo anti-tumor activity. B7-H4 appears to be expressed independently of HER2 or PD-L1 expression in breast cancers, the anti-B7-H4/CD3 bispecific antibody may be a therapeutic tool for immune checkpoint blockade or anti-HER2 antibody-unresponsive cancer patients.

P-1144

Chemotherapy enhances the efficacy of anti-PD-1 antibody by reducing intratumoral myeloid-derived suppressor cells

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Background: Antibody which inhibits PD-1/PD-L1 axis showed a promising effect in several solid tumors but the effect is limited in some subset of patients. Recently, the favorable effect of the combination of anti-PD-1 antibody and chemotherapeutic agents was reported, although the mechanism by which cytotoxic drugs regulate the effect of anti-PD-1 antibody is still unknown.Method: We utilized syngeneic mouse tumor models of lung cancer and malignant pleural mesothelioma, and assessed the therapeutic effect of anti-PD-1 antibody in combination with chemotherapeutic agents. Host cells infiltrated to the tumor tissue were assessed by immunohistochemistry. Results: The combination therapy significantly inhibited the tumor growth compared to each single treatment. Treatment with anti-PD-1 antibody increased intratumoral CD8+ T cells. On the other hand, treatment with chemotherapeutic agents decreased intratumoral myeloid-derived suppressor cells (MDSCs), possibly by the suppression of vascular endothelial growth factor expression in the tumor cells. Conclusions: Chemotherapeutic agents enhance the effect of anti-PD-1 antibody by inhibiting MDSCs in the tumor microenvironment.

▲TOP

[P-1151] P14-1 [English/Japanese]
Esophageal cancer (1)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Hiroki Sasaki / Dept. Translational Oncol, Natl. Cancer Ctr. Res. Inst.

P-1151

Clinical Significance of Programmed Death-1 Ligand-2 in Esophageal Cancer: Comparison with Programmed Death-1 Ligand-1

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Background: Immunotherapies targeting the PD-1/PD-L1 pathway have emerged as promising therapeutic strategies for many cancers. PD-L1 and PD-L2 are ligands for PD-1 which are cell-surface glycoprotein. The clinical and prognostic features of the PD-L1 have been widely reported for various cancers, but those of PD-L2 remains unclear in esophageal cancer. Methods: Using a database of 301 curatively resected esophageal cancer from April 2005 to Jun 2013, we evaluated PD-L1 and PD-L2 expression by immunohistochemical staining. Results: Out of 301 cases of esophageal cancer, 48 (15.9%) showed PD-L2 positive and 253 (84.1%) showed PD-L2 negative expression. PD-L2 positive cases were significantly associated with age (p=0.045), histological type (p=0.039), and postoperative therapy present (p=0.012). Compared with PD-L2 negative cases, PD-L2 positive cases showed significantly worse overall survival (p=0.018). PD-L1 positivity was also significantly associated with poor prognosis (p=0.001). Importantly, there was no correlation between PD-L2 and PD-L1 expression. Conclusion: PD-L2 expression was significantly associated with prognosis, supporting their roles as a prognostic biomarker.

▲TOP

P-1152

The significance of PD-1 expression of tumor infiltrating lymphocytes in patients with esophageal cancer

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Introduction:Recently, many clinical trials for programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) signal-blockade agents have demonstrated dramatic antitumor efficacy in various cancer. However, there were few reports clarifying the relationship between PD-1 expression of tumor infiltrating cells(TILs) and clinicopathological features.Methods:A retrospective study was conducted on 241 patients who underwent esophagectomy for esophageal cancer between March 2005 and June 2013. We performed immunohistochemistry for PD-1 and PD-L1, and evaluated the density of TILs at invasive front with HE staining sections.Results:PD-1 positive patients (n=77, 31.9%) were significantly associated with advanced tumor stage (P=0.01). Interestingly, PD-1 expression was significantly related to PD-L1 expression of tumor cell and density of TILs (P=0.0004, P=0.0043, respectively). Furthermore, PD-1 positive patients tend to experience poor prognosis in cancer specific survival (P =0.08).Conclusion:Given the significant interest in cancer immunotherapies targeting PD-1/ PD-L1 pathway, the current findings should have considerable clinical implications.

P-1153

The concentration of PD-L1 in the peripheral blood could be a useful biomarker for esophageal squamous cell carcinoma

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BACKGROUND:
The correlation of PD-L1 concentrations in the peripheral blood and PD-L1 expression in the tumor tissue remain unclear.
METHODS:
Blood samples and resected specimens were collected from 30 patients with histologically proved ESCC. Serum levels of PD-L1 were measured using enzyme linked immunosorbent assays. Expression of PD-L1 in the resected specimen were revealed by the immunohistochemistry using the antiPD-L1 antibody (ab205921) for a preoperative untreated resected specimen and confirm the PD-L1 expression in the resected specimen.
RESULTS:
The average concentration of soluble PD-L1 in patients with high expression of PD-L1 in tumor tissue was 15.3pg/ml (n=9), and the average concentration of PD-L1 in patients with low expression of PD-L1 in tumor tissue was 11.6pg/ml (n=21). Average concentration of soluble PD-L1 was significantly higher than patients with low expression of PD-L1 in resected specimen (p=0.015).
CONCLUSION:
Our data showed that the patients with high expression of PD-L1 in resected specimen were high concentration of soluble PD-L1 in peripheral blood.

P-1154

Software-based IHC imaging cytometry of tumor-associated macrophages in the ESCC tissues

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Macrophages that invade tumor tissue are called tumor associated macrophages (TAMs). We previously demonstrated that there were elongated TAMs in the human esophageal squamous cell carcinoma (ESCC) tissue, which was consistent with our previous in vitro studies. In this study, we analyzed M2 macrophage marker CD163 immunohistochemistry (IHC) images of an ESCC tissue, in which we set 10 to 12 regions of interests in non-tumor epithelial mucosa (Area A), pT1a-EP (M1) squamous cell carcinoma region (Area B), pT1a-LPM (M2) squamous cell carcinoma (Area C) and non-tumor submucosa (Area D), by an image analysis software. Comparing the Area A with the Area B, there was no difference in the nuclear area and the CD163-positive area, but the nuclear area, CD163 mean intensity and the CD163-positive rate were increased in the Area B ($p < 0.001$). Both the nuclear area and the CD163-positive area were increased in the Area C ($p < 0.05$), but there were no difference in the average of mean intensity of CD163 and the CD163-positive rate ($p < 0.05$) in the Area C than those in the Area B.
This system is useful for quantitative assessment of macrophage cytometry in the ESCC tissues.

▲TOP

P-1155

Analysis of upregulated genes in cancer-associated fibroblasts of the ESCC microenvironment

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Cancer-associated fibroblasts (CAFs) are known to be involved in the progression of various cancers. We have reported the association of high expression levels of CAFs markers (α SMA and FAP) with poor prognosis in esophageal squamous cell carcinomas (ESCCs) via immunohistochemistry. However, the roles of CAFs in the ESCC microenvironment remain unclear. We previously co-cultured human bone marrow-derived mesenchymal stem cells (MSCs) with ESCC cell lines and confirmed the induction of α SMA and FAP in MSCs, which we defined as CAF-like cells. To investigate the roles of CAFs, we performed cDNA microarray analysis between MSCs and CAF-like cells and found several genes that were highly expressed in CAF-like cells when compared to MSCs. Now, we are investigating the roles of these genes in tumor promoting effects of CAFs on ESCC cell lines.

P-1156

TDO2 expression is related with cancer stem cells and prognosis in esophagus squamous cell carcinoma

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Objective: Esophagus cancer is one of the deadliest cancers in the world. Expression of tryptophan 2,3-dioxygenase (TDO2), an enzyme involved in tryptophan catabolism, has been linked with tumor survival and poor prognosis of brain and breast cancer. Here we explored the expression and biological significance of TDO2 in esophagus squamous cell carcinoma (ESCC). Methods: TDO2 expression was evaluated in 90 ESCC tissue samples by immunohistochemistry. TDO2 function in ESCC cell lines and spheroid colony formation was evaluated using RNA interference (RNAi). Results: TDO2 overexpression was associated with recurrence status and CD44 in ESCC. TDO2 expression was correlated with the outcome of ESCC patients. Inhibition of TDO2 expression by RNAi in ESCC cell lines reduced both the number and the size of spheroid colonies as well as cell proliferation. Knockdown of TDO2 expression also induced inactivation of the EGFR signaling pathway. Conclusion: Our results imply that TDO2 could play an important role in the progression of ESCC.

P-1157

Glutathione S-transferase Pi 1 predicts anticancer drug resistance in esophageal squamous cell carcinoma

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BACKGROUNDS: Esophageal squamous cell carcinoma (ESCC) is one of aggressive malignancies and Glutathione S-transferase Pi 1 (GSTP1) has reported as a predictor of malignancy or anticancer drug resistance in some cancers. Here we investigated the association of GSTP1 expression with malignancy or drug resistance in ESCC. METHODS: The proliferation and sensitivity to cisplatin regarding GSTP1 expression were examined in ESCC cell lines, KYSE170 and TE13. Apoptosis assay was also performed. In addition, immunohistochemistry (IHC) assay of tissue samples was performed in 72 ESCC patients with neoadjuvant chemotherapy (NAC). RESULTS: The proliferation of GSTP1 knockdown (siGSTP1) cells was significantly decreased ($p<0.01$). Similarly, the frequency of early apoptosis in siGSTP1 cells was increased ($p<0.05$). Viability of siGSTP1 cells was decreased by cisplatin treatment. Moreover, the frequency of early apoptosis in siGSTP1 cells was markedly increased by cisplatin treatment. ($p<0.01$). In IHC assay, GSTP1 expression was significantly associated with clinical downstaging by NAC($p=0.042$). CONCLUSIONS: GSTP1 expression related to the tumor progression and drug resistance in ESCC.

▲TOP

[P-1165] P14-3 [English/Japanese]
Esophageal cancer and GIST

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Masahiko Yano / Dept. Gastroenterol. Surg., Osaka International Cancer Institute

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P-1165

CCL3 produced from TAMs promotes migration of ESCC cell line via Akt and ERK pathways

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Tumor associated macrophages (TAMs) are known to contribute progression of various cancers including esophageal squamous cell carcinomas (ESCCs).We found that CC chemokine ligand 3 (CCL3) was highly expressed in TAM-like macrophages (Mφs) differentiated from human blood monocytes-derived Mφs stimulated with conditioned media of ESCC cell lines. CCL3 has been reported to promote progression of some cancers. We investigated that the significance of CCL3 for TAMs on ESCC.CCR1 and CCR5, known CCL3 receptors were expressed in ESCC cell lines. Treatment of ESCC cell lines with recombinant human CCL3 induced cell migration of ESCC cell lines with induced Akt and ERK phosphorylation. Moreover, Akt or ERK inhibitor suppressed CCL3-induced cell migration of ESCC cell lines. These results suggest that CCL3-CCR1 and/or CCL3-CCR5 axis is involved in the progression of ESCCs.Now we are investigating the effect of small interfering RNAs or inhibitors to CCR1 and CCR5 on the Akt and/or ERK signaling pathways and cell migration of ESCC cell lines.

▲TOP

P-1166

Interaction between macrophages and esophageal squamous epithelial cells enhances G-CSF signaling

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We previously reported that increased infiltrating number of macrophages (MΦs) was associated with poor prognosis of human esophageal squamous cell carcinomas (ESCCs). We also observed that infiltrating MΦs were upregulated in ESCC precancerous lesions when compared to normal squamous epithelium. Based on these findings, we established a coculture assay using human esophageal epithelial cells (Het-1A) and human acute monocytic leukemia cells (THP-1)-derived MΦs to study the roles of MΦs in esophageal carcinogenesis. In this study, we performed cDNA microarray analysis between monocultured Het-1A and co-cultured Het-1A with THP-1-derived MΦs, and found that G-CSF expression was upregulated in both Het-1A and THP-1-derived MΦs under co-culture conditions. Het-1A was found to express G-CSF receptors. Recombinant human G-CSF (rhG-CSF) induced cell migration and modulated GSK-3α/β-β-catenin signaling in Het-1A. Moreover, phospho-β-catenin (Ser675) expression levels were increased, and translocation of this protein from the cytoplasm to the nucleus was induced by rhG-CSF. These results indicate that G-CSF signaling may contribute to early esophageal carcinogenesis.

P-1167

The combined effect of PRIMA-1MET and chemotherapy in esophageal squamous cell carcinoma

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【Introduction and objective】PRIMA-1 has been shown to restore the function of mutant p53 protein. Since mutation of p53 is clinically associated with resistance to chemotherapy in esophageal cancer, combination use of PRIMA-1MET with chemotherapy is expected to improve for chemotherapy-resistant.【Materials and methods】We use KYSE410, 960 as p53 wild type, TE 1,4,5,8,10,11 as missense mutant, TE 9,14 as frameshift/nonsense mutant of esophageal squamous cell carcinoma (ESCC) cell lines. Cell viability was evaluated by MTT assay 48 hours after the administration of PRIMA-1MET or anticancer drug (any one of DTX/CDDP/5-FU). The Isobologram method with combination Index (CI) was used (CI＜0.8 as synergistic effect).【Result】ESCC with p53 missense mutation showed additive or more effects. In particular, synergistic effects in combination with 5-FU (CI=0.58, 0.71, 0.72) was identified in TE1, TE4, TE8. On the other hand, the combination use was less effective in p53 nonsense/frameshift mutant cells and wild type cells as compared with missense mutation.【Conclusion】The significant combined effect of PRIMA-1MET and chemotherapy was observed in ESCC with p53 missense mutation.

P-1168

Role of CLIC1 in human esophageal squamous cell carcinoma

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Recent studies have reported important roles for chloride intracellular channel 1 (CLIC1) in various cancers. The aim of the present study was to investigate the role of CLIC1 in human esophageal squamous cell carcinoma (ESCC). The cells of ESCC cell lines strongly expressed CLIC1. The depletion of CLIC1 using siRNAs inhibited cell proliferation, induced apoptosis, and promoted cell migration and invasion. The microarray analysis revealed that CLIC1 regulated apoptosis via the TLR2/JNK pathway. Immunohistochemical analysis performed on 61 primary ESCC samples showed that CLIC1 found in the cytoplasm of carcinoma cells. Although the very strong expression of CLIC1 was not related to any clinicopatholgical features, the very weak expression was related to histological type. Moreover, the very strong or very weak expression of CLIC1 was an independent poor prognostic factor. The present results suggest that the very strong expression of CLIC1 enhances tumor survival, while its very weak expression promotes cellular movement, and provide an insight into the role of CLIC1 as a switch among tumor behaviors in ESCC.

▲TOP

P-1169

IDO1 hypomethylation is associated with a poor prognosis in patients with esophageal cancer

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【Background】 IDO1 plays a major role in tumor immunology and is a potential immune-based therapeutic target. Recently, it has been reported that IDO1 expression is regulated by the methylation of IDO1 promotor. Thus, the aim of this study is to examine the relationship between IDO1 expression, IDO1 promotor methylation and clinicopahological feature in esophageal cancer.【Methods】 By treating 5AZA, we confirmed the change of IDO1 level in vitro. Using 146 FFPE samples from resected esophageal cancer, we evaluated the relationship between IDO1 expression level (IHC), IDO1 promotor methylation (bisulfite pyrosequencing) and clinicopathological features.【Result】 Treating 5AZA to cell lines, hypomethylation induced significantly higher IDO1 expression in cell lines. In FFPE samples, IDO1 methylation levels have also been inverse correlated with IDO1 expression level by immunostaining (P=0.047). Furthermore, IDO1 hypomethylation group (n=30) have poor prognosis compared with IDO1 hypermethylation group (n=116) (overall survival P=0.002).【Conclusion】 IDO1 promotor hypomethylation regulated IDO1 expression and have been associated with poor prognosis in esophageal cancer.

P-1170

Phosphorylation of transferrin receptor 1 can be associated with tumor activity in esophageal squamous cell carcinoma

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Background: Despite the recent improvements in multimodal therapies for esophageal squamous cell carcinoma (ESCC), the prognosis remains poor. The identification of disease specific molecular therapeutic targets is required for developing new molecular target therapy. Methods: We used normal two esophageal epithelial cell lines and six esophageal cancer cell lines. We performed quantitative tyrosine phosphor-proteomic analysis method, which combined immunoaffinity enrichment of phosphor-tyrosine-containing peptides with iTRAQ technology. Results: We identified 16 proteins, which overexpressed in cancer cells and focused on transferrin receptor 1 (TFRC1) as a novel molecule. Phosphorylated(p-) TFRC1 was increased >2-fold in all 6 ESCC cell lines compared with normal esophageal epithelial cell lines. In addition, we confirmed that the expression of p-TFRC1 was observed in TE-5, TE-9 in western blotting. Now, we are trying to show the function of p-TFRC1 in esophageal cancer.Conclusions: We showed the possibility of the targeting p-TFRC1 in ESCC therapy. Further study of this novel molecule might provide a new insight into the role of TFRC1 in cancer biology.

P-1171

The role of Aquaporin 1 in Esophageal Squamous Cell Carcinoma

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The objectives were to investigate the role of AQP1 in the regulation of genes involved in tumor progression and the clinicopathological significance of its expression in Esophageal Squamous Cell Carcinoma.50 human ESCC samples were categorized into two groups according to the expression of AQP1 in each of cytoplasm and nuclear membrane. Apoptosis assay was performed.The gene expression profiles of AQP1-depleted TE5 cells was analyzed. In an immunohistochemical analysis, AQP1 was primarily located in the cytoplasm and/or the nuclear membrane of carcinoma cells. The 5-year survival rate of patients with the “cytoplasm dominant” expression of AQP1 (47.1%) was significantly lower (p=0.028) than other patients (83.2%). A multivariate analysis of the 5-year overall survival rate showed that the pT categories, venous invasion and cytoplasm dominance groups of AQP1 were independent prognostic factors (p=0.0423, 0.0473 and 0.0058, respectively). The depletion of AQP1 using siRNA induced apoptosis in TE5 and TE15 cells. The results of microarray analysis revealed that Death receptor signaling pathway-related genes were changed in AQP1-depleted TE5 cells.

▲TOP

[P-1179] P14-5 [English/Japanese]
Novel biotherapy and molecular analysis of gastric cancer

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-1179

Induction of Apoptosis and Autophagy via Sirtuin1-mediated Pathways by Momordica charantia in Gastric Cancer Cells

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Sirtuin1 (SIRT1), one of the sirtuin family of proteins, was known to regulate cellular stress response and cell survival through deacetylation of its target proteins and modulation of apoptosis and autophagy. This study aimed to investigate the effects of *Momordica charantia* (MC) on apoptosis and autophagy and the underlying mechanisms which SIRT1 is involved in gastric cancer (GC) AGS and SC-M1 cells. MC extract (MCE) had potent apoptotic and autophagic effects in both GC cells. The MCE-induced inhibition of p38 mitogen-activated protein kinase and activation of 5'-AMP-dependent kinase (AMPK) lead to the pro-autophagic activity in cells. MCE-induced autophagy was found by the expression of autophagy-related proteins, LC3-II and p62. SIRT1 was significantly downregulated by MCE. The enhanced cell death by SIRT1 activator, SRT1720, indicated the regulatory role of SIRT1 on MCE-mediated cell death in GC cells. The pretreatment of z-VAD-FMK indicated that apoptosis induced by MCE in both GC cells was caspase-independent. Additionally, MCE-inhibited cell proliferation was confirmed by a zebrafish xenograft model, hence, implying the potential of MC for anticancer drug development.

▲TOP

P-1180

Jianpi Yangzheng Xiaozheng Decoction inhibit Gastric Cancer by regulating Tumor Associated Macrophage

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Jianpi Yangzheng Xiaozheng Decoction (JPYZXZ) is an empirical compound, which can improve the life quality of gastric cancer patients and prolong their survival. Here, we investigate the effect of JPYZXZ and its decomposed formulas Jianpi Yangzheng Decoction (JPYZ) for Qi-invigorating, spleen strengthening part and Xiao zheng san Jie Decoction (XZSJ) for stasis removing part on inhibiting progression and modifying tumor associated macrophages (TAMs) phenotype in gastric cancer.JPYZXZ, JPYZ and XZSJ were administered to the 615 mice transplanted with gastric cancer. Flow cytometry and immunohistochemical assay were used to test the phenotype of TAMs. PI3K/AKT pathway and Epithelial mesenchymal Transition (EMT) which are targets of TAMs were measured by qPCR, immunofluorescence and Western blot. We demonstrate that, JPYZXZ and XZSJ inhibited tumor weight and volume. JPYZXZ, JPYZ and XZSJ could reduce M2-TAM, inhibited PI3K/AKT pathway and EMT. And on the whole, the effect of JPYZXZ in inhibiting tumor progression is better than JPYZ and XZSJ.

P-1181

Analysis of a mechanism that initiates stemness in inflammation-driven gastric cancer cells

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Gastric cancer is a complex disease that often arises in a setting of chronic inflammation. In various type of human tumors, cancer stem cells, tumor-resident cells with stem cell characteristics are thought to be resistant to conventional anti-cancer therapies.
Recently, we have identified epithelial stem cell marker, Lgr5 positive chief cells serve as reserve stem cells to effect epithelial renewal following oxyntic atrophy in corpus stomach. Furthermore, these stem cells are likely to contribute to the gastric tumor initiation in vivo. However, whether and how Lgr5 positive cells serve as cancer stem cells which reside in the chronic inflammation-driven gastric cancer is largely unknown.
Here, we have found that a transcription factor, Sox9 which expresses in Lgr5 positive reserve stem cells was also upregulated in gastric tumor cells in Gan mice which develop tumors under the chronic inflammation in the corpus stomach. To evaluate the role of Sox9, we introduced the ex vivo organoid system and found that Sox9 could regulate the stemness in both normal and cancer cells.
We are further analyzing how Sox9 is differently regulated and contributes to the stemness in those cells.

P-1182

Epigenetic dysregulation in AFP-producing gastric cancer

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AFP-producing gastric cancer (AFPGC) is a highly malignant subclass of the gastric cancer. Most of studies demonstrated that AFPGC had more aggressive clinical behavior than non-AFPGC due to high incidence of lymphatic invasion, venous invasion and liver metastasis. Furthermore, high incidence of distant metastasis led to relatively poor overall prognosis. The underlying molecular mechanisms, which could explain why AFPGC shows hepatoid differentiation and more aggressive behavior, have not yet been elucidated. The aim of this study is to clarify the mechanism of transcriptional dysregulation which would be a key of its tumorigenesis and aggressive behavior in AFPGC. AFPGC cell lines and non-AFPGC cell lines were used to identify significant transcription factors by integrating RNA-seq, ChIP-seq, and ATAC-seq data. Furthermore, by comparing them to RNA-seq and ChIP-seq data from human live cancer cell lines, we observed that Foxa1 was overexpressed in AFPGC, indicating the cause of AFPGC. We are currently exploring the candidate genes targeted by Foxa1 and the mechanism for aberrant activation of Foxa1 in gastric epitherial cells.

▲TOP

P-1183

Neutrophil extracellular traps(NETs) on postoperative peritoneal surface may support the tumor recurrence on peritoneum

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Background: Peritoneal recurrence often happens after abdominal surgery in digestive malignancies. LDN (low density neutrophils) contained in the postoperative exudate in peritoneal cavity can produce NETs which enhances peritoneal metastasis in nude mice (Kanamaru R et al. Sci Rep.2018). Here, we examined the detailed mechanism. Method: LDN were seeded on monolayer of human peritoneal mesothelial cells (HPMC). NETs were visualized by the addition of SYTOX-green. The cytotoxicity of LDN against tumor cells and HPMC were measured with the Calcein-AM staining method. Results: LDN produced NETs on plastic plate in 2hrs incubation, but not on HPMC monolayer, except the bare area where the HPMC was scraped off. After 3hrs, LDN showed moderate cytotoxicity (16.6±1.2%,E/T ratio=20) against HPMC but little against tumor cells. LDN induced gap formation in confluent HPMC after 24hrs.Intraperitoneal injection of the LDN produced NETs-like structure on mouse peritoneum, especially at the area with mechanical damage. Conclusion: LDN has cytotoxicity against HPMC and subsequently produces NETs formation, which may cause the enhancement of peritoneal recurrence after abdominal surgery.

▲TOP

[P-1189] P14-7 [English/Japanese]
Novel biotherapy for gastric cancer: new antibody therapeutics and oncolytic virus therapy

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Tetsuo Ushiku / Dept. Pathology, Tokyo Univ.

P-1189

Translational approach of oncolytic herpes simplex viruses for human scirrhous type of gastric cancer

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Introduction: One of the promising therapeutic strategies for gastric malignancies is an oncolytic virotherapy. We have conducted a study of oncolytic herpes simplex viruses (oHSVs) against gastric cancer, and have previously reported a preliminary data about an armed oHSV expressing thrombospondin-1 (T-TSP-1). Purpose and Method: To overcome the resistance for T-TSP-1, we examined the antitumor effect of fourth-generation oHSVs, which contains ICP6 gene driven human TERT (telomerase reverse transcriptase) promoter (T-hTERT). For human gastric cancer cell line, we examined the expression of ribonucleotide reductase (RR) activity and their telomerase activity in gastric cancer cell line, and also evaluated the cytotoxicity of T-hTERT. Result: Almost gastric cancer cell line showed RR expression. We detected telomerase activity and hTERT mRNA in all gastric cancer cell lines. For freshly dissected scirrhous gastric cancer specimen, however, T-hTERT reduced cytotoxicity compared with T-TSP-1. Conclusion: For oncolytic virotherapy using oHSVs against scirrhous gastric cancer, we need to modify an additional modality and approach.

▲TOP

P-1190

Therapeutic Efficacy of a Third Generation Oncolytic HSV-1 G47 δ in Multiple Mouse Models of Gastric Carcinoma

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Antitumor effect of a third generation oncolytic herpes simplex virus type 1, G47 δ , was studied for gastric carcinoma (GC). Cytotoxic effect, infectivity and replication capability of G47 δ were investigated in vitro using 6 human GC cell lines. In vivo, subcutaneous tumors of MKN45 or MKN74 cells in athymic mice were treated with intratumoral injections with G47 δ (2×105 pfu) or mock, and the tumor size was observed. Also, athymic mice with peritoneal dissemination of MKN45-luc cells were treated with intraperitoneal injections with G47 δ (1×106 pfu) or mock, and followed by observation and measurement using in vivo imaging system. G47 δ showed good cytotoxic effect, good infectivity and good replication capability in all cell lines. In subcutaneous tumor models, the treatment with G47 δ significantly suppressed the tumor growth compared with mock treatment. In the peritoneal dissemination model, G47 δ treatment significantly decreased the total bioluminescence from disseminated tumors ($P<0.01$) and prolonged survival compared with mock treatment ($P<0.01$). These results demonstrate that G47 δ may be useful for the treatment of advanced GC with peritoneal metastases.

P-1191

Antitumor effects of the antiparasitic agent ivermectin via inhibition of YAP 1 expression in gastric cancer

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Background: Intranuclear yes-associated protein 1 (YAP1) acts as an oncogene and its high expression is associated with poor prognosis in gastric cancer (GC). We previously identified ivermectin as a YAP1 inhibitor. We aimed to clarify whether ivermectin had antitumor effects on GC.Materials and Methods: We evaluated the antiproliferative effects of ivermectin on human GC cells. We explored the mechanism through which ivermectin regulated YAP1 expression or localization by immunoblotting and RT-qPCR for YAP1 and the downstream gene CTGF. The clinical significance of YAP1 expression was examined using three GC datasets.Result: We found that MKN1 cells were most sensitive to ivermectin. In MKN1 cells, ivermectin suppressed tumor growth, and the sensitivity to ivermectin was decreased by YAP1 knockdown. Ivermectin decreased YAP1 mRNA expression, thereby inhibiting the nuclear accumulation of YAP1. Low YAP1 mRNA expression was associated with a better prognosis in three GC datasets.Conclusion: We identified ivermectin as a potential antitumor agent and found a promising novel therapeutic strategy for inhibition of GC by blocking YAP1 expression.

P-1192

Trifluridine/tipiracil overcomes the resistance against human gastric 5-fluorouracil-resistant cells

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Trifluridine/tipiracil (FTD/TPI) is approved for the treatment of unresectable advanced or recurrent colorectal cancer. The phase 3 TAGS trial is ongoing to evaluate FTD/TPI efficacy in metastatic gastric cancer patients refractory to standard treatments. Since only a few findings were reported on the effect of FTD for 5-fluorouracil (5-FU)-resistant gastric cancer cells, we evaluated the in vitro and in vivo efficacy of FTD and FTD/TPI using 5-FU-resistant MKN45/5FU, MKN74/5FU, and KATOIII/5FU human gastric cancer cells overexpressing thymidylate synthase (TS). Only MKN45/5FU cells showed partial cross-resistance (3.7-fold) to FTD in vitro. Conversely, oral FTD/TPI administration twice daily sufficiently inhibited tumor growth in MKN45/5FU and MKN45 xenografts. The amount of FTD DNA incorporation was comparable, although deoxyuridine monophosphate levels were 3-fold higher in MKN45/5FU cells than in parental cells after 24-h FTD treatment. These results suggest that FTD/TPI in vivo was effective despite cross-resistance because FTD was incorporated into DNA regardless of TS levels. Our results suggest that FTD/TPI might be useful for the end line chemotherapy of gastric cancer.

▲TOP

P-1193

Anti-tumor effects of farnesyltransferase inhibitors on gastric cancer

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Farnesyltransferase inhibitors (FTIs) are capable of inhibiting proliferation of many cancers. We revealed the expression of hypoxia inducible factor-1 α (HIF-1 α) is suppressed by FTI, tipifarnib. Here, the relationship between the anti-tumor effect by FTI and HIF-1 α was examined in gastric cancer (GC) cells.MKN74, MKN45 and KATOIII cells were used. Tipifarnib inhibited cell proliferation in MKN45 and KATOIII, but not in MKN74. Interestingly, the expression of HIF-1 α was almost none in MKN74. The inhibitory effect of tipifarnib on HIF-1 α was observed in a dose-dependent manner in MKN45, KATOIII. We focused on Rheb which is one of farnesylated proteins and largely involved in the mTOR pathway. In all cell line, farnesylation of Rheb was inhibited by Tipifarnib and mTOR pathway was inactivated. Furthermore, ROS production was increased by Tipifarnib in all cell lines.Our results were showed that FTI inactivated mTOR pathway and suppressed HIF-1 α expression via inhibiting farnesyration of Reb protein in GC cells, therefore, FTIs may have anti-tumor effect depending on HIF-1 α expression. In addition, FTIs increased ROS production and it could be involved in antitumor effect.

P-1194

Oncolytic reoviral therapy in combination with diagnosis of peritoneal metastasis from gastric cancer in animal model

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Background. Development of new effective diagnosis and treatment for peritoneal metastasis from gastric cancer are necessary. The aim of this study was to investigate whether oncolytic reovirus could have not only anticancer for peritoneal metastasis from gastric cancer but also visibility for gastric cancer cells.Methods. Four gastric cancer cell lines and reovirus serotype 3 were used in this study. We evaluated the cytopathic effect of reovirus in vitro. Oncolytic effect of reovirus for peritoneal metastasis was also evaluated in vivo. The visibility of reovirus labeled with Alexa Fluor 488 in cancer cell line was examined by immunofluorescence microscopy. Results. Cytopathic effect of reovirus was observed in only cancer cell lines. Significant anticancer effect for peritoneal metastasis in reovirus group was observed compared to control group. The labeled reovirus was visualized in cancer cell line.Conclusions. Reovirus could be useful as a new modality against peritoneal metastasis in gastric cancer. In addition, the Alexa Fluor 488 labeled reovirus might act as a novel tracer for surgery of gastric cancer.

▲TOP

[P-1202] P14-9 [English/Japanese]
Prognostic biomarkers in gastric cancer

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Michitaka Fujiwara / Clin. Simulation Ctr., Nagoya Univ. Grad. Sch. of Med.

P-1202

Clinical significance of RNF126 in gastric cancer

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Background: Ring finger protein 126 (RNF126) has been associated with cancer cell proliferation. However, clinical significance of RNF126 in actual cancers remains largely unknown. Methods: We investigated the RNF126 expression in primary gastric cancer tissues from 170 patients by immunohistochemistry, and explored its clinical relevance and prognostic value. Furthermore, the effect of RNF126 expression on cancer cell proliferation was analyzed in vitro using a siRNA silencing technique. Results: There was a significant difference in the expression level of RNF126 in terms of the tumor depth and venous invasion. The postoperative overall survival rate was significantly lower in patients with RNF126-high tumors than in patients with RNF126-low tumors. Multivariate analysis identified the RNF126 status as an independent prognostic factor. RNF126 gene silencing significantly inhibited the proliferation of gastric cancer cells in vitro. Conclusions: The RNF126 expression has a significant prognostic value in gastric cancer. RNF126 might play an important role in regulating the proliferation of gastric cancer cells and promoting the development of postoperative recurrence.

▲TOP

P-1203

Clinical significance of MAGE-A3 expression in gastric cancer

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Background: Although it has recently been found that melanoma-associated antigen (MAGE) may play an important role in tumor malignancy, its role in gastric cancer remains uncertain. Methods: We investigated the MAGE-A3 expression in gastric cancer specimens from 174 patients by immunohistochemistry. In addition, the impact of MAGE-A3 expression on proliferation was analyzed in vitro using siRNA silencing technique. Results: The mean percentage of MAGE-A3-positive tumor cells was 62.9 ± 29.8. There was a significant difference in the expression level of MAGE-A3 in terms of the tumor depth (P = 0.018), pathological stage (P = 0.009), lymphatic invasion (P = 0.003), and venous invasion (P = 0.006). The postoperative overall (P = 0.005) and relapse-free survival (P = 0.003) rates were significantly lower in MAGE-A3-high group than low group. The multivariate analysis identified the MAGE-A3 status as an independent prognostic factor. The MAGE-A3 gene silencing inhibited the proliferation of human gastric cancer cells in vitro. Conclusion: The MAGE-A3 expression may have a significant prognostic value in gastric cancer, and be involved in the proliferation of gastric cancer cells.

P-1204

Functional analysis and prognostic value of sodium iodide symporter (NIS) in gastric cancer

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The objectives of the present study were to investigate the role of the sodium iodide symporter (NIS) in the regulation of genes involved in tumor progression and the clinicopathological significance of its expression in gastric cancer (GC). NIS was strongly expressed in MKN45 and MKN74 cells. The depletion of NIS using siRNA inhibited cell proliferation, migration, and invasion and induced apoptosis. The results of the microarray analysis revealed that various interferon (IFN) signaling-related genes, such as STAT1, STAT2, IRF1, and IFIT1, were up-regulated in NIS-depleted MKN45 cells. Furthermore, the down-regulation of NIS affected the phosphorylation of MAPKs and NF-kB. Immunohistochemical staining performed on 145 primary tumor samples showed that NIS was primarily located in the cytoplasm or cell membranes of carcinoma cells, and its expression was related to the histological type or venous invasion. Prognostic analyses revealed that the strong expression of NIS was associated with shorter postoperative survival. In conclusion, NIS regulates tumor progression by affecting IFN signaling, and its strong expression is related to a worse prognosis in patients with GC.

P-1205

MLH1 expression is associated with PD-L1 expression, chemosensitivity and prognosis in gastric cancer

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Background: Microsattelite instablity (MSI) in gastric cancer (GC) was reported as one subtype of molecular classification and considered mainly caused by dysfunction of MLH1. This study aimed to investigate the association between MLH1/PDL1 expression and chemosensitivity and prognosis in GC. Methods: A total of 271 patients who underwent gastrectomy for GC were retrospectively analyzed. The relationship between MLH1 and PDL1 expression and the pathological effect of neoadjuvant chemotherapy (NAC) was examined, and RFS was compared separately with and without NAC.Results: Low MLH1 expression (M-L) was observed in 10.7% and high PDL1 expression (P-H) in 25.8%. There was a significant association between M-L and P-H. M-L was significantly associated with low chemosensitivity in patients with NAC. M-L had significantly better RFS than that with M-H in patients without NAC, however no significant difference was observed in patients with NAC. PDL1 expression was not significantly associated with RFS in patients with and without NAC.Conclusion: Low MLH1 expression may be associated with high PDL1 expression and better prognosis, however also associated with low chemosensitivity.

▲TOP

P-1206

Podoplanin expression as a prognostic factor in gastric cancer

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Background/Aim: Several recent studies have suggested that podoplanin (PDPN) expression correlates with malignant potential in various types of cancer. We investigated the expression pattern of PDPN and its clinical significance in gastric cancer.Materials and Methods: We assessed immunohistochemical expression of PDPN in 91 gastric cancer tissues of patients who underwent curative surgery and evaluated the relationship between the expression levels and clinicopathological factors.Results: The tumor cells themselves revealed no expression of PDPN. However, PDPN was expressed in spindle-shaped stromal cells surrounding tumors in some cases. PDPN-positive stromal cells significantly correlated with a larger tumor, advanced T- and N-stages, and lymphatic and vascular invasion. Multivariate analysis indicated that PDPN expression in spindle-shaped stromal cells (p=0.030) as well as N-stage (p=0.023) are independent prognostic factors.Conclusion: Our findings demonstrated the PDPN expression in spindle-shaped stromal cells of patients with gastric cancers has prognostic significance. Intensive chemotherapy and close follow-up should be recommended for these patients.

P-1207

BRCA2 mutation is a favorable prognostic indicator in surgically resected gastric cancer

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Clinical implication of BRCA1 and/or BRCA2 (BRCA1/2) mutations remains unclear in gastric cancer (GC). A total of 130 patients (92 men and 38 women, with a median age of 68 years) were enrolled. We analyzed genetic alterations in surgically resected tumor tissues by using panel-based sequencing. BRCA1 and BRCA2 mutations were found in 6 (4.6%) and 18 (13.8%) patients, respectively. These mutations were not significantly associated with clinicopathological factors including pathological (p) TNM stage. The frequency of BRCA2 mutation was significantly higher in MSI-high tumor than in MSI-low or MSS tumor (33.3% vs. 6.3%, P<0.01). Five-year overall survival (OS) rate after surgery was higher in patients with BRCA2 mutation than in patients without it (94.4% vs. 65.9%, P=0.01). BRCA1 mutation was not significantly associated with OS. Multivariate analysis revealed that BRCA2 mutation was an independent prognostic factor [Hazard ratio (HR) 0.1, 95% confidence interval (CI) 0.02-0.80] in addition to age (≥68, HR 2.4), pStage (III/IV, HR 3.9) and residual tumor (R1/2, HR 2.6). BRCA2 mutation contributes to favorable patient outcome after surgery in GC.

P-1208

Expression and distribution of RCAN-2 in gastric carcinoma

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The Regulator of Calcineurin (RCAN) proteins are important endogenous modulators that interact with calcineurin and alter its function by interfering with calcineurin-NFAT (Nuclear Factor of Activated T cell) signaling pathway. We previously reported that RCAN2 was specially expressed in colorectal cancer and had an inhibitory role in cancer cell proliferation. The aim of present study is to investigate the biological significance of RCAN2 in Gastric cancer (GC). Immunohistochemically, 110 (53%) of the 207 GCs were positive for RCAN2. The prognosis of patients with positive RCAN2 expression was significantly worse in all stages and stage II/III/IV. RCAN2 expression was also associated with Helicobacter pylori infection. Multivariate analysis revealed that RCAN2 expression was an independent predictor of survival in patients with GC. RCAN2 participated in cell invasion and cell proliferation in GC. We analysed the phosphorylation of EGFR, Akt and Erk in GC cells with RCAN2 inhibition. The levels of phosphorylated Akt and Erk were lower in RCAN2 siRNA1-, siRNA2- and siRNA3-transfected MKN1 cells than in control cells.

▲TOP

[P-1216] P14-11 [English/Japanese]
Epigenetics in gastric cancer

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Motohiro Hirao / Dept. of Surg. Natl. Hosp. Organization, Osaka Natl. Hosp.

P-1216

Novel epigenetic markers for gastric cancer risk stratification in individuals after Helicobacter pylori eradication

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Risk stratification of Helicobacter pylori (HP)-eradicated healthy people for gastric cancer (GC) is an urgent issue. For this, the assessment of aberrant DNA methylation accumulated in gastric tissues is a promising strategy. We aimed to establish novel epigenetic risk markers that are unaffected by contaminating blood cells in biopsies and informative among HP-eradicated people. Gastric mucosa were collected from healthy volunteers without HP infection (G1), healthy people (G2), and GC patients (G3) after HP eradication. Eight G1, 12 G2, and 12 G3 samples were analyzed by a beadarray, and two screening algorithms yielded 57 candidates unmethylated in G1 and differentially methylated in G3 compared with G2. For 9 of the 57 candidates, validation was conducted by bisulfite pyrosequencing of 63 G2 and 82 G3 samples, and all of them had significantly higher methylation levels in G3 than in G2. Their methylation levels were highly correlated, indicating that they reflect epigenomic damage throughout the genome. The candidates had sufficient performance (AUC: 0.7–0.8) with high odds ratios (5.4–23.4). Our novel epigenetic markers are used for our ongoing prospective cohort study.

▲TOP

P-1217

Development of gastric cancer-specific markers based on methylome analysis

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Dynamic reprogramming establishes tissue-specific epigenetic profiles during organ differentiation. The aim of this study was to develop novel markers of gastric carcinoma (GC) based on tissue-specific DNA methylation for detecting the primary organ in cases of cancer of unknown primary site. First, we analyzed single-institutional methylome data of 509 samples of cancerous tissue (T) obtained from stomach, lung, kidney, breast and liver by Infinium bead chip assay. Significant DNA hypermethylation on GC was observed in 25 probes compared with other 4 organs. We confirmed their GC-specificities using dataset of The Cancer Genome Atlas, and focused on Gene X which was able to discriminate GC from other cancer, especially from colorectal cancer. Then we verified and validated DNA methylation status of GCs and from other organs on Gene X by pyrosequencing. The optimal cutoff value of DNA methylation rate generated by the Youden index was able to discriminate GC samples from T samples from other organs in the validation set with high sensitivity and specificity. DNA hypermethylation on Gene X may be a useful marker of GC.

P-1218

DNA methylation of microRNA genes in gastric mucosae of gastric MALT lymphoma patients

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H. pylori (Hp) infection is known to be involved in the pathogenesis of gastric MALT lymphoma (GML) and gastric cancers. However, there are few reports concerning complication by both of these conditions. Of the 81 cases diagnosed with Hp-positive GML at our hospital between August 1996 and May 2012, 9 cases were determined to involve gastric cancer. The annual incidence of gastric cancer following eradication of Hp infection-associated gastritis was 0.1-0.3%, and the onset rate of gastric cancer after Hp eradication for GML was considered to be significantly higher (0.78%). As such, DNA was extracted from biopsy specimens collected from mucosal tissue after Hp eradication for GML and background gastric mucosa after Hp eradication for chronic gastritis, methylation-specific polymerase chain reaction (MSP) was conducted, and miR-124a-3 methylation levels of each tissue specimen were compared. The degree of methylation was determined to be significantly higher in the GML Hp eradication group. The incidence of gastric cancers tended to be higher following GML Hp eradication, and it was considered to result from the high degree of methylation in the background gastric mucosa.

P-1219

Identification and characterization of a long non-coding RNA associated with chronic gastritis and gastric caner

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We aimed to understand the role of long non-coding RNAs (lncRNAs) in the inflammation-related carcinogenesis. To this end, we searched for lncRNAs associated with H. pylori-related gastric cancer (GC). Genome wide profiling of histone H3 lysine 4 trimethylation (H3K4me3) identified elevated expression of LUGGC1 (LncRNA Upregulated in Gastritis and Gastric Cancer 1) in the gastritis mucosa of GC patients as well as in GC cells. Knockdown of LUGGC1 suppressed GC cell proliferation, migration, invasion and in vivo tumor formation, suggesting its oncogenic role. Microarray analysis revealed that LUGGC1 knockdown suppressed expression of interferon-stimulated genes (ISGs), while LUGGC1 overexpression upregulated ISGs. We identified that LUGGC1 interacts with PURA and YB1, which are multifunctional DNA/RNA binding proteins involved in transcriptional and translational regulation. We found that depletion of PURA and YB1 also suppressed GC cell proliferation, suggesting that LUGGC1 may play an oncogenic role by interacting these proteins. Our results suggest that upregulation of LUGGC1 may be associated with the development of GC, and could be a potential therapeutic target.

▲TOP

[P-1225] P14-13 [English/Japanese]
GIST

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Masaaki Motoori / Dept. Surg., Osaka Ganeral Med. Ctr.

P-1225

Clinical significance of ZFP57-IGF2 transcription system in GIST

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We found that the ZFP57 gene as a new oncogene. We clarify the role of the ZFP57 gene in gastrointestinal mesenchymal malignant tumors (GIMT) such as GIST and report on whether it can be a novel target molecule for therapy. [Study 1] Transplantation experiment of HT1080 into mice found that ZFP57-overexpression increased proliferative capacity and pulmonary metastatic potential. On the other hand, it was found that ZFP57-knockdown decreased proliferative capacity and metastasis ability. [Research 2] The expression of ZFP57-IGF2 was immunohistologically evaluated using resected specimens of GIMT including GIST. Subjects are 51 GIST patients and 6 other mesenchymal malignancies. In the GIMT including GIST, the ZFP57-overexpression and the IGF2-overexpression had significantly higher relapse rates and lower survival rate. In multivariate analysis, it was suggested that overexpression of ZFP57 and IGF2 is a prognostic factor independent of Fletcher risk classification. [Conclusion] The ZFP57-IGF2 transcription system is an important biomarker of GIMT including GIST, suggesting that ZFP57 may be a target molecule for therapy.

▲TOP

P-1226

The efficacy of novel HSP90 inhibitor, TAS-116, against gastrointestinal stromal tumors

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【Background】GISTs have the gain-of-function mutations in the KIT gene. Despite its effectiveness of imatinib mesylate(IM), half of GISTs treated with IM develop resistance, due to the additional mutations accompanied by concomitant re-activation of the KIT tyrosine kinase. Heat shock protein 90 (HSP90) is one of chaperon molecules required for the proper folding of KIT. The aim of this study is to clarify the efficacy and mechanism of new HSP90 inhibitor, TAS-116, against IM resistant GIST. 【Material and Method】 We used the established human GIST cell line T1, and IM-resistant cell lines (R8 and R9), which had additional mutations in KIT. We investigated the cytotoxicity, signaling inhibition, immunofluorescence assay. T1, R8, and R9 xenograft models were also treated by IM and TAS-116. 【Result】TAS-116 showed growth inhibition and apoptosis induction. In addition, TAS-116 showed inhibition of phosphorylated-KIT. Treatment with TAS-116 decreased colocalization of Kit with GM130. In xenograft mice models, TAS-116 resulted in tumor growth inhibition in R8 and R9, whereas was not effective for IM. 【Conclusions】TAS-116 showed the anti-tumor efficacy for IM resistant GISTs.

P-1227

FBXW7 can associated with tumor progression regulating C-MYC in GIST

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Objective:Gastrointestinal stromal tumor (GIST) is a rare mesenchymal tumor. FBXW7 is known as tumor-suppressor gene in various malignancies. C-MYC is an oncogene that plays a role in cell cycle progression, apoptosis. The aim of this study to evaluate correlation between FBXW7,C-MYC expression and clinicopathological characters in GIST. Methodology: i)We examined the FBXW7 and C-MYC expression in 90 resected primary GIST samples by immunohistochemical staining. ii)We examined cell proliferation and C-MYC expression by Cytotoxicity assays in GIST-T1 cells suppressed by FBXW7-specific siRNA. Results: i)There are significantly more high risk cases in modified-Fletcher classification in low FBXW7 expression cases of immunohistochemistry ($P < 0.01$). In cases of low FBXW7 expression, there is a tendency for poor prognosis for RFS ($P=0.08$). FBXW7 and C-MYC expression are significantly inverse correlated ($P<0.01$, $R=0.36$). ii)The GIST cells suppressed FBXW7 expression by siRNA have higher cell proliferation rate and high C-MYC expression compared to control GIST cells ($P<0.01$). Conclusion: This study suggested that FBXW7 may be associated with malignant potential of GIST regulating C-MYC.

P-1228

SOCS1 gene therapy has antitumor effects in imatinib-resistant gastrointestinal stromal tumor

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【Background】Despite the revolutionary effects of imatinib, some GIST patients become resistant because of acquisition of secondary mutations in KIT. This study investigated the antitumor effects of SOCS1 gene therapy, which targets several signaling pathways. 【Material and Method】 We used GIST-T1 (imatinib-sensitive) and GIST-R8 (imatinib-resistant) cells. We infected both cell lines with an adenovirus expressing SOCS1 (AdSOCS1) and examined antitumor effect and mechanisms of its agent.【Result】The latter harboured with secondary KIT mutation and had imatinib resistance 1000 fold higher than the former cells. We demonstrated that AdSOCS1 significantly decreased the proliferation and induced apoptosis in both cell lines. Moreover, SOCS1 overexpression inhibited the phosphorylation of signal transducer and activator of transcription 3 (STAT3), AKT and focal adhesion kinase (FAK) in both of them.【Conclusions】Our results indicate that the activation of FAK signalling is critical for proliferation of both imatinib-sensitive and -resistant GIST cells and the interference with FAK/AKT pathway might be beneficial for therapeutic target.

▲TOP

[P-1008] P2-2 [English/Japanese]
Animal models for cancer (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Daisaku Yamada / Dept. Gastroenterological Surg., Osaka Internatinal Cancer Inst.

P-1008

Development of Vγ9Vδ2T cell based chemo-immunotherapy in bladder cancer

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INTRODUCTION: In 75th JCA annual meeting, we reported that human γδT cells exerted potent cytotoxicity toward urinary bladder cancer (UBC) cells and that low dose gemcitabine (GEM) pretreatment enhanced the cytotoxicity of human γδT cells against UBC cells through MICA/B-NKG2D axis in in vitro cytotoxicity assays. In the present study, to achieve maximum cytotoxic effect by Vγ9Vδ2T cells in in vivo, we focused on bladder infusion approach of γδT cell and investigated the efficacy of intravesical γδT cell treatment combined with GEM. METHODS: Apoptosis of UBC cells was evaluated using a flow cytometer in vitro. The efficacy of ex vivo expanded γδT cell immunotherapy was examined in an orthotopic xenograft model using In Vivo Imaging System (IVIS). RESULTS: IVIS analysis revealed that intravesical administration of γδT cells had potent cytotoxicity and GEM pretreatment significantly increased the cytotoxicity of γδT cells in vivo. CONCLUSIONS: Intravesical γδT cell immunotherapy exerted potent cytotoxicity against UBC and γδT cell immunotherapy combined with low dose GEM may be a promising strategy in UBC.

▲TOP

P-1009

The CRISPR-Cas9-mediated gene knockout system to identify tumor suppressor genes in basal-like breast cancer mouse model

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Basal-like breast cancer, a malignant subtype, lacks expression of therapeutic targets such as ER, PR and ErbB2. To identify possible therapeutic targets for basal-like breast cancer, one must establish the in vivo tumor model in which tumor formation can be enhanced by knocking out candidate genes downregulated in basal-like breast cancer specimens. In this study, we established the CRISPR-Cas9 mediated-in vivo gene knockout system in C3(1)/SV40TAg mice (C3 mice). C3 mice generally develop mammary adenocarcinoma similar to human basal-like breast cancers by 6 months. To verify the effect of CRISPR-Cas9 mediated-gene knockout on tumorigenesis, we generated lentivirus vector expressing gRNAs for Pten together with Cas9 and injected them to mammary ducts. Eleven out of 85 tumors had gRNA cassettes and Pten mutations, while no gRNA cassette was detected in 34 tumors derived from C3 mice injected with virus expressing control gRNA. These results suggest that lack of Pten enhanced tumorigenesis in C3 mice. Taken together, our method is useful to identify tumor suppressor genes in development of basal-like breast cancers

P-1010

Blocking CXCLs-CXCR2 axis in tumor-stromal interaction contributes to the survival in a mouse model of pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by dense stromal reaction (desmoplasia). We have previously reported that mice having conditional $Kras^{G12D}$ mutation and knockout of TGF-β receptor type Ⅱ (Tgfbr2), PKF mice, develop PDAC with desmoplasia modulated by CXC chemokines produced by PDAC cells through the tumor-stromal interaction. In this study, we further found that PDAC and cancer-associated fibroblast (CAF) accelerated the invasion and migration of each other through the CXC chemokines and the receptor (CXCLs-CXCR2) axis. Heterozygous knockout of Cxcr2 in PKF mice (PKF2h mice) prolonged the survival and inhibited tumor angiogenesis and PDAC microinvasion. Infiltration of neutrophils, myeloid-derived suppressor cells (MDSCs) and arginase-1^+ M2-like tumor-associated macrophages (TAMs) significantly decreased in the tumor of PKF2h, whereas inducible nitric oxide synthase $(iNOS)^+$ M1-like TAMs and apoptotic tumor cells markedly increased. These results suggest that blocking of the CXCLs-CXCR2 axis in the tumor-stromal interactions would be a therapeutic approach against PDAC progression.

P-1011

Establishment of novel murine pleomorphic rhabdomyosarcoma cell lines with KrasG12V expression and disruption of p53

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Pleomorphic rhabdomyosarcomas arise predominantly in the skeletal musculature of adult and are typically associated with poor prognosis. Here we established murine tumor generating cell lines, designated RMS3, RMS310 and RMSg2. RMS3 was directly from primary culture of a K-rasG12V; p53-/- tumor induced in the femur. RMS310 was established as RMS3 derivative by limiting dilution of tumor cells from lung metastatic colony of RMS3 and RMSg2 was RMS310 derivative established by limiting dilution of tumor cells from lung metastatic colony of GFP expressing RMS310. These all cell lines had stable tumorigenicity and gave rise to pRMSs featured with bizarre giant cells, positive for desmin by subcutaneous inoculation. RMSg2 cell line have highly metastatic ability and colonized in the lung from orbital vein injected cells or subcutaneously implanted tumor mass. RT-PCR revealed that all of these tumors generating cells in culture displayed gene expressions for FAPs/MSCs markers; Sca-1, PDGFRα, Adam12, and Tcf4 but negative for satellite cell markers; Pax7 and Myf5, suggesting that these K-rasG12V; p53-/- tumor cell lines have mainly FAPs/MSCs like feature but not satellite cells.

▲TOP

P-1012

Insights into the histogenesis of Barrett's esophagus from a study using a mouse duodenal contents reflux model

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Barrett's esophagus (BE) is the normal esophageal squamous epithelium is replaced with metaplastic columnar cells due to chronic damage of gastroesophageal reflux. To study the histogenesis of BE leading to adenocarcinoma, several rat duodenal contents reflux models have been developed. In this study, we carried out esophago-jejunostomy after cutting esophagogastric junction for inducing reflux using male C57BL/6 mice and examined the morphological changes using immunohistochemical stainings of various markers including CDX2 and CK7 at 60 weeks after surgery. Although we have been reported that BE with goblet cells has been reported to originate from stem cells located in the basal layer of esophageal squamous epithelium in the previous models, most are developed in the mucosal wound-healing response in this study. However, we found a case with CK7 positive cells in the mid-esophagus far from the anastomosis. Unfortunately, we did not detect any cases with esophageal carcinoma because of milder inflammation compared with the previous models using rats. These findings suggested that a native esophageal stem or progenitor cell could be the cell origin for BE up to the environment.

P-1013

UTX deficiency promotes inflammatory microenvironment and develops bladder cancer by cooperating with p53 inactivation

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Epigenetic deregulation is deeply implicated in human cancers. Recent studies revealed that Utx, encoding a demethylase for histone H3 lysine 27, is frequently mutated in bladder cancer (BC), but underlying molecular mechanism(s) remains largely unknown. To address this issue, we created urothelium-specific Utx-deficient mice. Although Utx-deficient mice did not show any obvious abnormalities, mice both deficient in Utx and heterozygous for p53, another gene highly inactivated in BC, developed dysplasia and carcinoma. Pathway analyses of RNA sequencing using the urothelium revealed that Utx deficiency induced activation of cytokine-cytokine receptor interaction with upregulation of pro-inflammatory factors, while p53 inactivation contributed to cell cycle progression. These results collectively demonstrate that Utx deficiency generates inflammatory microenvironment, and additional genetic alterations, such as p53 inactivation, promote cell proliferation and finally develop BC. These results provide a novel insight into the multistep carcinogenesis in BC and find application in developing a molecular-targeted therapy for BC patients with Utx deficiency.

P-1014

Development of a vaccine against adenovirus-conjunctivitis in a mouse model

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[Background] : Adenovirus (Ad) infection is a major factor for conjunctivitis. However, Ad vaccine and animal models of acute conjunctivitis have not been developed. The aim of this study is to establish a mouse model and develop a vaccine for adenovirus-conjunctivitis. [Methods] : BALB/c mice were immunized by Ad-GFP vector twice with 2 weeks interval by intramuscular or intranasal route. Four weeks and 10 months after last immunization, mouse eyes were challenged with Luciferase-expressing Ad vector (Ad-Luc). The eyes were surgically removed and luciferase activity in the eye lysis was detected 3 days post challenge.[Results] : 1) Vaccination by both intramuscular route and intranasal route strongly induced adenovirus-specific IgG and IgA antibodies in both sera and eye-washing solution. 2) Vaccination greatly protected from Ad infection performed in either 4 weeks or 10 months post immunization. [Conclusion] : We have developed a mouse model for Adenovirus-conjunctivitis. Vaccination of Ad vector can protect from Adenovirus-conjunctivitis.

▲TOP

[P-1022] P2-4 [English/Japanese]
Animal models for cancer (4)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Hiroshi Suemizu / Lab. Anim. Res. Dept., CIEA

P-1022

The effect of cancer cachexia on myocardial tissue

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Disorders of various tissues are evoked due to excess of catabolism in cancer progression, which impair tolerance to chemotherapy and quality of life in patients. However, there are few reports on the direct impact of cancer on myocardium. In this study, the effect of cancer on myocardium was examined using mouse cachexia model. As a result, body weight and cardiac weight were significantly lower in the tumor-burden mice than those in control mice. Histopathological examination revealed myocardial atrophy and dilatation of the left ventricular cavity in the tumor group. It was also confirmed that oxidative stress was accumulated to the nuclei in the cardiomyocytes of the tumor group. These findings suggest that heart failure was induced by cancer cachexia. Although it has been reported that myocardial injury is caused by chemotherapy for cancer, this study suggested that cancer itself may also cause myocardial injury. We will clarify the mechanism in the future and examine the possibility of therapeutic intervention.

▲TOP

P-1023

Effects of Alcohol consumption on DMH-induced rat colon cancer

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Although alcohol is responsible for considerable morbidity and mortality, an epidemiological study explains cardioprotective effect of low to moderate alcohol intake. Our objective is to investigate the effect of low to high alcohol on DMH-induced rat colonic cancer.Material and Method;40 Male F344 rats were randomly divided into 4 groups : Ⅰ group (water , control group) , Ⅱ group(1% ethanol group), Ⅲ group(2%) and Ⅳ group(5%). They were injected 1, 2-dimethylhydrazine (DMH, 20 mg/kg /BW) once a week for consecutive 8 weeks from 5 weeks of age. All the rats were sacrificed at the end of week 28, and were histopathologically examined for aberrant crypt foci(ACF), tumors of the colon and proliferation index of tumor cellsResult and conclusion; Low 1% ethanol Ⅱ group compared with control group, 2% and 5% ethanol group showed statistically lower number of adenocarcinoma of the colon. Ⅱ group showed lower number of differentiated adenocarcinoma. The J-shaped relationship between alcohol consumption and colon cancer risk was confirmed in DMH-induced rat colon tumor with low taking alcohol group.

P-1024

The genetic polymorphism in p19Arf confers resistance to tumor progression

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Previous study, we successfully mapped highly significant QTLs for late stage papillomas (<6 mm) and identified Stmm3 (Skin tumor modifier of MSM 3) locus on Chromosome 4 by crossing a resistant Japanese wild-derived inbred strain MSM/Ms with a susceptible strain FVB/N and subjected to the two-stage skin carcinogenesis protocol using DMBA/TPA. Recent study, we identify the cyclin-dependent kinase inhibitor gene Cdkn2a/p19^{Arf} as a major responsible gene for Stmm3 locus. We provide evidence that the function of Stmm3 is dependent on p53 and p19$^{Arf\ MSM}$ confers stronger resistance to papillomas compared with p16$^{Ink4a\ MSM}$ in in vivo. In addition, we generated p19$^{Arf+/-}$ (FVB/N×MSM/Ms) F1 mice to investigate the function of p19^{Arf} in in vivo. And we subjected these mice to the two-stage skin carcinogenesis protocol using DMBA/TPA. Skin carcinogenesis experiments showed that p19$^{Arf\ MSM}$ allele confers resistance to larger papillomas. In this study, we show the genetic polymorphism in p19^{Arf} between MSM/Ms (Leu) and FVB/N (Val) can modify susceptibility to skin carcinogenesis.

P-1025

Effect of oral administration of lauric acid on heart muscle in mouse model

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Lauric acid (LAA, C12:0) is a medium chain saturated fatty acid, which is translocated into mitochondria in a carnitine-independent manner. LAA is converted to acetyl CoA by processing with beta-oxidation and is used to oxidative phosphorylation. Administration of LAA might be expected to alter cancer energy metabolism, which is known as Warburg effect. Based on these characteristics, LAA may provide relevant effect on mitochondria-rich organs. Here, we examined the effect of oral intake of LAA on the heart. Five weeks old male BALB/c mice were fed with LAA-added CE-2 control diet. It is no differences between groups in calorie intake. The 10% group showed a significant weight loss after LAA feeding and became moribund on the 5th day. Body weight and cardiac weight at sacrifice were significantly reduced in 5% and 10% LAA groups in comparison with those in control group. Histological examination of the heart revealed loss of myocardial volume, increased oxidative stress and autophagy activity in the 10% LAA group. From these findings, it was suggested that intake of excess LAA cause myocardial impairment.

▲TOP

P-1026

Involvement of histamine in the invasion of mTOR-inhibitor resistant intestinal tumors

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Distant metastasis is the leading cause of disease-related deaths among patients with colorectal cancers, necessitating the identification of novel preventive or therapeutic targets for their invasion and metastasis. We previously reported that treatment with the mTOR inhibitor AZD8055 failed to block the invasion of the intestinal adenocarcinomas in cis-Apc/Smad4 mice, despite efficient suppression of the tumor expansion. To gain insights on the mechanism by which the intestinal tumors acquire the mTOR-inhibitor resistance, we conducted metabolome analysis. The CE/MS-based profiling revealed significant increase of the histamine level in the mTOR-inhibitor resistant tumors compared with the control tumors. Because both histamine H1 receptor (H1R) and H2R were expressed in the control and the mTOR-inhibitor resistant tumors, we tested the effects of blocking H1R and H2R on their growth and invasion. Intriguingly, combination of H1R and H2R antagonists significantly suppressed the invasion but not the expansion of mTOR-inhibitor resistant tumors. These results indicate a key role of histamine in the invasion of mTOR inhibitor-resistant intestinal adenocarcinoma.

P-1027

Effect of combination intake of glucose and lauric acid on tumor growth and skeletal muscle atrophy in CT26 mouse model

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Skeletal muscle volume is associated with prognosis of cancer-burden patients. Maintenance of skeletal muscle is an essential issue in cancer treatment. In nutritional intervention, it is important to focus on differences in metabolism between tumor and skeletal muscle. We examined the influence of oral intake of carbohydrate and medium-chain fatty acid on tumor growth and skeletal muscle atrophy in a mouse peritoneal metastasis model. Glucose loading suppressed skeletal muscle atrophy; however, it promoted tumor growth. Administration of lauric acid, a medium-chain fatty acid, did not alter muscle atrophy; however, it suppressed tumor growth by producing ROS. Glucose is promoting tumor growth, however; it inhibits atrophy of skeletal muscle. From these findings, it is important to improve the combination of glucose and medium-chain fatty acid in oral intake.

P-1028

Roles of intestinal epithelial MyD88 in intestinal tumor formation in Apc mice

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Mutations in the APC gene are frequently involved in the onset of the colorectal adenoma-carcinoma sequence. Although LOH of the Apc gene in intestinal epithelial cells (IEC) of $Apc^{+/\Delta 716}$ (Apc) mice leads to activation of the Wnt pathway and initiates polyp formation, the Wnt pathway activation is not sufficient for the polyp expansion; it requires activation of the JNK-mTORC1 signaling. However, the upstream signaling that activates the JNK-mTORC1 axis remains elusive.
We found that IL-1, an inflammatory cytokine up-regulated in the intestinal polyps, caused activation of the JNK-mTORC1 pathway in organoids derived from the Apc mutant polyps. Conditional knockout of the MyD88 gene in IEC significantly reduced the number of intestinal tumors in Apc mice, accompanied by attenuation of the JNK-mTORC1 signaling. Furthermore, induction of MyD88 deletion in the intestinal polyp-derived organoids, but not in the normal IEC-derived organoids, caused apoptotic cell death and reduction of the organoid growth. These results indicate that intestinal epithelial MyD88 functions in the maintenance and growth of the intestinal tumors of Apc mice.

▲TOP

[P-1036] P4-1 [English/Japanese]
Novel oncogenes in solid tumor

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Tatsuo Hata / Dept. Surg., Tohoku Univ., Sch. Med.

P-1036

CGRP-CRLR/RAMP1 signal regulated by EVI1 promotes leukemogenesis

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Ecotropic viral integration site 1 (EVI1) is highly expressed in around 10% of AML including chromosomal abnormality at 3q26, which is associated with poor prognosis. Identification of novel therapeutic targets for EVI1high AML is urgently needed. Here, we identified calcitonin receptor like receptor (CRLR) as a potential therapeutic target for EVI1high AML. We found that CRLR was specifically expressed in EVI1high AML cells, and that EVI1 directly activated the CRLR promoter. Since receptor activity modifying protein 1 (RAMP1) was specifically expressed in EVI1high AML, the RAMP1-CRLR complex was thought to function as a receptor for calcitonin gene-related peptide (CGRP). CGRP treatment enhanced cell growth via cell cycle progression in EVI1high AML cell lines and EVI1high leukemia cells from MLL-AF9-transduced mouse models. Moreover, inhibition of CGRP signaling by CGRP8-37 abrogated CGRP-stimulated cell growth. In summary, CGRP-CRLR/RAMP1 signal plays an important role in EVI1high AML and can become a therapeutic target for EVI1high AML.

▲TOP

P-1037

Molecular mechanisms by which GRWD1 downregulates p53 to transform cells

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GRWD1 is a Cdt1-binding protein that promotes MCM loading through its histone chaperone activity. Previously, we demonstrated that GRWD1 acts as an oncogene by downregulating p53 and GRWD1-mediated p53 inhibition may occur via GRWD1 binding to and sequestering RPL11 from MDM2. Recently, we comprehensively identified GRWD1-binding proteins and found that GRWD1 interacts with various proteins involved in transcription, translation, DNA replication and repair, chromatin organization, and ubiquitin-mediated proteolysis. We also found that GRWD1 regulates RPL23 protein levels via the ubiquitin-proteasome system and showed that EDD, an E3 ubiquitin ligase that was identified as a GRWD1-interacting protein, is involved in RPL23 degradation. Further experiments suggested that the promotion of RPL23 proteolysis may play a role in GRWD1-mediated p53 downregulation and tumorigenesis. In addition, we found that GRWD1 might directly regulate p53 transcriptional activity. We will discuss these mechanisms by which GRWD1 downregulates p53 to transform cells.

P-1038

BRCA1-interacting protein OLA1 interacts with BARD1 to regulate centrosome number

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Germ line mutations of BRCA1 result in hereditary breast and ovarian cancer syndrome. BRCA1 forms heterodimer with BARD1 to regulate DNA repair and centrosome number. We previously identified Obg-like ATPase (OLA1) as a component of BRCA1/BARD1-containig complex and found that OLA1 functions in the regulation of centrosome number, together with BRCA1. However, the precise mechanism of centrosome regulation by OLA1 remains to be clarified. We constructed plasmids to express OLA1 with missense mutations at candidate residues of phosphorylation, acetylation, and ATP-binding. We identified six mutants that are deficient in the regulation of centrosome number. Three of them, S36A, F127A, and T325A, did not bind to BARD1. By contrast, BARD1 V695L mutation which reported in cancer decreased binding to OLA1, centrosomal localization, and activity of regulation of centrosome number of BARD1. In tertiary structure, OLA1 T325 located close to the binding surface to BARD1. Because both S36 and F127 of OLA1 closely located around ATP-binding pocket, their mutations may affect binding to ATP. These data suggested that OLA1-BARD1 interaction is important for the regulation of centrosome number.

P-1039

Stomatin like protein 2 promote liver metastasis through regulating mitochondrial induced EMT in pancreatic cancer

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【Background】Recent study highlighted that Mitochondrial retrograde signaling pathway can drive EMT. Stomatin like protein(SLP-2), a protein on mitochondrial inner membrane, is associated with mitochondrial respiratory function. The malignant potential of SLP-2 has been reported in several cancers. 【Aim】Through analyzing the function of SLP-2 in pancreatic cancer, elucidate the mechanism of how mitochondrial membrane protein drives malignancy in pancreatic cancer. 【Method】The SLP-2's effect on phenotype was measured in vitro. Splenic injection model using SCID mouse was performed to analyze SLP-2 function in vivo. Immunohistochemical study was also executed. 【Result】In SLP-2 silencing cells, the abilities of migration and invasion were down-regulated, furthermore E-cadherin expression was enhanced, while Vimentin expression was down-regulated. SLP-2 silencing cells demonstrated significantly low number of metastatic tumor compared with control cells. In immunohistochemical study, SLP-2 high group demonstrated malignant prognosis.【Conclusion】SLP-2 is strongly involved in EMT-mediated migration and invasion of cells which promote liver metastasis in pancreatic cancer.

▲TOP

P-1040

High expression of the MAF1 is a poor prognostic marker in colorectal cancer with MSI

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Colorectal cancer (CRC) with microsatellite-instability (MSI) shows distinct characteristics such as relatively better prognosis than non-MSI cases, low sensitivity of fluorouracil-based chemotherapy and benefit from immune checkpoint blockade. The aim of this study is to identify the gene involved in poor prognosis of CRC with MSI and clarify its functional significance. First, we analyzed the TCGA database to examine the significant difference in prognosis between MSI and non-MSI CRCs and identified MAF1 Homolog, Negative Regulator of RNA Polymerase Ⅲ (MAF1) is closely associated with poor prognosis only in MSI cases, but not in MSS cases. Multivariate analysis indicated that MAF1 was independent prognostic factor for overall survival in 187 CRC cases with MSI. In vitro loss of function assays revealed that MAF1 confers chemoresistance and migratory ability in CRC cancer cells. Immunohistochemical analysis in independent dataset of 145 CRC cases also revealed that high expression levels of MAF1 were associated with poor prognosis. Overall, our results indicate that MAF1 plays an important role in CRC progression, especially in MSI cases.

▲TOP

[P-1045] P4-3 [English]
Novel oncogenes / tumor suppressor genes [English]

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Jun-Ya Kato / Biosci. NAIST

P-1045

The heparin binding motif of CHI3L1 in tumor angiogenic activity

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Chitinase 3 like 1 (CHI3L1) play crucial role in controlling tumor angiogenesis and was shown high binding affinity for heparin, the property that is essential for angiogenesis. However, molecular mechanism for heparin binding capacity of CHI3L1 is still poorly understood. In this study, we examined the ability of CHI3L1 to bind heparin and drive the tumor angiogenesis. We created point mutations of C–terminal KR–rich domain in residues 334–345 and evaluated the ability of these mutant proteins to form endothelial cell–mediated vascular tubes in Matrigel. We found that wild type KR–rich domain induced increased human microvascular endothelial cells tube formation compared with the control and mutant proteins group ($P<0.05$). Moreover, the mice receiving wild type KR-rich domain MDA–MB–231 cells developed tumors that were larger than those observed in animals bearing control and other mutant bearing tumors MDA–MB–231 cells ($P<0.05$). These data strongly support that C–terminal KR–rich domain (residues 334–345) plays a central role in heparin binding activity. Our findings may bring the CHI3L1 as a novel and effective target anti–angiogenic therapy for solid tumors and other diseases.

▲TOP

P-1046

Down-regulation of ROR2 promotes prostate cancer metastasis through regulation of miRNAs on PIAS3 expression

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Prostate cancer (PCa) ranked the 5th most common cancer in the world. Recently, ROR2 had been reported to be a regulatory protein in non-canonical Wnt signaling. Wnt signaling played important role in regulation of PCa metastasis, we examined the role of ROR2 in PCa metastasis. Oncomine database and IHC revealed that both mRNA and protein level of ROR2 decreased in PCa tissues, while ROR2 mRNA level was the lowest in metastatic PCa. Moreover, PCa patients with lower mRNA level of ROR2 had poor survival rate and higher recurrent frequency. Over-expression of ROR2 in DU-145 and PC-3 PCa cells significantly suppressed cell migration, invasion and EMT markers. Micro-Western Array revealed that over-expression of ROR2 elevated protein expression of PIAS3, but decreased the abundance of Stat3, NF-&kappaB, and phosphorylation of Akt in PCa cells. Orthotopic xenograft mice model demonstrated that ROR2 can suppress PCa cells metastasis to lung. Over-expression of ROR2 suppressed expression level of miR199a-5p, and knockdown PIAS3 by over-expression of miR-199a-5p restored ROR2 mediated suppressive effect on PCa cell migration. Taken together, ROR2 is a novel tumor suppressor in PCa.

P-1047

Identification of a novel oncogenic fusion, VAPA-Rab31 in lung cancer

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Fusion genes have been discovered and identified as novel oncogenes in solid tumors. Here, we identified a fusion gene comprising coding region of Rab31 and VAPA (VAMP-associated protein A) gene and investigated its oncogenic effects in lung cancer. Rab31 belongs to the Ras superfamily of small GTPase and its promotive effect on tumor progression has been reported in several cancers such as glioblastoma, breast cancer and hepatocellular carcinoma. Exogenous expression of VAPA-Rab31 fusion increased colony forming activity and cellular proliferation. Also, enhanced tumorigenicity by VAPA-Rab31 fusion was confirmed using mouse xenograft model. Overexpression of VAPA-Rab31 upregulated anti-apoptotic protein Bcl-2 as well as mRNA level. Increased Bcl-2 protein contributed to inhibit apoptosis after bortezomib treatment. The proportion of subG1 phase cells was much smaller and phosphorylated CREB was sustained elevated level in fusion expressed cells even after bortezomib treatment. Our data suggest the oncogenic function of a novel fusion, VAPA-Rab31 that contributes to the resistance to apoptosis via up regulated Bcl-2 by transcriptional activation of CREB in lung cancer.

P-1048

Validating the relationship between ZBTB20 and CTNNB1 in human liver cancer cell lines

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Liver cancer is a major health problem worldwide, with the most common type of liver cancer being hepatocellular carcinoma (HCC). Currently, identifying the various molecular mechanisms of HCC is critical for improving the prognosis and treatment of this deadly disease. Importantly, the molecular mechanisms associated with disease initiation and progression remain unclear. In our previous findings, Zbtb20 was identified as a candidate oncogene in a insertional mutagenesis forward genetic screen for liver cancer genes. Interestingly, ZBTB20 overexpression in human liver cells resulted in increased levels of CTNNB1. In order to elucidate the role of ZBTB20 in the canonical WNT signaling pathway, we generated ZBTB20 knockout cell strains from the human liver cancer cell lines using CRISPR/Cas9 technology. Deletion indels were detected at both the genomic and transcriptional level of ZBTB20. In these ZBTB20 knockout cell strains, CTNNB1 was slightly decreased at the transcriptional level but was significantly reduced at the translational level. These results indicate ZBTB20 role in regulating CTNNB1 expression at the translational level in human liver carcinogenesis.

▲TOP

P-1049

Oncogenic fuinctions of THG-1/Tsc22D4 in squamous cell carcinoma development

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Carcinoma cells exhibit a high level of robustness against environmental stresses, metabolic disorders and therapeutic efforts. Here, we provide a novelmechanism of the squamous cell carcinoma development by THG-1, a Tsc-22 family protein. THG-1(TSC22D4), a member of TSC-22 family, is expressed in the basal layer of normal squamous epithelium and overexpressed in squamous cellcarcinomas. THG-1 is phosphorylated by Ras-ERK pathway, which promotes cellproliferation, invasion and tumorigenesis. However, molecular functions andphysiological roles of THG-1 have not been clear. Therefore, we identified the THG-interacting proteins using proteomic approach. THG-1 interacts with several factors that regulate the cell proliferation, cytoprotection, metabolism and microenvironment. Our resutls highlight the pivotal roles of THG-1 as a novel regulator of tumorigenssis under the oncogenic signaling pathway.

P-1050

Discovery of novel RET fusion gene, DCTN1-RET, as an oncogenic driver in papillary thyroid cancer

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Objectives: RET gene fusion is a major class of alterations in papillary thyroid cancer. Accumulating studies have identified various RET fusion gene partners with oncogenic activities. Here, we describe a novel oncogenic RET fusion gene, DCTN1-RET, in thyroid cancer and assess the anti-cancer activity of a selective RET inhibitor, TAS0286, in cells expressing DCTN1-RET.Results: A DCTN1-RET fusion was identified in an NGS analysis of RNA derived from 16 thyroid cancer samples. The findings were validated using Sanger sequencing and droplet digital PCR. We also investigated the frequency of DCTN1-RET using FISH in a thyroid tissue microarray. Next, we established cell lines expressing DCTN1-RET to examine whether the DCTN1-RET fusion functions as an oncogenic driver. The stable expression of DCTN1-RET led to a remarkable increase in the phosphorylation of RET, AKT and ERK. Cell growth and phosphorylation were both inhibited by TAS0286.Conclusions: A novel fusion gene, DCTN1-RET, was identified as an oncogenic driver, the enhanced proliferative activity of which was suppressed by TAS0286. This result suggests that TAS0286 might be effective for cancers harboring the DCTN1-RET fusion.

P-1051

Establishment of the high-throughput screening system for identification of novel oncogenes

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Assays for cellular transformation, such as focus formation assay and soft-agar colony formation assay, are classic but robust assays for identification of novel oncogenes. We established the high-throughput screening system with these assays, which could evaluate the cellular transformation. Combination of highly effective retroviral gene transfer and automated time-lapse cell culture observation system (BioStation CT, Nikon) enabled us to detect foci and colonies in small-scale culture such as 96-well plate. In this study, we selected more than 1000 genes highly expressed in various cancers and used them for the screening. Full-length cDNAs constructed in the retroviral vector were individually introduced to NIH3T3 mouse fibroblast cells in 96-well plates. The cellular transformation was evaluated by time-lapse recording of the cells, and we identified several genes that induce focus formation and soft-agar colony formation significantly. We will characterize these genes to verify this high-throughput screening system and discuss their function on the cellular transformation.
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▲TOP

[P-1066] P5-3 [Japanese]
MicroRNAs (3)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Takaaki Masuda / Dept. Surg., Kyushu Univ., Beppu hosp

P-1066

Oncogenic miRNAs induced by copy number gains in squamous cell carcinoma of the lung

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Background: Copy number gains in cancer genomes have been shown to induce oncogene expression and promote carcinogenesis. Our aim was to identify oncogenic microRNAs (onco-miRNAs) induced by copy number gains in human squamous cell carcinoma (Sq) of the lung.Methods: We performed a genome-wide screen of onco-miRNAs from 245 Sqs using data sets from RNA-sequencing, comparative genomic hybridization, and the corresponding clinical information from The Cancer Genome Atlas. Results: Among 1001 miRNAs expressed in the samples, 231 were correlated with copy number alternations, with only 11 of these being highly expressed in Sq compared to adenocarcinoma and normal tissues. Of eleven miRNAs, the high expression of 3 miRNAs was significantly associated with poor prognosis. The multivariate analysis, using the cox proportional hazard model showed that miRNA expression and smoking were independent prognostic factors, and associated with poor prognosis. Furthermore, miRNA mimics of the three miRNAs repressed mRNA expression of the anti-metastatic gene.Conclusion: We found that copy number gains in Sq of the lung induce onco-miRNA expression that is associated with poor prognosis.

▲TOP

P-1067

Role of secretory microRNA-518c-5p on the metastasis of oral cancer cells

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We have demonstrated that CXCR4 system is involved in the metastatic process of oral cancer and interact with their microenvironmental cells via extracellular vesicles, such as exosomes. In this study, we examined the role of miR-518c-5p as a secretory miRNA on the metastasis of oral cancer. We transfected miR-518c expression vector into oral cancer cells, B88 and CAL27, and isolated stable transfectants, B88-518c and CAL27-518c, respectively. The growth and migration of both cells were significantly enhanced in compared with those of mock cells. LNA-based miR-518c-5p inhibitor significantly impaired the enhanced cell growth and migration of these cells. When miR-518c transfectants were inoculated into the masseter muscle or the blood vessels of nude mice, tumor volume, lymph nodes metastasis, and lung metastasis were significantly increased in compared with those of mock transfectants. Next we examined the miR-518c-5p expression in the conditioned media in these transfectant cells. The growth and migration of endothelial cells were enhanced using conditioned media. We now analyze the role of exosomal miR-518c-5p on their microenvironmental cells via extracellular vesicles.

P-1068

Up-regulation of BLU tumor suppressor gene by miR-34 family

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miR-34a is well known to strong tumor suppresser microRNA. However, the mechanisms of tumor suppression by miR-34a remain still unclear. To elucidate the mechanisms of tumor suppression by miR-34a, we performed microarray analysis of miR-34a transfected cells. In general, microRNA(miRNA) negatively regulated target gene using RNA interference. However, the number of up-regulated genes was larger than down-regulated genes by the transfection of miR-34a. Among the up-regulated gene by miR-34a, tumor suppresser BLU (also known as ZMYND10) was strongly up-regulated by miR-34a and it has been reported that BLU is down-regulated in non-small cell lung cancer (NSCLC) compared with normal lung tissue. Therefore, we investigated that whether miR-34a inhibits lung cancer development through inducing BLU expression. We found two binding sites of miR-34a in the BLU promoter locus, and we identified the lncRNA from the BLU promoter named this non-coding RNA. Moreover, siRNA against the lincRNA could induced BLU expression. These results suggest that miR-34a binds novel lincRNA and this complex led to induced tumor suppresser BLU through the binding to the promoter-associated lncRNA.

P-1069

The functional analysis of miR-200 family in renal cell carcinoma

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Expression profiles revealed that the expression levels of any of 5 EMT-related microRNAs, i.e. miR-141, miR-200a, miR-200b, miR-200c and miR-429, called as miR-200 family, were reduced in 98% of the examined 95 renal cell carcinoma (RCC). Quantitative RT-PCR analysis verified the levels of ZEB1 and ZEB2, which were downstream targets of miR-200 family, were significantly increased, and their target CDH1 expression was further decrease in RCC. In order to clarify the effect of miR-200 family in RCC cells, transfection assay was performed using mimics or inhibitors for miR-200 family. The levels of ZEB1 and ZEB2 were significantly reduced, and that of CDH1 was elevated in the transfected 786-O cells with miR-141 or miR-200c mimics. The other hand, the level of ZEB1 was significantly elevated, and that of CDH1 was reduced in the transfected ACHN cells with five mixed inhibitors. Moreover, transwell invasion assay using the transfected 786-O cells with miR-200c mimic revealed that the invasive capacity was significantly decreased in the transfected cells. These data suggested that miR-200 family may participate in the malignant progression of RCC, via regulation of the EMT pathway.

▲TOP

P-1070

Analyses of miR-25 that was downregulated in micrometastatic cancer stem cells in a human breast cancer xenograft model

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Cancer stem cells (CSCs) are involved in tumor initiation, progression and metastasis. However, molecular characteristics of CSCs that initiate metastases are not elucidated. In this study, we analyzed the microRNA (miRNA) expression of CSCs isolated from the patient-derived breast cancer xenograft mice. We found that expression levels of all miRNAs transcribed from miR-106b-93-25 cluster were significantly lower in micrometastatic CSCs in the liver than in CSCs at the primary site. We then focused on miR-25, and evaluated its role in metastases by injecting the equal numbers of control and miR-25-expressing breast cancer MDA-MB-231 cells into the spleen of immunodeficient mice. Liver metastases were much more efficiently formed by miR-25-expressing MDA-MB-231 cells than control cells. Furthermore, the results of luciferase assays confirmed that miR-25 targeted F-box and WD-40 domain protein 7 (FBXW7), a suppressor of cell proliferation and metastatic ability. These results suggest that initiation of micrometastases in the liver was not initiated by the downregulation of miR-25, but more likely to be mediated by other miRNAs within this cluster, namely miR-106b and miR-93.

P-1071

Growth suppression of synthetic miR-143 in HER2-positive gastric cancer

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【Background】HER2 overexpression in gastric cancer is reported to be seen in 7-34% and the enhanced activation of PI3K/AKT signal pathways is well known in HER2-postive gastric cancer.MiR-143 silences KRAS and its effector signaling molecules, AKT and ERK, in several cancers, however the role of miR-143 in HER2-positive cancer is still unknown.【Aim】We investigated the effect of syn-miR-143 to the cell growth of gastric cancer cell lines【Methods】The expression levels of miR-143 were examined by RT-PCR. Also, the expression levels of KRAS and its effector molecules were evaluated by Western Blotting (WB) and RT-PCR.【Results】Remarkable cell growth suppression was observed by transfection with syn-miR-143 in the gastric cancer cell lines, MKN-7 and KATO-3. The axis of HER2/KRAS/PI3K was downregulated in both cell lines after the transfection, which induced apoptosis. The expression levels of effector molecules of KRAS were also decreased by the transfection with syn-miR-143.【Conclusion】Syn-miR-143 inhibited the cell growth of HER2-positive gastric cancer cell lines through suppressing KRAS/PI3K/AKT, ERK/MAPK signal pathways.

P-1072

Identification of 5-FU resistance-related microRNAs in colorectal tumors

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Aim: Drug resistance makes treatment difficult in cancers. The aim of this study is to determine the miRNA expression profiles in the 5-FU resistant cells.
Materials: We analyzed miRNA expression profile by miRNA array, and found the drug resistance miRNAs. We examined the identified miRNA expression levels in 90 colorectal tumor samples from the patients.
Results: We identified 12 miRNAs to be involved in 5-FU resistance by miRNA arrays. We investigated the relationship between miR-X that was one of these miRNAs, and drug resistance. Anti-miR-X caused significantly growth inhibition in DLD/F cells, which is 5-FU resistant colon cancer cell lines DLD-1, expose of 5-FU, while mimic miR-X caused a significant 5-FU resistance in DLD-1 cells. When we expose high dose of 5-FU to DLD-1 or DLD/F cells, the expression levels of miR-X were higher than control cells. The expression level of miR-X was positively associated with the grade of clinical stage of colorectal tumors.
Conclusion: In this study, four types of 5-FU resistant colon cancer cell line were established. We identified miR-X that is involved in 5-FU drug resistance and associated with clinical stages.

▲TOP

[P-1080] P5-5 [Japanese]
Signal transduction (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Noriaki Kitamura / Bristol-Myers Squibb

P-1080

Trogocytosis of ligand-receptor complex and its intracellular transport in CD30 signalling

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CD30, which is expressed in classical Hodgkin lymphoma (cHL), is thought to transduce signals by ligation of trimerised CD30 ligand (CD30L) on the surface of surrounding cells and recruitment of downstream molecules. We propose a new mechanism for CD30 signaling by its ligand.

We prepared two stable transformants, CHO cells expressing CD30L fused to mCherry and HeLa cells expressing CD30 fused to GFP. Co-culture of these cells triggered clustering of CD30 and CD30L at the cellular interface, formation of multiple CD30L-CD30 complexes, internalization of these complexes with a portion of the plasma membrane into the HeLa cells, and intracellular transport to the lysosomal compartment. The internalization process was inhibited by actin polymerization inhibitors. The CD30L-CD30 interaction was found to trigger active signaling processes and similar mechanisms were observed using cHL cell lines.

These results suggest that CD30 extracts CD30L from CD30L-expressing cells by actin-mediated trogocytosis, resulting in the generation of signalosomes, intracellular signaling and lysosomal degradation. These observations provide new insights into the biological roles of CD30.

▲TOP

P-1081

Endosomal Src promotes exosome secretion and tumor progression

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Cancer cells aberrantly secrete exosomes, lead to cancer progression. Although multi-functions of exosomes against recipient cells have been reported so far, the mechanisms or influences on host cells underlying aberrant secretion have not been fully elucidated. Here, we show that exosome secretion is upregulated by c-Src, and aberrant exosome secretion may be correlated with maintenance of cancer cell homeostasis. c-Src, frequently overexpressed or hyper-activated in various human cancers, is a membrane-associated tyrosine kinase and localized to the plasma membrane. In this study, we found that c-Src was also localized to the endosomal membrane, and interacted with the ESCRT-associated protein Alix. The interaction between c-Src and Alix activated ESCRT-mediated intra-luminal vesicles formation, resulting in the upregulation of exosome secretion. Furthermore, we observed a correlation between malignant phenotypes and Alix-dependent aberrant exosome secretion in Src-upregulated cancer cells. Our findings provide a unique mechanism for the upregulation of exosomes in cancer cells, as well as new insights into the significance of exosome secretion in cancer progression.

P-1082

Activation of the tumor suppressive Hippo pathway by high-molecular-weight hyaluronan and its breakdown in breast cancer

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High-molecular-weight hyaluronan (HMW-HA), a major component of the extracellular matrix, exhibits anti-oncogenic action whereas low-molecular-weight hyaluronan (LMW-HA), the degradation product of HMW-HA by HYAL2 hyaluronidase, acts oncogenically. However, the mechanisms underlying the size-dependent opposite actions of hyaluronans remain unclear. We found that treatment with HMW-HA activates Hippo signaling in mammary epithelial cells. Conversely, LMW-HA and ectopically expressed HYAL2 antagonize the HMW-HA-mediated Hippo signal activation and thereby activates pro-oncogenic transcriptional coactivator YAP. Thus, an alteration of size of hyaluronan by HYAL2 negatively regulates Hippo signaling. To gain pathophysiological relevance of hyaluronan-mediated Hippo signal regulation, we investigated functional relationship between HYAL2 expression and YAP activity in both of various breast cancer cell lines and clinical breast cancer tissues, and found that elevated HYAL2 inhibits Hippo signaling and thereby deregulates YAP activity in aggressive breast cancers. Hence, elevated HYAL2 causes the development of aggressive breast cancer via inhibition of the Hippo pathway.

P-1083

Transcriptional repression of IL-10 by K+ channel activators in human T-cell lymphoma HuT-78 cells

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Potassium ion (K^+) channels can regulate cytokine expression and production. Our recent study showed that the intermediate-conductance Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ activators significantly suppressed Interleukin 10 (IL-10) transcription and production in human T-lymphocyte lymphoma cell line HuT-78. IL-10 enables tumor escape from immune surveillance. The objective of this study is to clarify the underlying mechanism. Applications of the $K_{Ca}3.1$ activators induced the hyperpolarization responses and subsequent intracellular Ca^{2+} elevation in HuT-78 cells. Treatments with $K_{Ca}3.1$ activators for 6 hr inhibited nuclear translocation of phosphorylated Smad2 (P-Smad2) in HuT-78 cells. In addition, $K_{Ca}3.1$ activator-induced transcriptional repression of IL-10 and prevention of nuclear translocation of P-Smad2 were disappeared by pre-treatment with the calmodulin kinase II (CaMKII) inhibitor. These suggest that inhibition of Smad signaling pathway by activation of CaMKII may be involved in $K_{Ca}3.1$ activator-induced transcriptional repression of IL-10 in HuT-78 cells. $K_{Ca}3.1$ activator is therefore a possible therapeutic option to suppress tumor promoting activities of IL-10.

▲TOP

P-1084

The role of Rheb-SmgGDS-mTOR signaling pathway in mesothelioma cells

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Rheb is a Ras family small GTPase that regulates diverse cellular function such as cell growth and autophagy. These function are mainly mediated via the activation of mTOR complex 1 (mTORC1). Rheb is localized at lysosomal membranes, where mTORC1 is concentrated in response to nutrients, including amino acids. However, it is largely unknown whether Rheb localization is regulated. We found that Rheb binds to SmgGDS. Knockdown of SmgGDS altered Rheb localization and inhibited mTORC1 activity assessed by the phosphorylation of mTORC1 substrate S6K1. To examine whether SmgGDS-Rheb-mTORC1 signaling pathway is involved in cancer cell growth, SmgGDS is knocked-down in malignant mesothelioma cells, which are tumor cells derived from mesothelial cells. Knockdown of SmgGDS significantly suppressed the mesothelioma cell growth, while the activation of mTORC1 by overexpressing Rheb promoeted the growth of immortalized mesothelial cells. These results suggest that SmgGDS may represent an attractive therapeutic target in malignant mesothelioma.

P-1085

Stiff substrates increase the nuclear localization of ATF5 via actin filament in pancreatic cancer cells

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Tumors often tend to be stiffer than the surrounding normal tissues. Furthermore, stiff substrates correlate with poor prognosis. In particular, pancreatic cancer shows remarkable stiffness. However, it is poorly understood how mechanical stimuli are transduced into transcriptional outputs. We previously reported that activating transcription factor 5 (ATF5) increases the invasiveness. The purpose of this study is to clarify whether stiff substrates activate ATF5 in pancreatic cancer cells. First, we cultured human pancreatic cancer cell line (AsPC-1) on a collagen-I gel to make a soft substrate or on a cover glass coated with collagen-I to make a stiff substrate. Next, by using immunostaining, we found that ATF5 accumulates in the nucleus of the cells on stiff substrates compared to that on soft substrates. Furthermore, we focused on actin filament, which is known to play a role in mechanotransduction. To prevent formation of actin filament, we treated the cells with cytochalasin D. The treated cells exhibited decreased localization of ATF5 at nucleus on stiff substrates. These results suggest that substrate stiffness regulates ATF5 localization via actin filament.

P-1086

LATS2 inhibits O-GlcNAcylation in malignant mesothelioma cells

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O-GlcNAcylation, which provide a GlcNAc unit to serine or threonine residues of nuclear and cytosolic proteins by O-GlcNAc transferase (OGT), is an important post-translational modification that regulates diverse cellular functions, such as protein interaction and degradation. Previous studies have shown that O-GlcNAcylation level is increased in many types of cancer and elevated O-GlcNAcylation activity is associated with acquisition of malignant phenotypes. However, in malignant mesothelioma (MM) cells, O-GlcNAcylation level and its regulatory mechanisms are unclear. To address this issue, we examined O-GlcNAcylation level in MM cell lines by western blotting. O-GlcNAcylation levels of several distinct proteins were significantly increased in the MM cell lines lacking LATS2 kinase, a key regulator of Hippo signaling pathway. Interestingly, inhibition of O-GlcNAcylation by a use of an OGT inhibitor induced cell death of these cells lines. These results suggested that loss of LATS2 may promote MM cell survival via activation of O-GlcNAcylation. In a poster session, we would like to discuss the mechanism of O-GlcNAcylation by LATS2 and the potential therapeutic target for MM.

▲TOP

[P-1094] P5-7 [Japanese]
Transcriptional regulation

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yoji Andrew Minamishima / Dept. Mol. Cell. Boil., Med. Inst. Bioreg., Kyushu Univ.

P-1094

HPF-4: the novel gene that links p53-deficiency to HIF-1 and induces malignant phenotypes of cancer cells

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Hypoxia-inducible factor 1 (HIF-1) has been recognized as a cause of malignant phenotypes of cancer. Functional deficiency of p53 is also known to exhibit positive impact on the progression of carcinogenesis. Both of them work in the late stage of carcinogenesis and thus a gene that links them could exist to promote malignancies, but has yet to be identified. We recently obtained HIF-1-promoting factor 4 (HPF-4) by screening novel activators of HIF-1. Luciferase assays confirmed that HPF-4 increased the transactivation activity of the regulatory subunit of HIF-1 (HIF-1α) in functional p53-deficient cancer cells. A co-immunoprecipitation assay and a split luciferase complementation assay demonstrated that HPF-4 forms a homodimer to upregulate HIF-1 activity. The overexpression of HPF-4 was confirmed to facilitate the invasion of cancer cells in vitro and promote subcutaneous tumor growth only when functional p53 was deficient. Finally, immunohistochemistry of clinical lung cancer samples revealed that the expression level of HPF-4 was associated with the poor prognosis. These results indicate that HPF-4 links p53-deficiency to HIF-1 and induces malignant phenotypes of cancer cells.

▲TOP

P-1095

Apoptosis induced by CROX (Cluster regulation of RUNX) in neuroblastoma cells

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Neuroblastoma (NB) is the most common tumor in children. Despite the development of new treatment options for high-risk NB, five-year survival remains at less than 50%. Therefore, identification of new therapeutic targets in NB is urgently required. It has been known that NB cell proliferation is sensitive to changes in levels of RUNX1 (Runt-related transcriptional factor 1). We suggest that gene regulation of RUNX1 suppresses two p53 inhibitory factors, TRIM24 and BCL11A, resulting in apoptotic cell death in NB cells efficiently. Moreover, we utilized the patient cohort in NB and identified that these two genes are poor prognostic markers. Therefore, we examined the efficacy suppressing RUNX1-TRIM24 and BCL11A in NB by using our novel RUNX inhibitor, Chlorambucil-conjugated pyrrole imidazole polyamide (Chb-M'), which were designed to specifically bind to the consensus RUNX1 sequence. The tumor growth of the xeno-transplanted NB cells in immunodeficient mice was well-controlled by weekly injections of Chb-M' without significant side effects. From these results, inhibitions of downstream signaling of RUNX1-TRIM24 and BCL11A axis would be a new strategy for NB patients.

P-1096

Molecular mechanism of transcriptional regulation of REV7, which is involved in the DNA damage tolerance mechanism

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REV7 is an accessory subunit of DNA polymerase ζ which enables bypass replication over a DNA damaged site to continue replication, and plays a major role in the damage tolerance system. REV7 expression is high in tumor cells and germ cells compared with the other normal cells, suggesting the cell type-specific expression of REV7. However, the mechanism of regulation of REV7 expression has not been understood. To elucidate the mechanisms, we analyzed the promotor region of the human REV7 locus to identify transcriptional regulators controlling REV7 expression. The transcriptional start point of the REV7 gene was determined by 5'-RACE using human testis cDNA. About 3 kb of the upstream region of the REV7 locus was isolated by genomic PCR and promoter activity was analyzed by luciferase reporter assay. Using several deletion mutants, we found that there are two transcriptional activation regions in the upstream of the REV7 locus. Bioinformatic analyses revealed several motifs of transcription factor binding sites in these regions, and we focused on one molecule as a candidate for the transcription factor controlling REV7 expression. We will present and discuss our further analyses.

P-1097

Inhibition of BCR-ABL expression through CROX(Cluster regulation of RUNX)

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Chronic myeloid leukemia (CML) is caused by oncogenic p210 BCR-ABL fusion protein. Although tyrosine kinase inhibitors (TKIs) are the most useful treatment for CML patients. Despite recent development of next-generation TKIs, a portion of them steadily become refractory to therapy mainly through acquired mutations in BCR-ABL gene, necessitating a novel strategy to treat TKI-resistant CML patients. Here we show that RUNX1 stringently controls the expression of BCR-ABL gene. We also found that treatment with our novel RUNX inhibitor (Chb-M') which specifically targets RUNX consensus binding DNA sequences induces profound CML cell death through attenuating the expression of BCR-ABL. Mechanistically, RUNX1 binds to the proximal promoter region of BCR and directly transactivates BCR-ABL expression in CML cells. RUNX1 knockdown significantly reduced the expression of BCR-ABL in CML cells and deteriorated their proliferation. These results underscore the vital role of RUNX1 both in the regulation of BCR-ABL expression and in the maintenance of CML cells, thus making RUNX1 as an ideal druggable target in the treatment of CML.

▲TOP

P-1098

Cluster regulation of RUNX induces apoptotic cell death through regulating gene X in acute promyelocytic leukemia(APL)

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Although ATRA and ATO are effective drugs against APL, drug resistance(DR) are recognized for patients with cancer recurrence. Therefore, new therapeutic treatment against DR-APL is highly in need. Toward this end, we identified the key factor to suppress DR-APL, RUNX1-gene X axis, using our novel RUNX inhibitor, Chb-M'. RUNX1 is well known tumor suppressor as a master regulator of hematopoiesis and paradoxically required for various malignancies. We first examined the efficacy of Chb-M', which specifically binds to the consensus RUNX1 sequence and shows inhibitory effect against NB4 cells. Chb-M' was remarkably effective, suggesting RUNX1 is essential for APL proliferation. shRNA-mediated knockdown of RUNX1 suppresses NB4 growth and increases the expression of cleaved form of caspase-3 and PARP. Next, we defined gene X is the most consistently up-regulated genes in RUNX1-high expression of APL derived from clinical dataset. Inhibition of RUNX1 down-regulated gene X, resulting in apoptotic cell death. Besides, silencing of gene X performed the same phenotype. Taking together, we showed a novel interaction of RUNX1 and gene X-apoptotic axis, offering a new strategy for DR-APL.

P-1099

The importance of novel CROX: Cluster regulation of RUNX approach for ErbB2/HER2 gastric cancer

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Although runt related transcription factor 1 (RUNX1) is famous for oncogene in many cancers, its function in gastric cancer is still unclear. To investigate RUNX1 significance, we made shRNAi-mediated RUNX1 knockdown, inducing cell death in gastric cancer cell line MKN-45 with HER2(+), but we couldn't confirm this suppression with HER2(-). Next, we analyzed public database by using GSEA and DAVID to identify some candidate genes, SOS1 and GRB2, because these proteins play a pivotal role as an adaptor protein regulating ErbB2/HER2 signaling. Silencing RUNX1 decreased SOS1 and GRB2, suggesting that RUNX1-SOS1/GRB2 axis is required in HER2(+) gastric cancer progression. In order to verify importance of this axis, we used our novel RUNX inhibitor (Chb-M'). Chb-M' treatment had killing effect on MKN-45 cells via suppressing SOS1/GRB2-HER2 signaling. This result was also confirmed in vivo model. In the end, RUNX1 is candidate therapeutic target in HER2 (+) gastric cancer.

P-1100

Two transcription activation mechanisms by nuclear receptor ERR through cofactor and basal transcription factor

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Nuclear hormone receptor (NHR) binds to small molecules called ligands, such as hormones and this binding induces the conformational change at C-terminus of NHR. This conformational change results in recruitment of transcription cofactors which modifies chromatin structure and promotes formation of general transcription factors complex at transcription start site for transcription initiation. Estrogen related receptor (ERR) belongs to NHR family is one of important transcription factors regulating metabolism and development, and also indicated to relate with breast cancer. Because any natural ERR ligand has not been identified so far, the transcription activation mechanism by ERR is still obscure. Our precise biochemical system revealed (1) ERR did not bind to regular transcription cofactors directly but could bind them indirectly thorough another cofactor PGC-1 α , and (2) ERR also bound to general transcription factor TFIIH directly. We also showed these two interactions were necessary for ERR-dependent transcription activation in vitro and also for self-renewal ability of embryonic stem cells.

▲TOP

[P-1107] P5-9 [Japanese]
Proliferation (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Keiji Miyazawa / Dept. Biochem., Univ. Yamanashi

P-1107

Analysis of the involvement of cancer-associated fibroblasts in the progression of malignant mesothelioma

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Malignant mesothelioma is an aggressive neoplasm. We pathologists often encounter mesothelioma cases with a profound desmoplastic reaction, suggesting the involvement of cancer-associated fibroblasts (CAFs) in their progression. The roles of CAFs have been extensively studied in other malignant tumors, leading to the notion that there are heterogeneous populations of CAFs in the cancer stroma. The role of CAFs in mesothelioma, however, has remained unknown. We previously showed that connective tissue growth factor (CTGF) derived from fibroblasts promotes mesothelioma invasion *in vitro*. Here, we examined the expression of CTGF in CAFs infiltrating in mesothelioma using 35 surgical specimens in our hospital. In addition, we also examined the expression of α-SMA, a well-known marker of cancer-promoting CAFs, and Ki-67, a marker of cell proliferation, and the extent of fibrosis. We have been also interested in the identification of markers of cancer-inhibiting CAFs and examined the expression of some of the candidate marker proteins. We will show some of our preliminary data to discuss how the expression of these marker proteins correlates with the prognosis of mesothelioma patients.

▲TOP

P-1108

Analysis of dual mechanism of IL-24 suppression in myxoid liposarcoma cells

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The great majority of myxoid liposarcomas (MLSs) have TLS-CHOP fusion oncoprotein that plays a key role in sarcomagenesis and sarcoma cell proliferation. Our previous studies have revealed that TLS-CHOP significantly reduces IL-24 mRNA level through induction of PRG4 expression (Br. J. Cancer 2012; Biochem. Biophys. Res. Commun. 2017). Because IL-24 is an anticancer-cytokine that induces apoptosis in various cancer and sarcoma cells, loss of IL-24 expression is essential for tumor cell viability. On the other hand, we have also demonstrated that TLS-CHOP induces PAI-1 expression, and knockdown of PAI-1 inhibits MLS cell growth (Biochem. Biophys. Res. Commun. 2012). In this study, we found that although double knockdown of PAI-1 and IL-24 canceled the cell growth inhibitory effects by the PAI-1 single knockdown, PAI-1 expression seemed to have no effects on IL-24 mRNA level. Furthermore, a proteasome inhibitor MG-132 inhibited MLS cell growth, but IL-24 knockdown reduced the effects. These results support the notion that PAI-1 may promote degradation of IL-24 protein. Currently, we further investigate the details of the dual mechanism of IL-24 suppression in MLS cells.

P-1109

Processing body protein ATXN2L maintains progenitor properties of CML cells

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Cytoplasmic processing bodies (P-bodies) are non-membranous structures found in somatic cells, which regulate mRNA in terms of degradation, translational repression, as well as RNA-mediated gene silencing. Former studies suggest that Ataxin-2-like (ATXN2L) may potentially regulate P-body formation. To investigate a role of ATXN2L in myeloid leukemia cells, we made knocked down cells using two Philadelphia chromosome-positive CML cell lines, K562 and KU812. Lentiviral transduction of both cell lines with ATXN2L shRNA ($K562^{ATXN2L\text{-}KD}$, $KU812^{ATXN2L\text{-}KD}$) resulted in a marked reduction of cell proliferation with partial increase in apoptotic cell population. Surprisingly, surviving cell populations from $K562^{ATXN2L\text{-}KD}$ and $KU812^{ATXN2L\text{-}KD}$ cells differentiated into megakaryocytes and mature basophil-like cells, respectively. Furthermore, both ATXN2L-knockdown CML cells exhibited attenuation of constitutive STAT5 phosphorylation and EZH2 expression. These results suggest that ATXN2L is required for maintaining progenitor properties of CML cells.

P-1110

Protein phosphatase 6 controls tumor progression of colon cancer

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Background: Protein phosphatase 6 (PP6) is a Ser/Thr protein phosphatase classified into a type 2A protein phosphatase. PP6 regulates various cell biological processes including mitosis and DNA repair. Previous reports showed that PP6 expression is upregulated in glioma and mesothelioma. These data suggest that PP6 plays an important role in cancer. However, it is not clear how PP6 regulates cancer development and there is no report about PP6 expression level and function in gastrointestinal cancer. In this study, we aimed to clarify the role of PP6 in colon cancer. Method and Result: We analyzed PP6 expression level in colon cancer by performing immunohistochemistry in tissue microarray and western blot in colon cancer tissues. It has been found that PP6 expression is significantly increased in colon cancer compared with normal colon. Next, we performed colony formation assay and MTS assay by using colon cancer cell lines suppressing PP6 expression by shRNA. We found that colony number and cell proliferation rate are significantly decreased by suppressing PP6 expression. Conclusion: It was suggested that PP6 plays an important role in the colon cancer.

▲TOP

P-1111

Myc is involved in DNA synthesis, but not in glycometabolic changes of cultured mouse hepatocytes

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Cultured hepatocytes undergo robust DNA synthesis when cultured in appropriate media, such as Williams' E (WE), supplemented with EGF. Our preliminary data demonstrated that DNA synthesis is accompanied by a Warburg effect-like glycometabolic status (aerobic glycolysis). Here, we investigated whether Myc, a crucial regulator for both cell proliferation and cellular metabolism, is involved in DNA synthesis and the metabolic alterations in cultured mouse hepatocytes. Hepatocytes cultured in WE showed marked DNA synthesis peaking after 4 days, while the DNA synthesis in those cultured in DMEM was retarded and weak. Myc expression was strongly induced at 3 to 4 days in WE, but not in DMEM. Knockdown of Myc expression by siRNA significantly suppressed DNA synthesis of hepatocytes cultured in WE. However, the changes in gene expression of the key enzymes in glycometabolism were almost comparable regardless of the media used and Myc knockdown did not exert discernible changes in their expression. Our data suggest that DNA synthesis of cultured mouse hepatocytes requires induced Myc, but the associated glycometabolic changes were initiated by Myc-independent mechanisms.

P-1112

AhR plays an important role in heregulin-induced cell migration in HER2-overexpressing breast cancer cells

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Epidermal growth factor receptor 2 (Erbb2/HER2) overexpression accounts for approximately 15-20% of all breast cancers. Recently, we have reported that HER2 induces expression of the aryl hydrocarbon receptor (AhR) in breast cancer cells. In this study, firstly, we found that in HER2-overexpressing breast cancer cell lines, AhR expression was up-regulated by treatment with the HER3 ligand heregulin (HRG). In addition, AhR was translocated into the nucleus by HRG treatment. To further investigate the role of AhR in HRG-HER2/HER3 signaling-mediated cell migration, we performed wound-healing assays using AhR knockout (KO) cells. Cell migration after HRG treatment was markedly promoted in wild type (WT) cells, but slightly in AhR KO cells. Furthermore, the mRNA expression levels of inflammatory cytokines interleukin (IL)-6 and IL-8, which have been reported to promote cell migration, were clearly increased by HRG treatment in WT cells, but not in AhR KO cells. These findings suggest that AhR is a key regulator of HRG-induced cell migration in HER2 overexpressing breast cancers.

P-1113

NRF3-POMP-20S proteasome axis enhances tumor growth

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がんの発生や進行は、増殖抑制の回避や細胞死への抵抗といった特徴的な細胞性質の獲得によって惹起される。またプロテアソーム阻害剤が抗がん作用を有することから、タンパク質恒常性（プロテオスタシス）の破綻もがん増悪の要因になることが推察されているものの、その分子基盤は不明であった。本研究では転写因子NRF3（NFE2L3）を出発点とした一連の解析から、腫瘍増大に寄与するプロテオスタシス制御機構を見出した。NRF3は、プロテアソーム構成因子を標的とする転写因子NRF1（NFE2L1）のホモログとして当研究室が同定し、腫瘍増大を引き起こすことを見出した。またNRF3は20Sプロテアソームのアッセンブリ因子POMPを直接転写することで、p53やRetinoblastomaのタンパク質分解を亢進し、細胞周期の停止やアポトーシス誘導を阻害していることを明らかにした。NRF3-POMP-20Sプロテアソーム経路とがん増悪の関連を支持する知見は、がん患者の遺伝子発現解析からも得られていることから、このプロテオスタシス機構の破綻は腫瘍増大に寄与する新たながん特性であると考えられる。

▲TOP

[P-1121] P8-1 [English/Japanese]
Cell death / DNA replication

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Takashi Suda / Div. Immnol. & Mol. Biol., Cancer Res. Inst., Kanazawa Univ.

P-1121
Withdrawn

No Abstract

▲TOP

P-1122

Ursolic Acid Induced Apoptosis in Huh-7 cells via regulated the PI3K/Akt and MAPK signaling pathway

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Ursolic acid (UA), a natural pentacyclic triterpene acid that is exhibiting in a wide biological properties. In this study, we determined the effects of UA on apoptosis and the proliferation of hepatocellular carcinoma Huh-7 cells. Huh-7 cells were treated with UA at different concentrations and we observed that cell viability was reduced in a dose- and time-dependent manner. Furthermore, UA induced chromatin condensation of nuclei was observed by using DAPI staining. Apoptotic cell population was detected bydouble staining assay. The results of western blot revealed that exposure to UA was associated with decreased expression of the anti-apoptotic proteins Bcl-2, Mcl-1, and TCTP and increased expression of the apoptosis-related proteins TNF- α , Fas, FADD, Bax, cleaved caspase-3, and PARP. Thereafter, immunocytochemistry staining showed that treatment of Huh-7 cells with 40 μ M UA resulted in increased expression of caspase-3. Moreover, exposure of Huh-7 cells to 60 μ M UA resulted in the inhibition of the p-Akt and p38 MAPK but increasing the p-ERK signaling pathways. These findings suggest that UA inhibits the proliferation of Huh-7 cells and induces apoptosis.

P-1123

Litchi Flower Ethanol Extract Inhibits Colorectal Cancer Growth

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Litchi flower extract have been proved rich in polyphenolic substances to resist oxidation and inflammation. Here, we further tested its anti-cancer efficacy in vitro and in vivo, and the relevant mechanisms. SW480 and HT-29 cells of colorectal cancer were treated with litchi flower ethanol extract and assessed the proliferation, colony formation and cell cycle distribution and the change of the associated proteins. The xenograft of these two cell lines were also treated. The results showed that the survival rate after treatment above 50 μ g/mL was lower than 50%. SW480 was more sensitive by means of colony formation than HT-29, and the cell cycle was dominated by G1 and S phase arrest. Cyclin A, B were suppressed. The tumor growth of the xenograft were inhibited by the extract. In conclusion, litchi flower extract could provide a novel agent for the treatment of colorectal cancer.

P-1124

The molecular mechanisms of GABA tea in colony formation and invasion of colorectal cancer cells

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Abstract Objectives: GABA(γ -Aminobutyric acid) is the inhibitor of major neurotransmitters in the central nervous system, it can assists stress relief by lowering anxiety levels. The objective of this study is asses the effects of GABA tea on colorectal cancer cell proliferation, colony formation and invasion. Methods: Colorectal cancer cell line HT-29 treated with GABA tea extract (12.5~200 μ g/mL) were assessed for viability by MTT assay and for their invasion potential by evaluating their ability to penetrate through a matrix gel-coated Boyden chamber. Calculate the ability ratio for each of the dose group and the control group to obtain the survival rate.Results: We assessed the effects of four different types of tea extracts. GABA tea extract exhibited slight effect on cell proliferation of HT-29. The colony formation were inhibited in both culture plate and Boyden chamber. Conclusions: GABA tea extract inhibited the colony formation and invasion of colorectal cancer cells. GABA tea is also a potential adjuvant agent for colorectal cancer therapy.Keywords: GABA tea; colorectal cancer; colony formation; invasion.

▲TOP

P-1125

Anti-neoplastic activity of Petasites japonicus extract

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Petasites japonicus extract (PJE) is an herbal product studied for an anti-inflammatory agent. However, effects of PJE on neoplastic diseases has yet to be elucidated. We presently evaluated the anti-cancer potential of PJE in several cancer models. PJE reduced viable cells up to 23-47% in various cancer cell lines (K562, T24, A2058, DLD-1). We identified four active ingredients (petasin, neopetasin, neo-S-Petasin, and S-petasin) and demonstrated that these ingredients had the identical cytotoxicity. None of these showed apparent cytotoxicity in fibroblasts. PJE increased PARP cleavage and transition of LC3B-Ⅰ to LC3B-Ⅱ, which suggests that PJE induces either or both apoptosis and autophagic cell death. Intraperitoneal administration of PJE to DLD-1 xenograft mice showed a significant growth suppression, whereas it showed no apparent adverse effect on the mice. We further initiated preliminary clinical assessment in canine spontaneous melanoma model. We orally administered PJE in three cases of canine melanoma, and one case showed regression of metastatic lung lesion. The present results suggest that PJE has the potential to be a chemopreventive agent for a broad range of cancers.

P-1126

To investigate the molecular mechanism and therapeutic roles of PSF1 in Leukemia

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Leukemia is a type of hematopoietic stem cell (HSC)-derived malignant tumor, commonly happened in children and teens. When leukemia develops, the accumulation of mutations in HSCs cause the abnormal proliferation and block the differentiation. Chemotherapy can temporarily alleviate the symptoms, but hard to maintain the effect for a long period. The relapse sometimes occurs. The recent study indicated that the recurrence of leukemia is the result of a culpable existence of LSCs. Direct contact with neighborhood stromal cells was essential for LSC escaped from chemotherapy. However, the mechanism of their interaction has yet to disclose. In order to clarify the mechanisms, LSC becomes the novel therapeutic research target. More and more studies indicated that the LSC-located microenvironment (niche) regulated the LSC proliferation and assisted the escape from chemotherapy. In this research, we would like to apply the animal model which labeled with LSC-specific EGFP signal, not only to disclose the LSC-dependent microenvironment but also investigate its mechanism of chemotherapy resistance in vivo. Further to establish the new therapeutic method of LSC-specific clinical treatment.

▲TOP

[P-1134] P8-3 [English/Japanese]
Cell death / cellular senescence

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Katsuya Ohta / Dept. Gastroenterological Surg., Higashiosaka City Med. Ctr.

P-1134

Rebamipide suppressed anticancer drugs-induced cell death via Akt/mTOR activation in oral mucosal keratinocytes

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<Purpose> Oral mucositis is a common adverse effect of chemotherapy that limits the required dose of chemotherapeutic agents. Recently, it has been indicated that rebamipide prevents chemoradiotherapy-induced oral mucositis in patients. However, the details of the underlying mechanism involved in the cytoprotective effect of rebamipide remain obscure. In the present study, we investigated the mechanism behind rebamipide cytoprotective effect in the oral mucosa using primary normal human oral keratinocytes (NHOK cells). <Methods> Cell viability was assessed by the trypan blue dye method. <Results> We found that rebamipide prevented 5-fluorouracil (5-FU)-induced cell death in NHOK cells. In addition, rebamipide increased the levels of phosphorylated Akt and mTOR, enhanced the Bcl-2 and Bcl-xL expressions, and suppressed the expression of Bax and Bim. This is in contrast to 5-FU-induced suppression of Akt and mTOR activation, Bcl-2 and Bcl-xL expressions, and the enhanced expression of Bax and Bim.<Discussion> These findings suggest that rebamipide can potentially be used for the protection of oral mucosa from chemotherapy-induced mucositis.

▲TOP

P-1135

Analysis of Paclitaxel-induced apoptosis in triple-negative breast cancer

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The microtubule targeting agent Paclitaxel is prescribed widely for various malignancies, including breast adenocarcinomas. When cancer cells undergo Paclitaxel-induced apoptosis, the Bcl-2 family proteins-dependent mitochondrial apoptotic pathway is activated. This activation is regulated by the balance between pro-apoptotic and pro-survival Bcl-2 family proteins. When these cancer cells are treated with Paclitaxel, cellular microtubule assembly was promoted and subsequent apoptosis. Furthermore, recent studies have reported that pro-survival Bcl-2 family protein Mcl-1 is an essential survival factor in TNBC cells. However, the precise mechanism of Paclitaxel-induced apoptosis is not clearly understood. From these findings, we used siRNA screening approach to identify genes involved in Paclitaxel-induced apoptosis. Here we identify that X gene which is required for the activation of pro-apoptotic Bcl-2 family proteins, Cytochrome c release from the mitochondria and play an important role of Paclitaxel-induced apoptosis. In this study we discuss the effects of X gene on microtubule dynamics and determine whether it becomes biomarker for the effect and susceptibility for Paclitaxel.

P-1136

Cytoplasmic DNA accumulation induces SASP in senescent cells

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Cellular senescence is a state of irreversible cell cycle arrest that can be induced by a variety of potentially oncogenic stimuli and has been considered to suppress tumorigenesis. However, it has recently become apparent that senescent cells increase secretion of various inflammatory proteins, termed the senescence-associated secretory phenotypes (SASP). In addition to secretory proteins, senescent cells also increase the secretion of exosomes, which are extracellular small vesicles. However, the biological roles of exosome secretion in exosome-secreting cells have remained largely unexplored. To enhance our understanding of exosome biology, we examined the effects of the inhibition of exosome secretion in senescent cells. As a result, we found that exosomes contain various lengths of chromosomal DNA fragments, indicating that exosome secretion maintains cellular homeostasis by removing harmful cytoplasmic DNA from cells. Furthermore, in senescent cells, the downregulation of DNases appears to cause the cytoplasmic accumulation of nuclear DNA, thus provoking SASP through the aberrant activation of cytoplasmic DNA sensing machinery.

P-1137

53BP1 is involved in the HSF1 depletion-induced senescence

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Heat shock factor 1 (HSF1) participates in tumorigenesis and age-related diseases, and regulates wide variety of genes under both proteotoxic stress and normal conditions. Recently, we showed that shRNA-mediated depletion of HSF1 induces the p53-p21 dependent cellular senescence in human fibroblasts (T Oda et. al, J Cell Sci, 2018). p53-binding protein 1 (53BP1) is a critical regulator of the cellular response to DNA double-strand break that facilitates DNA repair by promoting non-homologous end-joining. Although the importance of the interaction between 53BP1 and p53 had not been elucidated, recent works show that 53BP1 enhances DNA-binding activity of p53 and genome-wide p53-dependent transcription. In this study, we examined whether 53BP1 is involved in the HSF1 depletion-induces senescence (HDIS). Depletion of 53BP1 reduced the proportion of morphologically senescent cells and SA-β gal positive cells in the HSF1-depleated human fibroblasts. Furthermore, expression of the senescence-associated secretory phenotype (SASP) was decreased in those cells. These results suggest that 53BP1 is involved in the HDIS mediated by regulation of the p53 transcriptional activity.

▲TOP

P-1138

BCR-ABL, an oncogene of chronic myeloid leukemia, can induce cellular senescence

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Oncogenic signals can frequently induce senescence, designated as oncogene-induced senescence (OIS). OIS was once presumed to exert anti-tumorigenic effects, but subsequent studies suggest that senescent cells can have pro-tumorigenic effects by exhibiting senescence-associated secretory phenotype (SASP) with robust expression of pro-inflammatory molecules. BCR-ABL is an oncogenic fusion gene, which is specific to chronic myeloid leukemia (CML). We observed that the expression of senescence-associated- β -galactosidase (SA- β -gal) is enhanced in leukemic cells in CML-bearing mice. Moreover, in vitro colony formation assay demonstrated that CML stem cell-derived colonies exhibited augmented SA- β -gal expression. Furthermore, qRT-PCR analysis revealed that the expression of senescence markers was upregulated in BCR-ABL^{+} CD150^{+} CD41^{+} leukemic megakaryocytes. Concomitantly, the expression of IL-6, a cytokine characteristic of SASP, was elevated in the same population. Given the capacity of IL-6 to impair normal hematopoiesis in CML patients, senescent IL-6-expressing leukemic megakaryocytes can be a novel target to treat CML.

▲TOP

[P-1145] P12-2 [English/Japanese]
Molecular target therapy (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Kazunori Kato / Dept. Biomed. Eng., Toyo Univ.

P-1145

Establishment of complete human IgG antibody expression system from recombinant Fab against CSPG4 on tumor cells

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CSPG4 is a proteoglycan expressed in many types of cancer and has attracted attention as a therapeutic target because of its limited expression in normal cells. We isolated human recombinant Fab (rFab) recognizing CSPG4 using phage display. To apply this rFab to cancer treatment, we investigated to construct expression system of complete IgG with effector function. *pIGG* vector was used for complete IgG expression. Antibody fragments of VH-CH1 and VL-CL cloned in *pComb3* were amplified, then digested with restriction enzymes, and cloned into *pIGG*. The resulting clones were transfected into 293T using Lipofectamine 3000. The culture supernatant was collected over time and antigen binding activity was examined by indirect fluorescence (IIF). Purification of IgG from culture supernatant was performed using Protein A column.Transfection was performed in 24 well plate format. At this time, 1.5 µl/well of Lipofectamine 3000 was added, and high antigen binding activity was confirmed in incubation for 72 hrs or more. In addition, a maximum of 6 µg of purified antibody was obtained from 1 mL of culture supernatant, and it was confirmed as a single band approximately 150 kDa by SDS-PAGE.

▲TOP

P-1146

Withdrawn

No Abstract

P-1147

Production of novel monoclonal antibodies recognizing SLC7A1 (CAT1)

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SLC7A1/ CAT1 (cationic amino acid transporter 1) is 14-pass transmembrane protein, whichtransports arginine, lysine and ornithine. Arginine is an important substance which is metabolized byvarious enzymes and involved in the biosynthesis of carbon monoxide, urea and glutamic acid.Deficiency of arginine-related metabolites is known to enhance the expression of SLC7A1, andcellular uptake of arginine promotes cell growth. In this context, SLC7A1 is upregulated in multipletypes of human cancers. Monoclonal antibodies (mAb) recognizing SLC7A1 on living cancercells were not reported, because of scarce extracellular region of SLC7A1. In this study, wereports successful production of anti-SLC7A1 mAb and extensive analysis towardsdiagnosis and therapy of human cancers. To develop mAb against SLC7A1, rats wereimmunized with RH7777 rat hepatoma expressing SLC7A1 fused to green fluorescent protein(GFP), and antibodies secreted from hybridomas were screened for the binding to transfectantsexpressing SLC7A1-fused to GFP, in a GFP expression-dependent manner in flow cytometry. Anti-SLC7A1 mAb reacted with human cancers from colon, breast, lung and pancreas. Collaborator:Kazue Masuko

P-1148

Temperature or fixation dependent reactivity of antibodies against multi-pass membrane proteins

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We succeeded in the production of a set of monoclonal antibodies (mAb) to surface epitopes of molecules containing amino-acid transporters, oncogene products and adhesion molecules expressed on various human cancers, by the immunization of rats with rat transfectants expressing target molecules fused to GFP. All established mAb could react with transfectants expressing GFP-fused target proteins in a GFP expression-dependent manner, however, some mAb, especially against epitopes in multi-pass membrane proteins have shown relatively weak reactions against cancer cell lines. In these cases, mAb might not efficiently access epitopes of target proteins, possibly because of the complicated structural topology of multi-pass proteins affected by differences in the surrounding molecules in a given cell line. In this study, we have analyzed the effect of temperature and fixation conditions on the binding of mAb against 12-pass (L-type amino acid transporter 1) and 7-pass (adenosine receptor 2A) membrane proteins, and demonstrated improved binding of these mAb to cancer cell lines in physiological (37 ° C) rather than experimental (4 ° C) temperature. Collaborators: Akari Hoshi, Daiki Hara

▲TOP

P-1149

Efficacy and safety study of anti-podoplanin cancer-specific monoclonal antibody, chLpMab-23

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Podoplanin (PDPN), the ligand of C-type lectin-like receptor-2, is involved in cancer metastasis. In this study, we aimed to produce cancer-specific anti-PDPN mAb, which could target only PDPN expressed in cancer cells although PDPN is highly expressed in both cancer and normal cells. We immunized mice with recombinant PDPN which was produced using LN229 glioblastoma cells and established cancer-specific anti-PDPN mAb (clone: LpMab-23). LpMab-23 reacted with PDPN-expressing many cancer cell lines whereas it weakly recognized normal cells such as lymphatic endothelial cells (LECs) and HEK-293T cells in flow cytometry. Furthermore, LpMab-23 reacted only with PDPN-expressing cancer cells, not with LECs in oral cancer tissues using immunohistochemistry. To evaluate the efficacy of LpMab-23 as a therapeutic antibody, we developed a mouse-human chimeric antibody of LpMab-23 (chLpMab-23). ChLpMab-23 exerted ADCC activity in vitro and anti-tumor effect in vivo. Intravenous injection of chLpMab-23 induced no adverse effects and tissue disorders in cynomolgus monkey. In conclusion, chLpMab-23 could be a safe and effective therapeutic antibody for targeting PDPN in cancer.

P-1150

Anti-cancer effects of novel anti-ASCT2 monoclonal antibody on human colorectal cancer

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ASCT2 is one of the primary glutamine transporters on mammalian cells, and is highly expressed in various human cancers originated from such as gastrointestinal, respiratory and urinary organs. Furthermore, experiments with inhibitors or targeted disruption of ASCT2 gene demonstrated inhibition of cell proliferation and tumor growth. Here, we developed novel anti-human ASCT2 monoclonal antibody (mAb), and examined whether this antibody had anti-cancer effects. Rats were immunized with RH7777 rat hepatoma cells expressing green fluorescent protein (GFP)-fused human ASCT2 proteins, and splenocytes from rats were fused with X63 mouse myeloma cells. Anti-ASCT2 mAb (Ab3) was selected from hybridomas using flow cytometry. Ab3 reacted with RH7777 and HEK293F cells expressing GFP-fused human ASCT2 protein in a GFP expression-dependent manner, but not with ASCT2-knockouted HEK293F and SW1116 human colorectal cancer cells. Ab3 inhibited phosphorylation of AKT and ERK, and proliferation of SW1116 cells. Furthermore, Ab3 inhibited tumor growth of SW1116 in nude mice. These results indicate that ASCT2 is a promising target for human cancer therapy. collaborators: Soushi Yoshimoto, Kazue Masuko

▲TOP

[P-1158] P14-2 [English/Japanese]
Esophageal cancer (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yoshifumi Baba / Dept. of gastroenterological Surg., Kumamoto Univ.

P-1158

TAMs down-regulated the expression level of miR-29c and stimulated migration of ESCCs by up-regulating GABRP

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Tumor-associated macrophages (TAMs) are involved in the tumor progression. We previously reported the association between high number of infiltrating TAMs and poor prognosis in esophageal squamous cell carcinomas (ESCCs). To study the interaction between TAMs and ESCCs, we conducted microRNA microarray analysis between TE-9 ESCC cells and TE-9 cells co-cultured with TAMs (TE-9co). As a result, miR-29c was down-regulated in TE-9co. We confirmed low expression level of miR-29c in TE-9co and TE-11co. We focused on *Gamma-aminobutyric acid receptor subunit pi* (*GABRP*) suspected as a target gene of miR-29c. We demonstrated the expression level of GABRP was up-regulated in TE-9co and TE-11co. We confirmed miR-29c inhibitor up-regulated expression level of GABRP and miR-29c mimic down-regulated it in TE-9 and TE-11. GABA agonist stimulated migration of TE-9 and TE-11 through phosphorylation of Erk1/2. GABAA receptor antagonist suppressed migration of them stimulated by GABA agonist. These results suggested that TAMs down-regulated the expression level of miR-29c and stimulated migration of ESCC cells by up-regulating the expression level of GABRP.

▲TOP

P-1159

Analysis of microRNAs downregulated in esophageal squamous cell carcinoma after co-culture with TAMs

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Tumor-associated macrophages (TAMs) are known to be involved in the progression of various cancers. We have previously reported the association between increased number of infiltrating CD204+ TAMs and poor prognosis of esophageal squamous cell carcinomas (ESCCs). MicroRNAs (miRNAs) which downregulate various genes contribute to tumor progression in tumor microenvironments. However, the role of TAM-mediated miRNA aberrant expression remains unclear in ESCC microenvironments. We constructed an in vitro differentiation system where peripheral blood monocyte-derived macrophages differentiated into TAM-like macrophages. Here, we also established co-culture assays using human ESCC cell lines and TAM-like macrophages and performed miRNA microarray analysis between mono-cultured ESCC cell lines and co-cultured ESCC cell lines with TAM-like macrophages. We are investigating several miRNAs downregulated in ESCC cell lines co-cultured with TAM-like macrophages when compared to mono-cultured ESCC cell lines.

P-1160

CARD9 high expression associates with cancer malignancy and poor prognosis in esophageal squamous cell carcinoma

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[Background] In many kinds of cancer including Esophageal squamous cell carcinoma (ESCC), inflammation induced by NF-κB is known to be very important in cancer progression. In this time, we focused on Caspase Recruitment Domain Family Member 9 (CARD9), which is a signal adapter essential for NF-κB activation, and we examined whether CARD9 can be used as a prognostic factor in ESCC.[Methods] The expression of CARD9 in the surgical specimen from the 108 patients was assessed by immunohistochemistry. The patients were divided into 2 groups as low and high expression group by the staining intensity and the proportion in the cancer area.[Results] Among 108 cases, CARD9 expression is low in 39 cases and high in 69 cases. Overall survival (OS) and disease free survival (DFS) were significantly poor in the CARD9 high expression group (Kaplan Meier and the log-rank test). Univariate analysis and multivariate analysis showed that high CARD9 expression was significant prognostic factor for OS in these ESCC patients.[Conclusion] High expression of CARD9 was significantly associated with a poor prognosis, and CARD9 expression may be a prospective prognostic biomarker in ESCC.

P-1161

Inhibition of the Src-YAP pathway is a candidate therapeutic target in esophageal squamous cell carcinoma

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Backgrounds: The prognosis of esophageal squamous cell carcinoma (ESCC) is still poor among gastrointestinal carcinoma. The Src-YAP (Yes-associated protein) pathway is important for inflammation, regeneration and carcinogenesis in colon, however, its clinical and biological significance in ESCC still remains unknown. Method: We reviewed 111 consecutive patients who underwent esophagectomy for ESCC in our department and examined the expression of YAP and phosphorylated Src by immunohistochemical staining. We evaluated the relation of YAP expression and clinical outcome, and the correlation of YAP and phosphorylated Src expression. The efficacy of YAP and Src suppression was analyzed by proliferation and migration assays using human ESCC cell lines in vitro. Results: The expression of YAP was significantly associated with poor overall survival of the ESCC patients, and the expression of YAP and phosphorylated Src was correlated. Suppression of YAP and Src attenuated the ability of proliferation and migration in human ESCC cells. Conclusion: YAP expression contributes the malignancy of ESCC. Inhibition of the Src-YAP pathway might be a novel therapeutic target in ESCC.

▲TOP

P-1162

Clinicopathological significance of Cullin4A in human esophageal cancer

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Background Cullin4A (CUL4A) is a component of SCF E3 ubiquitin ligase. The role of CUL4A in esophageal cancer remains unknown. Methods We investigated the CUL4A expression in primary esophageal cancer tissues from 120 patients by immunohistochemistry, and explored its clinical relevance and prognostic values. Furthermore, the effect of CUL4A expression on cancer cell proliferation was analyzed in vitro using a siRNA silencing technique. Results Higher CUL4A expression was significantly associated with deeper depth of tumor invasion ($P<0.001$) and presence of venous invasion ($P=0.014$). The postoperative disease specific survival rate was significantly lower in patients with CUL4A-high tumors than in patients with CUL4A-low tumors ($P=0.001$). Importantly the CUL4A status was identified as an independent prognostic factor for esophageal cancer ($P=0.045$). Furthermore, CUL4A gene silencing significantly inhibited the proliferation of esophageal cancer cells in vitro. Conclusion The CUL4A expression has a significantly prognostic value in esophageal cancer. CUL4A might play an important role in regulating the proliferation of esophageal cancer cells.

P-1163

Expression and role of Na+/K+-ATPase in human esophageal squamous cell carcinoma

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Recent studies have reported important roles for Na^+/K^+-ATPase, such as involving in uptake of CDDP, in various cancers. The aim of the present study was to investigate the role of Na^+/K^+-ATPase in human esophageal squamous cell carcinoma (ESCC). Immunohistochemical analysis performed on 53 primary ESCC samples showed that Na^+/K^+-ATPase primarily found in the cell membrane of carcinoma cells. The expression of Na^+/K^+-ATPase in the invasive front of tumor (IF) was related to pT category ($p=0.039$), and the weak expression of Na^+/K^+-ATPase in IF had a worse prognosis ($p=0.023$). In in-vitro experiments, the cells of ESCC cell lines expressed Na^+/K^+-ATPase. The depletion of ATP1A1, Na^+/K^+-ATPase α 1 subunit, using siRNAs reduced cell death induced by CDDP or staurosporine. Moreover, the depletion of ATP1A1 promoted cell migration and invasion in human ESCC cell lines. These results suggest that the weak expression of Na^+/K^+-ATPase in ESCC may affect cellular invasion and be related to a worse prognosis in patients with ESCC.

P-1164

HMGB1 is involved in esophageal squamous cell carcinoma progression

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High-mobility group box-1 (HMGB1) mediates a broad range of inflammatory responses as a secretory form and is also involved in the progression of various types of malignancies. However, the roles of HMGB1 in the progression of esophageal squamous cell carcinoma (ESCC) is unclear. The aim of this study was to investigate the significance of HMGB1 in ESCC. Frozen tumor and paired non-cancerous tissues were collected from ESCC patients and plasma samples were also obtained from preoperative ESCC patients and healthy volunteers. The expression level of HMGB1 in ESCC tissue was significantly higher than that in paired non-cancerous esophageal mucosa tissue. Plasma HMGB1 level was slightly higher, but not significant, in ESCC patients than in healthy volunteers. It was significantly higher in ESCC patients with neoadjuvant chemotherapy (NAC) than in those without NAC. In ESCC cells with high HMGB1 expression, knockdown of HMGB1 using specific siRNAs inhibited the cell proliferation, migration and invasion. These findings suggest that overexpression of HMGB1 will play a crucial role in tumor malignant potential in esophageal squamous cell carcinoma.

▲TOP

[P-1172] P14-4 [English/Japanese]
Esophageal cancer (3)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Hiromi Kataoka / Dept. Gastroenterology & Metabolism, Nagoya City Univ., Grad. Sch. Med.

P-1172

Efficacy of neoadjuvant chemotherapy on micrometastasis in lymph nodes for esophageal cancer patients in OGSG1003 trial

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Backgrounds：We have reported that DCF (DTX+CDDP+5FU) therapy chemotherapy was associated with prolonged RFS for patients with resectable advanced esophageal cancer (EC) compared with A(ADM)CF by randomized study (OGSG1003). On the other hand, it has been reported that lymph nodal micrometastasis (MM) is important prognostic factor in EC. The aim of this study was to clarify the effect of neoadjuvant chemotherapy (NAC) on nodal MM according to regimens: ACF and DCF.Methods:　A total of 102 patients with EC underwent esophagectomy followed by NAC with ACF (n=52) or DCF (n=49). Resected lymph node were examined the MM by cytokeratin (CK)(AE1/AE3) immunohistochemistry. Positive staining for CK antibody of pathologically negative node was considered as MM involvement. Results: MM was observed in 24 patients (24%). By regimen, MM occurred in 17 patients (33%) in ACF and in 7 patients (14%) in DCF (p=0.036). By pN status, the proportion of MM of ACF or DCF was 37%/22 % in pN0, 33%/17% in pN1, 30%/6% in pN2 and 20%/0% in pN3, respectively. Conclusions: Regulation of the MM by DCF may be one of the reasons that DCF was more effective than ACF as NAC for EC patients.

▲TOP

P-1173

Chemotherapy for esophageal cancer ~Off-Target effect & Biomarker

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Purpose: In this study, we obtained peripheral blood before and after the DCF therapy, analyzed various immune related cells by flow cytometry and tried to identify indicators to predict its therapeutic effects.Methods: Peripheral blood were obtained from 24 esophageal cancer patients before and after preoperative DCF therapy. Peripheral blood was collected before and after the surgical treatment followed by. By flow cytometry, CD11b+CD14+CD33+HLA-DRlow M-MDSC (monocyte-myeloid derived suppressor cell) was observed. The effectiveness of NAC was determined hisitopathologically in accordance with the grading system of the Japanese Classification.Results: Of 24 patients with esophageal cancer, 11patients were responders and 13 patients were non-responders to the DCF therapy. Before the DCF therapy, the ratio of M-MDSC to monocytes was observed in 6.73% in the non-responders and 23.9 % in the responders (p=0.058). The numbers of M-MDSC calculated were 24.7 / μ L in the non-responders and 132.5 / μ L in the responders (p=0.021). Conclusion: Patients with better pathological or clinical responses showed lower ratio of M-MDSC to monocytes and fewer number of M-MDSC in peripheral blood.

P-1174

A 17-molecule set as a predictor of pathological complete response to NAC-DCF in esophageal cancer

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[Background] Recently, NAC-DCF was identified as a novel strong regimen with a high rate of pathological complete response (pCR) in locally advanced esophageal cancer (LAEC) in Japan. The purpose of this study was to identify a novel predictor of pCR in LAEC treated with NAC-DCF.[Patients and Methods] A total of 32 patients who received NAC-DCF followed by esophagectomy between 2013 and 2016 were enrolled in this study. We divided the patients into the following 2 groups: pCR group (9 cases) and non-pCR group (23 cases), and compared gene expressions between these groups using DNA microarray data and KeyMolnet Analysis. Subsequently, a validation study was performed in 7 additional cases.[Results] Seventeen molecules (E2F, TCF, Src, IRF-1 1, TSase, cyclin B, CDK 4, CDK, caspase-1, VDR, HDAC, MEK, Bax, RUNX1, BLIMP-1, PDGFR, and IL-1) were identified as candidate molecules. The molecules were mainly associated with pathways, such as transcriptional regulation by SMAD, RB/E2F, and STAT. The validation study indicated that 12 of the 17 molecules matched the trends of molecular expression.[Conclusions] A 17-molecule set that predicts pCR after NAC-DCF for LAEC was identified.

P-1175

Intraoperative photodynamic diagnosis of lymph node metastasis in esophageal cancer patients using 5-aminolevulinic acid

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[Background] In patients with esophageal cancer, the establishment of a simple and rapid intraoperative diagnostic method of lymph node metastasis is essential. Exogenous application of 5-aminolevulinic acid (ALA) causes a selective accumulation of protoporphyrin IX, which is a fluorescent substrate, in cancer cells. The purpose of this study is to evaluate the feasibility of photodynamic diagnosis using ALA (ALA-PDD) for lymph node metastasis in patients with esophageal cancer. [Methods] Eight patients with esophageal squamous cell cancer were administered ALA orally prior to surgery. Excised lymph nodes were cut in half and examined by spectrometer. [Results] A total of 292 lymph nodes were analyzed Among them, 19 nodes (6.5%) were histologically metastatic and 21 nodes (7.2%) were PDD-positive. The sensitivity and specificity of ALA-PDD were 84.2% (16/19) and 98.2% (268/273), respectively. The area of cancer nests of the PDD-negative lymph nodes was <2 mm^2. Metastatic lymph nodes, including cancer nests>4 mm^2, were correctly diagnosed by ALA-PDD. [Conclusion] ALA-PDD for lymph node metastasis in patients with esophageal cancer is feasible.

▲TOP

P-1176

In vitro and in vivo preclinical studies on esophageal cancer by the combination usage of palbociclib and erlotinib

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Esophageal cancer in man is the sixth most common cancer worldwide. The patients in East Asia consist mainly of esophageal squamous cell carcinomas (ESCCs). The 5-year survival rate of locally advanced ESCC is still, at best, 55%. Development of new therapeutic modality is awaited. Recently, comprehensive genome analyses showed G1/S transition- or EGFR-RAS/AKT pathway-related gene alteration in about 80% ESCC. Therefore, CDK4/6- and/or EGFR-targeted therapy is thought to be most promising. Based on genetic background of 52 ESCC cell lines, we selected 28 cell lines by CCND1, CDKN2A, KRAS and/or RB1 status and conducted in vitro and in vivo preclinical studies. Palbociclib (CDK4/6 inhibitor) provided from Pfizer could suppress in vitro cell growth of most cell lines without RB inactivation, and the combination effect between palbociclib and erlotinib (EGFR inhibitor) was also observed without any relation to CDKN2A/p16, CCND1, EGFR, or RAS status. Although palbociclib alone was enough to inhibit tumor development through reduction of proliferating cells in vivo, most ESCC patients without RB1 inactivation (>90%) may become targets of palbociclib mono- or the combination-therapy.

P-1177

The significance of SCC and CEA mRNA in the pleural cavity after lymphadenectomy in esophageal cancer patients

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Background: This report focuses on the surgical manipulation and spread of cancer cells. Little is known regarding the clinical significance of the lavage procedure in esophageal cancer patients who undergo preoperative treatment.Methods: A cohort of 94 patients with squamous cell carcinoma of the esophagus who underwent esophagectomy was prospectively analyzed for free cancer cells in the pleural cavity after mediastinal lymphadenectomy. RT-PCR was performed to detect free cancer cells in the pleural lavage fluid by measuring squamous cell carcinoma-related antigen (SCC) and carcinoembryonic antigen (CEA). Results: Forty-two patients (44.7%) were positive for SCC after thoracic lymphadenectomy, and 15 patients (15.9%) were positive for CEA. SCC positivity was significantly associated with venous invasion (p=0.037) and with the clinical response to preoperative treatment (p=0.001). Furthermore, SCC positivity was associated with poor prognosis compared with negative SCC (p=0.026). Conversely, positive CEA was not associated with any clinicopathological finding, response, prognosis.Conclusions: Tumor spillage as evaluated by SCC mRNA was associated with a poor prognosis.

P-1178

Prediction of the neoadjuvant chemotherapy for esophageal cancer by the IgG

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[Background] IgG is used as monitoring of the disease such as an infection or tumor. Previously, we reported that the high level of IgG in preoperative patients with esophageal cancer is poor prognosis. In addition, neoadjuvant chemotherapy for esophageal cancer is effective for prolonging prognosis. We analyzed the relationship IgG values and the effectiveness of neoadjuvant chemotherapy.[Methods] We examined 25 patients whose IgG values were measured both before and after neoadjuvant chemotherapy from 2012 to 2016.[Results] The average age is 66.5 years old. There were 23 males and 2 females. All patients were squamous cell carcinoma with high dose FP therapy. IgG values in 14 cases were reduced after chemotherapy. 14 cases were evaluated as PR, 10 cases as NC and 1 case as PD. Comparing with PR group, the pre-treatment IgG value in the NC/PD groups was significantly higher (P value = 0.0134). [Conclusions] In about 60% of patients who underwent neoadjuvant chemotherapy, the tumors were reduced. In cases with high level of IgG before treatment, it was considered possible that neoadjuvant chemotherapy may make it difficult to reduce the tumor.

▲TOP

[P-1184] P14-6 [English/Japanese]
Clinical practice in gastric cancer

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-1184

Postoperative gastric stasis and migrating complex after pylorus-preserving gastrectomy for early gastric cancer

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Background/Aims: The demerit of pylorus-preserving gastrectomy (PPG) is gastric stasis in the remnant stomach (GSRS). We investigated the relationship between GSRS and interdigestive migrating motor complex (IMMC) in PPG patients. Methodology: A total of 24 patients (16 men and 8 women; average age, 61.2 years) 1 year after PPG for early gastric cancer were divided into 2 groups (Group A, 12 patients without GSRS; Group B, 12 patients with GSRS). The relationship between GSRS and IMMC was studied. Results: IMMC from the duodenum was significantly more common in group A than in group B (p=0.0005). Appetite was significantly more common in group A than in group B (p=0.0264). Postprandial abdominal fullness (PAF) was significantly more common in group B than in group A (p=0.0041). Reflux esophagitis (RE) and body weight loss were found in group B more than in group A. Endoscopic gastritis in the remnant stomach was found significantly more in group B than in group A (p=0.0031). Conclusions: Postoperative QOL in PPG patients with IMMC positivity was better than that in those with IMMC negativity. GSRS may occur by the absence of IMMC from the duodenum.

▲TOP

P-1185

Prognostic factor for gastric cancer patients undergoing neoadjuvant chemotherapy

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Background: Prognostic factor for gastric cancer patients undergoing neoadjuvant chemotherapy (NAC) has not been established yet. We compared the validity of response criteria and other histological factors in gastric cancer patients who underwent NAC.Methods: The target population in this study was 97 gastric cancer patients who underwent NAC followed by curative gastrectomy between January 2007 and March 2017. Hazard ratio (HR) for RFS in responders according to each factor was estimated, and survival curves were compared between responders and non-responders using log-rank test.Results: The number of patients in each regimen was as follows: DCS, 42; DOS, 23; CS, 22; SOX, 5; DS, 5. HRs were 0.616 (p=0.118) in the endoscopic response criteria, 0.485 (p=0.091) in the histological response criteria, 0.397 (p=0.005) in differentiated type, 0.409 (p=0.051) in ypT0-1, and 0.122 (p=0.001) in ypN0, respectively. ypN0 (p=0.006) was independent prognostic factor for survival ion a multivariate analysis.Conclusions: Pathological ypN status was the best prognostic factor of RFS for gastric cancer patients undergoing NAC. Patients with ypN0 would be expected for long relapse-free survival.

P-1186

Prognostic value of neutrophil-to-lymphocyte ratio in gastric cancer

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The neutrophil-to-lymphocyte ratio (NLR) have been reported to be a prognostic factor in various cancer. The study was performed to determine the prognostic value of NLR in gastric cancer (GC)Method: A total of 130 gastric cancer patients, diagnosed between January 2010 and December 2012 in our hospital were enrolled in this study. The clinicopathological parameters, laboratory analyses, and outcomes were collected. The optimal cutoff value of NLR was estimated by the receiver operating characteristics (ROC) curve. The association between NLR and clinicopathological characters was analyzed with univariate and multivariate analyses.Results: NLR was significantly related to higher CA19-9 and CEA values, tumor depth, distant metastasis, advanced stage, and possibility of curative surgery. The overall survival (OS) was poor in the High-NLR group (OS: not reached vs 17.8 months, p<0.01). Multivariate analyses indicated age, liver metastasis, peritoneal metastasis, clinical stage and NLR were independent prognostic factors. Conclusion: Elevated NLR was associated with poor prognosis in GC patient before treatment. The NLR was an independent prognostic factor for OS.

P-1187

Our experience of Nivolumab for gastric cancer

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Background: Nivolumab, an immunocheckpoint inhibitor, was insured in September 2017 and has recommended for tertiary treatment in the gastric cancer treatment guidelines revised in January 2018. We report on its safety and effectiveness based on experience with gastric cancer at our hospital.Materials and Methods: We experienced administration of Nivolumab for six unresectable recurrent gastric cancer cases from October 2017 and examined the clinical characteristics.Results: The average age was 68 years (61-75), and all of them were men. The clinical stage were one case in stage IIIb, two cases in stage IIIc, and three cases in stage IV. Postoperative recurrence was three cases (one case was p-IIIb, two cases were p-IIIc cases). The treatment line was three cases of 3rd, one case of 4th, two cases of 5th. The best overall response was two case of PR and four cases of PD. Adverse events were observed in four out of six cases (67%). Grade 3 skin rash were observed in only one case. Adverse events less than Grade2 were pruritus, fatigue, constipation and hypothyroidism.Conclusion: We plan to further accumulate cases, study safety and efficacy, and explore biomarkers.

▲TOP

P-1188

Prognostic risk factor of pStage 3 gastric cancer after gastrectomy

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Objective:The aim of this study was to identify the prognostic risk factors for overall survival in patients with of pStage 3 gastric cancer after gastrectomy. Methods:From 2010 to 2016, 94 patients underwent gastrectomy for pStage 3 gastric cancer, whose prognostic risk factors for recurrence were retrospectively studied. Results:Their average age was 65.5, and 21 of them were female. 70 patients underwent laparoscopic surgery. The overall D2 lymph node(LN) dissection rate was 81.9%. The mean operation time and blood loss were 288.2 minutes and 420.3 ml. The tumor diameter was 71.6 mm, depth was T2:T3:T4=7:49:38, LN metastasis was N0:N1:N2:N3=1:26:20:47 and histological findings were tub1/tub2:por/sig:others=30:60:4. By univariate analysis, LN metastasis (p=0.033) was the only significant factor for poor prognosis, while multivariate analysis depicted the number of metastatic LN and degree of LN dissection as independent prognostic factors. Conclusion:For stage 3 gastric cancer, number of metastatic LN and the degree of LN dissection correlated with overall survival, while surgical approach did not, which suggested that laparoscopic gastrectomy can be safely applied to that.

▲TOP

[P-1195] P14-8 [English/Japanese]
Cell-to-cell interaction in gastric cancer

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-1195

Extracellular vesicles from gastric carcinoma induce mesothelial-mesenchymal transition of peritoneal mesothelial cells

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Background: Human scirrhous gastric carcinoma (SGC) is known as highly peritoneal metastasis. We previously reported that SGC cells effect pre-metastatic niche formation of peritoneal mesothelial cells (PM cells). Extracellular vesicles (EVs) play cell-cell interactions. We investigated the effect of EVs from SGC cells on the pre-metastatic niche formation of the peritoneum.Methods: Four SGC cell line, 2 gastric cancer cell line and PM cells were used. PKH26-labeled EVs derived from SGC were intravenously injected into nude mice and examined by a fluorescence microscope. The morphology and gene expression of PM cells was investigated in the addiction of EVs by microscope and RT-PCR. Clinical significance of CD9 and CD63 evaluated on human gastric cancer specimens.Results: Labeled EVs were frequently distributed to the peritoneum. PM cells change to spindle shape and increased mRNA expression of MMPs and mesenchymal markers, such as N-cadherin. The high expression of CD9 and CD63 was significantly correlated with distant metastasis.Conclusion: EVs from gastric cancer cells might induce a pre-metastatic niche at peritoneum by mesothelial-mesenchymal transition of mesothelial cells.

▲TOP

P-1196

Protocadherin B9 is associated with peritoneal dissemination in human gastric cancer

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Gastric cancer (GC) is one of the most common human cancers. Genes expressed only in cancer tissue, especially on the cell membrane, may be useful biomarkers for cancer diagnosis and therapeutics. In the present study, we focused on the PCDHB9 gene, which encodes transmembrane protein protocadherin B9. Immunohistochemical analysis revealed that 62 (36%) of 173 GC cases were positive for protocadherin B9. Although PCDHB9 knockdown or forced expression of PCDHB9 did not change cell growth and invasion activity in a GC cell line, cell adhesion to fibronectin was significantly reduced by PCDHB9 knockdown and significantly enhanced by overexpression of PCDHB9. Furthermore, both the number and size of spheres were significantly decreased by PCDHB9 knockdown and significantly increased by overexpression of PCDHB9. In a peritoneal dissemination mouse model, the weight of the total disseminated nodules of MKN-74 cells was significantly increased by forced expression of PCDHB9. These results indicate that protocadherin B9 plays an important role in the progression rather than pathogenesis of intestinal type GC.

P-1197

Loss of E-cadherin expression is the morphological determinant of human gastric signet ring cell carcinoma

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Recent genomic profiling has revealed that diffuse type gastric cancer frequently has loss-of-function type aberration in the CDH1 gene that encodes E-cadherin. We have reported that disruption of CDH1 in gastric stem cells expressing MIST1 transcription factor resulted in development of signet-ring cell carcinoma (SRCC) in a mouse model (Ariyama et al. Cancer Cell, 2015). To test the role of CDH1 in human SRCC, we established a human gastric organoid (hGO) culture system. The hGO contained a small population of $MIST1^{+}$/$Pepsinogen1^{-}$ cells that can be maintained for a long-term. We then specifically disrupted CDH1 in hGO by using the CRISPR/Cas9 technology. Interestingly, the cultured hGO cells turned into atypical cells with eccentric nucleus and PAS positive mucin that resembles SRCC cells. These cells expressed MIST1, showed increased migration ability in vitro, and gradually underwent apoptotic cell death 2 weeks after CDH1 KO. These results suggest that a loss of E-cadherin is sufficient for hGO to achieve morphology and migration ability of SRCC, but additional genetic alteration or supports from niche is required for malignant transformation.

P-1198

Adipose tissue derived stem cells promote gastric cancer growth

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Little is known regarding the influence of adipose tissue derived stem cells (ASCs) in gastric cancer (GC).We investigated the potential effects between GC cell lines (MKN45, OCUM2MD3, MKN74) and ADSC. ASCs were isolated from surgical specimens of human omentum. The sphere-forming efficiency of GC cells was significantly increased while directly co-cultured with ASCs. MKN45 cells generated subctaneous tumors increased in size after co-injection with ASCs, in comparison with MKN45 alone in mouse model. Immunohistochemistry of xenografts tissue revealed the expression of α SMA was increased in tumors generated by co-injection of MKN45 with ASCs in comparison with control tumors, which corresponds to the significant increase of fibrous area in Azan staining. The expressions of α SMA and FAP on ASCs were confirmed by fluorescent immunostaining. We also examined whether ASCs is associated with the chemo- sensitivity of MKN45 using 5-FU. ASCs enhance sphere formation and in vivo tumor growth of GC cells. ASCs also induce increased fibrous stroma through EMT-like change and 5-FU-resistance, which suggests ASCs acts as CAF in tumor microenvironment of GC.

▲TOP

P-1199

Annexin A10 induces gastric mucin phenotype via pancreatic duodenal homeobox-1 in gastric cancer tissue and organoids

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Annexin A10 (ANXA10), a member of annexin family, is a calcium-/phospholipid-binding protein; however, little is known about its functions. In the present study, we performed immunohistochemistry to evaluate the expression of ANXA10, PDX1, and mucin phenotype markers in 142 GC samples and assessed the correlation with clinicopathological factors. In addition, we tried to further scrutinize the correlation between ANXA10 and PDX1. ANXA10 was detected in 68 (48%) of 142 GC cases and preferentially detected in the GC cases with the gastric mucin phenotype. The loss of ANXA10 was significantly correlated with poor clinical outcomes in GC. PDX1 was significantly correlated with ANXA10 in GC cases. In GC cell line, PDX1 was concurrently repressed in ANXA10-knockdowned cells. As a further investigation, we generated organoids derived from human GC and identified the duplication of mucin phenotypes of GC by immunohistochemistry. The repression effect that was observed in the ANXA10-knockdown cell lines was also observed in human gastric organoids. ANXA10 could cooperate with PDX1 to introduce the gastric mucin phenotype in gastric type GC.

P-1200

Characteristics of tumor-associated stromal cells in scirrhous gastric cancer progression

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Cancer associated fibroblasts (CAFs) play a crucial role for tumor progression in a variety of malignancy. Activated CAFs frequently express α-smooth muscle actin(SMA) and are classified as myofibroblasts. However, the role and significance of CAFs in gastric cancer progression is still unknown. We investigated the biology of CAFs in scirrhous gastric cancers. We examined immmunohistochemical staining of SMA, fibroblast activation protein(FAP), and HGF in primary sites of scirrhous gastric cancer. Of the 24 scirrhous primary gastric tumors, 7(29.1%) scored positively for SMA. FAP scored positively in 21(87.5%). HGF scored positively in 20(83.3%). Interestingly, FAP markedly enhanced cell migration activity in human scirrhous gastric cancer cell lines in vitro. Moreover, the mean concentration of FAP protein was markedly higher in malignant ascitic fluid than nonmalignant peritoneal exudates. Our results suggest that the CAFs in scirrhous gastric cancer consists of highly FAP- and HGF-, but not SMA-expressing fibroblasts. We will present a mechanism of HGF production in CAFs of scirrhous gastric cancer.

P-1201

Association of Helicobacter pylori infection and hematological parameter among Japanese general population

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Helicobacter pylori (H. pylori) infection is a major risk factor for gastric cancer. Although studies showed associations between H. pylori infection and hematological parameter, evidence is still limited. This study aimed to examine the associations of H. pylori infection with serum hemoglobin (Hb), hematocrit (Ht) and red blood cell (RBC) levels in the Japanese. Subjects are 7,398 Japanese adults (4,051 males and 3,347 females) who were recruited as participants of the Japan Multi-Institutional Collaborative Cohort (J-MICC) study. H. pylori infection was determined by the anti- H. pylori IgG levels, with positive results defined as a value 10U/mL or higher. Multivariate linear regression analysis was used to assess the association of H. pylori infection with Hb, Ht and RBC levels after adjustment for age, sex and BMI. The mean age of subjects was 58.0±10.7 years old. H. pylori infection was significantly associated with lower Hb and Ht levels in female (Hb: $P<0.0001$ for female, Ht: $P<0.001$ for female). This study showed that H. pylori infection was associated with lower Hb and Ht levels in Japanese females.

▲TOP

[P-1209] P14-10 [English/Japanese]
Pathological features in gastric cancer

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Shunsuke Kagawa / Minimally Invasive Therapy Ctr, Okayama Univ. Hosp.

P-1209

Characteristics of super-minute gastric cancers

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Background: In previous reports, minute gastric cancer has been defined by a diameter of 5 mm or less. Recent progress in endoscopic techniques has enabled examination of very minute gastric cancers. Therefore, we aimed to describe super-minute gastric cancer (SMC), defined by a diameter of 1 mm or less, which is the earliest cancer stage detectable. **Materials and Methods**: A total of 24 SMCs, 72 microscopic cancers (diameter > 1 to 5 mm or less), and 304 non-microscopic cancers were examined in terms of clinicopathological features. **Results**: SMCs frequently showed macroscopic type 0-IIb, low tumor grades, p53 overexpression and less frequently showed nuclear beta-catenin expression, with statistical significance. **Discussion**: The p53 pathway, rather than the Wnt/beta-catenin pathway, may contribute to carcinogenesis of SMCs, which is less likely the case in cancers with a diameter greater than 1 mm.

▲TOP

P-1210

Histological diversity in gastric cancer and their characteristic molecules

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The histological features of gastric cancer (GC) may differ widely from area to area within the same tumor due to tumor heterogeneity. The aim of this study was to clarify the relationship of histological numbers and composites with clinicopathological parameters as well as tumor-associated molecules in 842 GC cases. As results, the total number of histological combination patterns between mucosal areas and invasive areas was 320, and GC with the higher T grade were more frequently composed of mixed histological composites or more histological numbers in invasive areas, compared to those in mucosal areas. Histological numbers and composites of GC, along with staging, were found to be poorer prognostic factors. In immunohistochemistry, cancer stem cell molecules and receptor tyrosine kinase molecules were expressed in GC with more histological numbers or mixed histological types significantly. These results indicate that histological diversity in invasive areas may be a useful predictor of GC with poor prognosis.

P-1211

KIF23 expression is frequently found in gastric cancer with intestinal mucin phenotype

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Spheroid colony formation is an effective model for characterization of cancer stem cell. Previously, we found that expression of Kinesin family member 23 (KIF23) were upregulated in gastric spheroid colonies. KIF23 is a member of microtubule-dependent molecular motors that transport organelles within cells and a key regulator of cellular cytokinesis. However, the significance of KIF23 in GC has not been studied. In the present study, we have analyzed the expression and distribution of KIF23 in human GC cases. qRT-PCR revealed that KIF23 expression in GC was higher compared with that in normal tissue. In addition, overexpression of KIF23 was observed in 9 (69%) of 13 GC cases. Next, immunohistochemistry was performed on 98 GC tissue samples. In non-neoplastic gastric mucosa, weak or no staining of KIF23 was observed in the foveolar epithelium. In contrast, GC tissue showed stronger, more extensive staining. In total, 33 (34%) of 98 GC cases were positive for KIF23. These results suggest that KIF23 may participate in pathogenesis of GC. KIF23 function in gastric spheroid colony formation should be analyzed.

P-1212

Clinical significance of diffuse-type histologic type coexistence in gastric cancer

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Background: Signet ring cell carcinoma (sig) and non-solid poorly differentiated adenocarcinoma (por2) are the histological forms of gastric cancer that is called diffuse type and is often observed in schirrous gastric cancer. Purpose: The purpose of the present study was to examine the influence of the coexistence of diffuse type on the prognosis of gastric cancer.Patients and Methods: We retrospectively analyzed the clinicopathologic data of 1050 gastric carcinoma patients who underwent gastrectomy between 2007 and 2016. Results: We observed that there were 202 cases (19%) with sig mixed (dominant 82 cases, inferiority 120 cases) and 211 cases (30%) with por 2 mixed (dominant 179 cases, inferiority 42 cases). We found no correlation of por2 mixture with prognosis in all pathological stages, however, patients with sig mixture had poor prognosis in stage Ⅲ. Among the patient who received the S-1 adjuvant chemotherapy, the prognosis of sig mixture was significantly poor. Conclusion: Our findings suggested that because sig mixed gastric cancer had high risk of recurrence even if chemotherapy was performed, a careful observation after surgery should be necessary.

▲TOP

P-1213

Long-term prognosis of Alpha-fetoprotein-producing gastric cancer defined as immunohistochemichal expression

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【Background】Alpha-fetoprotein producing gastric cancer (AFP-GC) is one of the primitive cancers and reported as a distinct variant of gastric adenocarcinoma, which shows aggressive clinical behavior.【Method】We have analyzed 95 tumors and 22 normal gastric tissues by RNA sequencing. We examined histological phenotype of tumors by evaluating immunohistochemichal expressions of AFP. AFP-GC was defined as immunohistochemichally high expression of AFP, GPC3, CLDN6, SALL4, or ALB.【Results】The incidence of AFP-GC was 14.7% (14/95). The median preoperative serum AFP level was 5.4ng/mL (2.1-1070). Three of 14 patients had high level of preoperative serum AFP. The pT, pN factor were pT2/T3/T4a: 1/4/9, pN0/N1/N2/N3a/N3b: 2/3/5/3/1, respectively. The 1y, 3y and 5y overall survival were 72.2%, 43.3% and 43.3%, respectively. Ten patients relapsed, and 3 of 10 patients survived after the recurrence by multidisciplinary treatment.
【Conclusion】Evaluating immunohistochemical expressions demonstrated that AFP-GC represent a larger subgroup of gastric cancer than the previous report. The prognosis might not as poor as the previous report by multidisciplinary treatment.

P-1214

Function analysis of Desmoglein1(DSG1) in gastric cancer

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Spheroid colony formation is a typically method to identify cancer stem cells (CSC). The growth of spherical colonies is considered indicative of self-renewal ability, and is consistent with a CSC phenotype. In the present study, we analyzed spheroid formation associated genes in gastric cancer (GC). Comprehensive gene expression profile of MKN-1 GC cell line that was grown as monolayers or spheroids was obtained by Affymetrix GeneChip, and we found several kinds of genes whose expression was up-regulated in cells that were grown as spheroids. DSG1 was up-regulated in MKN-1 cells that were grown as spheroids, however, expression and significance of DSG1 was not analyzed in GC. Therefore, we analyzed the expression and function of DSG1 in human GC. Immunohistochemical analysis of DSG1 in GC tissues revealed that 33 (42%) of 78 GC cases were positive for DSG1. Expression of DSG1 was associated with lymphovascular invasion and vascular invasion. DSG1-positive GC cases were frequently found in intestinal type GC than in diffuse type GC. Knockdown of DSG1 inhibited sphere formation and cell growth. These results suggest that DSG1 plays an important role in cancer stem cells.

P-1215

Overexpression of claspin and its clinicopathological significance in gastric cancer

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Gastric cancer (GC) is one of the leading causes of cancer-related death worldwide. Spheroid colony formation is a useful method to identify cancer stem cells (CSCs). In the present study, we analysed the microarray data in spheroid body-forming and parental cells, and CLSPN gene was overexpressed in the both GC cell lines MKN-45 and MKN74. Claspin is nuclear protein related to damage response. The expression and biological significance of claspin in human GC is still unclear. Therefore, we analyzed the expression and significance of claspin in human GC. Quantitative RT-PCR analysis revealed that claspin mRNA was overexpressed in GC cell lines. Immunohistochemical analysis of claspin in GC tissues revealed that 94 (47%) of 203 GC cases were positive for claspin. Claspin expression was associated with higher T grade, N grade, tumor stage, lymphatic invasion and poor prognosis. In addition, we revealed that claspin expression was co-expressed with CD44, HER2 and p53. Furthermore, claspin siRNA-transfected GC cells showed significantly reduced cell growth and cell invasion. These results indicate that claspin expression might be a key regulater in tumor progression of GC.

▲TOP

[P-1220] P14-12 [English/Japanese]
Translational research in gastric cancer and GIST

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Tsuyoshi Takahashi / Dept. Surg, Osaka Univ. Sch. Med

P-1220

Extraction and functional analysis of extracellular vesicles derived from gastric juice

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Recently, gene products of extracellular vesicles(EVs) are expected as new biomarkers. EVs are observed in body fluid. In this study, we focused on the EVs of gastric juice. EVs were extracted from two gastric cancer cell lines and fifteen gastric juices of gastric cancer patients by the ultracentrifuge methods. Extraction of EVs from gastric juice was difficult because they were covered with mucous ingredient. Finally, we were successful in extraction of EVs from gastric juice by making all kinds of efforts.The protein expressions of anti-CD81, CD9, TSG, CD40, and integrin 5 were used as surface marker of the EVs by Western blotting analysis. Also, we used Nano Sight, scanning and transmission electron microscope to prove existences of EVs in gastric juice. In addition, we examined the expression levels of microRNA(miR) in extracellular vesicles by the real-time PCR method. In order to examine the function of gastric juice-EVs, our gastric juice-EVs from gastric cancer patients and EVs from gastric cancer cell lines exposed normal fibroblast ASF-4 cells. As a result, promotions of ASF-4 cell growth were observed in both extracts tested.

▲TOP

P-1221

The thorough characterization of cancer cells in patients' ascites

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We have established over 100 cancer cell lines from the ascites in 7 kinds of cancers (85 cell lines from 47 gastric cancers, 29 from 22 pancreatic cancers, 6 from 6 ovarian cancers, and each one from esophageal-gastric junction, gallbladder, peritoneal mesotheliomas, and liposarcoma) in the past 8 years, and reported about them previously. It is hard to establish conveniently the cell lines from patients' ascites because a large majority of cancer cells lack self-propagating ability followed by slow growth and small colony formation, and because some cancer cells show mesothelium- or fibroblast-dependent growth. To characterize the ascetic cancer cells thoroughly, we investigated expression of EpCAM, some cancer stem cell markers and Ki-67 by flow cytometry analysis, and also evaluated sensitivity against some anticancer drugs and molecular target drugs. Furthermore, to clarify clonality of the ascetic cancer cells, we compared mutation profiles among EpCAM-positive fraction and the matched cell line including its sub-cell lines. Here we will present those results and argue about the characteristics of the ascetic cancer cells.

P-1222

Enhancement of paclitaxel uptake into gastric cancer cells via the hypotonicity-induced cell volume regulation

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Backgrounds. Paclitaxel (PTX) is a key drug of systemic and intraperitoneal chemotherapy for gastric cancer (GC). This study examined whether hypotonic stimulation enhances PTX uptake into GC cells.Methods. MKN45 cells (human GC cell line) were incubated with 1 μM Flutax-2 (Oregon green 488 PTX) in the iso- or hypotonic condition, and the cellular uptake of PTX was determined by fluorescence intensity. The change of mRNA expression of influx (OATP1B3) and efflux (MDR1) transporters for PTX, and the influence of Rifampicin, a blocker of OATP1B1/3, on cellular uptake of PTX were examined under hypotonic stimulation.Results. Fluorescence intensity of a MKN45 cell was significantly higher in the hypotonic condition with increasing cell volume. Further, the blockade of chloride transports with 200 μM NPPB inhibited the occurrence of regulatory volume decrease (RVD), and significantly enhanced PTX uptake in GC cells. Meanwhile, hypotonic stimulation did not alter the expression and function of influx and efflux transporters for PTX.Conclusion. Hypotonic stimulation enhances PTX uptake into GC cells via the regulation of cell volume.

P-1223

Regulatory Volume Decrease was suppressed by hypothermia stress in gastric cancer cells

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Background:Cancer cells avoid rupture under mild hypotonicity through regulatory volume decrease (RVD), which is homeostatic volume regulation by water transport via the activation of ion conductance. This study examined whether hypothermia stress affects cell volume change in gastric cancer (GC).Methods and Results: The three human GC cell lines (NUGC4, Kato III, and MKN45) were exposed to mild hypotonic solutions at 37°C or 24°C, and cell volume change was measured. Hypothermia stress inhibited RVD on all three GC cells, and cell volume at 24°C was larger than at 37°C after hypotonic stress. Next, we analyzed the expression of membrane transport proteins regulating cell volume. The cell membrane proteins were isolated. The expression of SWELL1 (volume-regulated anion channel) was decreased and AQP5 (water channel) was increased at 24°C in NUGC4 cells. RVD was suppressed in SWELL1-depleted NUGC4 cells. Cells swelling after hypotonic stress was larger in AQP5-overexpressed NUGC4 cells. Conclusions: Hypothermia stress could suppress RVD after hypotonic shock in gastric cancer, regulating expression of membrane transport proteins, SWELL1 and AQP5.

▲TOP

P-1224

Detection of PD-L1 amplification using circulating cell-free DNA in gastric cancer patients

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(Background) Programmed death-ligand 1 (PD-L1) is one of the key immune checkpoint molecules. The immune checkpoint inhibitor was used for advanced gastric cancer, but a useful predictor has not been reported. (Aim) To detect PD-L1 amplification in circulating cell-free DNA (cfDNA) of patients with gastric cancer (GC). (Method) 1) Evaluation of tumor PD-L1 expression and amplification in GC tissue. 2) Detection of plasma PD-L1 amplification in cfDNA of GC patients by rt-PCR and ddPCR. 3) Plasma PD-L1 amplification in patients with immune checkpoint inhibitor. (Result) 1) Tumor PD-L1 amplification was detected in 11% of patients. There was no relationship between PD-L1 expression and PD-L1 amplification in tissue sample. 2) Plasma PD-L1 amplification was detected in 13% of patients and significantly correlated with tumor PD-L1 amplification (p=0.04), pN factor (p=0.01), and pStage (p=0.02). 3) Plasma PD-L1 amplification was evaluated in 8 patients before and after the treatment by immune checkpoint inhibitor. (Conclusion) The present study has demonstrated the clinical utility of circulating cfDNA to detect PD-L1 amplification.

▲TOP

[P-1229] P14-14 [English/Japanese]
Molecular analysis of cervical cancer and uterine sarcomal in clinical senario

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-1229

α GlcNAc and α 4GnT are favorable prognosis markers for cervical gastric-type tumors

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Cervical gastric-type adenocarcinoma (GAS) is a non-HPV- related rare tumor and often involves metastasis, resulting in poor prognosis. Gastric gland mucin contains unique *O*-glycans having terminal α1,4-linked N-acetylglucosamine (αGlcNAc) attached to MUC6, formed by α1,4-N-acetylglucosaminyltransferase (α4GnT). Recently, we revealed α4GnT-deficient mice spontaneously develop gastric adenocarcinoma, indicating αGlcNAc as a tumor suppressor. Here, we analyze expression of αGlcNAc, MUC6, and α4GnT in 86 cases of cervical glandular tumors including a GAS-precursor lobular endocervical glandular hyperplasia (LEGH). MUC6 was expressed in GAS and LEGH as well as normal cervical glands. By contrast, αGlcNAc and α4GnT expression were observed in GAS and LEGH, but rarely observed in normal cervical glands. In addition, expression of α4GnT and αGlcNAc in GAS were significantly decreased compared to LEGH. Notably, GAS patients strongly expressing α4GnT and αGlcNAc exhibited better prognosis compared to those with lower expression. Such significance was not observed in MUC6. These results indicated that αGlcNAc and α4GnT were favorable prognosis biomarkers for cervical gastric-type tumors.

▲TOP

P-1230

Clock gene DEC1 regulates the expression of stem cell marker genes SOX2 and c-MYC in cervical cancer

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Clock genes CLOCK, BMAL1/2, Differentiated embryonic chondrocyte gene (DEC)1/2, PER1/2/3 and CRY1/2 play important roles in the regulation of cell proliferation, apoptosis and epithelial to mesenchymal transition (EMT). We have shown that DEC1 is highly expressed in pancreatic cancer and oral cancer cells compared with non-tumor cells. However, the expression of clock genes in cervical cancer is not well understood. In this study, we focused on the expression of DEC1 in human cervical cancer tissues. As a result, DEC1 was highly expressed in tumor cells compared with adjacent non-tumor cells. Especially, the positive cells were observed in the front lesions of the tumors, suggesting that DEC1 is associated with invasion. In addition, we examined the expression of stem cell markers SOX2 and c-MYC in cervical cancer tissues. They were highly expressed in tumor cells compared with the adjacent non-tumor cells. Interestingly, DEC1 regulated the expression of SOX2 and c-MYC under cisplatin induced apoptosis. These results suggest that clock gene DEC1 may promote progression of cervical cancer, regulating SOX2 and c-MYC expression.

P-1231

IGF2R acts as a poor prognostic biomarker and promotes survival of cervical cancer cells

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Given that the number of both morbidity and mortality of cervical cancer is increasing in younger age and that clinically significant subtype has not been reported, the predicting biomarker or subtype discovery from transcriptome and clinical outcome data becomes much important in cervical cancer. Recently, we revealed that SIM2, which controls cell differentiation and angiogenesis, is a good prognostic biomarker for radiotherapy in squamous cell carcinomas. Also, it is very important for new drug development to investigate a poor prognostic biomarker and its function. We searched for a new prognostic biomarker from databases and found that Insulin-like growth factor 2 receptor (IGF2R) was aberrantly expressed in cancerous part and its high expression was correlated with worse prognosis. Although IGF2R has been reported to act as tumor suppressor in many kinds of cancers, IGF2R knockdown induced apoptosis and suppressed cell growth of cervical cancer cells via up-regulation of tumor suppressor. Taken together, our results show that IGF2R can be established as a new poor prognostic biomarker and its biological insight may lead to new therapeutic target discovery in cervical cancer.

P-1232

Proteomics reveals similar protein expression profiles of uterine cervical and lung small cell carcinoma

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Objective: The phenotypic and pathological features of small cell cervical carcinoma (SMCC) and small cell lung carcinoma (SCLC) are very similar, thus the chemotherapy regimens used for the rare SMCC have been routinely based on regimens used for common SCLC. However, protein expression profile similarities between these two cancers have yet to be unexplored. Methods: Protein expression analysis was performed for three SMCC, and one example each of SCLC, mucinous adenocarcinoma of the cervix (MACC), lung mucinous adenocarcinoma (MACL), and squamous cell carcinoma of the cervix (SCC). We used cancer-tissue-originated spheroids (CTOS) with isobaric tags for relative and absolute quantitation (iTRAQ)-based comprehensive and quantitative protein expression analysis. Expression in corresponding clinical samples was verified by immunohistochemistry. Results: Rather than organ of origin-specific patterns, the SMCC and SCLC samples revealed remarkably similar protein expression profiles. Conclusions: We demonstrate a overlapping similarity of protein expression profiles of lung and cervical small cell carcinomas, despite the differences in their organs of origin.

▲TOP

P-1233

Over expression of Carbonyl reductase 1 induces MET by suppressing TGF beta signaling in uterine leiomyosarcoma cells

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Background: Uterine leiomyosarcoma (ULMS) is one of high grade malignancy tumor. This study investigated whether carbonyl reductase1 (CBR1) induces mesenchymal epithelial transition (MET) and inhibits malignant behaviors of UMLS. Method: (1) We established the clone overexpressing CBR1 of ULMS cell line, SKN cells. Activities of cell proliferation, migration, and invasion were evaluated. Expressions of MET-related markers and TGF β production were analyzed. (2) To investigate whether suppression of TGF β signaling induces MET, SKN cells were treated with TGF β receptor blocker (SB431542). Result: (1) CBR1 overexpression suppressed the activities of cell proliferation, migration, and invasion, and TGF β production. Expressions of epithelial markers of MET were increased while mesenchymal markers were decreased by overexpression of CBR1. (2) SB431542 increased E-cadherin expression with the decrease in snail expression, indicating TGF β signaling suppresses MET. Conclusion: Overexpression of CBR1 induces MET by suppressing TGF β signaling, which may be involved in the inhibition of the malignant behavior in ULMS. This study provides a therapeutic strategy targeting CBR1 for ULMS.

P-1234

Clinical sequencing by Todai OncoPanel (TOP) for uterine sarcomas

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(Background) Uterine sarcomas are rare and heterogeneous, and pathological diagnosis and therapeutic strategies are not established. Molecular profiling is a fundamental component of cancer precision medicine, enabling the identification of genomic alterations for targeted therapy. However, their efficacies for sarcomas are controversial. (Methods) In the University of Tokyo Hospital, an original clinical sequence assay, named Todai OncoPanel (TOP), was launched in February 2017 under the approval of ethics committee. Nine cases of uterine sarcoma were enrolled. (Results) We obtained oncogenic gene information for 8 cases, and detected 3 homologous recombination deficiency (HRD) cases (somatic mutation of RAD51, homozygous deletion of BRCA2, and BRCA2 germ line variant), which may be effective to both platinum-based chemotherapy and PARP inhibitors. Two novel fusion genes were detected, which helped to amend the initial pathological diagnosis. (Conclusion) Clinical sequencing was useful for pathological diagnosis and treatment decision in certain patients with uterine sarcoma. HRD may be a promising therapeutic target for uterine sarcomas.

▲TOP

[P-1235] P14-15 [English/Japanese]
Molecular analysis of uterine cancer

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-1235

Comprehensive Sequencing Analyses of Uterine and Ovarian Carcinosarcoma

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Carcinosarcoma (CS) of the uterus or the ovary is a rare biphasic neoplasm composed of malignant epithelial and mesenchymal elements. Since CS has been considered as a derivative of adenocarcinoma, the treatment regimen follows to that for carcinoma counterparts. Nevertheless, CS exhibits more aggressive nature and poorer prognosis. To explore actionable targets for this aggressive disease, we performed targeted sequencing of CS with 596-gene panel in combination with methylome and transcriptome analyses. Genomic profiling identified four molecular subgroups: POLE-mutated (POLE), microsatellite instable (MSI), copy number high (CNH) and copy number low (CNL). Each subtype shows correlation with clinico-pathological and/or molecular markers; POLE and MSI subtypes were significantly correlated with better prognosis and PTEN inactivation, whereas CNH tumors were linked with poor outcomes, serous histology and TP53 mutation. Comparative genomic analyses of the carcinoma and sarcoma elements confirmed clonal origin with retention of driver events in each histology. This study would provide deep insights into the development of novel diagnostic and personalized treatment for CS patients.

▲TOP

P-1236

Clinical implications of microsatellite instability and HLA Class I downregulation in endometrial cancer

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Aims: Microsatellite instability (MSI) is the favorable prognostic factor in colorectal cancers and associated with downregulation of HLA class I molecules. MSI is a frequent phenomenon also in endometrial cancers (EC). In this study, we retrospectively investigated microsatellite status and level of HLA class I in 127 cases of EC. Methods: Cases were recruited from EC treated in our hospital from 2005 to 2009, and observed till December 2017. Lesions were stained immunohistochemically for MSH6/PMS2 (mismatch repair proteins) and EMR8-5, HLA Class I antibody. Expression of these molecules were statistically analyzed with clinical factors and prognosis. Results: MSI was detected in 33 cases and not correlated with HLA class I levels (p=0.55). MSI group showed significantly higher rates of myometrial invasion (p=0.016). Lower HLA class I was highly detected in cases with lymph-vascular space invasion (p=0.03). In multivariate analysis, MSI and lower HLA class I were not independent prognostic factors. Conclusions: Lower HLA class I and MSI did not impact for prognosis of EC unlikely to colorectal cancers. But these factors were associated with local invasion of EC.

P-1237

Myeloid-derived suppressor cells (MDSC) induce cancer stem cells (CSC) in G-CSF producing endometrial cancer

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[Objective] To investigate the role of MDSC in the induction of CSC in endometrial cancer. [Methods] CSC was defined as cells with high aldehyde dehydrogenase (ALDH) 1 activity. 1) To investigate the role of G-CSF from tumor, G-CSF-expressing and Mock-expressing endometrial cancer cell lines were subcutaneously inoculated into nude mice. The frequencies of MDSC and CSC were examined. 2) MDSC isolated from tumor-bearing mice were co-cultured with endometrial carcinoma cell line, then the frequencies of CSC were examined. 3) Tumor samples and clinical data from surgically-treated endometrial cancer cases were collected. Then, the relationship between the immunoreactivities for G-CSF, CD33, or ALDH1 in tumor, pretreatment white blood cell (WBC) counts, and their prognosis were examined. [Results] 1) Increased MDSC and CSC were observed in G-CSF-expressing endometrial cancer cell derived tumors. 2) MDSC induces CSC through the production of PGE2. 3) A positive correlation was demonstrated between the immunoreactivities for G-CSF, ALDH1, CD33 (MDSC marker), and pretreatment WBC count. [Conclusions] MDSC induced by tumor derived G-CSF promote CSC expansion via the production of PGE2.

P-1238

Elevated expression of PIM1 can be a poor prognostic indicator of endometrial serous carcinoma

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Background: PIM1 is a serine/threonine kinase involved in signal pathways such as JAK/STAT and reported as a poor prognostic factor of breast cancer. In this study, the expression of PIM1 and its prognostic significance in endometrial carcinoma (EC) were examined.Methods: Immunohistochemical expression of PIM1 in 133 cases of EC [103 endometrioid carcinomas (EECs, Grade1:62, Grade2: 21, Grade3: 20) and 30 serous carcinomas (ESCs)] surgically removed at Shinshu University Hospictal from 1997 to 2017 was evaluated using H-score (histo-score) and an analytical software, SPSS.Results: The expression of PIM1 was observed in the nucleus. H-score of PIM1 in ESC was significantly higher than that in EEC with Grade1 ($p < 0.001$) and Grade2 ($p = 0.031$). In ESCs, the cases with strong expression of PIM1 showed significantly shorter progression-free ($p = 0.003$) and overall survival ($p = 0.001$) than those with weak PIM1.Conclusions: The elevated expression of PIM1 can be a useful marker predicting a poor prognosis of the ESC patients.

▲TOP

P-1239

NOCTH signaling pathway in endometrial cancer stem cells

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Cancer stem cells (CSCs) possess the ability for self-renewal, differentiation and tumorigenesis, and play a role in cancer recurrence and metastasis. We sorted side population (SP) cells from a human endometrial cancer cell line using the FACSAria system and analyzed the biological properties of these cells. We reported that the SP cells exhibited self-renewal and higher tumorigenicity.
The NOTCH signaling pathway is an evolutionarily conserved pathway and plays an important role in cell fate determination, proliferation, differentiation and survival. Aberrant NOTCH signaling activity is highly implicated in several types of malignant tumor development. In the SP cells, NOTCH2, JAG1 and HES1 genes were expressed more highly than in the main population (MP) cells. Moreover, when the NOTCH signaling pathway was suppressed by NOTCH inhibitor DAPT, colony formation activities were suppressed in both SP and MP cells. While, KRAS transcription was decreased in SP cells. Thus, it was suggested that the NOTCH signaling pathway may participate in the regulation of KRAS expression in endometrial cancer stem cells.

▲TOP

[P-1246] P14-17 [English/Japanese]
Acute myelocytic leukemia

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-1246

A New Role for MEIS1 in the Immune Evasion of Myeloid Leukemic Cells

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In Acute Myeloid Leukemia, the transcription factor MEIS1 is critical for engraftment and propagation of HOXA9-transformed leukemic cells, notably through expression of Sytl1 and Syk. As reported, overexpression of Syk in HOXA9-transformed leukemic cells is sufficient to bypass MEIS1 absence, by restoring engraftment capacity and propagation of leukemic cells in irradiated mice.
However, in non-irradiated mice, we found that HOXA9/Syk-overexpressing cells are unable to propagate and die, demonstrating that restoring engraftment capacity is not sufficient for leukemia onset and suggesting that MEIS1 may control other critical functions.
Inoculation of HOXA9/Syk-overexpressing cells into non-irradiated immune deficient mice allowed these cells to expand to leukemia, demonstrating that these cells are normally eliminated by the immune system in non-irradiated wt mice. Therefore, MEIS1 confers the ability for leukemic cells to evade immune surveillance and protects AML cells from immune attack.
The discovery of MEIS1-target genes involved in this process may lead to the design of new strategies for AML therapy, aimed at annihilating escape of leukemic cells from immune attack.

▲TOP

P-1247

Trib1 functions as a critical epigenetic regulator in AML

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Trib1 pseudokinase acts as a collaborator of Hoxa9/Meis1 and its overexpression induces AML through enhancement of MAPK signaling and degradation of C/EBPa. To clarify Trib1's functional role as a cooperator of Hoxa9, we have generated Hoxa9-expressing leukemic cell lines with Trib1 overexpression or loss, $Trib1^{hi}$ and $Trib1^{null}$, respectively. Proliferation and cell cycling of leukemia were increased by Trib1 overexpression. Combination of ChIP-seq analyses and gene expression profiling identified novel Hoxa9 target genes whose expression was altered according to Trib1 expression. Moreover, we identified Trib1-induced modification of super-enhancer signals of certain genomic loci which are associated with Hoxa9-binding peaks. Among these loci, Erg and Ptgds, were identified as up-regulated genes by Trib1 overexpression. Modification of super-enhancer was abolished by deletion of the MEK1-binding motif of Trib1 as well as treatment with a MEK1 inhibitor U0126. These results indicate that the cooperative function of Trib1 with Hoxa9 is achieved by modulation of global transcriptional regulation by chromatin remodeling via MAPK signaling.

P-1248

Bcl11a promotes Trib1-induced myeloid leukemia development

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Bcl11a/Evi9 is a zinc-finger protein that is associated with B-cell development and malignancy, hemoglobin switching, and chromatin remodeling. Although Bcl11a was initially identified as a disease gene of murine acute myeloid leukemia (AML), Bcl11a's role in myeloid leukomogenesis remains unclear. Previous study identified that Bcl11a was a candidate cooperative gene that located near retroviral integration sites in Trib1-induced AML. In addition, Bcl11a promoted the development of Trib1-induced AML. To further analyze the cooperative activity between Trib1 and Bcl11a, we established Trib1-/Bcl11a-expressing leukemia cell lines. Bcl11a expression did not affect the leukemia phenotypes and had a moderate impact on cell proliferation in vitro, however, Bcl11a promoted leukemia engraftment into the bone marrow, accelerating leukemia development. These data strongly suggest that Bcl11a plays a crucial role in promoting leukomogenesis. We will present the mechanisms of transcriptional regulation by Bcl11a using gene expression profiling and ChIP-seq, and will uncover Bcl11a function in myeloid leukemogenesis.

P-1249

CX5461, a selective Polymerase I inhibitor, induces autophagy and suppresses the growth of leukemia cell lines

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Despite recent advances in therapeutic strategies of acute leukemia, the prognosis remains poor. Therefore, new effective treatments are needed. Increased transcription of ribosomal RNA genes by Polymerase I is a common feature of cancer. Thus, we examined the effect of CX5461, a selective Polymerase I inhibitor, for leukemia cell lines, HL-60, THP-1, KG-1. CX5461 exhibited growth inhibitory effect and induction of apoptosis. Because treatment of solid tumor with CX5461 has been reported to cause an autophagic cell death, we investigated the relationship between CX5461 and autophagy on leukemia cell lines. Analysis by western blotting revealed that using CX5461 cause an increase in autophagy related proteins. We then examined the effect of combination of Chloroquin (CQ), an autophagy inhibiter, and CX5461. Treatment of leukemia cell lines with CQ and CX5461 promoted apoptosis and growth suppression. Thus, we determined that autophagy induced by CX5461 contribute to the cell survival on leukemia cell lines. CX5461 may have a potential for a novel therapeutic approach to the treatment of leukemia.(Non-member contributor; Masumi Itadani, Chikage Kawai, Futoshi Kuribayashi)

▲TOP

P-1250

Involvement of impaired ribosome biogenesis in leukemogenesis

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The DDX41 gene, encoding a DEAD-box type ATP-dependent RNA helicase, has been shown to be mutated in a variety of myeloid diseases. The aim of this study was to clarify the roles of DDX41 in leukemogenesis. Transduction with mutant DDX41 in cord blood-derived CD34-positive cells significantly decreased cell proliferation compared with WT cells, which was accompanied by the negative enrichment of cell cycle-promoting genes regulated by the RB-E2F axis. In addition, data from cord blood cells and leukemia cell lines indicated that DDX41 mutation led to an improper ribosomal biogenesis thorough the impaired pre-ribosomal RNA processing, which produced free ribosomal proteins not incorporated into ribosomes. Although this mechanism would at least partially account for the slow growth rate of hematopoietic cells, how the mutation induces leukemia development remains unclear. To address this issue, currently a ribosome profiling assay using a leukemia cell line is underway. Understanding possibly altered global translation in DDX41 mutant cells using this assay would give insight into how dysregulated ribosome biogenesis affect leukemogenesis.

▲TOP

[P-1256] P14-19 [English/Japanese]
Malignant lymphoma

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-1256

Absolute peripheral CD4+ T-cell count predicts prognosis of patients with diffuse large B-cell lymphoma

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Absolute peripheral blood lymphocyte count at diagnosis is known to be a strong prognostic factor in patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP), but it remains unclear as to which peripheral blood lymphocyte population is reflective of DLBCL prognosis. In this cohort, 355 patients with DLBCL treated with R-CHOP from 2006 to 2013 were analyzed. The low absolute CD4+ T-cell count (ACD4C) at diagnosis negatively correlated with the overall response rate and the complete response rate significantly (P<0.00001). An ACD4C<343 × 106/l had a significant negative impact on the 5-year progression-free survival and the overall survival as compared with an ACD4C>=343 × 106/l (73.7% (95% confidence interval (CI)=66.7-79.5) versus 50.3% (95% CI=39.0-60.6), P<0.00001 and 83.3% (95% CI=77.1-88.0) versus 59.0% (95% CI=47.9-68.5), P<0.00000001, respectively). Multivariate analysis revealed that the ACD4C was an independent prognostic marker (hazard ratio=2.2 (95% CI=1.3-3.7), P<0.01). In conclusion, a low ACD4C at diagnosis served as an independent poor prognostic marker in patients with DLBCL.

▲TOP

P-1257

Ranolazine is a potential anti-tumor reagent against refractory cases in malignant lymphoma

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We've detected a protein Trifunctional Protein (=HADHA) which was frequently detected in high grade follicular lymphoma by shotgun proteomics, and HADHA is an independent prognostic factor in Diffuse large B-cell lymphoma (DLBCL). HADHA forms heterodimer with HADHB and they work as fatty acid beta oxidation enzymes, however, little is known about the relationship of the expression in both proteins. In immunohistochemistry, both HADHA and B tended to be overexpressed in high grade lymphoma subtypes. 68% (86/126) of HADHA and 65% (83/126) of B overexpressed in DLBCL and both overexpression of these protein correlated worse prognosis. We next used shHADHA knockdown system and shHADHA cells grew up less rapidly. Recent reports have shown that aberrant beta oxidation has been supportive in cancer cells. Furthermore, shHADHA cells were more susceptible to Ralonazine which was an inhibitor of fatty acid beta oxidation. Taken together, trifunctional proteins may be novel targets for anti-lymphoma therapy and Ranolazine may have a potent reagent for refractory malignant lymphoma.

P-1258

Antiproliferative effects of MYC/PLK1 inhibitions in a cell line derived from lymphoma with MYC/BCL6 rearrangements

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Double-hit lymphoma with MYC and BCL2 rearrangements that forms the great majority of DHLs is associated with inferior prognosis. However, there are far less data available for DHL with MYC and BCL6 rearrangements (MYC/BCL6 DHL). We established a novel MYC/BCL6 DHL cell line, designated DH6, which was genetically consistent with primary tumor cells. By utilizing this cell line, we evaluated the potential of MYC- and BCL6-targeted strategies and in combination with certain chemotherapeutic agents targeting molecules associated with cell proliferation. Treatments with a bromodomain and extra-terminal (BET) inhibitor (+)-JQ1, an inhibitor of the MYC transcription, efficiently inhibited cell growth of DH6 cells when combined with a PLK inhibitor. Furthermore, knockdown of PLK1 as well as BCL6 and MYC by gene-specific siRNA significantly suppressed cell proliferation. Specifically, reduction of PLK1 expression induced both apoptosis and cell cycle arrest in DH6 cells. These findings suggest that PLK1 may have a key role of cell survival for aggressive MYC/BCL6 DHL and that (+)-JQ1 in combination with the PLK inhibitor could be a useful therapeutic strategy for advanced MYC/BCL6 DHL.

P-1259

Evaluation of artesunate for the treatment of primary effusion lymphoma

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Primary effusion lymphoma (PEL) caused by KSHV is characterized by lymphomatous effusion in body cavities and poor prognosis. The anti-malaria compound artesunate (ART) reportedly has anti-cancer potential, and we aimed to test the effects of ART on KSHV-infected PEL cell lines. ART suppressed cell viability and proliferation of PEL cells, while the effect of ART on PBMCs was less pronounced. ART arrested cell cycle in G1 or G2/M phase by inhibiting cyclin D1/D2 and Cdk2/6. ART also increased apoptosis by the activation of caspase-3/8/9. It did not reactivate KSHV lytic genes. ART increased intracellular ROS and the DNA damage marker γ-H2AX. ART-induced cytotoxicity was partly decreased by pretreatment with a pan-caspase inhibitor, a ROS scavenger or a necroptosis inhibitor, suggesting the involvement of both caspase-dependent and caspase-independent pathways of killing. ART induced downregulation of Bcl-2, Bcl-xL, survivin, XIAP and c-IAP1/2, as well as upregulation of apoptotic Bak. Furthermore, ART suppressed NF-κB and AP-1 through inhibition of phosphorylation of IκBα and expression of JunB and JunD. Our findings indicate that ART is a potential drug for treatment of PEL.

▲TOP

P-1260

Clinicopathological analysis of breast lymphoma

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<Background>Lymphomas of the breast are rare. The distinction of lymphoma from breast cancer is important, because the treatment and prognosis for these two lesions are different. The aims of this study were to investigate the clinicopathological characteristics of cases with breast lymphoma.<Methods>Between 2011 and 2018, we retrospectively analyzed the data of 6 cases of breast lymphoma. <Results>Of the 6 patients, 5 were women with an average age of 56 years (range 50-68 years). All the patients were B-cell lymphoma. Five cases were primary breast lymphoma. An elevated LDH level was observed in 4 patients. Two patients that had large sized tumor were high soluble interleukin-2 receptor levels. Ultrasound image showed hypoechoic mass that was well-circumscribed, and elastoscore was low. The type of primary breast DLBCL was determined by immunohistochemistry. Three cases were GCB and 1 case was non-GCB by Hans classifier. All patients were alive at the point of searching.<Conclusion>We described the cases of breast lymphoma. Breast lymphoma having a good prognosis should be correctly diagnosed.

▲TOP

[P-1267] P14-21 [English/Japanese]
Head and neck cancer (1)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Yohei Miyagi / Kanagawa Ca Ctr Res Inst

P-1267

The roles of macrophages in the early oral carcinogenesis

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Macrophages (Mφs) are the most abundant cancer stromal cells. Previous studies suggested that Mφs are involved in the progression and development of oral squamous cell carcinomas. However, the role of Mφs in the early oral carcinogenesis remain unclear. Here, we investigated the significance of Mφs in oral leukoplakia, the most common oral potentially malignant disorders. We counted $CD163^+$, $CD204^+$ or $CD206^+$ Mφs by immunohistochemistry in 24 cases of surgically resected tongue leukoplakia and compared them with the clinicopathological factors. The number of $CD163^+$ Mφs showed a significant positive correlation with the degree of atypia, Ki-67 abnormal expression and cytokeratin 13 loss in tongue leukoplakia. Conditioned media of CD163-expressing human acute monocytic leukemia cell line THP-1 demonstrated increased mRNA expression of immune suppressive molecules such as interleukin-10, programmed cell death 1 ligand 1 and programmed cell death 1 ligand 2 in human oral keratinocytes. These results suggest that $CD163^+$ Mφs might play roles in early oral carcinogenesis by the immunosuppression in oral epithelial cells.

▲TOP

P-1268

The role of YAP expression in oral squamous cell carcinoma

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Yes-associated protein (YAP), serve as key mediators of the Hippo pathway that regulates organ size, cell proliferation and cell fate determination. Although YAP overexpression has been reported in various cancers, the role of YAP of invasive front of oral cancer has not been fully elucidated. We performed immunohistochemical analysis of YAP expression and localization in 70 oral squamous cell carcinoma (OSCC) cases. Well differentiated and moderately differentiated type had tendency to have low expression and localize in the cytoplasm, while poorly differentiated type had tendency to have high expression and localize in both nucleus and cytoplasm. Cases showing infiltrative invasion pattern (Yamamoto Kohama classification-4C and -4D) at invasive front had also tendency to have high expression of YAP and localize in the nucleus and cytoplasm. Further, our preliminary analysis showed that YAP localization, both nuclear and cytoplasmic, had tendency to correlate with E-cadherin loss and high slug expression. These findings suggest that YAP may play a role in regulating tumor aggressiveness of OSCC through EMT at the invasive front.

P-1269

Expression of cytosolic malic enzyme (ME1) is associated with disease progression in human oral squamous cell carcinoma

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Malic enzyme 1 (ME1) is a multifunctional protein involved in glycolysis, the citric acid cycle, NADPH production, glutamine metabolism, and lipogenesis. It is overexpressed in various cancers. We examined the expression of ME1 in 119 oral squamous cell carcinomas (OSCCs) via immunohistochemistry. ME1 expression was moderate to strong expression in 57 (48%) OSCCs and correlated with pT, pN, clinical stage, and histological grade. In 37 cases with prognostic evaluation, moderate to strong ME1 expression indicated a worse prognosis than did weak ME1 expression. ME1 knockdown or inactivation by lanthanide inhibited cell proliferation and motility and suppressed the epithelial-mesenchymal transition in HSC3 human OSCC cells. ME1 knockdown also shifted energy metabolism from aerobic glycolysis and lactate fermentation to mitochondrial oxidative phosphorylation, and the redox status from reductive to oxidative. In a mouse tumor model, lanthanide administration suppressed tumor growth and increased survival time. These findings seem that ME1 is a valid target for molecular therapy in OSCC.

P-1270

Therapeutic potential of targeting BDNF/TRKB signaling in poorly differentiated oral squamous cell carcinomas

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BDNF and the receptor, TRKB tyrosine kinase receptor, are both overexpressed in some types of cancers and has reported to promote cancer progression. However, the role of TRKB in patients with cancers is not fully elucidated. Here, we analyzed the correlation between the expression levels of TRKB and/or BDNF, and clinicopathological characteristics including tumor differentiation, tissue invasion, and disease-free survival in oral squamous cell carcinoma (OSCC) patients. The expression levels of TRKB/BDNF, were significantly higher (TRKB/BDNF^{high}) in moderately/poorly-differentiated (MD/PD) OSCCs than in well-differentiated (WD) OSCCs. Moreover, the TRKB/BDNF^{high} OSCC showed poor disease-free survival. In an orthotopic transplantation mouse model of human OSCC cell lines (PD-OSCC and WD-OSCC), administration of a TRKB-specific inhibitor, ANA-12, significantly suppressed the tumor growth in TRKB/BDNF^{high} PD-OSCC but not in WD-OSCC. In vitro cell culture assays, ANA-12 also inhibited BDNF-induced cell migration and invasion in PD-OSCC but not in WD-OSCC. These data suggest that BDNF/TRKB signaling could be a potential therapeutic target for OSCC, especially for PD-OSCC.

▲TOP

P-1271

Association of PD-1, PD-L1 and PD-L2 expression with clinicopathological factors in tongue squamous cell carcinoma

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[Background] The immune checkpoint pathway through PD-1/PD-L1/PD-L2 molecules play a critical role in immune escape mechanisms of cancer. The clinical application of the immune checkpoint inhibitor using anti-PD-1 antibody and the anti-PD-L1 antibody has been pushed forward in a many types of cancer, and it can also be utilized in oral cancer. It is well-known that PD-L1 is a predictive biomarker for response in cancer immunotherapy in various cancer types, but there are not many studies on PD-L2.[Purpose] We clarify the association between expression of PD-1/PD-L1/PD-L2 and clinicopathologic factors in tongue squamous cell carcinoma(TSCC).[Method] We performed immunohistochemical staining of TSCC tissues in primary cases surgically operated in our department between 2005 and 2016 using anti-PD-1 mAb, antiPD-L1 mAb, antiPD-L2 mAb.【Result and Discussion】Expression of PD-1/PD-L1/ PD-L2 was statistically associated with poor clinical outcomes in TSCC, suggesting that not only PD-L1 also PD-L2 expression analysis could provide beneficial information in cancer immunotherapy against TSCC.

P-1272

Antitumor effect of focal adhesion kinase inhibitor in head and neck squamous cell carcinoma

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Background: Focal adhesion kinase(FAK) is a nonreceptor type tyrosine kinase, which is involved in tumor cell proliferation and infiltration and is highly expressed in multiple solid cancers. There is a report that high expression was seen in tumors of patients with head and neck squamous cell carcinoma(HNSCC). In this study, the expression of FAK in HNSCC cells and the antitumor effect of FAK inhibitor were investigated. Materials and Methods:Expression of FAK in HNSCC cells was examined by immunostaining. Anticancer effect of FAK inhibitor on HNSCC cells was evaluated by growth inhibition assay, cell cycle assay and apoptosis assay. Results: High expression of FAK was observed in HNSCC cells. The FAK inhibitor showed antitumor effect in a concentration dependent manner on HNSCC cells. In FACS analysis, increase of SubG1 and induction of apoptosis were observed. In immunostaining as well, the induction of apoptosis by FAK inhibitors is confirmed. Conclusion: The FAK inhibitor is useful as an antitumor drug in HNSCC by inhibiting proliferation of HNSCC cells through novel mechanism.

▲TOP

[P-1279] P14-23 [English/Japanese]
Head and neck cancer (3)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Nobuhiko Oridate / Dept. Otolaryngology-Head & Neck Surg., Yokohama City Univ, SchMed

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P-1279

Multiple coagulation factor deficiency protein 2 as a crucial component in metastasis of human oral cancer

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Multiple coagulation factor deficiency protein 2 (MCFD2), a binding partner of lectin mannose binding 1 (LMAN1), causes combined deficiencies of coagulation factors V and VIII. The aim of the current study was to investigate the states of MCFD2 in oral squamous cell carcinoma (OSCC). The expression of MCFD2 was up-regulated significantly in all cell lines examined. Evaluation of the cellular functions associated with tumoral metastasis showed that MCFD2 knockdown (shMCFD2) cells exhibited significantly lower cellular invasiveness and migration and higher cellular adhesion compared with shControl cells. Of note, shMCFD2 cells also showed weak immunoreactivity of LMAN1 and a lower secretion level ofgalactoside-binding soluble 3 binding protein (LGALS3BP). In addition to in vitro validation, clinical data on 70 patients with OSCC indicated that state of MCFD2 expression level is associated with regional lymph node metastasis. Altogether, we have demonstrated that MCFD2 promotes cancer metastasis by regulating LMAN1 and LGALS3BP expression levels. Hence, MCFD2 may represent a promising candidate for a novel therapeutic target for patients with metastatic OSCCs.

▲TOP

P-1280

Clinical implications of podoplanin and plasma soluble podoplanin in early-stage oral squamous cell carcinoma

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Background: Podoplanin (PDPN) is expressed on many malignancies and is involved in subsequent metastasis. Recently, it has been reported that the soluble podoplanin (sPDPN) in plasma is a novel marker for the diagnosis of tumor occurrence and metastasis. However, the predictive values of PDPN and sPDPN have not been fully elucidated in early-stage oral squamous cell carcinoma (ESOSCC).Methods: The biopsy or resected specimens obtained from patients with ESOSCC were used for immunohistochemical analysis for evaluating PDPN expression. In addition, we collected pre- and post-operative plasmas from patients and analyzed the concentration of sPDPN by using ELISA.Results: The highly expression of PDPN in tissues was associated with tumor invasion and subsequent metastasis. The plasma sPDPN level of the post-operative group was lower than that of the pre-treatment group. Moreover, the sPDPN level of the subsequent metastasis group was higher than that of the non-subsequent metastasis group.Conclusion: These results suggest that PDPN and sPDPN could be related with tumor invasion and subsequent metastasis, and could be a potential predictive marker of subsequent metastasis in ESOSCC.

P-1281

Clinical significance of serum p53 antibody in oral squamous cell carcinoma

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Background: Serum antibodies directed against p53 protein (S-p53-Abs) have been detected in some patients with malignant tumors. But, the clinical value of S-p53-Abs has not been fully elucidated. The aim of this study is to investigate the clinical significance of S-p53-Abs in patients with OSCC. Methods: S-p53-Abs concentrations were determined by an enzyme-linked immunosorbent assay (ELISA) before and after surgery in patients with OSCC. The relationships between S-p53-Abs level and clinicopathological variables were examined. Results: Among a total of 50 primary OSCC cases, 35% were positive for s-p53-Abs. The majority of positive cases were patients with advanced OSCC. S-p53 Abs titers varied from 0.36-8.7 (mean, 3.09) in preoperative samples and from 0.23-6.19 (mean, 1.65) U/mL in postoperative samples, respectively. Among patients positive for S-p53 Abs before surgery, the S-p53 Abs levels were reduced after surgery in most cases. But, in patients with Stage 3-4 OSCC, the antibody titer showed a higher positive rate than the other cases both before and after surgery. Conclusions: These results suggest that S-p53 Abs could be a useful biomarker in patients with advanced OSCC.

P-1282

Screening for long noncoding RNAs associated with oral cancer reveals the potentially oncogenic actions of lnc-A

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In the present study, we identified lncRNAs functionally associated with OSCC. By analyzing RNA-seq datasets obtained from primary head and neck squamous cell carcinoma (HNSCC), we identified 15 lncRNAs aberrantly expressed in cancer tissues. We then validated their expression in 18 OSCC cell lines using qRT-PCR and identified 6 lncRNAs frequently overexpressed in OSCC. Among those, we found that knocking down lnc-A strongly suppressed OSCC cell proliferation. Lnc-A knockdown also suppressed migration, invasion and xenograft formation by OSCC cells, which is suggestive of its oncogenic functionality. Microarray analysis revealed that lnc-A knockdown significantly affects expression of a number of cancer-related genes in OSCC cells, including HAS3, CD44 and TP63, suggesting that lnc-A regulates HA-CD44 signaling. Expression of lnc-A was elevated in 71% of primary OSCC tissues, and high lnc-A expression was associated with shorter overall survival of HNSCC patients. These data suggest that elevated lnc-A expression contributes to OSCC development, and that lnc-A may be a useful therapeutic target in OSCC.

▲TOP

P-1283

The role of collagen IV in progression of tongue cancers: Examination by using a new 3D cell culture system

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Collagen IV has been reported to play an important role in angiogenesis and tumor invasion during carcinogenesis. We investigated the role of collagen IV in progression of tongue cancer, by using a new three-dimensional cell culture system; Cellbed. Cellbed is made of nonwoven fabric sheets of silica glass fibers. Tongue cancer cell lines (HSC-3, HSC-4, SCC-15) were cultured for approximately 3 weeks by using Cellbed coated with Cellmatrix IV and non-coated Cellbed. The horizontal and vertical sections of the Cellbed were observed using HE staining and the width of infiltration of cancer cells in the vertical section was measured. Comparison of cell-proliferative activity was carried out using MTT assay. HE staining confirmed the presence of squamous cell carcinoma-specific formation of a laminar structure. Cancer cells cultured using coated Cellbed showed a greater width of infiltration than using non-coated Cellbed. However, cell proliferative activity was no significant difference by MTT assay. Our findings revealed that coating Cellbed with collagen IV increased the infiltration capacity of cancer cells, and suggested that collagen IV might be involved in tumor development.

P-1284

Overexpression of AIM2 enhances the invasion of OSCC cells by inducing EMT via activation of the TGF-β/Smad3 pathway

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The interferon-inducible gene AIM2 is overexpressed in oral squamous cell carcinoma and associated with the growth of OSCC. Since the expression of AIM2 is highly up-regulated in OSCC with lymph node metastasis compared with non-metastatic OSCC, we investigated whether AIM2 is involved in metastastic potential of OSCC. In the OSCC cell line HSQ89 with low AIM2 expression, ectopic expression of AIM2 enhanced the cell migration and invasion capacity and conversely, knocking-down AIM2 in SAS/OSCC cell line with high AIM2 expression reduced the cell migration and invasion. Importantly, the expression of AIM2 enhanced the lymph nodes metastasis of OSCC which was transplanted in the tongue of NOG mice. The enhancement of the migration capability by AIM2 was accompanied by the induction of EMT, as indicated by cell scattering with increased expression of EMT-related genes. Moreover, we found that AIM2 interacts with the TGF-β activated kinase 1 (TAB1)/Smad3 complex and enhances the phosphorylation of Smad3 to activate the TGF-β signaling. Collectively these results suggest that overexpression of AIM2 contributes to the invasion of OSCC cells through the activation of TGF-β pathway.

▲TOP

[P-1240] P14-16 [English/Japanese]
Molecular and cellular characteristics of ovarian cancer

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-1240

Inhibition of BET bromodomains reduces growth and invasive characteristics of chemoresistant ovarian carcinoma cells

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Epithelial ovarian cancer (EOC) is the most lethal gynaecological malignancy worldwide. Development of chemoresistance and peritoneal dissemination are the major reasons for low survival rate. The BET proteins are epigenetic "readers" and their inhibitors are novel epigenetic strategies for cancer treatment. Epigenetic modifications have critical roles in the development of EOC and overexpression of the BET family is a key step in induction of important oncogenes. Here, we examined the mechanistic activity of I-BET151, a pan-inhibitor of the BET family, in therapy-resistant EOC cells. Our findings showed that I-BET151 diminished cell growth, clonogenic potential and induced apoptosis. I-BET151 inhibited cell proliferation through down-modulation of FOXM1 and its targets Aurora kinase B and cyclin B1. I-BET151 attenuated migration and invasion of the EOC cells via down-regulation of EMT markers fibronectin, ZEB2, and N-cadherin. I-BET151 synergistically enhanced cisplatin chemosensitivity via down-regulation of survivin and Bcl-2. Our data suggest that BET inhibition has potential as a therapeutic strategy in therapy-resistant EOC.

▲TOP

P-1241

MITF contributes to cell migration/invasion in ovarian carcinoma cells

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MITF (microphthalmia-associated transcription factor) represents a melanocytic lineage-specific transcription factor whose role is profoundly extended in malignant melanoma, and its level is associated with increased cell migration, metastasis and poor prognosis. Here we investigated whether MITF expression and its roles in ovarian carcinoma cells (OCCs). Several ovarian carcinoma cell lines expressed MITF and these cell lines had high malignancy in mouse xenograft model. We analyzed whether MITF contributes to migration/invasion using conventional transwell assays. After transfection with MITF specific siRNA, these cells showed significant reduction in migration/invasion properties. In addition, we found that MITF positive OCCs expressed CD146, which is also known as melanoma cell adhesion molecule and was initially identified as a marker of melanoma progression and metastasis. Interestingly, the expression of CD146 was decreased after transfection with MITF specific siRNA. Our findings show that MITF may play an important role for cell migration/invasion through CD146 in OCCs and that MITF can be a potential therapeutic target in ovarian cancer.

P-1242

Subcellular localization of MCM2 correlates with the prognosis of ovarian clear cell carcinoma

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Highly malignant tumors overexpress the minichromosome maintenance 2 (MCM2) protein in the nucleus, and this is associated with an advanced tumor grade, advanced stage, and poor prognosis. In this study, we show that MCM2 is highly expressed in clinical samples of ovarian clear cell carcinoma. Although MCM2 expression was localized to the nuclei in a majority of cases as in other cancers, a fraction of the cases exhibited cytoplasmic localization of MCM2. Surprisingly, tumor samples with cytoplasmic MCM2 demonstrated excellent prognosis, showing 100% survival during the observation period of more than 200 months. However, cases with nuclear expression of MCM2 exhibited an approximately 78% 5-year-survival rate. In clinicopathologic analysis, the cases with cytoplasmic MCM2 showed independent prognostic predictor in clear cell carcinoma. We are analyzing the molecular detail of cytoplasmic MCM2 expression in ovarian cancer cells using in vitro system.

P-1243

Expression of estrogen receptor subtypes in clear cell carcinoma and high- grade serous carcinoma of the ovary

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[Background] Estrogen receptor beta (ER- β) and G protein-coupled estrogen receptor-1 (GPER-1) were genetically different from ER- α, and the expressions in clear cell carcinoma of the ovary (CCC) have not yet been clarified. [Materials and Methods] FFPE tissues were collected from 41 patients with CCC and 44 with high grade serous carcinoma of the ovary (HGSC), treated at Tottori University hospital between 2006 and 2014. Samples were subjected to immunohistochemistry for expression of ER- α, ER- β, and GPER-1. The association of these expressions with histologic subtype and survival were evaluated.[Results] ER- α expression was more frequent in HGSC than CCC (76% vs. 3.7%, P<0.0001). Positivity of ER- β expression was higher in CCC than HGSC (94% vs. 80%, P=0.02). GPER-1 expression was comparable between CCC and HGSC. Patients with GPER-1-negative HGSC showed significant shorter overall survival than positive cases (P=0.004) while there was no difference in CCC. Neither ER- α nor ER- β expression was associated with survival in CCC and HGSC.[Conclusions] ER- β expression was more frequent in CCC than HGSC. Negative GPER-1 expression was associated with worse prognosis in HGSC.

▲TOP

P-1244

The HNF-1beta-USP28-CLASPIN is the important pathway to upregulate DNA damage induced Chk1 phosphorylation in OCCC

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Objective:Transcription factor hepatocyte nuclear factor (HNF)-1β enhances checkpoint kinase 1 (Chk1) activation and accumulation of G2/M cell cycle phase in ovarian clear cell carcinoma (OCCC). However, the underlying mechanism still remains largely unknown.Material and Methods：SiRNAs targeting HNF-1β, CLASPIN and USP28 were transfected to TOV-21G(OCCC). Ubiquitination and stabilization of CLASPIN protein by HNF-1β was assessed by immunoprecipitation. Cell viability were detected by MTT assay after transfection.Results:Loss-of-function studies indicated that HNF-1β facilitated the CLASPIN expression after a genotoxic agent bleomycin, resulting in accumulation of phosphorylated Chk1 (p-Chk1). Because HNF-1β has no effect on expression of CLASPIN mRNA, we find that USP28, a de-ubiquitinase crucial for CLASPIN expression, is one target gene of HNF-1β. Knockdown of endogenous USP28 suppressed the CLASPIN and p-Chk1 expression and cell proliferation. Discussion:Our findings identify a novel pathway of the HNF-1β-USP28-CLASPIN-Chk1 axis in checkpoint signal amplification in response to DNA damage. Targeting this pathway may be a novel, anticancer strategy in OCCC.

P-1245

Novel therapeutic strategies for ovarian cancer: iPS cell-derived myelomonocytic cells producing interferon-β

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The microenvironment of disseminated ovarian cancer is composed of fibroblast, adipocyte, and immune cells including macrophage (Mφ), the most abundant cell type. Mφs are differentiated from myelomonocytic cells and also can exert diverse functions from anti-tumor (M1) to pro-tumor (M2) effects. We focused on the cell-to-cell interactions between Mφs and ovarian cancer cells. This study aimed to evaluate the therapeutic effect of induced pluripotent stem (iPS) cell-derived myelomonocytic cells producing interferon-β (iPS-ML/IFN-β) towards disseminated ovarian cancer. iPS-ML/IFN-β was confirmed to express Mφ marker such as CD68. We developed an orthotopic xenograft model of intraperitoneal ovarian cancer by injecting SKOV3 human ovarian cancer cells. After confirmation of the engraftment of the cancer, iPS-ML/IFN-β was administered by intraperitoneal injection. Consequently, iPS-ML/IFN-β not only inhibited the growth of disseminated ovarian cancer cells, but also suppressed the ascites accumulation. This study suggests the therapeutic potential of the iPS-ML/IFN-β in patients with advanced ovarian cancer.

▲TOP

[P-1251] P14-18 [English/Japanese]
CML and post-transplant leukemia / lymphoma

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Yoshikane Kikushige / 1st Dept. Int. Med. Kyushu Univ.

P-1251

Antitumor effects of IL-27 against chronic myeloid leukemia in a mouse model

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We here investigated whether IL-27, one of the antitumorigenic cytokines with ability to act on hematopoietic stem cells, would exert antitumor effect against hematologic tumors such as CML, or IL-27 would rather augment their growth by promoting expansion and differentiation of the leukemic stem cells as in the case of HSCs. We used a mouse CML model, which was established with retroviral transduction of *BCR/ABL-GFP* followed by transplantation. First, the bone marrow LSK cells of deficient mice for WSX-1, one of the IL-27 receptor subunits, were transduced with *BCR/ABL-GFP*, and then transferred to irradiated WT mice. Similarly, the role of IL-27 in the CML was also examined using IL-27-transgenic mice and deficient mice for EBI3, one of the IL-27 subunits. Collectively, the present results suggest that IL-27 shows antitumor activity rather than protumor activity towards CML. We presume that IL-27 exerts the antitumor activity by directly killing leukemic stem cells or augmenting the generation of CTLs against *BCR/ABL*, whose possibilities are currently under investigation. Thus, therapeutic applications of IL-27 targeting hematologic tumors could be beneficial as well.

▲TOP

P-1252

Biological significance of nascent BCR-ABL revealed by modeling translocation (9;22) using CRISPR/Cas9 system

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BCR-ABL fusion protein caused by t(9;22) plays the central role in pathogenesis of CML and its expression level is a critical determinant for the disease progression. Although BCR-ABL has also been detected in healthy subjects, its significance remains to be solved. In order to investigate the role of endogenous formation of BCR-ABL itself, we took advantage of CRISPR/Cas9-mediated genome editing in this study. Guide RNAs (gRNAs) were designed to target frequent break points in BCR and ABL genes. A human GM-CSF-dependent leukemic cell line, UT7 was transiently transduced with vectors expressing Cas9 and gRNAs. We obtained 17 $p210^+$ clones and 16 $p190^+$ clones.
While mRNA level of BCR did not differ among the cell groups, the transcripts of ABL (exon 4-5) were expressed at slightly but significantly higher level in $p210^+$ and $p190^+$ cells when compared to control cells. $p210^+$ and $p190^+$ cells showed mildly enhanced proliferation only at lower GM-CSF concentration and survived even in the absence of GM-CSF.
These data suggest that nascent BCR-ABL provide cells with survival and growth advantage and this might be the basis for further accumulation of mutations which cause leukemia onset.

P-1253

Combination therapeutic strategy with tyrosine kinase inhibitors targeting energy metabolic alteration in leukemia cells

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[Purpose] Cancer cells obtain most of their energy from glycolysis. We previously reported that low-concentrated imatinib (IM) alters energy metabolism in chronic myelogenous leukemia (CML) cells with maintaining their survival, and enhances the anti-cancer effect of tyrosine kinase inhibitors (TKIs). Here, we examined whether the anti-tumor effect of TKIs is enhanced by combination of energy disruptors in vitro and vivo. [Methods] We evaluated activity of intracellular signaling related to energy metabolism (S6K1 and AMPK) in CML cells exposed to low-concentrated IM. . Next, we expose rapamycin (a mTORC1 inhibitor) and metformin (an AMPK activator) to CML to disrupt these signaling, and examined whether the anti-cancer effect of TKIs is enhanced. Finally, we established Ba/F3 cells that constantly expresses BCR/ABL protein and luciferase, and injected it in the mice to evaluate the utility of the combination strategy by in vivo imaging system. [Results/Discussion] Using drugs altering intracellular energy metabolism enhanced anti-cancer effect of TKIs against CML in vitro and vivo. Here, we propose energy disruptors plus TKIs is a useful therapeutic strategy for CML treatment.

P-1254

Pathogenic role of leukemia cell-derived extracellular vesicles in donor cell-derived leukemia after BM transplantation

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BM transplantation (BMT) is a curative treatment strategy against hematologic malignancies including chronic myeloid leukemia (CML). However, some patients develop donor cell-derived leukemia (DCL), such as secondary leukemia or myelodysplastic syndrome (MDS), originating from malignant transformation in donor cells. The lack of an experimental model is an obstacle to the elucidation on the pathophysiological mechanism of this weird disease. We observed that donor-derived MDS-like pathology developed when CML-bearing mice were transplanted with congenic healthy BM cells following sublethal irradiation. In some recipient mice, donor-derived cells harbored recipient cell-derived BCR-ABL gene and expressed its protein product. Moreover, leukemia cell-derived extracellular vesicles (EVs) abundantly contained BCR-ABL gene as well as CML cell-derived double-stranded DNA fragments. Thus, we established DCL model, which can enable the elucidation of its pathophysiological mechanism, especially the involvement of EV-mediated horizontal transfer of malignant phenotypes.

▲TOP

P-1255

Clinicopathological analysis of LPD that developed in patients with RA receiving calcineurin inhibitors

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Post-transplantation lymphoproliferative disorder （LPD）develops in patients receiving calcineurin inhibitors such as tacrolimus (TAC). Currently, approximately 10% of patients with rheumatoid arthritis (RA) receive TAC. However, it is unclear whether TAC relates to LPD development like methotrexate (MTX)-associated LPD. We analyzed 70 LPD patients with RA who were administered TAC and who developed LPD and compared the clinicopathological characteristics of patients to those with MTX-LPD. The median age at LPD onset was 70 years, and the male:female ratio was 1:2.7. Approximately 50% patients were diagnosed with diffuse large B-cell lymphoma: nine with Hodgkin lymphoma and eight with polymorphic LPD. The EBER-1 positivity rate was 56% and 27% of TAC-LPD had developed LPD within one year of initiating TAC. The 5-year survival rate in TAC-LPD (79.9%) was significantly worse than that in MTX-LPD (91.1%). Multivariate analysis revealed TAC was an unfavorable prognostic factor. With the exception of earlier development and unfavorable prognosis, TAC-LPD showed similar clinicopathological characteristics to MTX-LPD.

▲TOP

[P-1261] P14-20 [English/Japanese]
Adult T-cell leukemia / lymphoma and multiple myeloma

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-1261

The roles of HGF/c-Met pathway in adult T-cell leukemia/lymphoma

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Adult T-cell leukemia/lymphoma (ATL) cells infiltrate into different systemic organs such as liver, lymph nodes, peripheral blood, bone marrow, and skin. ATL cells in lymph node lesion (LN, LN-ATL) tends to be resistant to drug treatment compared to those in peripheral blood (PB-ATL). The aim of this study is to clarify the molecular differences underlying between LN-ATL and PB-ATL. Using three PB-ATL cell lines (MT-1, TL-Om1 and ATN-1) and one LN-ATL cell line (HUT102), we found that HUT102 showed higher expression of HGF compared to PB-ATLs. Downstream of HGF/c-Met pathway was constitutively active, together with high proliferation and invasion activity in HUT102. Intriguingly, high level of HGF was closely associated with aberrant histone acetylation in HGF regulatory region. Treatment of JQ1, an inhibitor of histone acetylation reader proteins, led to an attenuation of HGF-mediated proliferation and invasion in HUT102. Our data indicate that epigenetic regulation of HGF expression might be a key mechanism for invasive LN-ATL and that targeting HGF/c-Met axis may be beneficial for treatment of this type of ATL.

▲TOP

P-1262

A case of refractory CTCL remitted by treatment based on artificial intelligence analysis of whole exome sequencing data

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A 74-year-old Indonesian gentleman was diagnosed as Sezary syndrome (T4N3M0B1b) in 2015. Although he received many treatments world widely between 2015 and 2016, the clinical outcome was disappointing.He was admitted to our hospital because of generalized erythroderma (Stage 3A) in Nov. 2016. He received Mogamulizumab and then achieved partial response (PR) as Stage 1A on Dec. 27, 2016. However, new lesions appeared (Stage2B) in Jan. 2017. After obtaining the informed consent, the whole exome sequencing of the biopsied skin tumor was done followed by analysis using AI of Watson. According to the results of AI analysis, lenalidomide was recommended among the candidate drugs. After obtaining the approval by our institutional ethical committee in Aug. 2017, he was started with lenalidomide and almost all of his skin tumors except for the one at the sternal region disappeared. As the sternal tumor grew bigger gradually, the tumor was irradiated with 20Gy in Dec. 2017. The 5th course of lenalidomide was completed on Feb 3, 2018 and the tumor disappeared completely. We recognized the clinical usefulness of analyzing whole exome sequencing data of patients' tumor using AI of Watson.

P-1263

Circulating serum microRNAs as a minimally invasive biomarkers for treatment response and prognoisis in multiple myeloma

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Serum of multiple myeloma (MM) patient contains sufficiently stable miRNAs, which can be valuable markers for patient management. In this study, through miRNA array analysis, we identified six microRNAs (miR-26a-5p, miR-29c-3p, miR-30b-5p, miR-30c-5p, miR-193a-5p and miR-331-3p) associated with treatment outcome and prognosis in relapsed/refractory MM (RRMM) patients receiving lenalidomide plus low-dose dexamethasone (Len-dex) treatment. Of note, lower expression of the six miRNAs was also significantly associated with shorter time to progression or poorer overall survival. For reliable prediction of treatment outcome, we developed a Len-dex treatment response prediction (LdTRP) model by combining miRNA markers and clinical factors. The LdTRP model showed much improved stratification power (AUC=0.933: sensitivity 90.0%, specificity 77.8% and accuracy 85.4%) compared with SVMs composed of either clinical variables only (AUC=0.670) or miRNAs only (AUC=0.675). Our results suggest the potential of circulating miRNAs as minimally invasive markers for treatment response and prognosis in RRMM patients, which may replace traditional invasive tumor cell examination by bone marrow biopsy.

P-1264

Platelets enhance Multiple Myeloma progression via IL-1beta upregulation

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The interplay between tumor cells and their microenvironment has been known to play a critical role in tumor malignancy. Platelets have been increasingly recognized as essential for promoting tumor growth and metastasis; however, these reports have been limited to solid tumors and the importance of platelets in supporting hematologic malignancies is unknown. Here, we show that multiple myeloma (MM) cells activate platelets as evidenced by increased P-selectin on the platelet surface as well as relevant pathways that were enriched via RNA sequencing of platelets from MM patients. The activated platelets promote MM cell proliferation in vitro and tumor engraftment in vivo mice models. RNA sequencing revealed that IL-1β was upregulated in MM cell lines co-cultured with platelets, whereas IL-1β knockout in MM cell lines abrogated the effects of platelets on MM cell proliferation and engraftment in vivo. Thus, our results reveal that platelet-mediated upregulation of IL-1β is important in disease progression and may represent a novel target for therapeutic intervention.
Collaborator: Irene M. Ghobrial, MD.

▲TOP

P-1265

Involvement of PDZ binding kinase in tumor growth in multiple myeloma cells

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Multiple myeloma <MM> is an incurable hematological malignancy, despite recent development of novel molecular-targeted drugs. Elevated expression of PDZ binding kinase <PBK> has been reported to link to a poor prognosis in solid cancers. Here, we investigated the significance of PBK in tumorigenesis of MM cells. Importantly, the public data showed that the MM patients with higher expression of PBK have a significant shorter survival time compared with those with moderate/lower expression of PBK. Therefore, we further examined the effect of PBK on the proliferation and tumorigenesis in MM cell lines. Of note, knockout of PBK in MM cells significantly suppressed the tumorigenesis in xenografted mice model. In addition, the migration activity was suppressed in the $PBK^{-/-}$ OPM2 and KMS-11 cells. Finally, we examined the effect of a specific PBK inhibitor OTS514 on tumor growth in vivo. Interestingly, we found that OTS514 treatment significantly reduced the size of KMS-11-derived tumor. Taken together, these results strongly suggest that PBK expression is closely associated with myeloma tumorigenesis; PBK may be a novel therapeutic target for treatment of MM.

P-1266

Elucidation of IMiDs-resistant mechanism in multiple myeloma

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Recently, Immunomodulatory drugs such as Lenalidomide (Len) play a central role in Multiple Myeloma (MM) therapy. Expanded application of Len has increased number of Len-resistant patients. However, Len-resistant mechanism has not been fully understood, and urgent elucidation of the molecular mechanism is needed. The purpose of our research is to unravel the molecular mechanism of Len-resistant.In our laboratory, Len-resistant cell lines, KMS21R,KMS27R and MUM24R have been established by long-term co-culture with low-dose Len. Using these cell lines, we examined expression of CRBN and the down stream molecules. Decreased expression of CRBN was observed in KMS21R while IKZF 1/3 expression was increased in KMS27R without alteration of CRBN level, suggesting occurrence of functional mutation in CRBN gene. Expression levels of the CRBN pathway molecules weren't significant changed in MUM24R ,indicating involvement of unknown CRBN-independent mechanisms.Our present data suggested diversity of Len-resistant mechanism in MM patients. Recent research suggests that genetic mutations induce the Len-resistance. In KMS27R and MUM24R, genetic mutation for Len-resistant will be also shown.

▲TOP

[P-1273] P14-22 [English/Japanese]
Head and neck cancer (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Takashi Saku / Fukuoka Dent. College

P-1273

Radio-sensitivity of IGFBP3 in oral squamous cell carcinoma cells

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We have previously demonstrated that ectopic insulin-like growth factor binding protein 3 (IGBBP3) expression enhanced lymph node metastasis of OSCC cells. However, the survival analysis of OSCC patients with high levels of IGFBP3 had an increased survival rate compared to those with low levels of IGFBP3. Furthermore, we found that ectopic IGFBP3 expression enhanced the radiation-induced cell-killing effect, indicating that the sensitive effects of IGFBP3 on radiation treatment might contribute to the better survival of OSCC after conventional treatment. In vivo, we discovered that ectopic IGFBP3 expression after 8-Gy radiation treatments can reduce tumor weight, volume and metastasis ability, but did not decrease tumor lymphangiogenesis. In vitro, ectopic IGFBP3 expression did not affect radiation-induced cell cycle arrest, but enhance radiation-induced cell death as evidenced by doubling staining with PI and annexin V. Additionally, we found that ectopic IGFBP3 expression increased the reactive oxygen species production and decreased mitochondrial membrane potential by flow cytometry. We will further investigate the roles of IGFBP3 in mitochondria upon radiation treatment.

▲TOP

P-1274

Nrf2, anti-oxidative stress-regulatory factor, controls resistance to radiation in oral squamous cell carcinoma

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Background: Recently, some studies have been shown that NF-E2-related factor 2 (Nrf2), anti-oxidative stress-regulatory factor, confers a resistance of chemotherapy and/or radiotherapy in many malignancies. The aim of this study is to explore the biological impacts that contribute to radioresistance of oral squamous cell carcinoma (OSCC) cells via Nrf2 anti-oxidant pathway. Methods: Biopsy samples obtained from OSCC patients were used for immunohistochemical analysis for phosphorylate Nrf2 (p-Nrf2). We examined the influence on radioresistace of OSCC cells via Nrf2 in vitro and in vivo using OSCC and clinically relevant radioresistant (CRR) cell lines. Results: The high expression of p-Nrf2 was significantly associated with the poor response for chemoradiotherapy. On multivariate analysis, p-Nrf2 expression was a significant prognostic factor. The p-Nrf2 stably expressed in cellular nuclei of CRR cells than the parent cells. The parent and CRR cells were sensitized radiation by suppressing the Nrf2 expression. Conclusion: To targeting Nrf2 could augment the treatment response in patients with radioresistant OSCC, thereby could improving the survival rate of patients with OSCC.

P-1275

Osteopontin in tumor microenvironment confers radioresistance on oral squamous cell carcinoma cells

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Introduction Osteopontin (OPN) is a secreted protein involved in various aspects of tumor progression. The purpose of this study is to explore the impact of OPN on radioresistance and clinical significance in OSCC patients.Materials and Methods We examined the OPN expression in OSCC cell lines by WB and ELISA. We analyzed the localization of OPN by IHC and the effects of OPN on radiosensitivity and DNA damage after X-ray irradiation in vitro. Results The various endogenous expression level of OPN were observed in all OSCC cell lines. But, the extracellular OPN was not detected in conditioned media by ELISA. The OPN expressions were mainly observed in tumor microenvironment that is rich in TAMs. Increased levels of exogenous OPN suppressed radiation-induced cell death, and the radioresistant effect of OPN was associated with decreased DNA damage after radiation. In the IHC analysis, the frequency of OPN high patient was significantly higher in cases who showed poor response for CCRT. Conclusion These results suggest that OPN provided by TAMs may plays an important role in radioresistance of OSCC, and may be a potential target of the treatment-resistant OSCC cells.

P-1276

Interleukin-6 confers radioresistance phenotype on Sq-1979 cells: mouse-derived oral squamous cell carcinoma cell line

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Background: IL-6 signaling plays a crucial role in cancer many malignancies including OSCC. The aim of this study is to establish the experimental models for the study of IL-6-mediated redioresistance mechanisms in vivo.Materials and Methods: Sq-1979; buccal mucosa cancer cell line established by C3H mouse was selected to establish preclinical model for evaluating the effect of tocilizumab, a humanised anti-human IL-6 receptor antibody, on radiosensitivity in OSCC. We examined the expressions of IL-6, IL-6R, and sIL-6R on Sq-1979 by using PCR assay, Western blotting, and ELISA. The effect of mouse IL-6 or, MR-16, anti-mouse IL-6 receptor antibody, or both on radiosensitivity of Sq-1979 was evaluated in vitro.Results: The expression of IL-6 and IL-6R were confirmed by PCR assay and Western blot. Moreover, we validated IL-6 and sIL-6R in culture media by ELISA. Increased levels of IL-6 suppressed radiation-induced cell death, and the blockade of IL-6 signalling by MR-16 sensitised tumor cells to radiation. Conclusions: These results suggest that Sq-1979 cells were suitable for establishing the in vivo model for the study of IL-6-mediated redioresistance in OSCC.

▲TOP

P-1277

Interleukin-6 released by cancer-associated fibroblasts is critical for angiogenesis in oral squamous cell carcinoma

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Recent studies have shown that interleukin-6 (IL-6) plays an important role in cancer development and progression. However, little is known about the clinical significance of IL-6 for oral squamous cell carcinoma (OSCC). To elucidate the function of IL-6 in OSCC, we cultured three pairs of normal fibroblast (NF) and cancer-associated fibroblast (CAF) from patients. The expression levels of IL-6 and vascular endothelial growth factor (VEGF) of NF and CAF were evaluated using quantitative RT-PCR and ELISA. CAF produced significant amounts of IL-6 and VEGF than NF. Moreover, IL-6 enhanced VEGF production in NF and CAF, thereby inducing angiogenesis. Subsequently, we examined the effect of anti-human/mouse IL-6 receptor (anti-IL6R) monoclonal antibodies on the growth of human OSCC cell line in vitro and in vivo. Neither humanized anti-IL6R monoclonal antibodies nor exogenous IL-6 influenced the proliferation rate of OSCC and CAF cells under the condition of each culture. However, the anti-IL6R monoclonal antibodies reduced the growth of OSCC xenograft tumors by 70%. These data suggest that targeting IL-6 appears a useful approach to OSCC therapy.

P-1278

Investigation of the effect of tocilizumab on radiosensitivity in oral squamous cell carcinoma

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Background:IL-6 signaling is considered to have an important role in tumor progression and treatment resistance phenotypes. However, the clinical value of IL-6 on radiosensitivity in oral squamous cell carcinoma (OSCC) remains largely unclear. The purpose of this study was to investigate the effect of IL-6 on radiotherapy in OSCC.Materials and Methods:Two OSCC cell lines were used. We examined the effects of tocilizumab, a humanised anti-human IL-6 receptor antibody on radio-sensitivity after X-ray irradiation in vitro and in vivo. In addition, we explored the possible mechanisms underlying tocilizumab based bio-radiotherapy in OSCC.Results: In vitro analyses, the autocrine manner of IL-6 secretion by increasing STAT3 phosphorylation was observed in OSCC cell lines.Tocilizumab significantly enhanced the sensitivity of irradiation OSCC cells in vitro and in vivo. The inhibition of STAT3 phosphorylation and down-regulation of the downstream targets STAT3 were confirmed in OSCC cells treated with tocilizumab.Conslusion: These results indicate that the blockade of IL-6 signaling combined with conventional radiotherapy could augment the treatment response in radioresistant OSCC cells.

▲TOP

[P-1292] P14-26 [English/Japanese]
Head and neck cancer (5)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Takahito Fukusumi / ORL-HNS. Med. Osaka Univ.

P-1292

Thyroglobulin after two weeks of thyroid hormone withdrawal in thyroid cancer can determine radioiodine dose

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Purpose Retrospective study was to determine radioiodine (RI) dose using thyroglobulin (preTG) measured after 2 weeks of thyroid hormone withdrawal in differentiated thyroid cancer (DTC). Methods preTG was performed before 1 week of RI therapy. Patients (Pt) received 30, 100 and 150 mCi of RI in consideration of preTG, pathologic stage, surgeon's opinion and Pt selection. After 6 months of RI, complete ablation (CA) was investigated. CA was defined as showing no uptake in I-131 scan and stimulated TG of less than 1. Pt with preTG less than 3.09 were divided into 30, 100 and 150mCi groups, and CA of each group was investigated statistically. Results 70 with preTG level of 3.09 or less were investigated. Pt were divided into 30 (n=7, mean preTG=1.11;0.04-3.09), 100 (n=51, mean preTG=0.80;0.04-3.09), 150 (n=12, mean preTG=1.23; 0.1-2.8)mCi groups. CA were 71.4%, 86.3% and 75.0%. When each group was compared through Kruskal-Wallis test, there was no difference in CA between groups (p=0.458). There was no difference between two groups in Mann-Whitney test (30 vs 100, p=0.313; 30 vs 150, p=0.868; 100 vs 150, p=0.340). Conclusion 30mCi is recommended if preTG is 3.09 or less than in DTC.

▲TOP

P-1293

JAK inhibitors suppress paclitaxel resistant anaplastic thyroid cancer cells via IL-6 reduction

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Background: Anaplastic thyroid carcinoma (ATC) has a poor prognosis despite multimodal treatment approaches. Innovative strategies are required for treatment. Materials and Methods: Differences in cDNA microarray gene expression profiles before and after treatment with paclitaxel were analyzed in two ATC cell lines: KTA-3 cells, which are sensitive to paclitaxel, and TTA-2 cells, which are resistant to paclitaxel. GO Analysis and PAGE were performed to detect significant differences in pathway genes and transcriptionally regulated genes. The effect of a JAK inhibitor on ATC cells was examined in a cytotoxic growth-inhibition assay. IL-6 was measured using an ELISA.Results: JAK-STAT pathway was significantly downregulated in KTA-3 cells compared to TTA-2 cells after treatment with paclitaxel. The JAK inhibitors suppressed growth of the paclitaxel-resistant TTA-2 cells. IL-6 was reduced by JAK inhibitors in TTA-2 cells.Conclusions: Paclitaxel inhibits cell growth via downregulation of the JAK-STAT pathway. JAK inhibitors may be new drugs for treatment of paclitaxel resistant ATC. IL-6 may be a potential biomarker for treatment of JAK inhibitors.

P-1294

BRAF(V600E) mutation is highly prevalent in the young population in Fukushima

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(Aim) According to the surveillance, new cases of thyroid cancers in the young population have increased. The thyroid ultrasound screening for children aged 0-18 was performed in Fukushima after the accident at the Fukushima Daiichi Nuclear Power Plant. In this study, we analyzed clinicopathological features of thyroid cancers in the young population in Fukushima. (Methods and Results) We analyzed 126 patients (42 males and 84 females) operated between 2013 and 2016 at Fukushima Medical University. The median age at operation was 18 years. The median size of tumor was 18.5mm in the Child, 12.8mm in the Adolescence and 13.4mm in the Young Adult. The majority of patients (90%) were diagnosed as papillary thyroid cancer (Classical type). We analyzed BRAF(V600E) mutation by direct DNA sequencing. BRAF(V600E) mutation was observed 65.9% in the thyroid papillary cancer. (Conclusion) The prevalence of the BRAF(V600E) mutation was comparable to Japanese adult cases, implying that the carcinogenesis mechanism may be similar between young population and adult papillary thyroid cancers.

P-1295

STUDY OF EXTRANODAL LYMPHOPROLIFERATIVE MALIGNANCY AS ONLY PAROTID SWELLING

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INTRODUCTION Tumor of salivary gland are only 2% of all head and neck cancers and 85% of it is found in parotid.Primary lymphoma constitute 1.7%-3.1% of all salivary gland tumor. NHL and HL are commonest head and neck tumor after squamous and thyroid cancer.Extranodal lymphoma has better prognosis than others.AIM is to study patients with parotid mass and underwent surgery and their clinical course,treatment .MATERIAL AND METHODS In our study, analysis and review of 19 patients in 10 years, found NHL except 1 CML. All details taken, underwent preop CECT head and neck.Superficial parotidectomy done and sent for HPE.The patients staged as per Ann Arbor staging system.After diagnosis patient were sent for CT RT with CHOP or R-CHOP.RESULT one case was found HIV status.CT scan suggested towards malignancy.FNAC could be avoided as it didnt gave definitive diagnosis CONCLUSION The patients having parotid lymphoma are clinically indistinguishable from other lesions.As FNAB has negative yield,it has lead that s.parotidectomy helps to treat and confirm parotid lymphoma.

▲TOP

P-1296

A case of gross recurrent sialolipoma of the parotid gland

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Lipomatous neoplasms of the parotid gland are rare tumours, accounting for less than 0.5% of all parotid tumors. Sialolipoma was identified as a rare but distinct histological variant of lipoma, characterized by well-demarcated proliferation of mature adipocytes with secondary entrapment of normal salivary gland elements. In the current report, the case of a of a 38-year-old female with a slow-growing mass in the right parotid gland with recurrence is presented. The initial clinical diagnosis was a benign salivary gland tumor. The tumor was situated between the right parotid gland and invade the right parapharyngeal space. A parotidectomy was performed. Histopathology revealed that the tumor was a sialolipoma of the parotid gland. Postoperative recovery as well as follow-up has been uneventful. The aim of this report is to report an unusual gross appearance case of sialolipoma and discuss its clinicopathological and morphological features.

▲TOP

[P-1304] P15-1 [English/Japanese]
Diagnostic biomarker and prognostic factors

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Kazufumi Honda / Dept. Cancer Early Detection, Natl. Cancer Ctr.

P-1304

Functional analysis of cancer derived exosomes in esophageal squamous cell carcinoma

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<Background< Exosomes, that contain proteins and microRNAs, have been focused on in cancer research, particularly as potential diagnostic markers. In our research, the quantification of exosomes isolated from the patient plasma revealed that esophageal cancer patients expressed higher exosome levels than non-malignant patients. Although there was no correlation between the tumor progression and the exosome levels, exosome number was the independent prognostic marker and low levels of exosome predicted a poor prognosis. As the next step, we focused the functional changes and the changes of the gene expression affected by exosomes released from cancer cell itself. <Methods< We administered exosomes extracted from the culture supernatant of the esophageal squamous cell carcinoma cell line to another cell line, and performed proliferation assay and migration assay. <Results< Proliferation assay revealed decreasing cell count, and wound healing assay showed the reduction of open area. <Consideration< The involvement of esophageal squamous cell carcinoma and host exosome is suggested, but the mechanism is a subject for further study. We report this topic with some literature review.

▲TOP

P-1305

Exosomes secreted from gastric cancer cells deliver anti-apoptotic signals to tumor microenvironment

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Gastric cancer (GC) is the third leading cause of cancer-related deaths worldwide. To reduce the mortality and improve early detection rate for GC, in particular diffusely infiltrative type-Ⅳ carcinoma (scirrhous type), we comprehensively explored novel biomarker proteins on circulating exosomes isolated from patient's sera (n = 58: 10 normal donors, 16 early GC, 18 advanced GC, and 14 scirrhous GC). Proteome-wide quantification of 832 proteins, 13 proteins showed significant up-regulation in GC exosomes. Especially, PN-1 protein was detected as a highly enriched protein cargo in GC exosomes. PN-1 strongly express in GC tissues, whereas no expression in normal mucosa. Further functional analysis indicated that potential contribution of PN-1^{+} exosomes to apoptosis resistance in GC cells via dysregulation of intracellular pH homeostasis. Furthermore, exosomal PN-1 induced anoikis resistance and metastatic potential to GC cells. These results provide the first evidence of the Exo-PN-1 as an effective biomarker for early detection of lethal type of GC, and indicate that Exo-PN-1 confers apoptosis resistance to tumor microenvironment during GC progression.

P-1306

Circulating tumor DNA is associated with tumor progression

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Liquid biopsy using circulating tumor DNA (ctDNA) has been spread world-wide. So far, we showed from a retrospective and a prospective study that ctDNA was frequently detected in lung cancer patients with distant metastasis, and detection of ctDNA was associated with poor prognosis. To investigate the significance of these clinical data, 130 plasma samples from 92 lung cancer patients, 18 benign pulmonary patients, and 20 healthy individuals were examined. We found difference of circulating free DNA (cfDNA) size between lung cancer and healthy individuals; former showed two peaks of 5 kb and 170 bp, and latter was single peak of 170 bp. The concentration of long fragment ctDNA was correlated with tumor progression. To investigate which fragment contained tumor-derived DNA, short and long fragment ctDNA were separately isolated, and EGFR mutation, L858R was examined. L858R was detected in both DNA fragments, indicating that both DNA fragments contain tumor-derived DNA. The short fragment DNA is well known as an apoptotic product, but the origin of long fragment DNA is still unclear. How the long fragment ctDNA is secreted, and what are biological functions are under investigation.

P-1307

Construction of prognosis prediction for pancreatic ductal adenocarcinomas by methylation analysis of mucins promoters

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Pancreatic cancer is still a disease of high mortality despite advanced diagnostic techniques. Mucins (MUC) play crucial roles in carcinogenesis and tumor invasion in pancreatic neoplasms. MUC1 and MUC4 are high molecular weight transmembrane mucins. These are overexpressed in many carcinomas, and high expression of these molecules is a risk factor associated with poor prognosis. We evaluated the methylation status of MUC1 and MUC4 promoter regions in pancreatic tissue samples from 169 patients with various pancreatic lesions by the methylation specific electrophoresis (MSE) method. These results were compared with expression of MUC1 and MUC4, several DNA methylation/demethylation factors and hypoxia biomarker. These results were also compared with clinicopathological features including time of overall survival of PDAC patients. We show that the low methylation of MUC1 and/or MUC4 promoters correlates with decreased overall survival. This is the first report to show a relationship between MUC1 and/or MUC4 methylation status and prognosis. Analysis of epigenetic changes in mucin genes may be of diagnostic utility and one of the prognostic predictors for patients with PDAC.

▲TOP

P-1308

Examination of induction of cancer cell-selective amino acid transporter LAT1 overexpression

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Boron neutron capture therapy (BNCT) is an attractive radiotherapy for cancer. Outcome from BNCT largely depends on the amount of intracellular accumulation of boron compound. L-type amino-acid transporter 1 (LAT1), through which boronophenylalanine (BPA) is transported into cells, is expressed in various types of tumor cells including glioblastoma but not in normal cells. We transfected pLAT-TRE/LAT1/IRES/tTA plasmids including a positive-feedback loop into glioblastoma cell line, T98G. The plasmids were designed to overexpress LAT1 gene in cancer cells selectively. We obtained several clones which stably overexpress LAT1 after lipofection of the plasmids. Intracellular incorporation of ^{14}C-BPA was examined by use of a RI tracer method in those clones.

P-1309

MyD88-activated form induces oncogenesis via NFkB - HIF1a

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Inflammation caused by pathogen infection is related to cancer growth. Pathogen infection is sensed by natural immunity via Toll-like receptors (TLRs). TLRs are expressed not only in immune cells, but are also expressed in non-immune cells (fibroblasts and epithelial cells). TLRs are also expressed in tumor cells, where they may influence tumor growth.
MyD88 is a key adaptor protein of TLRs and MyD88 L265P, a gain-of-function mutation, was found in diffuse large B-cell lymphoma and lymphoplasmacytic lymphoma. To examine how MyD88 L265P initiates tumorigenesis, we introduced MyD88 L265P into p53 knockout mouse epidermal fibroblasts (p53KO MEFs). In the MyD88 L265P expressing cells, transcription factor Hif-1a and glycolysis related genes known as downstream genes of Hif-1a were upregulated. Also, glucose uptake and lactic acid production were increased. Knocking down p65 NF-kappaB or Hif-1a expression made glucose uptake and lactic acid production attenuated. Xenotransplantation of MyD88 L265P expressing cells into nude mice formed tumors, but Hif-1a knockdown MyD88 L265P expressing cells did not.
Taken together, MyD88 L265P induces neoplastic transformation via Hif-1a in p53KO MEFs.

▲TOP

[P-1316] P15-3 [English/Japanese]
Cancer screening

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Hiroshi Fujiwara / Dept. Personalized Cancer Immunother., Mie Univ.Grad. Sch.Med.

P-1316

A new early cancer detection biomarker using multivariate index of the serum macroelements and trace elements

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Identification of biomarkers for risk assessment and early detection is always a challenging issue. We developed a novel elemental analysis for evaluating cancer development via precise profiling of serum trace and major elements. Under IRB-approved protocols, we assembled a cohort of 375 colon (225/150 male /female), 97 prostate, 157 female breast cancer cases along with 518 cancer-free individuals (270/248 M/F) in the Tokyo Bay area of Kanagawa and Chiba prefectures, collected serum samples and performed ICP-MS to characterize levels of 18 trace elements including Na, Fe, Cu, Zn and Se. Statistical analyses and subsequent validations revealed a robust cancer predictor model for colon, breast and prostate cancers, with high sensitivity and specificity repeatedly confirmed through cross-validation analysis. Form these results, we believe that Metallo-Balance is a promising screening method for the detection of various cancer types, even if the disease is in its early stages.

▲TOP

P-1317

Clinical validation of plasma amino acid-based cancer screening test, AminoIndex Cancer Screening, in multicenter study

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A novel cancer screening test, AminoIndex Cancer Screening (AICS), has been developed by multivariate analysis of plasma amino acid between various cancer patients and healthy subjects and utilized in medical examination. In this study, the potential of AICS for cancer detection by follow-up periods was validated in multicenter study. The AICS tests were examined in 10,460 subjects at Chiba Cancer Center, Mitsui Memorial Hospital, and Saihaku Hospital and the examination of cancer incidence were investigated. At 95% specificity, the sensitivities of AICS for gastric cancer, lung cancer, colorectal cancer, prostate cancer, breast cancer and gynecological cancer were 83% (10/12), 50%(2/4), 46%(6/13), 50%(8/16), 41%(7/17) and 50%(1/2), respectively within 1year after AICS examination. The total cancer detection rate by AICS was 0.33%(34/10,460). Whereas, the sensitivities for the corresponding cancers were 52%(15/29), 18% (2/11). 29% (8/28), 36%(8/22), 28%(9/32) and 33%(2/6), respectively during up to 5.7 years follow-up periods. AICS was demonstrated to be a more useful cancer screening marker for evaluation of a probability of cancer incidence than for prediction of cancer onset.

P-1318

Quantification of protein heterodimers by using fluorescent nanoparticles for breast cancer diagnosis

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The members of human epidermal growth factor receptor (HER) family are overexpressed on many kinds of human cancer cells and play a pivotal role for cancer progression. Many researchers in this field revealed that the formation of HER2/HER3 hetero-dimer was the most potent signaling pair in HER2 positive breast cancer, leading the bad prognosis. However, previous developments of fluorescence imaging of HER2/HER3 heterodimers using human cancer tissues did not achieve the level of clinical application due to interference of tissues autofluorescence. Tissue autofluorescence is strong and has an intensity comparable to the fluorescence intensity of commercially available quantum dots (QDs). Two kinds of fluorescent nanoparticles were employed as a pair of fluorescent probes. We performed detecting HER2/HER3 hetero-dimers by using QDs as energy donor and PIDs as energy acceptor which induce fluorescence particle-to-particle energy transfer. The efficiency of fluorescence energy transfer depends on the distance between the pair of particles. The energy transfer based PID fluorescence can be detected only in the case of heterodimer formed.

P-1319

A low-cost CTC isolation device

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A small, portable, no-need-for-power-supply, and very low-cost device to isolate viable circulating tumor cells (CTCs) was developed.
CTCs probably contain the origin of lethal metastatic disease, therefore an easy-to-use method to isolate CTCs has a potential to be used in precision medicine, next-generation diagnostics, fundamental metastasis research, etc. We newly developed a device which could isolate CTCs from blood samples using size-based deterministic lateral displacement technique. The device was tested using blood sample spiked with cultured cancer cells, and 98 percent or more of cancer cells were successfully isolated and recovered from blood sample.
The device has following features which is useful for systematic and large-scale analyses of CTCs: The device can isolate viable cells in suspension (not bound to the device), which is compatible with cell proliferation, next-generation sequencing, cytopathological and RNA-based characterization, etc. The device can isolate CTCs without known tumor marker, and is not require sample preparation steps, such as RBC lysis. The device can drastically reduce the cost of CTC isolation, and can be used at any place.

▲TOP

P-1320

Circulating tumor cells (CTCs) in malignant pleural mesothelioma (MPM) with the novel CTC-chip system

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Circulating tumor cell (CTC) is potentially useful marker in the diagnosis and treatment of malignant tumors. The CellSeach, EpCAM-based CTC detection system is most commonly used, but provided insufficient sensitivity in malignant pleural mesothelioma (MPM) because MPM cells may not express EpCAM. We developed a novel microfluidic system (CTC-chip) to which any antibody to capture CTCs can be conjugated, and showed that MPM cells were captured with an antibody against podoplanin that is abundantly expressed on MPM cells in previous studies. In the current study, we first achieved higher cell-capture efficiencies (around 80%) enough for capturing rare CTCs. The optimized CTC-chip showed superior cell-detection performances over the CellSearch in experimental models and in clinical samples. The CTC-count detected with the CTC-chip in MPM patients was significantly higher in advanced stages of patients. A ROC analysis showed that the CTC-test provided a significant diagnostic performance in discrimination of un-resectable diseases from resectable diseases. These results indicate that the CTC-test is potentially useful in the diagnosis and decision-making of treatment in MPM.

P-1321

Heterogenous circulating tumor cells detected by a size-based method in the blood of breast cancer patients

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Circulating tumor cells (CTCs) in cancer patients provide valuable informations: Heterogeneity of cancer cells, response to systemic therapy, early recurrence and poor prognosis. The concomitant detection of epithelial CTCs and EMT-like CTCs is important for understanding of metastasis and drug resistance. Using a method for size-based CTC enrichment, we isolated the cells from blood of metastatic and relapsed breast cancer patients, and those of healthy volunteers, with informed consent. Epithelial CTCs are characterized as (CK+, CD45- and DAPI+)-cells by immunocytochemical staining using mixed antibodies (CK) against pancytokeratin, cytokeratin 8, 18, 19, and EpCAM, and an antibody for leukocytes (CD45) along with DAPI. And EMT-like CTCs are (GCDFP+, vimentin+ and DAPI+)-cells using the antibody for mammary epithelium (GCDFP-15), and an anti-vimentin antibody. The epithelial CTCs and EMT-like CTCs in relapsed breast cancer patients were found in the ranges of 3-6 cells/ml, and 0-1.5 cells/ml, respectively, whereas no CTCs were detected in blood of healthy volunteers. Other markers for detecting heterogenous CTC are also discussed.

P-1322

Unique ubiquitination reaction of artificial E3 ligases in cancer cells

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Ubiquitination is undertaken through an enzymatic cascade comprising ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-ligating (E3) enzymes. E2 enzymes are associated with various diseases, such as breast cancer, leukemia, and gastric cancer. An artificial RING finger (ARF) was previously designed as an artificial E3 ligase, and E2 activities were conveniently estimated based on ARF reactivities. In this study, to extend the use of ARF in cells, we have constructed TAT-ARF using a cell-penetrating peptide TAT (49-57). In vitro ubiquitination assay, auto-ubiquitination of TAT-ARF was observed via its the TAT region, and the replacement of Lys residues with Arg in TAT-ARF led to the increasing ubiquitination activities. Furthermore, TAT-ARF was translocated into MCF7 breast cancer cells in a time- and dose-dependent manner, and then TAT-ARF was ubiquitinated upon itself via its ARF. This strategy is extremely simple and convenient, and the present detection system could be widely applied to specific E2s for various types of cancers.

▲TOP

[P-1330] P15-5 [English/Japanese]
Genetic diagnosis (1)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

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P-1330

Clinical application of KRAS exon 2 mutation measurement of plasma circulating DNA to diagnosis of colorectal cancer

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【Introduction】In recent years, some reports indicate the usefulness of measuring KRAS mutations of plasma circulating DNA (cDNA) as a minimally invasive biomarker for evaluating prognosis and the effect of drugs. However, there is no report of KRAS mutation measurement as an early diagnostic marker for colorectal cancer (CRC). In CRC, KRAS mutations are recognized in 40% of cases and most of them appear in the region of exon 2. Therefore, KRAS exon 2 mutation measurement of cDNA could be a marker to diagnose CRC.
【Materials and Methods】One hundred eighteen patients with CRC undergoing surgery from April 2017 to March 2018 in our department were collected. KRAS exon 2 mutation analyses were performed by digital PCR using plasma cDNA obtained from 5 mL of preoperative blood collection.
【Results】KRAS exon 2 mutations were identified in a total of 46 patients (39.0%), and 3 of them (6.5%) occurred in early cancer. The clinicopathological factors were also examined, but no significant difference was observed between mutant type and wild type.
【Conclusion】It was suggested that KRAS exon 2 mutation measurement of cDNA by digital PCR may be useful for early diagnosis of CRC.

▲TOP

P-1331

A nested multiplex PCR method for enrichment and detection of gene fusions by next generation sequencing

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Gene fusions are strong driver mutations in cancer. Recently, a number of novel fusions have been identified that can potentially be targets for molecular therapy. Although gene fusions can be detected by FISH and RT-PCR assays, these methods are generally labor-intensive and low-throughput. An optimal solution, especially in the clinical setting, is to utilize Next Generation Sequencing (NGS) to achieve high throughput, high accuracy and high sensitivity assays. In the new method we developed, after cDNA is generated from minimum 10 ng of total RNA by SMART technology, a nested multiplex PCR amplifies targeted cDNA using the combination of gene specific primer and universal primer.
Next, we assessed the ability to detect gene-fusions from FFPE reference samples that harbor ALK-, NTRK1-, RET-, and BRAF-fusions.Our new method accurately detected all gene-fusions without the information regarding the partner genes.
Taken together, we conclude that our NGS-based method would be a robust technology for detection of fusion transcripts from FFPE samples. The robustness of this method in applying to clinical field will be further discussed in detail.

P-1332

Enrichment of targeted genes by Multiplex PCR and detection of somatic mutations by next generation sequencing

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Detection of somatic mutations is a critical part of cancer research and diagnostics, and thus has been intensely investigated in recent years. Next generation sequencing (NGS) provides high accuracy, ease of use, and deep sequence data for detection of multiple variants. In this study, we developed a PCR-based enrichment method using ThruPLEX Tag-Seq kit, which technique can add molecular barcodes (UMT) to each DNA molecule.In our method, after ThruPLEX stem-loop adapters with UMT are ligated to the fragmented dsDNA, target genes are enriched by PCR amplification using multiplex primers Then, DNA libraries are generated by adding sequencing adapters. To estimate the performance of this method, we designed a custom gene panel for gastrointestinal cancers, and assessed the enrichment efficiency of this method by both qPCR and NGS. The results suggested that the novel method overall captured comparative or more DNA molecules with sufficient efficiency to detect somatic mutations, compared to the conventional hybridization-based method.Finally, we will demonstrate the whole workflow for target sequencing using our enrichment method and informatics pipeline.

P-1333

NGS-based fusion gene detection in sarcoma using RNA from FFPE tumor samples

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Many fusion genes have been identified in sarcoma, and a large part of them are known to be important as diagnostic biomarkers or drug targets. To evaluate the feasibility of next generation sequencing (NGS)-based fusion gene testing from FFPE samples, we analyzed 48 sarcoma patients, including 31 patients predicted to have specific fusion genes by the preceding FISH and other analyses. RNAs were extracted from their FFPE tumor tissue samples (prepared between 2000 and 2016) and were subjected to target capture RNA sequencing. DV200 (percentage of >200 nucleotide RNA) values of the extracted RNAs and duplication rates and error rates of the NGS reads were varied but clearly correlated with when the FFPE blocks were prepared. We were able to detect the correct fusion genes from 27 of the 31 samples but failed in the other 4 samples whose NGS data qualities were low. It was likely that long-time storage of FFPE blocks enhanced RNA degradation and increased duplication rates and error rates of NGS reads, resulting in failure to detect fusion genes. We will provide our data regarding RNA and NGS data qualities required for fusion gene detection.

▲TOP

[P-1285] P14-24 [English/Japanese]
Head and neck cancer (4)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Masashi Shiiba / Dept. Med. Oncology, Grad. Sch. Med., Chiba Univ.

P-1285

S100A10 promotes the cell migration and invasion of head and neck squamous cell cancer

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Altered expression of S100 family proteins is found in various cancers. Recently, through a proteomics approach, we screened for molecules relevant to cancer cell function, and identified S100A10 as a possible candidate. S100A10 is known to play an important role in the extracellular function of several cancers, however its intracellular function remains largely unexplored. Strong expression of S100A10 protein was observed in some clinical specimens of head and neck squamous cell cancer (HNSCC). To investigate the role of S100A10 in HNSCC, we knocked down or knocked out S100A10 in a hypopharyngeal cancer cell line HPCM2. In vitro, proliferation of these cells was slower than that of wild type cells (WT). In vivo, knockdown or knockout of S100A10 significantly suppressed tumor growth. Migration and invasion abilities were clearly decreased in these cell lines, which seems to be related to the dysregulated actin cytoskeleton organization. These results suggest that S100A10 is highly required for cell motility, and is involved in malignant properties of HNSCC.

▲TOP

P-1286

The roles of ZEB and ETS family proteins in head and neck squamous cell carcinoma cells

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The EMT is believed to be a critical step in the progression of cancers from both the pre-invasive to invasive state and organ confined to metastatic disease. EMT in cancer cells is also thought to promote resistance to anti-cancer drugs, and generate circulating tumor cells and cancer stem cells. We reported previously that levels of the δEF1 family proteins (ZEB1 and ZEB2), key regulators of the EMT, are positively correlated with EMT phenotypes and aggressiveness of breast cancer, and that Ets1 induces ZEBs expression and activates the ZEB1 promoter. In addition, ESE1, a member of the ETS transcription factor family, represses expression of ZEBs through ETS1 downregulation. Here, we investigated the roles of ZEBs and ETS family proteins in head and neck squamous cell carcinoma cells (HNSCC). We found that ZEBs are highly expressed in mesenchymal phenotypes of HNSCC, and that one of the ETS family proteins is negatively correlated with these phenotypes.

P-1287

FOSL1 promotes regional metastasis of head and neck squamous cell carcinoma

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Background: We initially performed the upstream and key nodes analysis together with whole gene microarray analysis characterized by regional metastatic potential with head and neck squamous cell carcinoma (HNSCC) cell lines and identified FOSL1, a member of the activator protein-1 (AP-1) family, as a key molecule in the regulation of the pathways related to regional metastasis in HNSCC. Methods: To study the role of FOSL1 on metastatic potential of HNSCC, small interfering (si) RNA and short-hairpin (sh) RNA mediated knockdown of FOSL1 in HNSCC cells were established and the abilities of cell invasion and migration in vitro were examined. The efficacy of knockdown of FOSL1 was also examined using an orthotopic mouse model of HNSCC. Results: siRNA and shRNA knockdown of FOSL1 in metastatic HNSCC cells significantly suppressed both cell invasion and migration in vitro. In addition, the knockdown of FOSL1 in metastatic HNSCC cells significantly repressed the incidence of regional metastases in vivo. Conclusion: These results suggested that FOSL1 could play an important role in promoting cell invasion, migration and regional metastasis in HNSCC.

P-1288

Suppression of CD82 reduces cisplatin and paclitaxel resistance in 3D culture model of head and neck cancer cell

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Micro cell aggregates including circulating tumor cell cluster (CTC cluster) or micro metastases of head and neck squamous cell carcinoma putatively cause later recurrence or metastasis. For this study, cancer spheroids were obtained from human oropharyngeal cancer cell line, T3M-1 through 3D culture to analyze resistance against chemo-radiotherapy in an in vitro model of micro cancer cell aggregates. Observations demonstrated that T3M-1 spheroids have significant resistance against cisplatin, paclitaxel, and radiation. The monolayer cell line T3M-1SMO was established with single cells separated from T3M-1 spheroids. T3M-1SMO was proven to lose resistance, suggesting that resistance of the spheroid is not irreversible. PCR array analysis demonstrated that expression of CD82 was increased in spheroid compared to monolayer T3M-1. Real-time PCR confirmed that the expression of CD82 was increased only in spheroids: not in T3M-1SMO cells. Suppression of CD82 by RNAi reduced resistance of spheroid against cisplatin and paclitaxel. CD82 is a feasible target to overcome chemoresistance of micro cancer cell aggregates including CTC cluster or micro metastasis in head and neck cancers.

▲TOP

P-1289

CD98hc as a marker of radiation resistance and cancer stem cell in head and neck squamous cell carcinoma

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Highly radiosensitive HNSCC generally has a good prognosis, but radioresistant forms of HNSCC also exist. Recently, CD98hc has been reported as a cancer stem cell marker. Moreover, CD98hc overexpression is strongly associated with cancers We hypothesized that irradiation contributes to CD98hc overexpression and cancer stem cell population expansion in HNSCC.5 cell lines were used. They were exposed to 60 Gy to establish cell lines that are stable when exposed to radiation.Using flow cytometry, CD98hc was almost 100% positive in all cell lines after radiation. In all parental cell lines, CD98hc-positive and negative cells were sorted by flow cytometer. CD98hc-positive cells exhibit an efficient ability to develop numerous spheres and a significantly high level of invasiveness. And CD98-positive cells of all cell lines shows significantly higher plating efficiency.We demonstrated that HNSCC that radioresitant strongly expressed CD98hc. CD98hc-positve cell has the ability of cancer stem cell. Moreover, there is a possibility that it can be used as a marker of radiosenstivity. Further investigations will contribute to the establishment of treatment methods suitable to all patients.

P-1290

Induction chemotherapy before CCRT for locally advanced nasopharyngeal carcinoma, the experience in south Taiwan

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Purpose: We tried to test if addition of induction chemotherapy (IC) to concurrent chemoradiation (CCRT) improving outcome for locally advanced (LA) nasopharyngeal carcinoma (NPC).Methods: We retrospectively collected LA NPC adult patients in Tainan. Most IC composed of PIF regimen while following CC was essentially cisplatin-based. For CCRT as upfront treatment, PF regimen was usually used in CC. Survival outcomes were accessed by Kaplan-Meier estimate with p value by Log-rank test to compare the survival distributions of IC or CCRT as the upfront treatment.Results: From 2007 to 2013, among 239 eligible LA NPC cases, 157 patients received CCRT and 82 received IC + CCRT as their upfront therapy. The 4-year overall survival (OS) was significantly better in IC group (89.3% vs 77.0%, p=0.03). After follow-up for near 12 years, trends only slightly toward IC group were noted for OS (72.1% vs 67.9%, p=0.14), and regional recurrence-free survival (94.9% vs 88.7%, p=0.18). However, there was significant difference in distant metastasis-free survival (DMFS) favoring IC (91.5% vs 79.4%, p=0.013).Conclusions: Addition of IC for LA NPC patients enhanced DMFS.

P-1291

Prognostic value of pretreatment serum lactate dehydrogenase level in nasopharyngeal carcinoma in early stage

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Objectives: About 15%-30% of nasopharyngeal carcinoma (NPC) patients in early stage would suffer treatment failure.In this study, we evaluated the association of pretreatment serum lactate dehydrogenase (S-LDH) level with prognosis of NPC patients with I and II stage, aiming to study its usefulness in predicting the outcome of the early-diagnose NPC. Methods: S-LDH levels in 399 NPC cases in I and II stage were measured before treatment, and their associations with OS and TFS were analyzed.The prognostic value of S-LDH was studied using univariate and multivariate analyses.Results: Univariate analysis showed that high S-LDH (>180U/L) was significantly associated with TFS of the NPC cases, and the multivariate analysis showed that the biomarker could be an independent predictor for TFS(OR =2.149,95%CI=1.247-3.704). No significance was found in the association of high S-LDH with OS in our univariate analysis. However, when this biomarker was mandatorily included in the multivariate analysis, the results showed that it was an independent predictor for OS(OR=1.754, 95%CI=1.005-3.061). Conclusion: Pretreatment S-LDH may be an useful predictor for the prognosis of NPC in early stage.

▲TOP

[P-1297] P14-25 [English/Japanese]
Head and neck cancer (6)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

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P-1297

DNA methylation of tumor-related genes in gargled fluid: A noninvasive method for detecting oral precancerous lesion

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Purpose: The early detection of oral squamous cell carcinoma (OSCC) is important, and almost all OSCCs arise from precancerous lesion. Therefore, in this study, the authors investigated the methylation status of tumor-related genes with the objective of establishing a noninvasive method for the detection of oral precancerous lesion. Material and Method: Oral rinse samples were obtained from 19 patients with precancerous lesion (leukoplakia) and from 55 healthy individuals (controls). The methylation status of tumor-related genes was determined by using methylation specific-multiplex ligation probe amplification (MS-MLPA) analysis. The accuracy as a tool for oral precancer detection was evaluated using a statistical analysis. Results: Of 26 types genes examined, ROC curves of 7 genes (RASSF1, DAPK1, CD44, BRCA2, FHIT, CDKN2A, and HIC1) showed the results of AUC > 0.8. 3 genes: RASSF1, CD44, and BRCA2, each having 80% or higher sensitivity and specificity, were found to be useful for oral precancer detection. Conclusions: The detection of methylated marker genes from oral rinse samples has great potential for the noninvasive detection of oral precancer.

▲TOP

P-1298

BRD4 is involved in high malignant potential in oral squamous cell carcinoma through the epigenetic regulation

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Oral squamous cell carcinoma (OSCC) has been increased morbidity, and its high malignant potential, including metastasis and therapeutic resistance affects patient survival. Bromodomain containing 4 (BRD4) associates with acetylated chromatin and facilitates transcriptional activation. BRD4 has been demonstrated to be involved in cell proliferation, metastasis and prognosis in several types of cancer. However, the role of BRD4 in OSCC remains to be elucidated. Herein, we investigated the role of BRD4 and possibility of a therapeutic target in OSCC. We performed ChIP-sequencing analysis using histone H3 lysine 27 acetylation (H3K27ac) antibody in OSCC cell lines. Global gene expression profiles were examined using microarray analysis in OSCC cell lines with BET inhibitor JQ-1. We extracted several genes associated with high malignant potential. ChIP assay showed JQ1 reduced the BRD4 binding to the H3K27ac-enriched sites in these gene locus. JQ1 suppressed the expression of the genes and cell proliferation, migration and invasion in OSCC cell lines. These results suggest that BRD4 may be a novel therapeutic target in OSCC.

P-1299

DNA methylation in circulating cell-free DNA of nasopharyngeal carcinoma

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The hypermethylation of CpG islands in gene promoter regions is a well-recognized epigenetic mechanism of carcinogenesis in malignancy through silencing tumor suppressor genes (TSGs). Nasopharyngeal carcinoma (NPC) is a prevalent malignancy of head and neck in Southeast Asia. Our recent study found that candidate TSGs were down-regulated with higher CpG methylation rates in NPC than in non-cancer nasopharyngeal epithelial (NNE) tissues using methylation-specific qPCR. Tumor- related methylated circulating cell-free DNA (ccf DNA) has been identified to be diagnostic biomarkers for various types of cancer in recent years. The purpose of this study is verifying whether our candidate hypermethylated genes could be potential epigenetic biomarkers for NPC diagnosis. We investigated the methods for DNA extraction from plasma specimens and quantification of DNA methylation rates of candidate genes in ccf DNA. Our results may provide novel convenient epigenetic marks for NPC diagnosis. [Collaborators: Dr. Y Mo, Dr. S Wang, Dr. W Zhao (Guangxi Medical University, China)]

P-1300

GDF10 is a candidate tumor suppressor gene inactivated by promoter hypermethylation in human nasopharyngeal carcinoma

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Growth differentiation factor-10 (GDF10), referred as BMP3b, is a member of the transforming growth factor- β (TGF- β) superfamily. It has been considered as a tumor suppressor, however, little is known about the molecular mechanism in nasopharyngeal carcinoma (NPC). We found that the expression of GDF10 in cancer tissues was significantly downregulated in various types of cancer than in non-cancerous tissues, as evidenced by The Cancer Genome Atlas (TCGA) database. Microarray data of NPC tissues and normal tissues was searched in GEO datasets. We obtained the results of low mRNA expression and promoter hypermethylation of GDF10. Furthermore, to investigate the methylation level of GDF10, we treated an NPC cell line (HK1) with 5-aza-2'-deoxycytidine (5Aza). The endogenous expression of GDF10 could be restored by the methyltransferase inhibitor 5Aza in NPC cell line. Our results suggest that GDF10 is frequently silenced by promoter CpG methylation in NPC and may play roles in cancer development. We will confirm the expression of GDF10 in NPC tissues by immunohistochemistry and its function by overexpression of GDF10 in NPC cell lines.

▲TOP

P-1301

The epigenetic feedback loop of the CpG demethylase TET family genes in head and neck cancers

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The aim of this study was to clarify the epigenetic regulation of ten eleven translocation protein (TET) family genes in HNSCC. We generated methylation profiles of TET1, TET2 and TET3 genes in tumor 233 samples. Promoter methylation was compared with various clinical characteristics and the TET methylation index (TE-MI). The TE-MI, representing the number of methylation events in TET family genes, was positively correlated with alcohol consumption, high-risk HPV status and disease recurrence. The simultaneous methylation analysis of TET family genes was correlated with reduced disease-free survival in unfavorable event groups (log-rank test, P = 0.026). In the multivariate Cox proportional hazards analysis, TET3 methylation in T1 and T2 tumor stages, oropharyngeal cancer, and oral cancer patients exhibited high association with poor survival (hazard ratio: 2.64, P = 0.014; 3.55, P = 0.048; 2.63, P = 0.028, respectively). A joint analysis of the tumor suppressor gene methylation index showed a significant trend toward a higher TE-MI. The methylation status of TET3 was independently associated with aggressive tumor behavior and a global effect on DNA methylation status in HNSCC.

P-1302

Targeted next-generation sequencing of 50 cancer-related genes in Japanese patients with oral squamous cell carcinoma

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Somatic mutation analysis is a standard of practice for human cancers to identify therapeutic sensitization and resistance mutations. We performed a multigene sequencing screen to explore mutational hotspots in cancer-related genes using a semiconductor-based sequencer. DNA from oral squamous cell carcinoma (OSCC) samples was used as a template to amplify 207 regions from 50 cancer-related genes. Of the 80 OSCC specimens from Japanese patients, including formalin-fixed paraffin-embedded (FFPE) samples, 56 specimens presented at least one somatic mutation among the 50 investigated genes, and 17 of these samples showed multiple gene somatic mutations. TP53 was the most commonly mutated gene (40 of 80; 50.0%), followed by CDKN2A (16.3%), PIK3CA (7.5%), HRAS (5.0%), MET (2.5%), and STK11 (2.5%). We also detected copy number variations (CNVs), in which segments of the genome could be duplicated or deleted from sequencing data. We detected the tumor-specific TP53 mutation in the plasma cell-free DNA (cfDNA) from one OSCC patient, and after surgery, the test for this mutation became negative. Our approach facilitates the simultaneous high-throughput detection of somatic mutations.

P-1303

Genomic mutational analysis of Japanese oral squamous cell carcinoma

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The aim of this study was to determine genomic alterations in Japanese oral squamous cell carcinoma (OSCC) using fresh frozen tumors and paired normal mucosae as controls. Selected cancer-related genes (502,696 bases, 48 driver mutation genes plus 28 genes which were frequently mutated in previous reports in head and neck squamous cell carcinoma (HNSCC) and OSCC in foreign countries) were evaluated. Materials and Methods: DNA paired samples obtained from 112 OSCC samples were analyzed using next-generation sequencing-based assay. Results: The average depth of coverage were 168.6× in tumors and 75.5× in normal mucosae. 368 exonic mutations (including 8 splicing and 22 frameshift indels) were observed. The common mutations were found in TP53 (41%), FAT1 (12%), NOTCH1 (11%), CDKN2A (11%), CASP8 (5%) and PIK3CA (4%). It was also notable that Japanese OSCC demonstrated frequent mutations in leukemia related genes (MLL3 (6%) and MLL4 (4%)) and genes in NOTCH signaling pathway (19%) as compared with previous reports for HNSCC and OSCC. Conclusion: This study has revealed the mutational profile of Japanese OSCC.

▲TOP

[P-1310] P15-2 [English/Japanese]
Novel cancer diagnostic tools and treatments (1)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Kiyotaka Shiba / Div. Prot. Engin., Cancer Inst, , JFCR

P-1310

Detection of carcinoma of the esophagogastric junction by topically spraying enzymatically activatable fluorescent probe

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Background: A fluorescent probe glutamylprolyl hydroxymethyl rhodamine green (EP-HMRG), which becomes fluorescent after cleavage by DPP-IV, has been recently developed, and we have reported that EP-HMRG is useful for the detection of superficial head and neck cancer. We investigated whether early carcinoma of the esophagogastric junction (EGJ) can be detected by spraying EP-HMRG.Methods: Fluorescence imaging of 12 cases resected by endoscopic submucosal dissection (ESD) was performed after spraying EP-HMRG, and the fluorescence intensity was measured for 10 min. Immunohistochemistry was performed to observe the expression of DPP-IV.Results: Fluorescence imaging of clinical samples demonstrated that tumor lesions, but not normal mucosa, became fluorescent within a few minutes after the application of EP-HMRG in 11 resected specimens. The fluorescence intensity in the tumor lesions was significantly higher than that in the normal mucosa 7 min after spraying of EP-HMRG. Immunohistochemical examination demonstrated that cancer lesions, but not normal mucosa, expressed DPP-IV in 11 out of 12 cases.Conclusions: EP-HMRG would be useful for fluorescent detection of early carcinoma of EGJ.

▲TOP

P-1311

ABCG2 gene expression defines the staining for 5-ALA in photodynamic diagnosis

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【Background】Photosensitizers are specifically accumulated in cancer cells, and photodynamic diagnosis (PDD) based on this trait has been focused as a novel diagnosis. We have been trying to establish an intraoperative cancer diagnosis method for gastrointestinal cancers using 5-aminolevulic acid (5-ALA) which is metabolically converted to photosensitizer protoporphyrin IX (PpIX).【Objective】We aim to improve PDD efficiency by analyzing the molecular basis of PDD using 5-ALA.【Method and Results】The fluorescence intensity of gastrointestinal cancer cells treated with 5-ALA was analyzed by flow cytometer. Most cell showed complete positive staining for PpIX, whereas PANC1 cells showed heterogenic staining pattern. To analyze the staining for PpIX at single cell level, 7 clone cells were established from PANC1 cells. PpIX positive rates in each clone cells were 11.8% to 50.4%. Quantity RT-PCR analysis revealed that ABCG2 gene showed tendency for relation with PpIX positive rate. Gene knockdown of ABCG2 using siRNAs resulted in an increase of PpIX positive rate in clone cells.【Conclusion】The expression of ABCG2 contributes to the PpIX fluorescence intensity.

P-1312

Development of acetylglucose-modified gefitinib derivatives as a novel radiosensitizer

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Introduction The aim of the present study is to design and synthesis of acetylglucose-modified gefitinib derivatives having inhibitory activity of autophosphorylation of EGFR and radiosensitizing activity. Results The gefitinib derivatives UTX-114, 115, and 116 was found to dissociate glycosidic bonds in the A431 cell, and inhibitory activity of EGFR autophosphorylation on A431 cells was confirmed. Furthermore, the Gefitinib derivative UTX-115 showed higher radiosensitizing activity than Gefitinib. Conclusion We succeeded in the development of Gefitinib derivative UTX-115 as a novel radiosensitizer having inhibitory activity of EGFR autophosphorylation. Co-authors Daisuke Miyamoto, Yuya Tanaka, Risa Kouzaki, Ayaka Hanyu, Mana Futawaka, Hisatsugu Yamada (Tokushima University).

P-1313

HDAC inhibitors sensitize well-differentiated colorectal cancer spheroid to X-ray irradiation

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Although most of the colorectal cancer patients are diagnosed as well- or moderately differentiated carcinoma, the effect of differentiation status on radio-sensitivity has not been well studied because of the lack of experimental platform. CTOS (cancer tissue-originated spheroid) method, which we recently established, enables us to recapitulate the characteristics of well-differentiated adenocarcinoma in vitro. We developed a novel radio-sensitivity assay evaluating focal regrowth of CTOS after X-ray irradiation. We previously reported that the Wnt activity of colorectal cancer CTOS was highly heterogeneous and plastic. The probability of CTOS regrowth was reduced by pre-treatment with Wnt inhibitors, indicating that cancer cells with high Wnt activity were resistant to irradiation. In addition, we found HDAC inhibitors, trichostatin A (TSA) and SAHA, severely prevented the CTOS regrowth after X-ray irradiation. The Wnt target genes were significantly downregulated in TSA treated CTOS in addition to the genes related to G2/M checkpoint or DNA repair. These results suggested that HDAC inhibitor increased the sensitivity to irradiation through reduction of Wnt activity in CTOS.

▲TOP

P-1314

Analysis of a novel mutation of ERBB2 in cancer of unknown primary

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A patient with cancer of unknown primary underwent an NGS-based multiplex gene assays (OncoPrime). As a result, a novel mutation of ERBB2 (E401G) which is a missense alteration located in the extracellular domain of the Her2 protein was detected. This alteration has not been reported (COSMIC, Jan 2018) and its effect on protein function is unknown. Although the variant allele frequency was relatively high (55.8%), Sanger sequence using genomic DNA from peripheral blood DNA sample showed that the variant was a somatic mutation. Immunohistochemistry using tumor specimen revealed over expression of Her2 protein. Accordingly we are planning to perform copy number variation analysis using Taqman® Assays and a functional analysis of the mutant Her2 protein. This study was approved by the Ethics Committee and the patient provided written informed consent for the use of genomic and clinical data for research purposes. Considering that clinical sequencing for cancer will be approved in near future, it is time to discuss how clinicians use these results for appropriate treatment decision.

P-1315

Biological evaluation of accelerator-based BNCT system in NCC

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Boron neutron capture therapy (BNCT) utilizes boron-carriers, such as 10B-boronophenylalanine (BPA). BPA is preferentially incorporated into cancer cells and alpha and 7Li particles were emitted by thermal neutron beam irradiation. An accelerator-based BNCT system with lithium target has been developed and installed in National Cancer Center (NCC) Hospital. We have investigated the relative biological effectiveness (RBE) of the neutron beam. Human cancer cell lines including HSG and SAS were irradiated with neutron beam or gamma-ray and cell survival was analyzed by colony formation assay. The frequency of micronuclei formation was also assessed. The dose-dependent decrease of cell survival after neutron beam irradiation was observed. The RBE of neutron beam was in the similar range reported for nuclear reactors used for BNCT. The dose-dependent increase of micronuclei formation was also observed. Taken together, the observed biological effects were within the range expected from the physical neutron flux measurement. Other co-authors from NCC: Satoshi Nakamura, Masashi Itoh, Hiroyuki Okamoto, Yoshihisa Abe, Jun Itami.

▲TOP

[P-1323] P15-4 [English/Japanese]
Screening assay

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Fumito Yamazaki / Dept. of Clin. Genomics, Natl. Cancer Ctr. Res. Inst.

P-1323

Identification of a gene set associated with poor clinical outcomes in prostate cancer patients

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We previously showed that androgen withdrawal led to increased alternatively spliced products in mouse Pten-deficient prostate tumors. RNA expression data from whole transcriptome analysis was used to identify candidate mRNA processing genes implicated in the progression to castration-resistant disease. A set of 43 unique RNA processing and splicing factor genes associated with the progression to castration-resistant disease were examined in prostate cancer patients (TCGA-Prostate Adenocarcinoma (PRAD). Further clustering identified 24 genes that were associated with decreased overall survival and disease-free progression times. This 23-gene set was examined in pan-cancer analysis of 30 TCGA cancer studies covering 26 human cancer types. Notably, this gene set showed a high specificity for lower disease-free survival in prostate cancer patients (P= 0.00427), with melanoma being the only other cancer type associated with decreased disease free-recurrence (P= 0.0487). Overall, we have identified a gene signature specific for prostate cancer and provide data to suggest that alterations in RNA processing genes lead to cancer-specific disease progression.

▲TOP

P-1324

Reduced serum miR-100 as a potential biomarker for cervical cancer

Zenta Yamanaka
OB

Co-author：Toru Sasaki、Hirotaka Nishi
OB

Objective: In this study, we measured the expression level of miR-100 in serum to investigate whether miR-100 can be useful as a biomarker in diagnosis of cervical cancer.Methods: We extracted total RNA from serum in 46 cervical cancer patients (CC), 64 cervical intraepitherial neoplasm patients (CIN) and 34 healthy volunteers (NC) after informed consent was obtained. The expression level of miR-100 was measured in each sample using quantitative real-time RT-PCR. The cut-off value of miR-100 in serum was set based on an ROC curve. We also examined the correlation between miR-100 levels and clinicopathological factors, such as pathology, lymph node metastasis and prognosis.Results: The relative levels of miR-100 in serum were 5.32±3.39 in the NC, 3.93±2.52 in the CIN and 1.84±1.72 in the CC, with significant difference between NC and CC ($p<0.001$). Those of miR-100 in cervical tissues were also significantly lower in the CC, compared with the NC. The level of miR-100 in the lymph node metastasis positive group was significantly lower than that in the negative (0.98±0.36 and 2.13±2.15, $p=0.01$). Conclusions: MiR-100 in serum may be useful as a biomarker for cervical cancer.

P-1325

Significance of surveillance for the early detection of gastrointestinal cancer with Crohn's disease

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According to the improvement of medical treatment, the control of Crohn's disease(CD) is improving. However, the risk of gastrointestinal(GI) cancers in CD patients is not decreasing and the prognosis is still poor. Further improvement of surveillance methods for early diagnosis and adequate treatments are needed. We investigated clinical features and prognosis of nineteen CD related GI cancer patients (7: diagnosed by surveillance, 12: without surveillance (non-surveillance)). Primary sites were rectum (including anal canal) in 9 patients, anal fistula in 3, colon in 2: stomach in 2, small bowel in 2 and other/unknown in 4. Curative resection could be performed in all surveillance patients. In contrast, curative resection was only five out of twelve in non-surveillance patients. Seven cases are in no relapse. Among twelve relapsed or without curative resection , eleven cases were died by cancer related reason in 23 months(1-142) (median(range)) of observation. Overall survival was significantly better in the surveillance cases. In conclusion, early diagnosis of GI cancers by surveillance was contributed to improving survival for patients with CD related GI cancers.

P-1326

Liquid biopsy monitoring HER2 amplification in plasma cfDNA using digital PCR system

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Digital PCR (dPCR) has the precision for absolute quantification, which enables detection of tumor-derived cell-free DNA (cfDNA). To establish an effective strategy for “liquid biopsy” in the diagnostics, a feasibility study is indispensable. Here, we optimized the dPCR assay to detect the HER2 amplification using Bio-Rad QX200 ddPCR system and evaluated the feasibility of the assay. We recruited breast and gastric cancer patients (n=21), quantified the HER2 copy number in plasma cfDNA, and compared those with the copy number of the reference gene. cfDNA collected from HER2-amplified patients (IHC 2+ and 3+) before the resection or the first chemotherapy, showed significantly higher levels of the HER2 : Reference ratio relative to the HER2-negative patients (IHC 0 and 1+). Furthermore, we monitored the HER2 amplification by the serial blood sampling and found the correlation between the re-rising of HER2 copy number with the poor prognosis in dPCR HER2-positive patients. Together, quantification of HER2 amplification in cfDNA using dPCR have potential in clinical practice even in localized cancers, offering a minimally invasive diagnostics.

▲TOP

P-1327

Peptidomics by new fully-peptide-solubilizing, non-enzymatic-cutting processing of whole blood of breast cancer patients

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Res 2 Gp, Cntrl Res Labs, Sysmex Co.

Breast cancer peptidomics, a basis of blood test with peptide markers (PMs), depends on completeness in discovery of PMs. But it remains poor, because practical processes of the discovery are still fragile: 1. Solubilizing/unfolding proteins/peptides (PPs) in water; 2. Cleaving PPs to fragments (FTs) with a controlled length and a uniform amino-acid residue (AR) at ends; and 3. Purifying/enriching FTs for mass spectrometry (MS). The completeness can be reinforced, if water converts to a good solvent, if proteases abandon their error-prone, AR-preferred cleavage, and if immiscible magnetic beads disperse to adsorb FTs richly.To reinforce, we have heated blood specimens (n=8, stage-2 patients; n=8, advanced; and n=8, healthy controls) with Zn at 433 K (90 s) to activate water in blood. The water solubilized and concurrently cleaved PPs specifically at L/S and D/P AR sites, producing 10-AR long FTs. This cleavage was faithful and absent in trypsinization. The FTs were purified at a high yield by facile mixing with beads of a nanopore-fabricated, FT-clathrating silica. MS identified PMs from insoluble, cell-motility-relevant titin and obscruin. The clinical potential will be discussed.

P-1328

Serum DNA testing by highly sensitive methylation assay to diagnose colorectal neoplasias

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Although methylated TWIST1 is a biomarker of colorectal neoplasia, its detection from serum samples is very difficult by conventional bisulfite-based methylation assays. Therefore, we have developed a new methylation assay that enables counting of even one copy of a methylated gene in a small DNA sample amount without DNA bisulfite treatment. We performed this study to evaluate the sensitivity and specificity of the new methylation assay for the detection of colorectal neoplasia. This study comprised 113 patients with colorectal neoplasia and 25 control individuals. For the new methylation assay, DNA was treated in two stages with methylation-sensitive restriction enzymes, followed by measurement of copy number of methylated TWIST1 by droplet digital PCR. The new assay had a sensitivity of 30.0% for advanced adenoma and 44.4% for colorectal cancer, and a specificity of 92.0%. Combination of the new assay and the fecal immunochemical test for hemoglobin increased the sensitivity to 45.7% and 72.2%, respectively, and resulted in a specificity of 84.0%. Combination of both tests may provide an alternative screening strategy for colorectal neoplasia.

P-1329

Diagnostics role of serum levels of novel multimarker in gynecological cancers

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For improve prognostication and treatment selection, identification of accurate biomarkers is necessary. We previously reported that novel serum biomarker candidates, such as B7-H3, EphA2 and EpCAM, were detected higher in patients with lung, pancreas or prostate cancers. The purpose of this study is to determine the diagnostic role of these novel biomarkers expressed in various gynecological cancers. Utilizing cell lines and patients serum derived from breast, ovarian or endometrial cancers, we examined the levels of soluble form of B7-H3, EphA2 and EpCAM by originally developed sandwich ELISA system. B7-H3 levels were higher in all gynecological cancers (ranged from 40 to 290 ng/ml, n=30) compared to healthy donors (from 10 to 60 ng/ml). In contrast, serum levels of EphA2 and EpCAM were only higher in patients with endometrial cancer. These data indicate that B7-H3 should be useful for common biomarker in gynecological cancers for a rationale of B7-H3 blockade therapy. Interestingly, we found strongly negative correlation between EpCAM and CA125, indicating that downregulation mechanism of EpCAM might be a new topic for diagnosis and prognosis of gynecological cancers.

▲TOP

[P-1334] P15-6 [English/Japanese]
Genetic diagnosis (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Toyomasa Katagiri / Div. Genome Med., Inst. Genome Res., Tokushima Univ.

P-1334

Single cell gene analysis of formalin-fixed circulating colorectal cancer cells

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【Background&Aim】Investigation of circulating tumor cells (CTCs) provides various information on disease status. Beyond staining of epithelial marker, single cell gene analysis is the most reliable to determine whether CTC like cell is a real cancer cell. We aimed to analyze gene mutations using single CTCs from colorectal cancer (CRC) patients.【Method & Result】Thirty-five cells were collected from 24 CRC patients. CTCs were fixed in 10% buffered formalin for the staining with the anti-cytokeratin (CK) or anti-CD45 antibody. After whole genome amplification (WGA), the products were subject to polymerase chain reaction (PCR) and Sanger sequence for mutations of KRAS, BRAF, and PIK3CA genes. Fourteen cells were CK positive and 21 cells were CK negative. Three CK positive cells had a point mutation in PIK3CA gene. On the other hand, one CK negative cell had a point mutation in KRAS gene and another CK negative cell had a point mutation in PIK3CA gene. These results were not always concordant with the gene status of primary or metastatic tumors.【Conclusion】Our data indicate that CTC like cells devoid of CK expression should be carefully evaluated as well as EpCAM(+) CTCs.

▲TOP

P-1335

Genomic profiling of circulating tumor cells collected by a label-free inertial microfluidics approach

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Circulating tumor cells (CTCs) provide valuable insights into cancer metastasis and treatment evaluations. CTCs were isolated using a label-free inertial microfluidics approach (LFMA), which can capture CTCs regardless of epithelial cell adhesion molecule (EpCAM) expression. We performed a prospective study using LFMA to establish the genomic profiling of CTCs by next-generation sequencing (NGS). In total, 31 patients with advanced head and neck cancer (HN) or gastrointestinal cancer (GC) were enrolled. CTCs were isolated using LFMA, and NGS of CTCs was performed after whole genome amplification. Circulating tumor DNA (ctDNA) was also extracted from plasma. The median CTC count was 14.5 cells/ml. The most frequently detected mutations in CTCs were those in EGFR, TP53, RB1, SMAD4, CDKN2A and CSF1R (9.1%) in HN, and RB1, SMAD4, ALK and GNAQ (20%) in GC; those in ctDNA were mutations in ALK and MET (9.1%) in HN, and in TP53 (44%) in GC. NGS was performed successfully using whole genome-amplified DNA from CTCs. We were able to capture effectively CTCs from HN and GC patients, and establish the genomic profiling of CTCs by NGS using LFMA without antibodies.

P-1336

Serum miRNA signature in luminal breast cancer at the acquisition of resistance to aromatase inhibitors

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Aromatase inhibitors (AIs) such as anastrozole, letrozole and exemestane are used for treatment of hormone receptor-positive breast cancer in postmenopausal women, but the efficacy is limited owing to the development of resistance to AI. Recently, a number of miRNAs have been reported to be deregulated in various cancers and have been suggested as potential biomarkers, including for breast cancer. However, serum miRNAs still have not been established as prognostic biomarkers of acquisition of AI-resistance. We analyzed serum miRNA profiles using human miRNA microarray for luminal breast cancers with acquired resistance to AI treatment, including luminal A, luminal B and luminal HER2, compared with healthy postmenopausal women. Serum samples were collected just after relapse was detected. We identified 58 miRNAs differentially detected in the serum between AI-resistant cases and controls, including 39 up-regulated and 19 down-regulated miRNAs. Analysis by qPCR also showed that some up-regulated miRNAs were detected at a higher level in the sera in AI-resistant cases. These miRNAs might be useful biomarkers for diagnosis of acquired AI resistance.

P-1337

Identification of gene sets inferring the survival of lung adenocarcinoma

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Lung cancer is the most common cause of cancer death. We tried to develop the risk score to predict the survival of lung adenocarcinoma patients using the gene expression data in The Cancer Genome Atlas (TCGA) dataset. We screened the prognosis indicator genes in combination with cox univariate regression and multivariate regression. The five genes were used to calculate the risk score. The patients with high risk score showed shorter overall survival and disease-free survival than the patients with low risk score. Multivariate analysis revealed that the risk score is an independent prognosticator compared with stage, smoking status, age, and gender. Furthermore, the risk score predicted the outcome of other datasets (GSE30219 and GSE50081). Taken together, we identified the risk staging model for lung adenocarcinoma patients using the expression levels of five genes.

▲TOP

[P-1338] P15-7 [English/Japanese]
Diagnostic imaging (1)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

Nakanishi Hayao / Lab. Path. & Clin. Res. Aichi Cancer Ct.

P-1338

AI beats specialists in radiographic detection of bone tumors

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Introduction: Radiographs are still one of the most influential diagnostic modalities for the prognosis of patients with bone sarcomas. Recently, AI (artificial intelligence) advanced with deep learning approach beat experts in many kinds of professions. The aim of this study is to compare AI with orthopaedic oncologists in predicting histological subtype on radiographs. Methods: Seventy-five patients with 78 common bone tumors around the knee treated between 2004 and 2017 were enrolled. There were 28 osteosarcomas, 20 giant cell tumors of the bone, 18 osteochondromas, 8 osteoid osteomas, 3 chondroblastomas, 2 aneurysmal bone cysts, and 2 non-ossifying fibromas. The subtypes were predicted on knee X-ray images at the first presentation by convolutional neural network and two orthopaedic oncologists. Results: Diagnostic scores of AI, senior, and junior oncologists were 56.4%, 53.8%, and 47.4%. Recognition rate of AI was significant better than junior specialist ($p<0.05$). Discussion: These findings suggested that AI might be a better interpreter to read radiographs of bone tumors than orthopaedic oncologists.

▲TOP

P-1339

Withdrawn

No Abstract

P-1340

Incidental breast cancer detected on computed tomography(CT)/magnetic resonance imaging(MRI) and FDG-PET/CT.

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【Introduction】CT and MRI are frequently used for diagnosis in many medical departments. Breast cancer is commonly found in subjective symptoms and cancer screenings, but asymptomatic breast cancer may be detected incidentally. 【Methods】 We investigated 20 cases that were diagnosed accidentally with CT, MRI/MRCP or FDG-PET/CT.【Results】All cases were female. The average age was 67.0 years (37-86). The mean diameter of the tumor was 17.7mm (7-60mm). The imaging modality for detection were underwent by CT:12, PET/CT:6 and MRI (MRCP):2 cases. Nine cases were secondary cancers. (Thyroid, Colon, Ovary: 2 cases. Oral, Kidney, Uterus: 1 case each). Pathological classification were 14 cases of invasive ductal carcinoma, and 4 cases of invasive lobular carcinoma. There were DCIS and apocrine carcinoma for each. 【Discussions】Breast cancer screening examination rate in Japan is still low. On the other hand, there are many opportunities to photograph CT and MRI tests for the purpose of searching for cancer etc. The number of PET-CT test is increasing in facilities to diagnose various cancers. It will be expected to increase the number of secondarily detected breast cancer.

P-1341

A RARE CASE OF NON-HODGKIN'S LYMPHOMA IN AJAMUNAPARI DOE IN BANGLADESH

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Dept. Genetics & Animal Breeding

Non-Hodgkin's lymphoma (NHL) is a cancer that originates in cells called lymphocytes, which are part of the body's immune system. NHL is common in animals and humans and is the most common type in dogs making up 83% of all haematopoitic cancer. To the author's knowledge no information on NHL has been found neither in cattle nor in goats. In this rare case study abnormally swollen superficial lymphnodes observed along with a history of nonresponsive to caseous lamphadenitis treatment. On physical examination the goat was found anemic but the condition was gradually deteriorating even with administration of haematinic and nutritional supplement. On gross and histo-pathololological examination the animal was eventually found to be affected with rare non-Hodgkin's lymphoma (small lymphocytic lymphoma). This report is unique in that it represents the recognition of lymphocytic lymphomas in goat. Body mass index (BMI),Various infectious factors as well as autoimmune and chronic inflammatory conditions have been implicated for NHL.

▲TOP

P-1342

X-ray CT Imaging of Micro Tumor in Mouse Model for Non-alcoholic Steatohepatitis-associated Hepatocellular Cancer

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Recently, the ratio of non-alcoholic steatohepatitis (NASH) associated hepatocellular carcinoma (NASH-HCC) has been increasing. NASH is caused by diabetes mellitus and fatty diet, and now spreading over the world. In Japan, about million people suffer from NASH and 2-5 % of the patients become NASH-HCC. In NASH-HCC, many factors including insulin resistance, inflammation, and lipid metabolism relate to the carcinogenesis. It's important to detect and analyze the early NASH-HCC for the elucidation of carcinogenic mechanism. In this study, to find the early HCC in NASH-HCC mice, we used the high resolution X-ray computed tomography (CT). We developed novel polyethylene glycol (PEG)-supported Au nanoparticles (Au/PEG) as contrast agents. The Au/PEG nanoparticles were injected into the tail vein of the mice and performed CT. We could find smaller tumors less than 1 mm by using the enhanced permeability and retention effect. Then, the positive tissue slice including micro tumor could be successfully-obtained with the assistance of X-ray CT image navigation. Moreover, we pathologically-analyzed the properties of micro tumors using fluorescence nanoparticles with ultra-high brightness.

▲TOP

[P-1348] P15-9 [English/Japanese]
Diagnostic imaging (3)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

Fumiaki Isohashi / Dept. Radiation Oncology, Osaka Univ., Sch. Med.

P-1348

Deep learning-aided drug sensitivity test for cancer cells: prediction of fluorescent labels from unlabeled cell images

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A variety of methods for measuring cell viability have been developed to screen anticancer drugs. MTT and ATP assays are the conventional methods but necessity of interruption of cell culture due to cell lysis to measure surrogate indicators for live cells is the major downside. Here we developed deep learning-aided non-invasive drug sensitivity test which is based on fluorescence label counting of live or dead cells predicted from unlabeled bright field images of drug -treated cancer cells. We trained the network with the supervised datasets consisting of pairs of bright field cell images and live (Hoechst 33342-stained minus propidium iodide (PI) -stained) or dead (PI-stained) cells-derived fluorescent labeled images. We then made prediction on withheld bright field cell images. The network was quite operative, generating fluorescence labeled images that are very similar to the corresponding right ones. These findings allowed us to establish the next-generation drug sensitivity test with the advantageous characteristics that are not only non-invasive, but also simple, fast, inexpensive, versatile, no need of fluorescent labeling and fully automatic.

▲TOP

P-1349

Automatic discrimination system for hematopoietic tumor-derived cell lines using Machine Learning

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Morphological images of cells contain extensive information, which help biologists to infer the type and state of cells to some degree based on their morphology. Convolutional Neural Network (CNN), a machine learning algorithm, is a powerful tool used for image recognition. However, whether it can be used to classify cells on the basis of their morphology remains unclear. In this study, we combined imaging flow cytometry (IFC) analysis and CNN to classify different populations of cells using their morphological features. We demonstrate that 10 different human hematopoietic tumor-derived cell lines (including acute myeloid leukemia, chronic myeloid leukemia, B-cell acute lymphoblastic leukemia, and myeloma) with similar morphology could be classified with over 90% accuracy using only their bright-field images analyzed using by CNN, although biologists found it difficult to discriminate between these cells. This novel and simple cell discrimination method must be widely applied for research works, such as clinical diagnoses.

P-1350

Development of radiogallium labeled folate and thieno pyrimidine derivatives for PET imaging of folate receptor

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The folate receptor (FR) is overexpressed in a variety of malignant tumors such as ovarian cancer but limited expression in normal tissues. In this study, we synthesized and evaluated novel radiogallium labeled folate derivative ($^{67/68}$Ga-NOTA-FL) and thieno pyrimidine derivative ($^{67/68}$Ga-NOTA-TP) as FR-targeting imaging agents. The K_D values of the ^{67}Ga-NOTA-TP and ^{67}Ga-NOTA-FL for FR were 25 and 46 nM determined in FR-positive KB cells. ^{67}Ga-NOTA-TP showed higher uptake than that of ^{67}Ga-NOTA-FL (50 and 29% at 4 h, respectively) in KB cells, while both radiotracers exhibited quite low uptake in FR-negative HT-1080 cells (0.45 and 0.52% at 4 h, respectively). Biodistribution studies of ^{67}Ga-NOTA-TP in tumor bearing mice demonstrated the uptake in FR-positive tumor was over 10 times higher than that of FR-negative tumor. PET studies revealed that high and specific uptake of ^{68}Ga-NOTA-TP in the FR-positive tumor and negligible uptake in the FR-negative tumor. Taken together, ^{68}Ga-NOTA-TP can be a promising PET ligand for FR-positive tumors.

P-1351

Prediction of stromal structural heterogeneity in lower rectal cancer by the diffusion image of MRI

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【Background】The stromal structural heterogeneity of cancer may be evaluated by DWI of MRI through quantification of water diffusional restriction in stroma. 【Method】Lower rectal cancer cases undergoing primary resection(PR) or surgery after CRT(nCRT) were included in this study. The proportion of stroma in tumor was calculated in five HPFs(x40) of HE-stained specimens by image analysis software. The intensity of tumor on DWI was measured. Heterogeneity in HE and DWI was quantified through substitution of the MAX and MIN value into followed formula. Heterogeneous score(HS) = (MAX-MIN)/(MAX+MIN). 【Result】In PR (29 cases), liner regression revealed that HS of DWI(dHS) was associated with pathological HS(pHS) (r^2=0.42). The number of T3 or deeper, or N(+) were larger in high dHS group. The AUC of ROC to predict the T3 or deeper, or N(+) by dHS were 0.79 and 0.72, respectively. In nCRT(37 cases), dHS before nCRT was significantly higher in CRT-therapeutic grade 1 cases($p<0.01$). The AUC of ROC to predict the grade by dHS was 0.80. 【Conclusion】The stromal heterogeneity could be predicted by dHS, which may be useful for predicting T, N stage, and the effect of CRT. .

▲TOP

P-1352

Detecting method for the regions of interest in colonic digital images via homology concept

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A region of interest (ROI) is a part of tissue that contains important information for diagnosis. To use many image analysis methods efficiently, a technique that would allow for ROI identification is required. For the colon, ROIs are characterized by areas of stronger color intensity of hematoxylin. Since malignant tumors grow in the innermost layer, most ROIs will be located in the colonic mucosa and will be an accumulation of tumor cells and/or integrated cells with distorted architecture.Homology is a mathematical concept that can quantify the contact degree. Due to the lack of contact inhibition of cancer cells, an area with unusual contact degree is expected to be a potential ROI.The current work verifies the accuracy of this method against the results of pathological diagnosis, based on the 50 WSI (whole slide image) colonic images provided by the Osaka International Cancer Center and University Hospitals Coventry & Warwickshire. Statistically, we obtained quite high accuracy results.In this presentation, we will explain the mathematical method and show the results. This system could be used to screen for colon cancer.

▲TOP

[P-1357] P15-11 [English/Japanese]
New biomarker (1)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

Masatoshi Inoue / Dept. Surg., Kindai Univ. Nara Hosp.

P-1357

Evaluation of serum ANGPTL2 level as a biomarker for pancreatic cancer associated with diabetes and inflammation

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Pancreatic cancer is one of the tumors with the worst prognosis. The number of patients suffering from pancreatic cancer in recent years has continued to increase dramatically. We focused on angiopoietin-like protein 2 (ANGPTL2), which has been reported to be related to chronic inflammation and Type 2 diabetes mellitus. In this study, whether ANGPTL2 can detect early pancreatic cancer was evaluated. It was found that the concentration of serum ANGPTL2 was significantly higher in pancreatic cancer patients and tumor stage 0-I patients than in healthy individuals ($P = 0.010$). In addition, the diagnostic capability of serum ANGPTL2 levels for pancreatic cancer was evaluated using receiver operating characteristic (ROC) curve analysis. The area under the ROC curve for ANGPTL2 was 0.906 ($P < 0.001$). In addition, serum ANGPTL2 levels were strongly correlated with inflammatory markers. In conclusion, this study suggested that an elevated serum ANGPTL2 level has the potential to be a biomarker capable of early detection of pancreatic cancer, and it was correlated with inflammation of the pancreas and the risk of developing diabetes mellitus.

▲TOP

P-1358

Relationship between preoperative serum IL-6/VEGF and pTNM Stage in 668 colorectal cancer patients

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Background: High serum IL-6 and VEGF has been reported as worse prognostic factors in colorectal cancer. However, relationship between them and TNM Stage at the period of surgery remains unclear. Methods: Preoperative serum IL-6 and/or VEGF were measured in 668 colorectal cancer patients who underwent surgery between 2009 and 2016 in our institution (IL-6 was measured in 666 and VEGF was measured in 653 patients). Their values were retrospectively compared among each pTNM factors and Stages. Results: Median IL-6 significantly increased in proportion to T factors except for among Tis/T1/T2, was higher in N1 and N2 than in N0, and was higher in M1 than in M0. In contrast, there were no differences in VEGF among all TNM factors. IL-6 generally increased in proportion to the Stage, however, it was the highest in Stage IIC. VEGF was the highest in Stage IIB and significantly higher than in Stage I/IIA/ IIIA/IIIB/IIIC/IV. Conclusions: Preoperative IL-6 was related to each pTNM factors at surgery while VEGF was not. Both markers were generally related to pStage but were higher in Stage II than in Stage III and IV. Stronger association between tumor depth and IL6/VEGF was suggested.

P-1359

The effect of primary tumor heterogeneity on circulating tumor DNA detection in colorectal cancer patients

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To verify the effect of tumor heterogeneity in regard to circulating tumor DNA (ctDNA), multiregional (three sites) sequencing of 14 primary colorectal tumors using a 151-gene ClearSeq panel followed by the ctDNA detection using digital PCR (dPCR) was performed. Among 76 mutations in 14 tumors, clonal mutations, common in the three regions, were observed in 78.6% (11/14); whereas all tumors exhibited at least one subclonal mutation. Mutant allele frequencies (MAF) of the clonal mutations were significantly higher than those of subclonal mutations ($p < 0.01$; 32.7% vs 20.9%). In preoperative patient plasma, ctDNA was detected in 85.7 % (12/14) of patients with the mean MAF of 2.1%. ctDNA was more frequently detected with clonal mutations than that of subclonal mutations ($p < 0.05$; 78.2% vs 50%). Of note, a tumor-unique mutation from one of the two tumors in a single patient with liver metastasis was detected in plasma. Proteomic analysis using reverse-phase protein array showed that the tumor with the mutation exhibited more aggressive cell proliferating patterns. These results suggest that clonal mutations with high MAF in a primary tumor are suitable for ctDNA analysis using dPCR.

P-1360

Exosomal microRNA profiles in peritoneal fluids as a therapeutic biomarker for peritoneal metastasis of gastric cancer

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Background: Peritoneal metastasis (PM) frequently occurs in patients with gastric cancer. Intraperitoneal chemotherapy (IPC) using taxane has demonstrated a remarkable clinical efficacy. In this study, we examined exosomal miRNA profiles derived from peritoneal fluids and explored possible biomarkers useful for IPC. Methods: Peritoneal fluids were collected from 2 groups; with or without PM. Total RNA including small RNA was extracted from exosomal fractions and expression analyses of miRNAs were performed using RT-PCR. Results: We comprehensively analyzed the expression of miRNAs between PM and without PM and identified 11 miRNAs which showed particularly different expression pattern between the 2 groups. Among them, 4 miRNAs (miR-21-5p, miR-223-3p, miR-342-3p and miR-92a-3p) were selected and further analyzed. Expression of these 4 miRNAs were significantly up-regulated in samples with PM which was consistent with former analysis. Conclusion: We identified several dysregulated exsosomal miRNAs, which were supposed to reflect the tumor burden on peritoneum. Exosomal miRNA profiles might be useful biomarkers to determine the presence of PM as well as the response to IPC.

▲TOP

P-1361

Anti-FIR δ exon2, a splicing variant of PUF60, antibodies are detected in the sera of gastrointestinal cancers patients

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(ABSTRACT) Anti-PUF60 autoantibodies are reported to be detected in the sera of dermatomyositis and Sjogren's syndrome but little is known whether it is detected in the sera of cancer patients. PUF60 is identical with FIR that is a transcriptional repressor of c-myc gene. In colorectal cancers, a splicing variant of FIR that lacks exon2 (FIR Δ exon2) is overexpressed as a dominant negative form of FIR. In this study, to reveal the presence and the significance of anti-FIRs (FIR/FIR Δ exon2) antibodies in cancers were explored in the sera of colorectal cancer patients. Anti-FIRs antibodies were surely detected in the preoperative sera of 28/87 colorectal cancer patients (32.2% of positive rates), and the detection rate was significantly higher than that in healthy subjects sera. Next, Alpha-LISA assay was performed in the sera of various cancers patients to confirm the results. Anti-FIR Δ exon2 antibodies were more sensitive than anti-FIR antibodies. The ROC analysis was carried out to evaluate the facility of these markers. ROC increases AUC. Therefore, the combination of antibodies with anti-p53 antibody and CEA is a potential approach for the diagnosis of digestive organ cancers.

P-1362

Exosomal expression analysis of serum pancreatic cancer miRNA markers

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Since the pancreas is surrounded by many adjacent organs, pancreatic cancer is not only difficult to detect early but also has adverse properties such as a lack of subjective symptoms, very rapid progression, and metastasis to other organs. Recently, circulating miRNAs have been demonstrated to have diagnostic potential as blood biomarkers for various tumors. In this study, we analyzed whether these serum markers for pancreatic cancer were present in a form of exosomes. From three pancreatic cancer cells (hTERT-HPNE, PANC-1, MIA PaCa2), exosomes and cultured supernatant were extracted, and comprehensive miRNA expression analysis was performed using microarray. Although many of these miRNA markers for pancreatic cancer were found both in intra- and extra-exosomes, those that were relatively enriched in exosomes were identified. The result indicates that some of these biomarkers are likely encapsulated in exosomes while circulate in peripheral blood. In the future, we will conduct functional verification and aim to elucidate the molecular mechanism of serum miRNA markers.

P-1363

Identification of a novel diagnostic antigen on circulating exosomes, toward sensitive early detection of colon cancer

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Early detection of colorectal cancer (CRC) is essential for improvement of prognosis by enabling therapeutic intervention at early stage. Recently, it has been shown that exosomes could have potential to be served as biomarker carriers in any body fluids. To explore CRC-specific antigens on EVs, we isolated exosomes from viable CRC or adjacent normal tissues, followed by global quantitative proteome analysis. Among 6,149 identified exosomal proteins, 393 proteins were significantly overexpressed ($p < 0.05$ and fold-change > 4.0) in CRC exosomes compared to those from normal mucosa. We especially focused on transmembrane protein TMAM ($p = 3.62 \times 10^{-5}$, fold change = 7.0) which was known to be a key regulator of cell growth and also overexpressed in CRC cells. Exosome sandwich ELISA confirmed significant elevation of plasma exosomal TMAM (Exo-TMAM) level even in stage-I CRC patients compared to normal donors. Interestingly, $TMAM^{++}$-exosomes decoyed its inhibitory ligand away from cancer cells, resulting in their outgrowth. These results indicate that Exo-TMAM should have great potential as a novel target for CRC diagnosis and therapy.

▲TOP

[P-1343] P15-8 [English/Japanese]
Diagnostic imaging (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

Hirofumi Hanaoka / Gunma Univ. Grad. Sch. Med.

P-1343

MRI based indication for lateral dissection for locally advanced rectal cancer treated with neoadjuvant chemotherapy

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Background: The problem of locally advanced lower rectal cancer is the control of distant metastasis and we are conducting preoperative chemotherapy with a strong regimen to ME + LPLND, a standard treatment for Japan, but regarding the positioning of LPLND in NAC cases There is no report.Objective: To consider the selection criteria of LPLND of NAC example.Patients and Methods: The subjects underwent ME + LPLND after NAC for Stage II / III lower rectal cancer. The relationship between lymph node diameter and pathological metastasis positive in MRI before and after NAC was retrospectively analyzed.Results: There were 56 subjects and 14 patients with pathological LPLN positivity. The short diameter was 4.3 / 3.2 mm before and after NAC, and the long diameter was 6/4 mm. In both cases there was a significant difference in diameter with or without pathological metastasis. The maximum AUC was obtained when the cutoff was 7.6 mm on the minor axis before NAC in the LPLN transition predicted ROC curve.Conclusion: There is a possibility of omitting LPLND after conducting NAC with strong regimen in case of negative metastasis based on short diameter before treatment.

▲TOP

P-1344

Development of a noninvasive imaging technique to detect the expression of CD73 mediating immunosuppression in cancer

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CD73 plays a crucial role in adenosine-mediated immune tolerance in cancer, therefore, anti-CD73 therapy is expected to be a next-generation immune checkpoint therapy. CD73 expresses in cancer and stromal cells but not in all patients, and the expression levels are variable in patients and lesions. Monitoring CD73 status in patients is important for optimizing regimens, and noninvasive imaging with radiolabeled anti-CD73 antibody can provide helpful information. We recently developed a new fully human anti-CD73 antibody with high affinity. In this study, the utility of the antibody with a gamma-emitting radionuclide In-111 was evaluated as a molecular imaging probe in nude mice bearing tumors, MIAPaCa-2 (CD73 high) and A431 (CD73 low). The biodistribution study showed significantly higher uptake of 111In-anti-CD73 antibody in MIAPaCa-2 tumors than in A431 tumors. The SPECT/CT imaging visualized the difference of CD73 expression between MIAPaCa-2 and A431 tumors. Our data suggest that the radiolabeled anti-CD73 antibody could be a promising molecular imaging probe to select patients appropriate for CD73-targeted therapy.

P-1345

Development of survivin-responsive fluorescent probes for cancer imaging

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Survivin is highly expressed in most human cancers, making it a promising target for cancer diagnosis. In this study, we developed several peptide probes composed of BOR-11, a high affinity survivin binding peptide, and peptide fragment of survivin (PFS) through peptide linkers as survivin-responsive fluorescent probes (SRFP). All conjugates were attached fluorescein (FAM) at C-terminal as a fluorophore and DABCYL at N-terminal as a quencher. We expected that the FRET quenching via intramolecular binding of BOR-11 with PFS could be diminished by the approach of survivin to SRFPs that dissociate the binding of BOR-11 with PFS and extend the distance between FAM and DABCYL. The binding assay using recombinant human survivin protein (rSurvivin) demonstrated the moderate to high affinity of SRFPs to survivin (K_D = 121-1740 nM). Under baseline conditions, the SRFPs had almost no fluorescence. SRFPs (0.5 μM) was treated with rSurvivin (0.1-2.0 μM), demonstrated dose-dependent increase in fluorescence intensity. A proline-rich SRFP showed the highest 2.7-fold fluorescence induction at 2.0 μM of survivin. Taken together, SRFPs could be used for survivin-responsive fluorescence imaging.

P-1346

Novel human anti-mesothelin scFv for cancer targeted therapy

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Mesothelin (MSLN) is a GPI-anchored differentiation-associated glycoprotein. MSLN is strongly expressed in various cancer cells, such as ovarian cancer, gastric cancer, pancreatic cancer, and mesotheliomas.In the present study, we established a single-chain fragment variable (scFv) from a human gene sequence. First of all, 120 clones were selected by ELISA from naive phage libraries derived from human tonsil lymphocytes. Fifteen phage scFv clones with a different gene sequence were selected for their high affinity for MSLN. In addition, 6 clones were confirmed to have high reactivity with the living cancer cell line by FACS. The humanized scFv was obtained by expressing the scFv gene synthesized from the gene sequence of the antibody V_L domain and V_H domain in CHO cells. The humanized scFv was analyzed with FACS and two MSLN-specific scFvs were established. PET imaging was performed on xenografted MSLN expressing cancer cells using ^{89}Zr-labeled humanized scFv to clearly visualize the tumor.This novel scFv is thought to be useful as a drug for targeted therapy.

▲TOP

P-1347

Management of incidentally diagnosed occult tumors by FDG- PET/CT as preoperative examination for primary lung cancer

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Typically, fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) is used as a preoperative examination for primary lung cancer. Although FDG-PET/CT rarely detects secondary tumors incidentally, the clinical significance of these tumors remains unclear. Between April 2010 and August 2017, 227 patients underwent surgery for primary lung cancer at Katsushika Medical Center; of these, we retrospectively examined 189 patients who acquired preoperative PET/CT. Extrathoracic incidental abnormal FDG uptakes on PET/CT were observed in 59 patients (31%); of these, 13 required other modalities for further assessment and 7 (3.7%) determined indications for treatments (colon polyp, 5; colon cancer, 1; thyroid cancer, 1). Five patients with colon polyp underwent endoscopic resection as a curative treatment, and those with thyroid and colon cancer underwent surgery first because their cancer was more advanced than primary lung cancer. This study infers that preoperative FDG-PET/CT could be a potentially useful modality to diagnose latent secondary tumors. Nonetheless, further investigation is warranted to elucidate the clinical significance ofthese tumors.

▲TOP

[P-1353] P15-10 [English/Japanese]
Pathological diagnosis

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

Daisuke Matsubara / Dept. Path., Jichi Med. Univ.

P-1353

Content-based histopathological image retrieval system for various cancer types

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Abundant accumulation of digital histopathological images has led to the increased demand for content-based image retrieval (CBIR) in pathology for educational or research purposes. However, there are no publicly available CBIR systems in digital pathology. We developed a novel web-based CBIR system, which enables pathologists, students, and researchers to retrieve cancer cases with histopathological images similar to those of the query image. The cases stored in the system are obtained from The Cancer Genome Atlas with rich clinical, pathological, and molecular information. We tested the system by querying typical cancerous regions from four cancer types, and confirmed successful retrieval of relevant images with both applications. This system will enable students, pathologists, and researchers to retrieve histopathological images of various cancer types similar to those of the query image.

▲TOP

P-1354

Vessel-derived muscular cushions at the peripheral zone of renal cell carcinomas

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Objectives: We have been engaged in morphology of human tumor capsule (Nakayama H, Enzan H, et al. Mod pathol 1999, J Clin Pathol 2002 and 2004). To elucidate a relationship between tumor capsular stromal cells and vessel walls, we examined vessel wall-related muscular cushions (VMC) at the peripheral zone of renal cell carcinomas (Sapino A, et al. Mod Pathol 1999;12:879-84). Materials and methods: A total of 24 surgically resected renal cell carcinoma and their surrounding non-neoplastic renal tissues were used; 17 with clear cell renal cell carcinomas (ccRCC), three with type 2 papillary renal cell carcinomas (pRCC), and four with chromophobe renal cell carcinomas (chRCC). We detected VMC by evaluating HE and EVG stain sections, and immunostaining for high molecular weight caldesmon and alpha-smooth muscle actin. Results: VMC were detected at the peripheral zone of 21 renal cell carcinomas; fifteen of 17 ccRCC, two of three pRCC, and four of four chRCC. Conclusion: Most of the renal cell carcinomas had VMC at the peripheral zone of renal cell carcinomas. There is a possibility that VMC are associated with tumor capsular smooth muscle cells in renal cell carcinomas.

P-1355

The association between VEGFA expression and intratumoral microvessel density in human soft tissue tumor

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Angiogenesis is recognized as an important aspect of tumorigenesis. Vascular endothelial growth factor A (VEGFA) is a key mediator of angiogenesis. Increased expression of VEGFA in a tumor cell is associated with poor prognosis in some cancers, but the mechanisms underlying the association remains elusive. In this study, we attempt to investigate the associations between the expression level of VEGFA protein and intratumoral microvessel density (IMD) in surgically resected undifferentiated pleomorphic sarcoma specimens. VEGF-A expression was investigated by immunohistochemical staining and the cytoplasmic staining of the gene was semiquantitatively scored for intensity on a scale of 0 to 3+. Quantitation of IMD was performed by counting 10 light microscopic fields at 200x of magnification, after the areas of highest microvessel density were identified at 40x magnification, and the average values for each field were calculated.

P-1356

Automated screening of breast cancer in liquid-based cytology using deep convolutional neural networks

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Diagnosis and treatment in breast cancer have been progressing and getting complex recently. Development of novel diagnostic technology is needed in breast cancer. In liquid based cytology (LBC), preparation of samples is more reproducible and inadequate samples decrease. However, diagnostic technique of LBC can be different from the one of traditional smear samples and the screener needs to have an abundant skill to reach high accuracy of diagnosis. Artificial intelligence generated by deep learning technology is an emerging research area in digital pathology. Deep convolutional neural networks (DCNNs) have proven to be very successful in detecting and classifying objects. Because LBC has thin layer of cells, indicating less cell overlapping, it could be suitable for computational image analysis. In this study, we investigated whether deep learning can be helpful for diagnosis of breast cancer in LBC. DCNNs were trained using whole slide images of LBC samples including malignancy or benign. The trained DCNNs showed high accuracy for diagnosis of malignancy. Deep learning-based computational approach can be beneficial for diagnosis of breast cancer with LBC.

▲TOP

[P-1369] P15-13 [English/Japanese]
New biomarker (3)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Takeshi Tomonaga / Lab. Proteome Res., Natl. Inst. Biomed. Innov. Health Nutl.

P-1369

Serum levels of soluble programmed death-ligand 1 in patients with metastatic melanoma

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抗PD-1抗体の効果予測バイオマーカーの確立が熱望されている。PD-L1の発現解析はその候補ではあるが、そのバイオマーカーとしての有用性は、がん種によって異なる。本研究では、可溶性PD-L1（sPD-L1）の血清中濃度を測定し、抗PD-1抗体で治療された患者において、バイオマーカーになりうるか、レトロスペクティブに解析した。治療前の血清sPD-L1濃度は、ステージⅠのメラノーマ患者において、in situ病変を有する患者や健常人コントロールに対して、有意に高値を示した。抗PD-1抗体で治療された患者に中で、レスポンダーとノンレスポンダーにおいて、治療前の血清sPD-L1濃度に有意差はなかった。一方、レスポンダーにおいてのみ、抗PD-1抗体で治療した前後で、血清sPD-L1濃度が有意に低下していた。腫瘍部のPD-L1染色が陽性だった患者と、陰性だった患者において、血清sPD-L1濃度を比較したところ、免疫染色が陽性だった患者の方が、有意に血清sPD-L1濃度は高かった。本研究の結果、血清sPD-L1濃度をモニタリングすることによって、抗PD-1抗体の効果を予測することができる可能性が示された。

▲TOP

P-1370

Investigation of origin of circulating free DNA: Are extracellular vesicles the carrier?

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The usefulness of circulating free DNA (cfDNA) for analysis of genetic alterations is widely accepted. We have reported there were large sized DNA fragments, around 5 Kb, which is longer than 170 bp of cfDNA conventionally detected in peripheral blood with advanced cancer patients. Extracellular vesicles (EVs) detected in peripheral blood has been reported to be involved in tumor progression through vesicle-mediated communication and EVs is considered as carrier of proteins, lipid and RNA, and DNA. The size of EVs-associated DNA is believed to have peak at approximately 6 Kb. To elucidate the origin of cfDNA in peripheral blood, we investigated relationship between cfDNA and EVs-associated DNA. We isolated both of cfDNA and EVs-associated DNA simultaneously, from patients' plasma with lung cancer, and compared the DNA yield and epidermal growth factor receptor (EGFR) mutation detection rate. The yield of EVs-associated DNA was about 29-44% of that of cfDNA. Despite a relatively small yield of DNA, EGFR mutations were detected from EVs-associated DNA as well as cfDNA. These results indicate EVs may be a carrier of tumor-derived DNA in the blood of patients with advanced cancer.

P-1371

Label-free identification of cells using quantitative phase microscope for negative selection of CTC

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Circulating tumor cells (CTCs) are used as a liquid biopsy target and most CTC detection technologies are based on their surface markers such as EpCAM. However, those positive selection methodologies appear to count only some CTCs because invasive tumor cells tend to change their surface markers during progression. Therefore eliminating WBCs from circulation is an efficient method to purify CTCs by negative selection. In this study, we developed a novel method for label-free image identification of live cells by computer vision technologies for pattern recognition based on the features of quantitative phase microscopy (QPM) images. First, we created an algorithm to differentiate WBCs from cell lines. The obtained algorithm successfully differentiated WBCs from cell lines. Next, we applied our image recognition methods to the cells flowing in the chamber, and properly differentiated WBCs from cell line cells (AUC=0.99). Finally, we applied this method to blood samples obtained from a healthy volunteer and a patient with advanced cancer, and identified CTC fractions which were much higher in blood of a cancer patient than that of a healthy volunteer.

P-1372

Magnetic Nanowire Networks for Ultrasensitive-Isolation and Detection of ctDNA

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Recent developments in genomic and molecular methods have revolutionized the range of utilities of tumor-associated circulating DNA in both basic and clinical research. Herein, we present a novel approach for ultrasensitive extraction of ctDNA at high yield and purity, via the formation of magnetic nanowire networks. We fabricated and characterized biotinylated cationic polyethylenimine conjugated magnetic polypyrrole NWs (PEI/mPpy NW). We applied these NWs to the extraction of ctDNA from the blood of 14 patients with lung cancer. We demonstrated reliable detection of EGFR mutations based on digital droplet PCR analysis of ctDNA from patients with lung cancer. The NW networks confined with a high density of magnetic nanoparticles exhibited superior saturation magnetization, which enabled rapid and high-yield capture whilst avoiding or minimizing damage and loss. The NW networks enabled the isolation of ctDNA of high quality and sufficient quantities, thus allowing the amplification of rare and low-prevalence cancer-related mutations. The simple, versatile, and highly efficient nanowire network tool allows sensitive and robust assessment of clinical samples.

▲TOP

P-1373

The validation of efficiency of ERO1 α as a novel endogenous marker of chronic hypoxia in human cancer cell lines

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Hypoxia is an important factor contributing to tumor aggressiveness and correlates with poor prognosis and resistance to conventional therapy. Therefore, it is useful to identify hypoxic environments within the tumors. Several studies have indicated CA9 as a reliable biomarker of hypoxia and a potential therapeutic target. Pimonidazole has also been identified as a hypoxia marker, with the pitfall of being an exogenous marker. Other reports, however, have suggested that CA9 is not directly induced by hypoxia and is not expressed in all cell lines. It is unquestionably relevant, if possible, to identify a marker that is both hypoxia-inducible and expressed in multiple cell lines. In this study, we focused on ERO1 α , an oxidase localized in the endoplasmic reticulum involved in the formation of disulfide bonds in proteins. Using in vitro and in vivo models, we found that ERO1 α expression is elevated under chronic hypoxia and our results indicate that ERO1 α , also detected in CA9-low cell lines, is a novel endogenous chronic hypoxia marker that is more reliable than CA9. Thus, ERO1 α can be used as a novel diagnostic biomarker and therapeutic target for cancer.

▲TOP

[P-1381] P17-2 [English]
Natural anticancer compounds (2) [English]

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Naohiro Nishida / Dept. Frontier Sci. for Cancer & Chemother., Osaka Univ.

P-1381
Withdrawn

No Abstract

▲TOP

P-1382

Distinct Anti-Cancer Activity of Genistein on HER2-Expressed and Highly Metastatic Breast Cancer Cells

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Genistein is a biologically active flavonoid that is found in high amounts in soy, which are widely known for their many benefits, such as anti-cancer. In this study, we evaluated the anti-cancer potential of Genistein on HER2-expressed and highly metastatic breast cancer cells. Under MTT assay, Genistein revealed cytotoxic effect on MCF-7/HER2 and 4T1 cells. Flow cytometry analysis demonstrated that Genistein 1 μM did not affect cell cycle progression, but interestingly Genistein 50 μM performed synergistic effect with Doxorubicin on G2/M arrest and apoptosis induction. DCFDA-staining assay revealed Genistein increased ROS level on MC-7/HER2 cells but decreased the ROS level on 4T1 cells. Genistein inhibited cells migration under scratch wound healing assay. Moreover, Gelatin Zymography and Western Blotting experiment revealed that Genistein inhibited the expression level of MMP-9, MMP-2 and Rac-1 proteins. Molecular docking result showed Genistein possibly interact with Akt proteins but not with GST and Glyoxalase. In conclusion, genistein exhibits anti-cancer activity on HER2-expressed and highly metastatic breast cancer cells with different mechanism.

P-1383

Nobiletin and 5-demethylnobiletin promote cell differentiation and exert anti-leukemic effects on human CML cells

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Chronic myeloid leukemia (CML) is a hematopoietic disease with a t(9;22) translocation resulting in the expression of the BCR-ABL fusion protein, a deregulated tyrosine kinase that activates several proliferative and anti-apoptotic signaling pathways. Nobiletin (NOB) and 5-demethylnobiletin (5-demethyl NOB), the citrus polymethoxyflavones, have been reported to possess the anti-oxidant, anti-inflammatory and anti-proliferative properties. In this study, we found that NOB and 5-demethyl NOB inhibited cell proliferation and increased expression of megakaryocytic differentiation markers including CD61, CD41 and CD42b on human CML cell line K562. Furthermore, we found that RAB27B, a Ras family protein related to platelet function, was upregulated in compounds-treated cells and probably involved in the megakaryocytic differentiation. Finally, we demonstrated that NOB and 5-demethyl NOB enhanced the imatinib-induced growth inhibition and synergized with imatinib administration in K562 cells. Our current findings provide a novel mechanism of NOB and 5-demethyl NOB as potential agents for differentiation therapy in CML.

P-1384

Discovering proteins for chemoprevention and chemotherapy by curcumin in liver fluke infection-induced bile duct cancer

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Curcumin exerts anti-inflammatory, anti- cholangiocarcinoma (CCA) and modulates protein activities. We performed proteomic approach to investigate the role of curcumin on protein dysregulation in CCA hamster model. Isobaric labelling and tandem mass spectrometry were performed to compare the protein expression profiles between the liver tissue from a hamster model of CCA and that with curcumin supplementation (CCA+Cur). These approaches were also extended to the liver tissue derived from cOpisthorchis viverrinifos(Ov)-infected hamsters and those with curcumin treatment (Ov+Cur). Among dysregulated proteins in CCA, 5 proteins including S100A6, lumican, plastin-2, 14-3-3 zeta/delta and vimentin, were upregulated in liver tissues of CCA hamsters but markedly downregulated by curcumin treatment (CCA+Cur). Similar fashion was also observed for plastin-2, 14-3-3 zeta/delta and vimentin expression in liver tissues of Ov-infected hamsters and curcumin-supplemented Ov-infected hamsters. Taken together, our results demonstrated the comprehensive proteomics change during Ov-induced CCA genesis and provide an insight into the possible protein targets for treatment of Ov-associated CCA.

▲TOP

P-1385

Cytotoxic effects of co–treatment of doxorubicin–curcumin and idarubicin–curcumin on KG1a and EoL–1 leukemic cell lines

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Acute myeloblastic leukemia is known as an aggressive blood cancer with high incidence to relapse due to drug resistance. To enhance efficacy of treatment and reduce toxicity of chemotherapeutic agents, this study aims to investigate effects of co–treatment with chemotherapeutic agents (doxorubicin and idarubicin) and curcumin on KG1a (leukemic stem cell or LSC) and EoL–1 (leukemic cell). Doxorubicin and idarubicin at various doses (IC_{10} – IC_{50} values) were cultured with low dose of curcumin at 1, 2, and 3 μg/mL and determined by MTT assay. Curcumin could increase cytotoxicity of both drugs in EoL–1 cells by dose dependent manner when compared to single drug treatment, while the drug–curcumin treatment exhibited cytotoxic effects on KG1a cell viability after increasing dose of curcumin to 4, 6, and 8 μg/mL. Thus, co–treatment could enhance efficacy of doxorubicin and idarubicin in leukemic stem cells and leukemic cells with different doses of curcumin. It may due to quiescent cell cycle status and drug efflux ability of LSCs.

P-1386

Antitumor effects of candidone, a natural flavanone derivative, in cholangiocarcinoma cells

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Cholangiocarcinoma (CCA), also known as biliary tract cancer, is the second most common hepatobiliary malignancy, with 5-year survival rates less than 20% and no curative chemotherapy as well as clinically-approved targeted therapies. For this reason, the therapeutic strategies need to be improved in order to overcome this fatal malignancy. Candidone is a flavanone derivative extracted from the fruit of Derris indica. Among many compound extracts from this plant, it is one of the most potent cytotoxic compounds against CCA cells. This study aims to evaluate antitumor effects of candidone on cell proliferation, apoptosis, and migration in CCA cells. To our findings, candidone exerted cytotoxic effect to CCA cells in a micromolar range. It suppressed CCA cell proliferation via upregulation of p21 expression. Moreover, the compound induced reactive oxygen species (ROS) generation and upregulated proapoptotic Bax expression in CCA cells, leading to induction of apoptotic cell death. These results suggest that candidone has potential clinical value for CCA treatment. The precise underlying antitumor mechanisms of candidone will be further elucidated.

▲TOP

[P-1393] P17-4 [English]
Anticancer drug resistance (1) [English]

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Yukinori Kurokawa / Dept. Gastroenterological Surg., Osaka Univ.

P-1393

The novel combination treatment of a HDAC inhibitor OBP-801 with eribulin for triple-negative breast cancer cells

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<Introduction> Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer. Eribulin has been frequently administered for metastatic breast cancer, and the efficacy of eribulin on TNBC was indicated. However, the prognosis of patients with TNBC is still poor. <Methods> To find a more effective treatment for TNBC, we investigated the synergistic effect of a novel HDAC inhibitor, OBP-801 and eribulin. The cell growth assay, the flow cytometry analysis, and Western blot analyses were conducted. <Results> The combination treatment of OBP-801 with eribulin showed the synergistic inhibition of cell growth in TNBC cells, involved with the enhancement of apoptosis. We, for the first time, found that eribulin up-regulated survivin, and also that OBP-801 could remarkably suppress the up-regulation of survivin by eribulin. Moreover, this combination potently suppressed Bcl-xL and the MAPK pathway compared with either single agent alone. <Conclusion> We found that the combination of OBP-801 and eribulin synergistically inhibited growth with apoptosis in TNBC cells, suggesting that this combination might be a promising novel strategy for treating TNBC patients.

▲TOP

P-1394

Adjuvant transarterial chemoembolization for patients with hepatocellular carcinoma involving microvascular invasion

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AbstractBackground: Microvascular invasion (MVI) has recently been reported to be an independent prognostic factor in patients with hepatocellular carcinoma (HCC). This study compared the outcomes of adjuvant transarterial chemoembolization (A-TACE) after hepatic resection (HR) in patients with HCC involving MVI.Methods: This prospective study involved 200 consecutive patients with MVI-HCC who underwent HR alone (n = 109) or HR with A-TACE (n = 91).The Kaplan-Meier method was used to compare disease-free survival (DFS) and overall survival (OS).Results: The two groups showed similar DFS at 1, 2, and 3 years (P = 0.077). The A-TACE group showed significantly higher OS than the HR-only group (P = 0.030). Subgroup analysis showed that A-TACE was associated with significantly higher DFS and OS among patients with a tumor diameter >5 cm or with multinodular tumors.Conclusions: A-TACE may improve postoperative outcomes for MVI-HCC patients, especially those with tumor diameter >5 cm or multinodular tumors.

P-1395

Role of SRPK1-modulated Alternative Splicing in Cisplatin Resistance in Breast Cancer Cells

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Cisplatin is a broadly used chemodrug that suppresses cancer growth. But, the acquisition of resistance by cancer cells is an inherent problem with the use of cisplatin. Hence, it is critical to elucidate the mechanism of chemoresistance to treat the cancers more effectively. In this study, we proposed that alteration in mRNA splicing landscape could be adopted by cancer cells to overcome cisplatin-induced cytotoxicity. We developed two different cisplatin-resistant breast cancer cell lines to compare the splicing profiling between sensitive and resistant cancer cells. In particular, SRSFs and their kinases SRPKs were found to be differentially expressed in response to cisplatin in sensitive and resistant cancer cells. Moreover, post-translational modifications, especially the acetylation of SRPK1 mediated by Tip60, was found to regulate cellular responses to cisplatin. The adaptive changes on acetylation of SRPK1 affects the RNA splicing such as BCL2L1 and MCL1 due to hyperactive SRSFs. Therefore, our study implies that SRPK1 and its relevant splicing regulation could be one of the key mechanisms that results in chemoresistance to cisplatin in breast cancer cells.

P-1396

Ursolic acid-induced autophagy reverses cisplatin resistance in gastric cancer via the PI3K/AKT/mTOR signaling pathway

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The prognosis of gastric cancer (GC) remains poor due to clinical drug resistance. Ursolic acid (UA), a pentacyclic triterpenoid which has been identified from a variety of fruits and herbs, was known to exhibit growth inhibition and used to combat cisplatin (CDDP) resistance in our laboratory on the SC-M1 cells, derived from a GC patient of Taiwan. Herein, UA exerted a specific cytotoxic effect on GC cells and CDDP-resistant GC cells in a concentration- and time-dependent manner. Combined delivery of UA and CDDP synergistically reduced cell proliferation and induced apoptosis in these cells by lowering COX-2, PCNA and Bcl-2 expression and by increasing the cleavage of PARP1, ICAD and lamins. The combined treatment induced apoptosis in a caspase-dependent manner, which might be related to the further depression of the PI3K/Akt/mTOR signaling pathway. Additionally, UA in combination with CDDP enhanced autophagy via the upregulated LC3II and downregulated p62. Moreover, cells were arrested at both S and G2/M phase by the upregulated p27 and downregulated CDK2. We identified that UA reverses cisplatin-resistance in SC-M1 cells through the PI3K/AKT/mTOR signaling pathway.

▲TOP

P-1397

Ectopic ATP synthase blockade overcomes gefitinib-resistance via CK2 α /TOP2A/GAS5 axis

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Gefitinib, an EGFR tyrosine kinase inhibitor, is commonly used as first-line treatment for lung cancer patients. However, some patients eventually become resistant to gefitinib and develop progressive disease. Here, we indicate that ecto-ATP synthase with the property of facing out-side the cell was considered as a potential therapeutic target for drug-resistant cells. Integrative analysis of quantitative phosphoproteomics and transcriptomics reveals that ecto-ATP synthase blockade evaluates cytotoxic effect via mediating cell cycle arrest and apoptosis. Moreover, topoisomerase II alpha (TOP2A) which was phosphorylated by CK2 α at serine 1106 plays a critical role in the cell death network. We show that TOP2A phosphorylation causes the double-strand DNA breakdown and further increases the expression level of long non-coding RNA GAS5 (lncRNA GAS5). Additionally, we also found that an important regulator of programmed cell death, p53 pathway, activates by lncRNA GAS5. Taken together, our findings suggest that ecto-ATP synthase blockade is an effective therapeutic strategy via regulation of CK2 α /TOP2A/GAS5 axis in drug-resistant lung cancer cells.

P-1398

Antipsychotic chlorpromazine suppresses YAP signaling and stemness properties in breast cancer cells

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The major obstacle in current cancer therapy is the existence of cancer stem cells (CSCs) which makes drug resistance and contributes to metastasis and relapse. Identification of reliable biomarkers is necessary for drug development and cancer treatment. In this study, we identified that the antipsychotic chlorpromazine (CPZ) exhibited potent anti-breast cancer and CSCs capability. Treatment with CPZ suppressed stemness properties including mammosphere formation, aldehyde dehydrogenase activity, and stemness-related genes expression in breast cancer and CSCs. Besides, CPZ increased susceptibility when combined with chemotherapies in MCF7 cells and drug resistant MCF7/ADR cells. Mechanistically, we identified that CPZ suppressed YAP through modulating the Hippo pathway. Elevated expression of YAP was further identified to be crucial for stemness-related genes expression, and was related with the invasiveness and stem-like signatures in breast cancer. Moreover, overexpression of YAP confers poor outcome of basal-like breast cancer particularly. Our data shows YAP as a promising therapeutic target for breast CSCs and the potential of repurposing drug in breast cancer treatment.

▲TOP

[P-1405] P17-6 [English/Japanese]
Anticancer drug resistance (3)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Yoshihiro Torimoto / Oncology Ctr. Asahikawa Med. Univ. Hosp.

P-1405

Lovastatin reduced viability of cisplatin-resistant cells by rapidly expressing KLF2, KLF6 and RHOB

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Statins, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase used to prevent hypercholesterolemia, have been reported to have antitumor activity in several cancers. In this study, cell viability of lovastatin was examined with cisplatin-resistant HCP4 cells and PCDP5 cells. HCP4 and PCDP5 cells were 13 times and 7 times more sensitive to lovastatin in comparison with parent cells, respectively. Lovastatin induced apoptosis of HCP4 cells more rapidly than parent cells, as assessed by flow cytometry and Western blotting analysis. To elucidate the mechanism of decreased survival rate against lovastatin, cDNA microarray analysis was performed and identified only three genes, KLF2, KLF6, and RHOB, were commonly induced more than 2-fold by lovastatin in HCP4 and PCDP5 cells. These mRNAs were strongly induced by lovastatin with transcriptional regulation in HCP4 cells. Consistent with transcription, protein expression of RHOB was also induced by lovastatin. Induction of these genes was associated with cell cycle arrest and apoptosis. These results suggest that statins may overcome cisplatin resistance as single agent therapy.

▲TOP

P-1406

Aripiprazole, an Antipsychotic and Partial Dopamine Agonist, Inhibits Cancer Stem Cells and Reverses Chemoresistance

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Background: There is a growing interest in repurposing antipsychotic dopamine antagonists for cancer treatment. However, antipsychotics are often associated with an increased risk of fatal events. The anticancer activities of aripiprazole, a partial dopamine agonist and an antipsychotic drug with an excellent safety profile, remain unknown. Materials and Methods: The effects of aripiprazole alone or in combination with chemotherapeutic agents on the growth, sphere forming ability, and stem cell/differentiation/chemoresistance marker expression of cancer stem cells, serum-cultured cancer cells from which they were derived, and normal cells were examined. Results: At concentrations non-toxic to normal cells, aripiprazole inhibited the growth of serum-cultured cancer cells and cancer stem cells. Furthermore, aripiprazole induced differentiation and inhibited sphere formation and stem cell marker expression of cancer stem cells while inhibiting their surviving expression, sensitizing them to gemcitabine, 5-fluorouracil, and cisplatin. Conclusion: Repurposing aripiprazole as an anti-cancer stem cell drug may merit further consideration.

P-1407

Aberrant activation of MET signaling induces acquired resistance to osimertinib in EGFR-TKI naïve NSCLC cells

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Osimertinib, a third-generation EGFR-TKI, is used to treat patients with NSCLC harboring EGFR T790M mutation. Although majority of patients respond to osimertinib, acquired resistances develop with several resistance mechanisms such as EGFR C797 mutation and alternative kinase activation (MET or HER2 amplification). Osimertinib also prolonged PFS in patients with treatment-naïve NSCLC with mutant EGFR, and development of acquired resistances has been reported. However, those resistant mechanisms have not been fully understood. In this study we generated osimertinib resistant cell clones of HCC827 which harbors EGFR activating mutation, and characterized those clones. One of the clones possessed MET activation with MET gene amplification. Treatment with MET inhibitor, TAS-115, or knockdown of MET could restore the sensitivity to osimertinib by suppression of EGFR/MET/HER3 signaling. These results suggest that MET activation is one of the resistance mechanisms to osimertinib in EGFR T790M-negative background and combination therapy of osimertinib with MET inhibitors is a potential strategy to treat first-line osimertinib resistant NSCLC patients with MET gene amplification.

P-1408

Oxphos inhibition downregulates p38 MAPK and mTOR activation in AML cells

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Acute myeloid leukemia (AML) cells frequently adapt to increased energy demand in the bone marrow microenvironment. Recent studies demonstrated that AML cells are highly dependent on oxidative phosphorylation (OxPhos) for survival. We investigate the molecular mechanisms of anti-leukemia efficacy of the OxPhos inhibitor (OxPhosi), utilizing OxPhosi-sensitive and -resistant primary AML samples and cell lines. First, we performed Cap Analysis of Gene Expression (CAGE), and detected mitochondrial metabolism related 4 genes that showed consistently higher expression in OxPhosi-resistant cells than in -sensitive cells. The IPA bioinformatics highlighted that mTOR, MAPK, Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2), erythropoietin (EPO), and IL3 were consistently activated in OxPhosi-resistant cells than in -sensitive cells. By immunoblotting, OxPhosi downregulated p-p38 MAPK and mTOR target p-S6 and p-4EBP1 more prominently in OxPhosi-sensitive cells than -resistant cells. These results indicate that kinase activation affects sensitivity to Oxphos inhibition, possibly through regulation of general cellular metabolic capacity.

▲TOP

P-1409

Bcl-2 inhibitor venetoclax augments cytotoxicity of cytarabine and clofarabine in drug-resistant leukemic cells in vitro

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Relapsed/refractory acute myeloid leukemia (AML) patients do not respond well to conventional salvage chemotherapies. We demonstrated the overexpression of antiapoptic protein bcl-2 in HL-60 AML cell lines resistant to nucleoside analogs in previous studies. The present study evaluated the cytotoxic effect of a novel bcl-2 inhibitor venetoclax in the cytarabine-resistant HL-60 variant, HL-60/ara-C, and the clofarabine-resistant HL-60 variant, HL-60/CAFdA20, in vitro. These variants showed bcl-2 overexpression. The addition of venetoclax augmented the growth inhibitory effects of cytarabine and clofarabine in these resistant variants as well as the parental HL-60. The lC_{50} values of cytarabine and cytarabine + venetoclax (minimally toxic concentration) were 0.7 μM and 1.1 μM, respectively in HL-60, and 6.9 μM and 2.3 μM, respectively in HL-60/ara-C. The lC_{50} values of clofarabine and clofarabine + venetoclax were 0.06 μM and 0.07 μM, respectively in HL-60, and 0.26 μM and 0.12 μM, respectively in HL-60/CAFdA20. The combination index revealed synergy in HL-60/ara-C and HL-60/CAFdA20.Thus, the inhibition of bcl-2 can be a promising strategy to treat relapsed/refractory AML.

P-1410

Olaparib resistant cells are sensitive to other PARP inhibitor, veliparib and rucaparib

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Recently, various PARP inhibitors (PARPis) have been developed, and olaparib, a PARP1/2 inhibitor is clinically available for maintenance treatment with platinum-sensitive recurrent ovarian cancer in Japan. In this study, we aimed to identify sensitivity factors of PARPi. We have isolated three olaparib-resistant cell lines (ola-R cl.3, cl.10, cl.15) from human ovarian cancer A2780 cells. These resistant cells showed 17-30-fold higher resistance to olaparib than the parental cells. In the resistant cells, PARP1 expression was down-regulated without the alteration of PARP2 expression, and cellular content of poly-ADP-ribosylated proteins in the resistant cells were lower than the parental cells. On the other hand, the resistant cells did not show resistance to other PARPi, veliparib and rucaparib. A2780 cells treated with PARP1 siRNA showed 3-4-fold higher resistance to olaparib, but not to veliparib and rucaparib. Our results suggest that PARP1 expression is one of the sensitivity factors of olaparib, but not of veliparib and rucaparib.

▲TOP

[P-1417] P17-8 [English]
Mechanism of action and resistance of anticancer drugs (1) [English]

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Ken Kato / Natl. Cancer Ctr. Hosp.

P-1417

Enhanced toxicity of gemcitabine by CD44-targeting curcumin-grafted hyaluronic acid nanoparticles for pancreatic cancer

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Pancreatic cancer (PACA) is one of the deadliest cancers due to poor prognosis and treatment. To improve therapy, CD44, hyaluronic acid (HA)-binding receptors, overexpressing on PACA cells can be used as a target ligand for specific delivery of anticancer drugs. In this study, the synergistic effect of gemcitabine (GEM) and curcumin (CUR) has been investigated for enhanced efficacy of cancer treatment. For this purpose, we developed nanoparticles based on polymer-drug conjugates for dual delivery of GEM and CUR to improve specificity and efficacy of GEM for PACA treatment. CUR-grafted HA was linked with GEM via pH-sensitive linker and assembled to nanoparticles. The drug release of GEM and CUR was found to be specific and fast in acidic microenvironment while retarded at physiological pH. In vitro cytotoxicity study in PANC-1 cell lines revealed enhanced toxicity of the nanoparticles with IC50 of 2-fold lower than free drugs. Their toxicity was decreased after CD44 blockade treatment compared to untreated cells suggesting CD44 specific delivery of GEM. Therefore, the developed polymer-drug conjugates potentially improve efficiency of GEM which would be beneficial for PACA therapy.

▲TOP

P-1418

Growth Suppression of Human Colorectal Cancer Cells with Mutated KRAS by 3-deaza-cytarabine in 3D Floating Culture

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Background/Aim: During screening for compounds which selectively suppresses growth of human colorectal cancer (CRC) spheroids with mutant (mt) KRAS, the uridine analogue, 5-bromouridine (BrUrd) was identified and its derivatives explored. Materials and Methods: DNA incorporation in two-dimensional (2D) and three-dimensional floating (3DF) culture was compared with uridine analogue, 5-ethynyl-2'-deoxyuridine (EdU). The area of HKe3 CRC spheroids expressing wild type (wt) KRAS (HKe3-wtKRAS) and mtKRAS (HKe3-mtKRAS) were measured in 3DF culture with 11 BrUrd derivatives. Results: EdU was strongly incorporated into newly synthesized DNA from HKe3-mtKRAS cells compared to HKe3-wtKRAS in 2D and 3DF culture. 3-deaza-cytarabine, which has properties of BrUrd and Cytidine, was the most effective inhibitor of HKe3-mtKRAS spheroids with the least toxicity to HKe3-wtKRAS. Growth suppression of 3-deaza-cytarabine was stronger than cytarabine in 2D culture, with toxicity was lower than gemcitabine in long-term 3DF culture. Conclusion: 3-deaza-cytarabine exhibits properties useful for the treatment of CRC patients with mtKRAS.

P-1419

Pentagamaboronon-0-sorbitol induces cell death and inhibits migration in two metastatic breast cancer cell lines

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Universitas Gadjah Mada, Indonesia synthesizes a new compound namely Pentagamaboronon (PGB)-0. A complex formation with sorbitol to increase its solubility is resulting PGB-0-sorbitol (PGB-0-So). The aim of this study was to investigate cytotoxic and antimetastatic activities of PGB-0-So toward metastatic breast cancer cell lines: 4T1 cells and HER2-overexpressed MCF-7 (MCF-7/HER2) cells. Boron distribution analysis using inductively coupled plasma showed that PGB-0-So was up taken by the cells greater than PGB-0. The IC_{50} values of PGB-0-So in 4T1 and MCF-7/HER2 were 39 and 35 μM, respectively. At the IC_{50}, PGB-0-So induced S-phase arrest and apoptosis in both cell lines. Intracellular reactive oxygen species level was increased after treatment with PGB-0-So in a dose dependent manner. Scratch wound-healing assay demonstrated inhibitory effects in the cell migration through the inhibition of matrix metalloproteinase-9 activities in 4T1 cells or the suppression of lamellipodia formation in MCF-7/HER2 cells. Taken together, PGB-0-So is potential to be developed as a boron-carrying chemotherapeutic agent for metastatic breast cancer cells.

P-1420

The in vivo study of antibody-drug conjugates against mouse tissue factor

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Tissue factor (TF), a 47-kDa transmembrane glycoprotein and an initiation factor of extrinsic blood coagulation, has been reported as a promising target for cancer therapy. The upregulation of TF-expression has been observed in various cancer cells as well as cancer stromal cells. We have already developed the antibody-drug conjugates (ADCs) against TF.
In the present study, we have been investigating the effects of anti-mouse TF ADC in mouse pancreatic cancer orthotopic models. We proposed that this study would reflect the clinical use of anti-TF ADC because anti-mouse TF ADC could recognize both TF-positive cancer cells and cancer stromal cells. To evaluate the effects of ADCs, we developed the orthotopic model of mouse pancreatic cancer cells derived from genetically engineered mice (LSL-$Kras^{G12D/+}$; LSL-$Trp53^{R172H/+}$; Ptf1a-Cre), which could form abundant stromal regions in tumor tissues. The in vivo study indicated that anti-mouse ADC could significantly prolong the survival time of the mice, compared with control ADC. Therefore, we concluded that anti-TF ADC would have a potential for cancer therapy.

▲TOP

P-1421

A Synthetic Cyclohexanone Curcumin Analog, BHMBC, Inhibits the Progression of Castration-Resistance Prostate Cancer

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The poor solubility and bioavailability of curcumin are the obstructions for its clinical use. Structural modification of curcumin is the one approach to solve the limitation. Our previous study showed that, 2,6-bis-(4-hydroxy-3-methoxy-benzylidene)-cyclohexanone (BHMBC) analog, exerted more potent multidrug resistance (MDR) reversing ability in human MDR cancer cells than curcumin. Therefore, we hypothesized that BHMBC could inhibit the progression of prostate cancer. In vitro studies showed that BHMBC significantly decreased proliferation and metastatic property of androgen-independent rat prostate cancer PLS10 cells. The in vivo pharmacokinetic study found that BHMBC could be detected in serum of mice at 1 hr after i.p. injection and the level was higher than that of curcumin. Moreover, BHMBC significantly decreased tumor growth in nude mice subcutaneously inoculated with PLS10 cells compared to the untreated group. Interestingly, the lung metastatic area of BHMBC-treated mice intravenously injected with PLS10 cells was significantly decreased compared to control group. Taken together, we could summarize that BHMBC shows promising ability to inhibit prostate cancer progression.

P-1422

Role of Mitochondrial Dysfunction in Acquired Resistance to Cisplatin in A549 cell-derived Cisplatin -resistant cells

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Background: Cisplatin-resistance is a major obstacle for effective cancer treatment, although this mechanism remains unclear. Cisplatin adducts to mitochondrial DNA (mtDNA), besides nuclear DNA, resulting in their damage. mtDNA damage leads to mitochondrial dysfunction. In this study, using human lung cancer A549 cell-derived cisplatin-resistant ACR20 cells, we examined whether mitochondrial dysfunction was involved in the acquirement of resistance to cisplatin. Methods: Expression of apoptosis-related proteins was examined by Western blot. Mitochondrial dysfunction was evaluated by mitochondrial oxygen consumption rate (OCR) and cytosolic reactive oxygen species (ROS) production. Results: Cisplatin induced caspase-3 cleavage in A549 cells but not in ACR20 cells. compared with A549 cells, expression of inhibitor of apoptosis proteins, phosphorylation of NF- κ B, and the number of ROS-positive cells were increased, while OCR was decreased in ACR20 cells. Conclusion: These results suggest that increased ROS production caused by mitochondrial dysfunction plays a role in acquired resistance to cisplatin.

P-1423

Nanomachine to deliver positively charged anticancer peptidic drug using crosslinked and pH responsive platform

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Cancer cells develop resistance to chemotherapeutics via mechanisms that deactivate or transport the drugs out of the cells before they can exert their effect, rendering them ineffective. Particularly, membranolytic anticancer peptidic drugs may unlock a new way towards future cancer therapy by solving that issue. But, this enthusiasm for peptide therapeutics was subsequently tempered by certain limitations of peptides, such as short plasma half-life and limited bioavailability. Nanomachine may can offer us a solution to solve those issues. Here, we synthesized nanomachine between positively charged anticancer peptide drug and triblock polymer where triblock was specifically designed to introduce stealth property, crosslink ability and to make the polymer negative charge for complexation (with positively charged peptide drug) respectively. We concluded that stable nanomachines are formed when concentration as well as mixing ratio of polymer and peptide drug are high. The nanomachine also disintegrate under acidic environment and release drug. Finally, we use the developed nanomachine for delivering anticancer peptide drugs to display its efficacy against different cancer cells.

▲TOP

[P-1429] P17-10 [English/Japanese]
Drug delivery system (1)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

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P-1429

Chemoprevention of liver cancer using nanoparticles with erlotinib on cell type-specific manner

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BackgroundHepatic myofibroblasts promote liver fibrosis that results in cirrhosis and development of liver cancer. Here we propose a strategy of DDS using molecular targeted agent to the effector cell type specifically. MethodsIn vitro, we made platelet-derived growth factor receptor-beta (PDGFRB) -targeting nanoparticles with erlotinib. First, we confirmed cellular uptake differences between in PDGFRB-expressing human myofibroblast cell lines, TWNT-4 and LX-2, and hepatoma cell line, HepG2, which lacks PDGFRB expression. Then we tested the chemo preventative effects in a rat model. ResultsIn vitro, the nanoparticles showed distinctly higher association with TWNT-4 and LX-2 cells compared to HepG2 cells.In vivo, with systemic administration of erlotinib, tumor burden was reduced to 31%, which was further improved to 21% by myofibroblast-targeted delivery even with 17% less total cumulative administered dose of erlotinib and less hepatocyte apoptosis. ConclusionOur strategy could facilitate chemoprevention of liver cancer, and contribute to substantial improvement of the dismal mortality.

▲TOP

P-1430

Identification of novel cell specific DDS peptides for prostate cancer by in vivo phage biopanning

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(Background) Prostate cancer may eventually result in castration resistance in most metastatic cancer patients. Various drugs are used to prolong the survival, but they fail in complete remission by appearance of systemic adverse reactions. (Purpose) To overcome the limitations of those current therapies, here we generate a cell specific drug delivery system (DDS) using in vivo phage biopanning.(Methods) Before the screening of homing peptides to prostate cancer cells, we have selected a population of phages including non-binding peptides to normal tissues in C57BL/6 mice and Cynomolgus monkey. Then we performed in vivo phage biopanning in SCID mice xenograft tumor model using the LNCaP cell lines to identify the specific peptides targeting cancer cells.(Results) By the phage biopanning, we identified three candidate peptides. These peptides were confirmed to react specifically with prostate cancer cell line by analysis of binding affinity to mouse organ. (Conclusion) We identified novel cell specific DDS peptides for prostate cancer by in vivo phage biopanning.

P-1431

Development of a tumor-penetrable drug carrier in response to tumor microenvironment

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Delivery of anti-cancer drugs into tumor cores comprised of malignant cancer cells can result in potent therapeutic effects. However, conventional nanoparticle-based drug carriers often exhibit inefficient tumor-penetrating properties. In this study, we focus on the interactions between cancer cells and the extracellular matrix (ECM), and demonstrate that liposomal drug carriers modified with slightly acidic pH-sensitive peptide (SAPSp-lipo) can penetrate tumor tissue and spheroids. We previously reported SAPSp-lipo, tumor microenvironment-sensitive liposomes, are effectively delivered to tumor tissue. Compared with conventional liposomes, SAPSp-lipo could be delivered to deeper regions within both spheroids and tumor tissues. Furthermore, tumor penetration was promoted at regions where actin depolymerization was induced by SAPSp-lipo. In addition, SAPSp-lipo attenuated the interaction between cancer cells and ECM, contributing to the penetration of SAPSp-lipo. These results suggest that SAPSp-lipo penetrates tumors via the interspace route and is accompanied by actin depolymerization. Taken together, SAPSp-lipo demonstrates potential as a novel tumor-penetrable drug carrier.

P-1432

Preclinical evaluation of Antibody/Drug-Conjugated Micelle with anti-tissue factor antibody

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Tissue factor (TF), an initiator in the extrinsic pathway of blood coagulation, is overexpressed in various cancers and known to cause the abnormal blood coagulation in patients with cancer. Furthermore, TF expression has been shown to be correlated with a poor prognosis in patients with cancer. We succeeded in producing a rat/human chimeric anti-TF IgG, clone 1849.Anticancer agent-incorporating micelles have a property of the passive targeting based on the enhanced permeability and retention effect. To enhance antitumor activity of the nanoparticles, we have developed Antibody/Drug-Conjugated Micelle (ADCM). The micelle is chemically conjugated with the anti-TF monoclonal antibody (anti-TF ADCM).In the study, we preclinically evaluated the anti-TF ADCM and also compared the antitumor activity with that of anti-human epidermal growth factor 2 (HER2) antibody conjugated ADCM (anti-HER2 ADCM) to reveal a difference in the characteristics between the ADCMs.

▲TOP

[P-1364] P15-12 [English/Japanese]
New biomarker (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

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P-1364

Identification of Circulating Exosomal Marker in Synovial Sarcoma

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Introduction:Although diagnosis based on fusion gene has been established, there is no useful blood-based marker in synovial sarcoma (SS). We investigated the exosomal surface marker to establish a new technology for liquid biopsy.Methods:Exosomes were purified by ultracentrifugation and size exclusion chromatography from culture media of human SS cell lines and SS patient serum samples. Global protein analysis of exosomes was performed by LC/MS, then we validated them and selected candidates by western blot (WB) and ELISA.Results:The exosomes derived from SS cell lines expressed CD81. The diameter was 30-100 nm by SEM and the peak size was approximately 100 nm by Nanosight. In proteomic analysis, we identified eight highly expressed candidate protein enriched in the exosomes, which distinguished those from control normal cells and healthy individuals. We identified one candidate marker by ELISA, which was closely associated with tumor status during multi-disciplinary treatment. Furthermore, expression of the marker was observed in all SS cell lines and exosomes.Conclusion:We identified a promising exosomal marker of SS, which was closely associated tumor status of patients.

▲TOP

P-1365

SNP (-617G>A) in ARE-like loci of the NRF2 gene: A new biomarker for prognosis of breast cancer

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Purpose: Accumulating evidence suggests nuclear factor (erythroid-derived 2)-like 2 (NRF2) plays an important role as cellular defense, tumor suppression, and oncogenesis. However, little information is available as to the genetic polymorphisms of the NRF2 gene and their clinical relevance. We aimed to validate the NRF2 gene as a prognostic biomarker in breast cancer.Experimental Design: We developed the rapid genetic testing method for detection of a SNP (-617C>A) in the ARE-like loci of the human NRF2 and CYP2A6*4 (whole gene deletion) genes that are involved in antioxidant effect.Results: Among a total of 107 breast cancer patients, whole deletion of CYP2A6 gene was detected in 4 (3.7%). We detected homozygous (A/A) and heterozygous (C/A) alleles with the SNP (c.-617C>A) in 6 (6.5%) and 42 (39.2%) patients, respectively. Multivariate logistic regression models including standard breast cancer factors revealed that the homozygous alleles (c.-617A/A) were significantly related to histological grade, CYP2A6 and lymph node metastasis. Conclusion: Our findings suggest that the polymorphism of human NRF2 and CYP2A6 genes provide a prognostic indicator in breast cancer.

P-1366

MicroRNAs, isomiRs and tRFs are promising biomarkers as liquid biopsy for breast cancer detection

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Breast cancer is one of the most common cancers in Japan. Small RNAs, such as microRNAs, isoforms of microRNAs (isomiRs) or tRNA-derived fragments have been discovered to be related to cancers. Small RNAs are known to be released into the peripheral blood from various cells. We investigated whether serum small RNAs can be used as biomarkers for breast cancer detection. Breast cancer patients (BC group) and cancer-free individuals (Control group) participated in this study. Small RNAs were extracted from serum using miRNeasy mini kit (Qiagen, CA). Small RNA profiles of each group were analyzed using next generation sequencer Ion Torrent S5 (Thermo Fisher Scientific, Waltham, MA, USA) and compared. The differential expression of some small RNAs, including isomiRs and tRFs in serum, was identified between breast cancer patients and control. Some small RNAs expressions were significantly up-regulated in BC group, which could validate with another independent cohort. The receiver operating characteristic curve was generated for validated small RNAs. The combination of isomiRs and tRFs was recognized as promising biomarkers for breast cancer screening.

P-1367

Novel urinary biomarker for Xp11.2 translocation renal cell carcinoma

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Xp11.2 translocation renal cell carcinoma (tRCC) is a group of kidney cancer caused by translocations involving a breakpoint at Xp11.2, which contains the TFE3 gene, forms a complex with various genes and leads to overexpression. It indicates poor prognosis and highly occurs pediatric.To establish a minimally invasive novel biomarker for the disease, we comprehensively analyzed the exosomal-miRNA contained in the urine of tRCC model mouse, and ascertained a significant increase in some miRNAs. The candidate miRNAs can be detected in any stage of tRCC model mouse, from 10-week-old in the pre-cancer state to 40-week-old in the terminal. In order to investigate whether the expression of candidate miRNAs confirmed in urine are altered in tumor cells, we isolated primary cancer cell (PCC) from the tRCC model mouse. The same alteration of miRNA expression was observed in the PCCs and its culture medium with high specificity. We also confirmed similar miRNA expression in human tubular cell lines conditionally over-expressing PRCC-TFE3. This study submits the potential of the miRNA released into the urine by overexpression of TFE3 as a novel urinary biomarker for Xp11.2 tRCC.

▲TOP

P-1368

Patients with nuclear expression of ERK5 had poor prognosis in renal cell carcionoma

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Objective. We have previously demonstrated that clear cell renal cell carcinoma (ccRCC) cell lines overexpressed ERK5 via disfunction of VHL and miR-143. Furthermore, inhibition of ERK5 suppresses anti-apoptotic proteins and cell viability, which causes apoptosis in ccRCC cell lines in vitro and in vivo. The purpose of this study is to examine the correlation between ERK5 expression and prognosis in ccRCC patients. Method. Primary tumor tissue from 248 ccRCC patients were immunohistochemically stained for ERK5 and estimated cytosolic and nuclear expression. The correlation between ERK5 expression and stage of pT, cN, or M or Fuhrman grade were estimated using Fisher exactly test. The cause-specific survival (CSS) was estimated by Kaplan-Meyer method and assessed by log-rank test. Result. Cytosolic and nuclear ERK5 expression was shown in 193 (77.8%) and 69 (27.8%) specimens. Cytosolic expression was statistically correlated with pT and Fuhrman grade, but not with cN and M stage. Nuclear expression was not correlated with these factors. However, nuclear expression related to worse CSS ($p = 0.0171$). Conclusion. Nuclear ERK5 expression related to worse CSS in RCC patients.

▲TOP

[P-1374] P17-1 [English]
Natural anticancer compounds (1) [English]

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

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P-1374

Antitumor effect of Nobiletin, a polymethoxylated flavonoid, against human colon cancer cells

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Flavonoids are phytochemicals found in fruits, vegetables, and teas. It has been reported that flavonoids show the effects of antioxidant, anti-inflammatory and anti-angiogenesis in several diseases. Nobiletin is a major polymethoxylated flavone in several citrus species, particularly in the peels of Shiikuwasha (*C. depressa*) and sweet orange (*C. sinensis*). Previous papers showed that nobiletin inhibited cell proliferation in a part of prostate and breast cancer cells. However, the mechanism is not well understood. In this study, it was aimed to investigate the effect of nobiletin in human colorectal carcinoma cell lines (HT-29, DLD-1, LS147T, SW480, and SW620). Cell viability was analyzed with WST-8 assays. Nobiletin decreased cell viability in four cell lines tested except SW480, with greater reduction in viability in HT-29 and DLD-1 cells. Nobiletin inhibited cell survival and colony growth in both cells. Flow cytometoric analysis demonstrated that nobiletin significantly induced Annexin V-positive apoptotic cancer cells. These results indicate that treatment with nobiletin efficiently induces the antitumor effect including growth inhibition of human colorectal cancer cells.

▲TOP

P-1375

Ginsenoside Rg5 induces apoptosis by activating two apoptotic pathways in human esophageal cancer cells

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We have recently found that Ginsenoside Rg5 (G-Rg5), a trace compound of red ginseng induces human esophageal cancer cell apoptosis. Under G-Rg5 treatment, Fas, an important membrane death receptors are remarkably upregulated. Moreover, the increases of Fas expression was temporally coincided with increases in Caspase-8 activity in ECA-109 cells upon G-Rg5 treatment. These results indicated that G-Rg5-triggered extrinsic apoptosis might rely on Fas over-expression. In the intrinsic apoptotic pathway, G-Rg5 induced a strong and immediate translocation of cytosolic Bax and Bak to the mitochondria, mitochondrial cytochrome c and Smac release, and subsequent caspase-9 activation. Fas expression and subsequent downstream caspase-8 activation as well as caspase-9 activation all contributed to the activation of the downstream effector caspase-3/-7, leading to ECA-109 cell death. Taken together, we suggest that G-Rg5 induces esophageal cancer cell apoptosis in a multi-path manner and therefore it might be a promising candidate for developing a drug for treatment with esophageal cancers.

P-1376

The anti-proliferative effect of CAPE on docetaxel-resistant prostate cancer cells

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The majority of patients with prostate cancer (PCa) receiving androgen ablation therapy will relapse castration-resistant prostate cancer (CRPC) within 1-3 years. Docetaxel is a FDA-approved chemotherapeutic drug for CRPC, however, PCa patient receiving docetaxel develop drug-resistant phenotype after a few months. Caffeic acid phenethyl ester (CAPE) is the main component for honey bee propolis. We previously showed that CAPE can suppresses growth of PCa tumors. In this study, we explored if CAPE can suppress docetaxel-resistant PCa cells. We observed that CAPE dose-dependently suppressed proliferation of docetaxel-resistant PC-3 cells, as well as growth of this docetaxel-resistant PC-3 xenograt in nude mice. We found that CAPE treatment promoted apoptosis-related proteins and thus induced apoptosis in docetaxel-resistant PC-3 cells. In conclusion, CAPE is an potential treatment for docetaxel-resistant PCa.

P-1377

Functional analysis of cancer stem cells inhibitor, catechol from aronia through lactic acid bacteria fermentation

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This study isolated and investigated a new cancer stem cell (CSC) inhibitor derived from lactic acid fermentation products using culture broth with 2% aronia juice. Activity-guided fractionation and repeated chromatographic preparation led to the isolation of one compound. Using nuclear magnetic resonance and ESI mass spectrometry, we identified the isolated compound as catechol. In this study, we report that aronia-fermented catechol has a novel inhibitory effect on human breast CSCs. Catechol inhibited breast cancer cell proliferation and mammosphere formation. This compound reduced the CD44high/CD24low subpopulation, ALDH-expressing cell population and the self-renewal-related genes. Catechol preferentially reduced mRNA transcript and protein levels of Stat3. Catechol inhibited Stat3 signaling by reducing Stat3 expression and the levels of secreted IL-6, a CSC survival factor. These findings support the novel utilization of catechol for breast cancer therapy via Stat3/IL-6 signaling

▲TOP

P-1378

Triterpene acid from aronia inhibits mammosphere formation of breast cancer through downregulation of c-Myc protein

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To investigate the effect of 3-O-p-coumaroyltormentic acid from aronia extracts on mammosphere formation of breast cancer cells and underlying mechanism. Mammosphere formation inhibition assay-guided fractionation and repeated chromatographic preparation over silica gel, preparatory thin layer chromatography, and HPLC using aronia extracts, lead to the isolation of one compound. Using 1H, 13C, 2-dimensional nuclear magnetic resonance, and ESI mass spectroscopy, the isolated compound is identified as 3-O-p-coumaroyltormentic acid. This compound inhibits breast cancer cells proliferation and mammosphere formation in dose-dependent manner. This compound reduces CD44high/CD24low subpopulation and ALDH expressing cell population and self-renewal-related genes, cd44, sox2, and oct4. 3-O-p-coumaroyltormentic acid induced c-Myc degradation and did not reduced mRNA transcripts of c-Myc. 3-O-p-coumaroyltormentic acid inhibits cancer stem cells (CSCs) formation through reducing level expression of c-Myc protein, CSC-survival factor. These findings support the novel utilization of 3-O-p-coumaroyltormentic acid for breast cancer therapy via c-Myc regulation.

P-1379

Rooibos Suppresses the Proliferation of Human Castration-Resistant Prostate Cancer Cells

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Androgen-deprivation therapy (ADT) is the standard treatment for metastatic PCa. Although it is very effective to induce tumor regression, the majority of PCa patients receiving ADT will develop CRPC within 1-3 years. Currently, there is no effective treatment for CRPC. We investigated if rooibos exhibits anti-cancer effect on CRPC. We used LNCaP 104-R1 (R1), a human AR-rich androgen-independent PCa cells, to mimic the clinical situation of CRPC. We demonstrated that rooibos treatment suppressed the proliferation of R1 cells, as determined by MTT and Hoechst dye-based proliferation assay. Flow cytometry analysis indicated that rooibos treatment increases cell population in sub-G1 and G2/M phase but decreases in S phase. Gavage of rooibos reduced tumor growth of R1 xenografts in nude mice experiments. Micro-Western Array revealed that treatment with rooibos decreased expression of proteins including Akt, Bcl-2, but increased protein expression of cytochrome C. Conventional Western blotting suggested that rooibos treatment increased cytochrome C, LC3, and activated caspase 3 expression. In conclusion, rooibos maybe an alternative therapy for CRPC.

P-1380

a novel tubulin targeted agent from natural substances

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Red wine, which consisted of various powerful antioxidants such as flavonoids and stilbenes including resveratrol, have been believed to be implicated in cancer prevention without obvious side effects. We found that a resveratrol analog, (E)-4-(3,5-dimethoxystyryl) phenyl acetate (CMPD1), could lead to cell cycle arrest at the M-phase. This cell cycle arrest occurred in the CMPD1 dose dependent manner and more than the threshold value of CMPD1 could induce cell death in cancer cells. CMPD1 was reported as a mitogen-activated protein kinase-activated protein kinase 2a. We found MK2 located on the tubulin in mitosis. We are developing CMPD as a novel tubulin targeted agent from natural substances and will prove the efficacy of red wine including resveratrol in tumor bearing patients.

▲TOP

[P-1387] P17-3 [Japanese]
Natural anticancer compounds (3)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Takehiro Noda / Dept. Gastroenterol. Surg., Osaka Univ.

P-1387

The anticancer activity of coffee diterpens kahweol and cafestol in prostate cancer

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【Background】Coffee diterpens, kahweol (KWO) and cafestol (CFO) have been reported to have anti-tumor effects on several cancers. We studied the effects of coffee components including KWO and CFO on human prostate cell lines.【Methods】The effects of KWO and CFO on the proliferation and migration of human prostate cell lines (PC-3, LNCaP, DU145, LNCaP-SF) were examined.【Results】KWO and CFO inhibited cell proliferation of human prostate cancer cells in a dose-dependent manner. Also, KWO and CFO inhibited cell migration of human prostate cancer cells. The combination of KWO 30 μM and CFO 30 μM showed a synergistic effect. In TUNEL assay, KWO and CFO induced apoptosis in a dose dependent manner. KWO and CFO inhibited the expression of Bcl-2 and Bcl-xl. KWO and CFO also stimulated the cleavage of caspase-3 and PARP. KWO and CFO inhibited the expression of twist, snail and slug. KWO and CFO inhibited the expression of AR and CCR2. On the other hand, caffeine have little anticancer activity for the prostate cancer.【Conclusions】Our study showed not Caffeine but KWO and CFO inhibited cell proliferation and migration. KWO and CFO may be exploited as chemotherapeutic drugs.

▲TOP

P-1388

Anti-oncogenic activity of a novel PDK4 inhibitor in bladder cancer by suppressing the H-Ras and cancer stemness

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The Warburg effect is a key metabolic hallmark of cancer cells. We found that cryptotanshinone (CPT), a natural compound extracted from the root of Salvia miltiorrhiza Bunge (Danshen), has potent activity to inhibit pyruvate dehydrogenase 4 (PDK4) which regulates the metabolic flux connecting glycolysis to the TCA cycle. PDK4 is an attractive target for cancer therapy by shifting glucose metabolism causing metabolic remodeling to inhibit tumor growth. In this study, we investigated anti-oncogenic effects of CPT for a bladder cancer cell line, T24. Low concentration of CPT (5-10 μM) significantly suppressed anchorage-independent growth, 3D-spheroid formation, and cell invasiveness of T24 cells. Rb phosphorylation and the expressions of cyclin D1, H-Ras, EpCAM and Oct4 were also suppressed by CPT. However, phosphorylations of Akt and Erk, were not affected by CPT treatment. Taken together, these results indicate that CPT is able to suppress the malignant phenotypes of human bladder cancer cells via the suppression of H-Ras expression and cancer stemness, suggesting that CPT could be a potential treatment of intractable human bladder cancer.

P-1389

Withdrawn

No Abstract

P-1390

The antitumor functions of the new polyethylene glycol derivative

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We have found recently that some of the polyethylene glycol derivatives (PEG-X), originally identified from bacterial extracts, strongly suppress the growth of tumor cells. In the present study, we conducted functional analyses of PEG-X to elucidate the mechanism of its anti-cancer effects. Our current data clearly demonstrated that PEG-X exhibit significant cytotoxicity on many types of tumor cells, regardless of their mutation status of oncogenes or tumor suppressor genes such as MYCN, P53. Cell cycle distribution analysis by using FACS revealed that PEG-X induced cell cycle arrest, but not cell death. When cells were treated with PEG-X in glucose depleted medium, however, dead cell population was notably increased. We also found that amount of intracellular ATP was significantly reduced by PEG-X treatment in glucose depleted condition, but not in normal culture condition. Global analysis of cell metabolite revealed that the amount of some TCA cycle metabolites, such as citric acid, 2-OG and succinic acid were reduced in PEG-X treated cells. These data indicate that PEG-X suppress cell viability by inhibiting energy producing pathway except glycolysis.

▲TOP

P-1391

Effects of EGCG on cellular differentiation in triple negative breast cancer cells

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Triple negative breast cancer (TNBC) lacks available targeted therapy and shows poor prognosis. In order to develop new strategies for treatments and preventions of TNBC, we investigated the effects of EGCG on differentiations of MDA-MB-231 cells. After treatments of EGCG for 14 days, expression levels of retinoic acid receptor (RAR) β 2 (RARB2) mRNA were increased in a dose dependent manner. EGCG decreased DNA methylation levels in the promoter region of RARB, suggesting that specific CpG site might be involved in increased expression of RARB2. On the other hand, no differences were observed in histone modifications, such as AcH3K9, AcH3K27 and H3K27me3, in the promoter region of RARB between control and EGCG-treated cells. All-trans retinoic acid (ATRA), a specific ligand of RAR, reduced the sphere formation capacity of EGCG-treated cells, but not control cells. Furthermore, EGCG treatments enhanced the reduction in expression levels of stemness markers, such as NANOG, SOX2, and POU5F1 (OCT4), by ATRA. These results suggest that EGCG enhances differentiations of TNBC with ATRA treatments by upregulation of RAR β 2 via epigenetic regulations.

P-1392

Mechanism of apoptosis induced by siphonodictoyal B, a derivative of terpenoids in human colon cancer cells

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Terpenoids are natural products formed from five-carbon isoprene units. Previous reviews reported that some terpenoids exhibit cytotoxicity and antitumor efficacy against a variety of tumor cells in preclinical animal models and that liphagal, one of the terpenoids, has PI3K inhibitory activity. Recently, we reported that an analogue of liphagal, siphonodictyal B also has PI3K inhibitory activity. Furthermore, we evaluated its inhibitory activity on other kinases and found that siphonodictyal B inhibits multiple kinases such as CDK4/6, CDK7 and PIM2. In the present study, we evaluated cytotoxic activity of siphonodictyal B against the human colon cancer cell line, HCT116. Siphonodictyal B exhibited cytotoxic effect more potently than liphagal. Siphonodictyal B exhibited the increase in sub-G1 fraction in FACS analysis which is the evidence of apoptosis induction. As a mechanism of apoptosis, we have found siphonodictyal B activates the p38 MAPK pathway which leads to enhance pro-apoptotic factors. Moreover, p38 siRNA or its inhibitor attenuated apoptosis induction by siphonodictyal B. These results suggested that siphonodictyal B might become a seed for the novel anticancer drug.

▲TOP

[P-1399] P17-5 [English/Japanese]
Anticancer drug resistance (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Yasuo Saijo / Dept. Med. Oncol., Niigata Univ., Sch. Med.,

P-1399

Clinical course and outcome of R1 resection of extrahepatic cholangiocarcinoma

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Background: Aim of this study is to retrospectively review the characteristics of patients who had surgical margin positive resection.Method: 136 extrahepatic cholangiocarcinoma (ECC) patients undergoing resection in curative intent in 2000 - 2017 was enrolled in this study. Clinicopathological parameters, prognosis, and recurrence pattern were compared between two groups; surigical margin positive group (SM+, n=25) and negative group (n=111). SM+ was determined as submucosal invasion at the margin of biliary tract and/or positive excisional wedge.Result: Forcusing on the treatment on the recurrence, patients receiving the regimen containing gemcitabine and cisplatin (GC) showed significantly decreased tumor marker ($p = 0.046$) and better response rate ($p = 0.041$). SM+ was correlated with left hepatic resection ($p = 0.041$), flat-infiltrating gross morphology ($p = 0.002$), large tumor size ($p = 0.004$), and poor OS ($p = 0.028$). Conclusion: Chemotherapy including GC is effective for recurrent tumor and that SM+ patients showed worse prognosis than others in ECC. Adjuvant or neoadjuvant setting of the regimen including GC should be debated for patients with high risk for SM+ surgery.

▲TOP

P-1400

Correlation between in vivo and in vitro assessment of drug resistance overcoming phenomenon using 3D culture scaffold

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Intrinsic or acquired multidrug resistance (MDR) of cancer cells is one of the major obstacles in the chemotherapeutic treatment against solid tumor. It is important to develop a cell-based in vitro system that can reflect in vivo MDR characteristics of the cancer cells. In this study, we examined the drug resistance overcome phenomenon in vivo to clarify an efficacy of a 3D culture on in vitro assay for the screening of drug resistance overcoming agents. The drugs used in this study, were doxorubicin (DOX) and verapamil (VRP) as a medicine of drug resistance overcoming, which were confirmed by in vitro experiments using a silicate fiber scaffold (Cellbed). We also examined the therapeutic effects of the combination of DOX and VRP on xenograft model mouse of carcinoma after the inoculation of HepG2 cells. The combination of DOX and VRP resulted in a marked inhibition of tumor growth compared with animals treated with DOX or VRP alone. The data indicate that the drug resistance overcoming phenomenon was obtained in vivo. Therefore, the data suggest that the phenomenon of the overcoming drug resistance in vitro using Cellbed could reproduce in vivo using the model mouse of carcinoma.

P-1401

Possible role of TNIK in JQ1-resistant HCT 116 cells

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BRD4 is a member of the bromodomain and extra-terminal domain (BET) family involved in histone code reading and transcriptional regulation. The first bromodomain inhibitor, JQ1, inhibits BRD4 and reduces c-Myc expression. We have established JQ1-resistant cells by treating human colon cancer HCT 116 cells with increasing concentrations of JQ1 (1~2 micromole/L) over a period of 6 months. In the JQ1-resistant clones, expressions of several c-Myc and E2F-target genes were downregulated under normal growing medium. In contrast, JQ1-induced hypo-phosphorylation of RB was reduced and Cyclin E expression was increased in the JQ1-resistant clones. Interestingly, TNIK expression is high in the JQ1-resistant clones, and TNIK overexpression enhanced Cyclin E promoter activation by E2F. These observations suggest that TNIK-associated Cyclin E expression might be involved in cell cycle progression in JQ1-resistant HCT 116 cells.

P-1402

Hsp70 inhibitors suppress androgen receptor expression in LNCaP95 prostate cancer cells

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Androgen deprivation therapy is considered as the standard therapy for patients with advanced prostate cancer. However, tumors become resistant to therapy which is termed castration-resistant prostate cancer (CRPC). Although Heat shock protein 70 (Hsp70) inhibitor is known to decrease the level of full length androgen receptor (AR-FL), its effects on CRPC cells expressing androgen receptor splice variant 7 (AR-V7) remains unknown. We therefore investigated the effect of Hsp70 inhibitors on the LNCaP-derived CRPC cell line LNCaP95. VER155008 and quercetin decreased cell growth and survival with suppression of both AR-FL and AR-V7. We next identified Hsp70 binding proteins by proteomic analysis, resulting that Y-box binding protein 1 (YB-1) was identified as one of the molecules regulating AR-FL and AR-V7. VER155008 decreased the phospholylation of YB-1 and its translocation to the nucleus, indicating that the involvement of Hsp70 in AR regulation might be mediated through the activation of YB-1. Collectively, these results suggest that Hsp70 inhibitors have potential anti-tumor activity against CRPC by decreasing both AR-FL and AR-V7 expression through YB-1 suppression.

▲TOP

P-1403

Downregulation of Bim via activation of signal molecules plays a central role in adriamycin resistant-myeloma cells

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<Purpose> The acquisition of anti-cancer drug resistance is a major limitation of chemotherapy for multiple myeloma (MM) and it is thus important to identify the mechanisms by which MM cells develop such drug resistance. In a previous study, we showed that multidrug resistance (MDR) involves the overexpression of MDR1 and survivin in adriamycin-resistant RPMI8226/ADM cells. However, the underlying mechanism of MDR remains unclear. <Methods> Cell viability was assessed by the trypan blue dye method. Signal molecules were determined by western blots. <Results> We found that RPMI8226/ADM cells exhibit increased levels of activated ERK1/2, Akt, and NF-κB than adriamycin-susceptible counterparts. In addition, the inhibition of ERK1/2, Akt, or NF-κB by inhibitors reversed the drug-resistance of RPMI8226/ADM cells via the enhanced Bim expression. <Discussion> These results indicate that decreased Bim expression via the activation of ERK1/2, Akt, and NF-κB plays a critical role in adriamycin resistance in RPMI8226/ADM cells. Our findings suggest that ERK1/2, Akt, and NF-κB inhibitors are potentially useful as anti-MDR agents for the treatment of adriamycin-resistant MM.

P-1404

The mechanism of apoptosis induced by eribulin in paclitaxel-refractory gastic cancer cell line

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Eribulin demonstrate the survival benefit for the patients with paclitaxel-refractory breast cancer. Eribulin inhibits polymerization of microtubule, by contrast paclitaxel inhibits depolymerization of microtubule. This difference might contribute to the anti-tumor effect of eribulin in paclitaxel refractory cancer. However, we are unable to identify patients who are sensitive to eribulin, and precise mechanism of eribulin induced anti-tumor effect in paclitaxel-refractory cancer is not well understood. We treated gastric cancer cell lines, MKN28 (p53 mutant type) and MKN45 (p53 wild type), with paclitaxel and eribulin. After 24hr exposure of 1nM eribulin, cell cycle was arrested at G2/M phase both in MKN45 and MKN28. But 1nM paclitaxel could not induce cell cycle arrest at G2/M phase in MKN45. In addition, 72hr exposure of 1nM eribulin induced apoptosis in MKN28, but not in MKN45. These results suggested that eribulin is effective even in paclitaxel resistant gastric cancer, and that cytotoxic effect of anti-microtubule agents is independent of TP53 status.Now, we analyze the mechanism of eribulin-induced apoptosis in paclitaxel resistant gastric cancer cell line.

▲TOP

[P-1411] P17-7 [English/Japanese]
Anticancer drug resistance (4)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Jun Inoue / Dept. Mol. Cytogent., Med. Res. Inst., Tokyo Med. & Dent. Univ.

P-1411

Evaluation of ACAT1 expression in biliary tract cancer and its relation to gemcitabine resistance

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Gemcitabine (GEM)-based chemotherapy is a standard treatment for unresectable biliary tract cancers (BTC), however, no other first- or second-line chemotherapies have not been available yet. So it is important to improve the GEM resistance in BTC. Acyl-CoA cholesterol acyltransferase 1 (ACAT1) is the enzyme which esterify cholesterol into cholesteryl ester. Recently, it is reported that ACAT1 expression is correlated with malignancy and prognosis in various kinds of cancers. We investigated the ACAT1 expression in BTC and its relation to GEM resistance. ACAT1 expression was evaluated in 71 resected BTC specimens by immunohistochemistry. In 19 patients who underwent adjuvant chemotherapy with GEM, recurrence free survival of ACAT1 high group was significantly shorter than that of low group. In vitro, we established GEM-resistant (GR) BTC cells, and GR cells expressed ACAT1 much higher than parent cell line, evaluated by polymerase chain reaction and western blotting. We performed that ACAT1 inhibitor resensitized chemoresistance of GEM in GR cell lines by growth inhibitory assay. These results suggested that ACAT1 expression might be correlated with GEM resistance.

▲TOP

P-1412

Overcoming antiestrogen-resistance in breast cancer by targeting activated YB-1 phosphorylation pathway

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[Background] Despite great advances in breast cancer treatments by endocrine therapeutics, appearance of antiestrogen resistant tumors often limits their therapeutic efficacies. Elucidation of novel mechanisms underlying such antiestrogen-resistance will contribute to further improvement of therapeutics for breast cancer patients. Herein, our present findings demonstrate the critical role of YB-1, and also novel approach to overcome antiestrogen-resistance. [Results] [1] Fulvestrant-resistant breast cancer cells showed enhanced expression of phosphorylated YB-1, accompanied by decreased ER α expression, and also by activated mTOR/Akt/S6K signaling pathway. [2] Overexpression of constitutive active YB-1 mutant (YB-1 S102E) conferred cancer cells resistance to antiestrogens. [3] Treatment with everolimus, an mTORC1 inhibitor, induced marked suppression of YB-1 phosphorylation, and overcame antiestrogen-resistance in vitro and in vivo.[Conclusions] Our present study presents the specific targeting activated pathway of YB-1 can be useful to overcome antiestrogen-resistant breast cancer. (Collaborator: Maki Tanaka (Kurume General Hospital), Yoshito Akagi (Kurume University Hospital))

P-1413

Src family kinases activation is a compensatory survival mechanism for osimertinib resistance in lung cancer cells

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[Purpose] Osimertinib, which is an irreversible 3rd generation epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), has improved therapeutic efficacies of patients with NSCLC harboring EGFR T790M resistance mutation. However, the appearance of tumors resistant to osimertinib has already been reported. We explored novel mechanism for osimertinib resistance in lung cancer.
[Results] We established and characterized osimertinib-resistant cells derived from H1975 cells harboring activating EGFR mutation and T790M mutation. [1] Resistant cells showed decreased expression of EGFR, HER2, HER3 and c-Met as compared with H1975 cells; [2] Constitutive Akt phosphorylation was observed in resistant cells; [3] Treatment with Src family kinase (SFK) inhibitors including dasatinib inhibited cell survival and AKT phosphorylation in resistant cells.
[Conclusion] Our study presents that SFK activation could be a mechanism responsible for acquired resistance to osimertinib. We will further discuss if SFK signaling pathway is useful to overcome osimertinib resistance in lung cancer.Collaborator: Daiki Kusakabe (Kyushu University)

P-1414

Analyses of the mechanisms of the resistance to neratinib in breast cancer

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[Background] Neratinib is an irreversible pan-HER tyrosine-kinase inhibitor. Neratinib was approved by FDA as additional adjuvant therapy in patients with early-stage HER2-positive breast cancer who have finished at least 1 year of post-surgery trastuzumab therapy. However, drug resistance is a clinical issue in these molecular-targeting drugs. Therefore, the development of the resistance to neratinib is also inevitable.
[Methods] We established various kinds of neratinib-resistant cell lines from HER2-amplified cell lines BT-474 and SK-BR-3 with changing the drug-exposing condition. Then we analyzed the mechanisms of the resistance and explored the effective therapy for the resistance.
[Results] Yes1, which is one of the Src family, was amplified in two neratinib-resistant BT-474 sublines. STAT3 was activated in two neratinib-resistant SK-BR-3 sublines. Furthermore, ABCB1 expression was up-regulated in a neratinib-resistant SK-BR-3 subline. Combination therapy of neratinib and Yes1 inhibitor dasatinib was effective to the Yes1-amplified cells.
[Conclusion] Yes1, JAK/STAT pathway and ABCB1 may play an important role in the mechanism of neratinib-resistance in breast cancer.

▲TOP

P-1415

Suppression of REV7, the regulatory subunit of Pol ζ , sensitizes drug-resistant germ cell tumors to chemotherapy

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REV7 is a multitasking protein involved in cell cycle regulation, replication past DNA lesions, and DNA repair. We previously reported that REV7 is highly expressed in the adult testis and plays an essential role in maintenance of primordial germ cells in mice. In this study, we evaluated the expression of REV7 in testicular germ cell tumors (TGCTs) and examined the effect of REV7 depletion on cell proliferation and chemosensitivity in TGCT cells. Strong immunohistochemical expression of REV7 was detected in the nuclei of tumor cells in embryonal carcinoma as well as seminomas. Depletion of REV7 in TGCT cells decreased cell proliferation and enhanced sensitivity to cisplatin and doxorubicin. After cisplatin treatment, phosphorylation of H2AX and cleaved-PARP were more increased in REV7 knockdown cells. Additionally, CRISPR/Cas9 inactivation of REV7 in TGCT cells enhanced cisplatin sensitivity of cisplatin-resistant cells as well as cisplatin-sensitive cells. These results indicate that depletion of REV7 enhances sensitivity to cisplatin in TGCT, suggesting that REV7 is a potential candidate of molecular target for TGCT therapy.

P-1416

The mechanisms acquiring drug resistance through the exosome-mediated cell-cell interaction in pancreatic cancer

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Gemcitabine (GEM) is a key drug for pancreatic cancer (PC). The interaction among cancer cells will play a dominant manner in acquiring drug resistance, and the exosome is recently emerging as a cell-cell interaction tool. For investigating the candidate factor involving GEM resistance, we employed GEM-resistant cells. The function of exosome is evaluated with exposure on PC cells. To clarify whether the factor brings drug resistance to PC cells by itself or via exosome function, we inhibited exosome secretion by transducing siRAB27B. GEM resistant cells secrete significantly high number of exosome, and the exosome brings GEM resistance. The transcriptome analysis showed miR-155 was target gene, and miR-155 overexpression increases the number of exosome and leads GEM resistance. The exosome derived from the cells overexpressing miR-155 bring GEM-resistance, and siRAB27B transfection decreases the exosome secretion and ameliorates induction of GEM resistance. We checked the miR-155 expression in resected specimen, miR-155 expression was a significant prognostic factor. The present results suggested that miR-155 have responsible for exosome secretion inducing GEM resistance.

▲TOP

[P-1424] P17-9 [English/Japanese]
Mechanism of action and resistance of anticancer drugs (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Daisuke Sakai / Dept Frontier Science for Cancer and Chemotherapy, Osaka Univ.

P-1424

Studies of compound exerting synthetic lethality in β-catenin mutated tumor cells

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β-catenin is known as a component of Wnt signaling pathway and plays a main role in this cell proliferation signaling pathway. When the β-catenin gene, *CTNNB1*, is actively mutated, Wnt signaling is hyperactivated, leading to aberrant growth. Although β-catenin is mutated in a wide variety of tumors, including 10% of colon cancer, effective therapeutic drugs have not yet been developed. Recent years, synthetic lethality is reported as a promising strategy for tumor treatment because of targeting respective gene mutation, and expected less side-effect for normal tissue. So, we searched for the compound which exerts synthetic lethality in β-catenin mutated tumors, in other words, induces cell death selectively in β-catenin mutated tumor cells. As a result, we found that DS23280164 (DS23), which is provided by Daiichi-Sankyo Pharmaceutical Company, showed such activity. DS23 induced cell death in β-catenin hetero-mutated parent HCT116 *CTNNB1* +/S45del cells, however, failed to induce cell death in mutant allele knockout HCT116 *CTNNB1* +/- cells. This result indicates that DS23 can induce mutated β-catenin-dependent cell death.

▲TOP

P-1425

Lipid peroxide accumulation enhances iron-dependent cell death ferroptosis in cancer cells

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The specific iron-dependent cell death is induced by a number of small compounds referred to as ferroptosis-inducers (FINs). During ferroptosis, inactivation of the glutathione peroxidase 4 (GPX4) causes accumulation of lipid reactive oxygen species that leads to cell death. A recent report suggested that a therapy-resistant state of cancer cells is characterized by vulnerability to ferroptosis induced by GPX4 inhibition.I assessed the contribution of lipid peroxidation activity of lipoxygenases (LOXs) to ferroptosis in oncogenic RAS-expressing cancer cell lines. Both specific inhibitors and siRNA-mediated knockdown of ALOX15 significantly decreased FIN-induced cell death. Immunofluorescence analyses revealed that the ALOX15 protein constitutively localizes to the cell membrane during the course of ferroptosis. Importantly, treatments of cells with ALOX15-activating compounds accelerated cell death at low doses of FINs, indicating that the combined use of these drugs could be effective in cancer therapy. The present results suggest that tumor ferroptosis is promoted by LOX-catalyzed lipid hydroperoxide generation in cellular membranes.

P-1426

Potential use of cladribine, an antileukemic drug, for the treatment of carcinoma

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Cladribine, 2-chloro-2'-deoxyadenosine, is a nucleic acid antimetabolite exclusively used for the treatment of hairy cell- and B-cell chronic lymphocytic leukemias. Cladribine is shown to be incorporated into the cells by a membrane transporter ENT1 and activated by conversion to 2-chloro-2'-deoxyadenosine monophosphate by deoxycytidine kinase (dCK). Since dCK is predominantly expressed in blood cells, cladribine is not used for the treatment of carcinoma. Unexpectedly, however, anticancer fingerprint across the JFCR39 human carcinoma cell line panel revealed that five of them from various origins exhibited hypersensitivity to cladribine. In contrast, gemcitabine, a deoxycytidine analog, is widely used for the treatment of carcinoma despite that it is also incorporated by ENT1 and activated by dCK. In fact, anticancer fingerprint of gemcitabine revealed that more than half of the 39 cell lines exhibited good responses. The results suggest that cladribine could be used for the treatment of selective carcinoma cells. In this presentation, we will discuss the difference between cladribine and gemcitabine sensitivity and the potential biomarkers for predicting their efficacies.

P-1427

2-Deoxy-D-glucose increases GFAT1 phosphorylation resulting in ER-related apoptosis in pancreatic cancer cells

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The glycolytic inhibitor 2-deoxy-D-glucose (2DG) causes energy starvation, affecting cell viability in a wide range of cancer cell lines. But, the mechanisms underlying the tumor growth inhibition of 2DG are poorly understood. Here, to elucidate the molecular mechanisms of 2DG, we carried out a proteomic analysis of the 2DG-treated pancreatic cancer cell line MIAPaCa2. We found that up-regulation of glutamine: fructose 6-phosphate aminotransferase 1 (GFAT1), which belongs to the hexosamine biosynthesis pathway (HBP) that produces uridine diphosphate N-acetylglucosamine to maintain glycoprotein, was determined. Unexpectedly, we found a reduction of total N-glycoproteins, induction of endoplasmic reticulum (ER) stress and phosphorylation of GFAT1 by AMP-activated protein kinase (AMPK) in 2DG-treated cells. In addition, an AMPK activator metformin (Met) synergistically enhanced the reduction of protein N-glycosylation and cell growth inhibition in the presence of 2DG. In conclusion, 2DG reduces N-glycosylation of proteins following the increase of phosphorylation of GFAT1 and results in the inhibition of cell growth mediated by ER stress in pancreatic cancer cells.

▲TOP

P-1428

Inhibitory action of NFAT pathway by a potential anti-cancer agent MO2455

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Poly(ADP-ribose) glycohydrolase (PARG) is a major enzyme for poly(ADP-ribose) (PAR) degradation. Dysfunction of PARG in particular cancer cells leads to enhanced cell death after treatments with alkylating agents or γ-irradiation, suggesting that PARG is a potential target for cancer therapy. By the screening of PARG inhibitors and PAR accumulators in cancer cells from in-house chemical libraries and further structural optimizations, we identified MO2455 that causes PAR accumulation and cytotoxicity to various cancer cell lines. The sensitivity spectrum of MO2455 is different from that of conventional anti-cancer agents. To clarify the mechanism of anti-cancer effect caused by MO2455, we investigated the effects of MO2455 on signal transduction pathways by using pathway screening method. MO2455 inhibited the B cell receptor directed activation of transcription factor NFAT (nuclear factor of activated T cells). Some of derivatives of MO2455 weakly inhibited NFAT pathway, whereas PARP inhibitor olaparib did not. Details of the action mechanisms are under investigation. (the other co-author: Kenji Matsuno)

▲TOP

[P-1433] P17-11 [English/Japanese]
Drug delivery system (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Jun Fang / Dept. Microbiol. & Oncol., Faculty Pharm. Sci., Sojo Univ.

P-1433

Specific induction of cell death in cells with mtDNA mutation by PI polyamide conjugated with TPP

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[Aim] Pyrrole-imidazole polyamide (PIP) is a synthetic small-molecule compound with N-methylpyrrole and N-methyl imidazole as the basic chemical structure, binds to the minor groove of the target DNA sequence and can slow down the DNA replication. We have designed and synthesized PIP specifically targeting mitochondrial DNA mutation. On the other hand, triphenylphosphonium (TPP) cation could transport conjugated molecules into mitochondria. We therefore synthesized PIP-TPP conjugates (TPP-PIP) targeting mtDNA and examined whether it suppresses the growth of cells harboring mutated mitochondrial DNA (mtDNA). [Methods] HeLamt3243 cybrids having A3243G mutation and HeEB1 cybrids having wild-type mtDNA were used. Cell viability was examined by WST assay. [Results] The IC_{50} of TPP-PIP in HeLamt3243 (having 82% of A3243G) and HeLamt3243 (having 55% of A3243G) was 8μM and 15μM, respectively. TPP-PIP did not induce drastic cell death of HeEB1 cells at 20μM. TPP-PIP-treated HeLamt3243 cells showed mitochondrial aggregation. [Conclusion] These results suggested that TPP-PIP designed to target mtDNA mutation can induce cell death of cancer cells perhaps in a mtDNA mutation-selective manner.

▲TOP

P-1434

Effect of pyrrole-imidazole polyamide conjugated with TPP on mitochondrial DNA replication

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Alterations in mitochondrial function are increasingly recognized to play an important role in cancers. To regulate mutated mitochondrial function by pyrrole-imidazole polyamide (PIP) targeting mitochondrial DNA (mtDNA), we used a model of HeLa cybrids cells with MELAS A3243G mutation (HeLamt3243), where 82% of mtDNAs have A3243G mutation. Initially we synthesized FITC labeled PIP to examine their effect on the replication of the mutant mtDNA. High fluorescence intensity in mitochondria was detected at 24 h after the FITC-PIP treatment, however it was eliminated in the subsequent 48 h. Next, we synthesized the mitochondria-targeting triphenirphosphonium (TPP) cation-conjugated PIP (TPP-PIP) and found that TPP-PIP was delivered to the mitochondria co-localizing with cytochrome c, and retained for more than 96 h after treatment. When the cybrid having 55% of A3243G mutation was treated with TPP-PIP, the proportion of the mutant mtDNA tended to decrease depending on TPP-PIP concentration. Thus, TPP-PIP can be used for suppressing the replication of mutant mtDNA in HeLa cells and is considered to be applicable to treatment of mitochondrial mutation related diseases including cancer.

P-1435

Photodynamic therapy using indocyanine green loaded on super carbonate apatite as minimally invasive cancer treatment

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Minimally invasive treatment is getting more and more important in an aging society. The purpose of this study was to explore the possibility of ICG loaded on super carbonate apatite (sCA) nanoparticles as a novel photodynamic therapy (PDT) against cancers. Using colon cancer cells, ICG uptake and anti-tumor effects were examined between the treatments of ICG and sCA-ICG. The temperature increase after laser irradiation was 27.1_C and 23.1_C in sCA-ICG and ICG, respectively (control DW:5.7_C). A significant increase in ROS generation was noted in cell cultures treated with sCA-ICG plus irradiation compared with those treated with ICG plus irradiation ($P<0.01$). Uptake of ICG in the tumor cells significantly increased in sCA-ICG compared with ICG in vitro and in vivo. The fluorescence signals of ICG in the tumor, liver, and kidney faded away in both treatments by 24 hours. Finally, the HT29 tumors treated with sCA-ICG followed by irradiation exhibited drastic tumor growth retardation ($P<0.01$), whereas irradiation of tumors after injection of ICG did not inhibit tumor growth. This study shows that sCA is a useful vehicle for ICG-based PDT.

P-1436

Pronounced intratumor diffusion of HPMA copolymer conjugates of pirarubicin

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Recently we have synthesized the HPMA copolymer conjugates of pirarubicin, P-THP. We have shown that P-THP accumulated and released the free THP in the slightly acidic tumor tissue. However extent of difusion or penetration of P-THP in the tumor tissues has not been known. In this study, we have examined the intratumor diffusion of P-THP in vitro using cell spheroid. In monolayer culture system, free THP was rapidly uptaken by cells, and it also showed much higher cytotoxicity than that of P-THP. In cell spheroid system, though cytotoxicity of free THP was also higher than P-THP, its difference becomes only 2-3 times, compared to 10 times difference in monolayer culture system. In consistent with the cytotoxicity, free THP was only accumulated at the surface of cell spheroid in contrast to deeper penetration of P-THP inside the cell spheroid. Penetration of free THP was improved by cell uptake inhibitor, amiloride, indicating the rapid cell uptake disturbs the free THP diffusion in the tissue. These result indicate that in in vivo, P-THP accumulate in the tumor tissue by EPR effect followed by pronounced penetration and cell kill in the tumro tissue.

▲TOP

[P-1437] P17-12 [English/Japanese]
Synthetic anticancer compounds

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Tomoya Kishimoto / Dept. Surg., Yao Municipal Hosp.

P-1437

Effects of pyrrole-imidazole polyamides targeting human TGF-&beta1 on the malignant phenotypes of liver cancer cells

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[1]Nihon Univ. Sch. Med.、[2]Chiba Cancer Ctr. Res. Inst.

Synthetic pyrrole-imidazole (PI) polyamides bind to the minor groove of double-helical DNA with high affinity and specificity, and inhibit the transcription of corresponding genes. In liver cancer, TGF-β expression is correlated with tumor grade. In order to evaluate the effects of TGF-β1-trageting PI polyamide on the growth of liver cancer cells, we analyzed TGF-β1 expression level after the administration of GB1101, a PI polyamide that targets human TGF-β1 promoter, and examined its effects on cell proliferation. GB1101 dose-dependently inhibited HepG2 colony formation by reducing the level of TGF-β1 mRNA. Although GB1101 did not substantially inhibit the proliferation of HepG2 cells, GB1101 significantly suppressed the invasion of HLF cells, which displayed high expression of CD44, a marker for and cancer stem cells. Furthermore, GB1101 significantly inhibited HLF cell sphere formation by inhibiting TGF-β1 expression, in addition to suppressing the proliferation of HLE and HLF cells. Taken together, GB1101 reduced TGF-β1 expression in liver cancer cells and suppressed cell invasion; therefore, GB1101 is a novel candidate drug for the treatment of liver cancer.

▲TOP

P-1438

Can MTHFR C677T SNP affect anti-mesothelioma effect via ER stress?

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Malignant mesothelioma (MM) is difficult to cure with current treatments. Therefore, establishment of effective treatments are required. We considered that effective effects can be obtained by proposing a treatment method according to single nucleotide polymorphism (SNP). In this study, we focused on methylene tetrahydrofolate reductase (MTHFR) C677T SNP. It promotes accumulation of homocysteine (Hcy) and endoplasmic reticulum (ER) stress. However, excessive stress induces apoptosis and autophagy. Therefore, the difference in Hcy concentration due to the SNP may cause a difference in anti-MM effects. In our laboratory, it was revealed that the tocotrienol succinate ether derivative (T3E) induces ER stress, but the association with the SNP is unknown. We investigated the difference on the susceptibility of cell death in MM cells based on the SNP. We analyzed expression of mRNA by qRT-real time PCR in H28 and H2452 cells. T3E caused ER stress. However there was a difference in induction of ER stress between both cells. We suggested that the presence of SNP is resistant to cell death via ER stress. Whether autophagy is involved in the cell death is under investigation.

P-1439

Analysis of the mechanism of action of RCOP8154 that inhibits glucose-independent cancer metabolism

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Cancer cells have metabolic variability and flexibility to sustain their growth and survival. Targeting the metabolic differences between cancer and normal cells holds promise as a novel anticancer strategy. We have discovered that human osteosarcoma MG-63 cells can grow even without glucose. By cell-based screening of our NPDepo chemical library, we found that RCOP8154 selectively inhibits glucose-independent growth in MG-63 cells. Bioenergetic analysis using a Seahorse flux analyzer showed that RCOP8154 decreases oxygen consumption rate (OCR). In RCOP8154-treated cells, the intracellular ATP level was significantly decreased. In addition, we found that RCOP8154 is a compound with autofluorescence and that it is localized in mitochondria. In vitro enzyme assays using semi-intact cells and purified mitochondria showed that RCOP8154 does not inhibit any of mitochondrial complexes (I - V). These results suggest that RCOP8154 targets mitochondrial respiration without directly inhibiting mitochondrial complexes. We are currently trying to identify RCOP8154-binding protein using RCOP8154-immobilized beads.

P-1440

No Abstract

▲TOP

P-1441

G2/M phase arrest and apoptosis induced by novel phenyl compounds, CCL360 and CCL361

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Anticancer effects of novel phenyl compounds, CCL360 and CCL361, were investigated using HeLa and Vero cells. Fluorescence-activated cell sorting (FACS) analyses revealed that CCL360 and CCL361 caused increasing of cells in G2/M phase both for HeLa and Vero cells. Increased expressions of p53, p21, p-histone H3, and cyclin B1 in CCL360- and CCL361-treated cell lines were induced. The cleaved PARP was more remarkable in CCL360- and CCL361-treated HeLa cells than Vero cells. Further detailed analyses of the effects of CCL360 and CCL361 on the cell cycle by fluorescence ubiquitin cell cycle indicator (Fucci) system revealed that 29% and 33% of CCL360- and CCL361-treated HeLa cells respectively were arrested in the M phase followed by apoptosis, and that extensions of M phase were observed in 42% and 67% of HeLa cells treated with CCL360 and CCL361 cells respectively. In contrast, G2 phase extensions were observed in 97% and 98% of Vero cells treated with CCL360 and CCL361 respectively. In conclusion, CCL360 and CCL361 can be a seed of anticancer agents.

P-1442

A novel peptide designed from GAPDH suppresses gastric cancer cell growth by cell cycle arrest

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Co-author：Manabu Kawada
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Fibroblast-like stromal cells (stromal cells) modulate the growth of cancer cells both positively and negatively through secreted factors as well as proteins of the extracellular matrix. We reported that Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is secreted by gastric stromal cells, Hs738, and secreted GAPDH suppresses gastric cancer MKN-7 and MKN-74 cell growth. We investigated the suppression of MKN-7 cell growth by partially deleted GAPDH recombinant protein and found that its N-terminal region is important for anti-cancer activity of GAPDH. Based on this result, we analyzed the activity of shorter peptides designed by N-terminal region of GAPDH sequence. As a result, we determined that a peptide composed of 10 amino acids (peptide 10-3) is the minimum units of the activity. Additionally, we found that phosphorylation of cdc-2 of gastric cancer cells was decreased by peptide 10-3 treatment. It suggested that peptide 10-3 suppresses the growth of gastric cancer cells through G2/M arrest.

▲TOP

[P-1450] P18-1 [English/Japanese]
Chemosensitivity (1)

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Naoyuki Nishiya / Div. Integ. Info., Dept. Clin. Pharm., Iwate Med. Univ. Sch. Pharm.

P-1450

1-methylnicotinamide can be a potential metabolic marker for trifluridine resistance in colorectal cancer cell, DLD-1

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Trifluridine (FTD) is an anti-tumor component of TAS-102, which was reported to improve the prolonged median OS of patients with metastatic colorectal cancer (mCRC) that were resistant to 5-FU based standard chemotherapy. Whereas FTD is a thymidine-based nucleoside analog and functions as antimetabolite, the detailed antitumor action and the mechanism of resistance to this drug remain to be elucidated. Here, we analyzed intracellular metabolic profiles in CRC cell lines, DLD-1, FTD-resistant DLD-1 (DLD-1/FTD) and 5-FU-resistant DLD-1 (DLD-1/5-FU) by capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS). We have found that several metabolites showed significant difference in DLD-1/FTD cells compared to DLD-1 and DLD-1/5-FU cells. 1-Methylnicotinamide (1-MNA), which is synthesized by nicotinamide N-methyltransferase (NNMT), was significantly upregulated in DLD-1/FTD cells. NNMT protein expression was also increased in DLD-1/FTD cells. We further examined the effects of exposure of 1-MNA and knockdown or overexpression of NNMT on susceptibility and resistance to FTD. In summary, the present results indicate 1-MNA can be a potential metabolic marker for FTD resistance.

▲TOP

P-1451

The investigation of predictor genes for chemoresistance in gastric cancer

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Prediction of chemoresistance is important for successful anti-cancer treatment. Here we examined two candidate genes for chemoresistance predictors. Reg4 is associated with 5-FU-based chemotherapy. Gene A, a family member of nonselective transient receptor potential ion channels, is expressed at higher levels in gastric cancer than those in the normal tissues. We investigated the association expression of Reg4 and/or Gene A with chemotherapeutic effect of 5-FU in gastric cancer. We examined these two genes for chemoresistance predictors. In four gastric cancer cell lines, the expressions of Gene A was inversely associated with growth inhibition by 5-FU , but Reg4 was not. We next investigated that association with expression of Reg4/Gene A and chemoresistance by using gastric cancer specimen. The expression of Gene A showed more significant correlation with chemoresistance than that of Reg4. These two factors and HMGB1 and Dyrk2, which we have reported were examined by cluster analysis, we got the clusters Reg4(+)/HMGB1(-)/GeneA(-)/Dyrk2(+) for a sensitive profile, GenaA(+)/HMGB1(+) for a resistant profile.

P-1452

Dysregulation of AR-miR-1 axis induced-ZBTB46 promotes metastatic castration-resistant prostate cancer

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Prostate cancer cells become resistance to androgen deprivation therapy and progress to castration-resistant prostate cancer (CRPC); however, the mechanism remains unclear and no curative therapy is available so far. We investigated the regulation of a novel oncogene, ZBTB46, by androgen receptor (AR) through microRNA-1 (miR-1), and the contribution to metastasis and androgen-independent proliferation. We showed that ZBTB46 expression was inversely associated with miR-1 and serves as a marker for prostate cancer progression. AR signaling negatively regulated ZBTB46 through miR-1-mediated downregulation. ZBTB46 promoted proliferation and metastasis in prostate cancer cell lines treated with both androgen deprivation and AR inhibitor. ZBTB46 transcriptionally regulates SNAI1, a key epithelial-to-mesenchymal transition (EMT) driver, which could contribute to the induction of EMT after androgen deprivation therapy and metastasis. Our findings are supportive of the model that disruption of AR function may predispose prostate cancer to progress to metastatic CRPC. Targeting both AR signaling and ZBTB46 could be a new approach for the treatment of recurrent prostate cancer and CRPC.

P-1453

Overcoming acquired resistance to photodynamic therapy using 5-aminolevulinic acid in gastric cancer cells

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Protoporphyrin IX (PpIX)-dependent photodynamic diagnosis (PDD) and therapy (PDT) using 5-aminolevulinic acid (ALA-PDD and ALA-PDT) are widely accepted as novel therapeutic strategies for the treatment of various cancers. Previously, we have studied the molecular mechanism of acquired resistance to ALA-PDT in human gastric cancer MKN-45 cells. The resistant MKN-45 cells showed strong resistance to ALA-PDT and high expressions of iron metabolism-related genes. In this study, we investigated the stimulating activity of deferoxamine mesylate, an iron chelator, on ALA-PDT in ALA-PDT-resistant cells. Consequently, we found that deferoxamine mesylate highly enhanced the effects of ALA-PDT and effectively improved the sensitivity of the resistant cells to ALA-PDT. These findings indicated that iron metabolism and recycling system are important target molecules for overcoming ALA-PDT resistance in cancer cells.

▲TOP

P-1454

Differences in the dependence of statin-sensitive and -resistant cancer cells on the mevalonate pathway

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Anticancer effect of statins [drugs that lower cholesterol by inhibiting the mevalonate (MVA) pathway] has drawn much attention recently. However, there may be statin-sensitive or -resistant cell lines. To investigate the factors determining the differences in sensitivity, we analyzed the dependence of both cell lines on the MVA pathway. We used HOP-92 and PC-3 as sensitive cells, and NCI-H322M and DU-145 as resistant cells. The HMG-CoA reductase (HMGCR), a rate-limiting enzyme in the MVA pathway, was suppressed by siRNA, and cell viability was measured. In addition, we analyzed the effect of co-treatment of atorvastatin and MVA pathway intermediates on cell viability. Downregulation of HMGCR was found to remarkably reduce the viability of sensitive cells than that of resistant cells. In the co-treatment tests, addition of geranylgeranyl pyrophosphate (GGPP) countered the growth inhibitory effects of atorvastatin. Suppression of GGPP synthase by siRNA led to a significant decrease in viability of sensitive cells than that of resistant cells. The above results showed that sensitive cells are more dependent on GGPP for survival than resistant cells.

P-1455

Primary cultures and chemosensitivity tests for esophageal cancer using organoid cultures

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抗癌剤の効果は腫瘍の遺伝子型など個体差に影響される。個々の症例に対し治療効果を予測する試みとして感受性試験がある。我々はOrganoids cultureにて食道扁平上皮癌(ESCC)を初代培養し、培養したESCC organoidsを用いた抗癌剤感受性試験を確立することを計画した。患者より採取した組織はDispaseおよび0.25% Trypsin+EDTAで10分間酵素処理した後、FACSチューブ(35μm)で通して回収したSingle cellを用いて培養した。培養Mediaは既存のOrganoids media内のいくつかのGFを削減し、食道扁平上皮癌の培養に適した新たなMediaを調整した。3D化学療法感受性試験は96 well plate内でWST-1を用いて行った。このMediaを用いた培養によりほとんどの症例でESCC organoidsの形成が可能となり、形成率(Organoids数/散布細胞数)は0.9%、day11時の大きさは約200μmであった。さらに正常食道上皮でも同様にOrganoidsの形成が可能であった。抗癌剤感受性試験はCell seeding後7日目で抗癌剤の接触を開始し、10日目にWST試薬を投与してViabilityを評価した。現在これまでに計3例の患者由来のESCC Organoidsに対し、5-FU単剤での抗癌剤感受性試験を行った。Organoids培養を用いた抗癌剤感受性システムを確立したので報告する。

▲TOP

[P-1462] P18-3 [English/Japanese]
Anticancer drug and side efficacy and toxicity

2018 / 9 / 27 (Thu) 16:30-17:15 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Masayuki Shiota / Res. Sprt. Platf., Osaka City Univ., Grad. Sch. Med.

P-1462

PD-L1 rs2282055 genotypes are oppositely associated with response to platinum-based chemotherapy and nivolumab treatment

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Recently, we have reported that PD-L1 single nucleotide polymorphisms are associated with response to nivolumab treatment. This study aimed to determine the response of platinum-based chemotherapy and nivolumab treatment versus PD-L1 SNPs in patients with NSCLC. A total of 115 NSCLC patients were treated with platinum-based chemotherapy, 70 patients treated with nivolumab, and were also evaluated for PD-L1 single-nucleotide polymorphisms (SNPs) in plasma DNA. Among the patients treated with platinum-based chemotherapy, the T/T genotype was more associated with response compared to both T/G and G/G genotypes, the median PFS time was 11.2 months (95% confidence interval [CI], 6.2 months to 24.8 months) for the T/T genotypes and 7.3 months (95% confidence interval [CI], 5.8 months to 8.4 months) for the C/G and G/G genotype (P=0.0199). The patients treated with nivolumab, the median PFS time was 3.1 months (95% confidence interval [CI], 1.9 months to 10.8 months) for the G/G genotypes and 1.9 months (95% confidence interval [CI], 1.4 months to 2.6 months) for the T/G and G/G genotype (P=0.0134). It can be used as a selection of cancer treatment.

▲TOP

P-1463

Genetic Variation in the ABCC10 gene is Associated with Neutropenia in Patients Treated with Docetaxel

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INTRODUCTION: Clinical predictive markers of docetaxel is not established. We examined whether polymorphism of ABCC10 could affect clinical outcome to docetaxel. METHODS: Using 18 NSCLC cell lines and CRISPR-based genome-edited HeLa cells, we analyzed whether genetic variants of ABCC10 (rs2125739, rs9349256) affected cytotoxicity to docetaxel. Subsequently, we analyzed genetic variants [ABCC10 (rs2125739), ABCB1 (C1236T, C3435T, G2677T/A), ABCC2 (rs12762549), and SLCO1B3 (rs11045585)] in 69 blood samples of NSCLC patients treated with docetaxel monotherapy. Clinical outcomes were evaluated between genotype groups. RESULTS: In the cell lines, one SNP (rs2125739) was significantly associated with docetaxel cytotoxicity, and this was confirmed in the genome-edited cell line. In the 69 patients, there were no significant differences related to rs2125739 genotype in terms of RR, PFS, or OS. However, this SNP was associated with grade 3/4 neutropenia (T/C group 60% vs. T/T group 87%; P=0.028). Furthermore, no patient with a T/C genotype experienced febrile neutropenia. CONCLUSION: Our results indicate that genetic variation in the ABCC10 gene is associated with neutropenia for docetaxel.

P-1464

Development of a drug-metabolizing enzyme panel for assessment of adverse drug reactions in anticancer treatments

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Inter-individual differences in drug and metabolite exposure are important causes of adverse drug reactions and lack of therapeutic response. In 2014, we launched 'Project HOPE' to evaluate the biological characteristics of cancer and the genetic predispositions of individual patients using integrated approaches. Currently, more than 4,000 cancer patients have been investigated by multiomics-based analyses. We have previously systematically identified genetic variants involved in drug response, such as drug metabolizing enzymes and transporters, by WES. However, further technical optimization is required to confirm the variants of genes with high sequence similarities, such as CYPs. Here, we have developed a drug-metabolizing enzyme panel (DMEpanel-SCC) based on long-range multiplex PCR target enrichment using an Illumina sequencer. The DMEpanel-SCC includes 20 target genes, such as CYPs, DPYD, CDA, and MTHFR and metabolizing enzymes related to alcohol and tobacco. We have successfully identified sequence variants of target genes for 276 patients with cancer. The application of DMEpanel-SCC will facilitate the development of personalized therapies for patients with cancer.

P-1465

Whole exome sequencing to identify genetic markers for trastuzumab-induced cardiotoxicity

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To identify a genetic marker(s) determining the risk of trastuzumab-induced cardiotoxicity, we performed whole exome sequencing of germline DNA samples from 9 patients with trastuzumab-induced cardiotoxicity, and conducted a case-control association study of 2,258 genetic variants between 9 cases (with trastuzumab-induced cardiotoxicity) and general Japanese population controls registered in Human Genetic Variation Database (HGVD). To further validate the result of the screening study, we carried out a replication study of 10 variants showing $P_{min} < 0.001$ in the screening study using 10 cases and 224 controls (without trastuzumab-induced cardiotoxicity). In the replication study, we observed that three variants had effect in the same direction as in the screening study (SNV1 in exon 2 of HERADR1, SNV2 in exon 2 of HERADR2 and SNV3 in exon 44 of HERLC). A combined result of the screening and the replication studies suggested an association of a locus on chromosome 6q12 with trastuzumab-induced cardiotoxicity (SNV3 in HERLC, combined- $P_{min} = 0.00056$, OR = 13.73). This finding provides new insights into personalized trastuzumab therapy for the patients with HER2 positive cancer.

▲TOP

P-1466

Association between plasma concentration and myelosuppression of S-1 in colorectal cancer model rats with SOX regimen

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SOX regimen with oxaliplatin (L-OHP) and S-1 is widely used for the treatment of patients with colorectal cancer (CRC). However, myelosuppression and neuropathies lead to the discontinuation of the chemotherapy. The management or monitoring of antitumor effect and adverse effect have to be controlled safely for the continuous medication. In this study, colorectal cancer model rats were treated with the SOX regimen (5 mg/kg of oxaliplatin on day 1 and 5 mg/kg of S-1) and S-1 monotherapy (5 mg/kg of S-1) for 14 days. Plasma anticancer drug concentrations and hematological toxicity were evaluated. The accumulation of S-1 in plasma were observed after both treatment in CRC rats. In the hematological toxicity, platelet counts were significantly decreased after the SOX regimen on Day 7 ($33.6 \pm 2.0 \times 10^4/\mu L$) compared to on Day 1, and then recovered on Day 14 ($113.5 \pm 8.5 \times 10^4/\mu L$), whereas not altered in S-1 monotherapy. These results suggested that the platelet count is the most sensitive and a key factor for myelosuppression. This may be useful experimental data for development of a pharmacokinetic and toxicodyanamic model of S-1 for continuous medication of colorectal cancer patients.

▲TOP

[P-1443] P17-13 [English/Japanese]
Clinical experience and supportive care

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Kazuhiro Noma / Dept. Gastroenterological Surg., Okayama Univ. Med. Sch.

P-1443

Hypothalamic arginine vasopressin-enhanced green fluorescent protein synthesis in cisplatin-administered transgenic rats

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Cisplatin is one of the most potent chemotherapy drugs widely used for cancer treatment, while several side effects induced by cisplatin could cause stress responses such as activation of the hypothalamic-pituitary adrenal (HPA) axis. Hypothalamic arginine vasopressin (AVP) as well as CRH in the parvocellular division of the paraventricular nucleus (pPVN) plays an important role in the stress-induced activation of HPA axis. We generated transgenic rats that express the AVP-enhanced green fluorescent protein (eGFP) fusion gene. Here, we evaluated the hypothalamic AVP synthesis after intraperitoneal (ip) administration of cisplatin (0.6 mg/mL/100g body weight), using AVP-eGFP transgenic rats. The eGFP fluorescent intensities in the pPVN but not the magnocellular PVN were significantly increased after ip administration of cisplatin. Immunohistochemistry revealed that almost all eGFP-positive neurons expressed FosB in the pPVN in cisplatin-administered transgenic rats. These results suggest that peripheral administration of cisplatin caused upregulation of AVP synthesis as well as increases of neuronal activity in the pPVN, which may induce activation of the HPA axis.

▲TOP

P-1444

Metformin augments panobinostat's activity by activating AMPK in bladder cancer cells

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Introduction: AMP-activated protein kinase (AMPK) is a novel target of cancer therapy. We evaluated the ability of metformin to augments panobinostat's antineoplastic activity by activating AMPK.Methods: Bladder cancer cells (UMUC3, J82, 5637, T24) were treated with panobinostat and metformin, and the combination's efficacy was evaluated.Results: Metformin enhanced panobinostat-induced apoptosis and the combination inhibited the growth of bladder cancer cells synergistically (combination index < 1). Metformin activated AMPK and inhibited panobinostat-caused activation of the mTOR pathway. Furthermore, we found that AMPK activation by metformin enhanced panobinostat-induced histone acetylation synergistically by decreasing the expression of acetyl CoA carboxylase and thereby causing acetyl CoA to accumulate.Conclusion: Metformin augments panobinostat's activity by activating AMPK in bladder cancer cells.

P-1445

Panobinostat and ixazomib induce endoplasmic reticulum stress and histone acetylation in bladder cancer cells

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Background: The pan-deacetylase inhibitor panobinostat hinders the refolding of unfolded proteins by suppressing heat shock protein 90. We thought that combining panobinostat with the proteasome inhibitor ixazomib would kill bladder cancer cells effectively by inhibiting the degradation of these unfolded proteins and thereby inducing endoplasmic reticulum (ER) stress.
Methods: Using bladder cancer cells, the efficacy of the combination of panobinostat and ixazomib was evaluated.
Results: The combination induced drastic apoptosis and inhibited bladder cancer cell growth synergistically. As expected, it caused ubiquitinated unfolded proteins to accumulate, and thereby induced ER stress. This ER stress induction was essential to the combination's cytotoxic action because inhibition of unfolded protein accumulation by the protein synthesis inhibitor cycloheximide markedly attenuated the combination-induced apoptosis. Interestingly, we also found that the combination induced histone acetylation synergistically, which is another important mechanism of action.
Conclusions: Panobinostat combined with ixazomib induces ER stress and histone acetylation cooperatively in bladder cancer cells.

P-1446

PKC inhibitor suppressed the anticancer drug-induced neuropathy

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<Purpose> Chemotherapy-induced neuropathy is a highly problematic, dose-limiting effect of potentially curative regimens of cancer chemotherapy. Recent reports suggest that chemotherapy-induced neuropathy is associated with signal transduction molecules, including PKC and mitogen-activated protein kinases. It is currently unclear whether PKC inhibition can prevent chemotherapy-induced neuropathy. In this study, we investigated whether administration of the PKC inhibitor tamoxifen suppress the anticancer drug-induced neuropathy in a mouse model. <Methods> Cold sensitivity was assessed with the hot/cold-plate analgesimeter. Mechanical allodynia and hyperalgesia were assessed using the von Frey hair filaments. <Results> We found that a PKC inhibitor, tamoxifen, inhibited anticancer drugs-induced neuropathy via the PKC/ERK pathway in lumbar spinal cords. Additionally, tamoxifen was shown to act in synergy with paclitaxel to inhibit growth in tumor cells-implanted mice. <Discussion> These results indicate that PKC inhibitors may be therapeutically useful in preventing anticancer drugs-induced neuropathy and could aid in combination antitumor pharmacotherapy.

▲TOP

P-1447

Roles of etoposide and cisplatin for CRPC in the post-cabazitaxel setting

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Introduction: The aim of this study was to explore an optimal treatment for CRPC in the post-cabazitaxel (CBZ) setting.
Methods: PC3, a metastatic CRPC cell line was used in this study. We incubated the cell line with gradually increasing concentrations of CBZ to establish CBZ-resistant cell lines. We analyzed the gene expression profiles of the CBZ-resistant cell line by microarray.
Results: We established a CBZ-resistant cell line, PC3CR. IC50 of CBZ in PC3 and PC3CR cells were 5.9 nM and 16.0 nM, respectively. We performed in silico screening for compounds overcoming CBZ resistance by CMAP analysis. Etoposide (VP16) was one of the candidate drugs which reverted gene expression pattern of CBZ-resistant cells into CBZ-sensitive cells. VP16 is ordinary used with cisplatin for neuroendocrine tumor, so we tested anti-tumor effect of VP16 and CDDP using PC3CR xenograft tumor model. Both single-agent treatments with VP16 and CDDP significantly inhibited PC3CR xenograft tumors. Moreover, VP16 and CDDP in combination use had a synergic effect for the xenograft tumors.
Conclusions: VP16 based chemotherapy may be an optimal treatment for CPRC in the post-cabazitaxel setting.

P-1448

Usefulness of TAS-102 as Third-line Chemotherapy for Metastatic Colorectal Cancer

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Background/Aim: The feasibility and oncological outcomes of treatment with TAS-102, which is recommended as third-line chemotherapy for patients with metastatic colorectal cancer (mCRC), remain unknown. Patients and Methods: Between 2013 and 2015, seven patients (five males, two females) with mCRC who were administered TAS-102 as third-line chemotherapy at our Institution were retrospectively studied. During the same period, seven patients with mCRC with Kirsten rat sarcoma viral oncogene homolog (KRAS) wild-type primary lesions who were administered irinotecan with panitumumab comprised the control group. Results: The duration of third-line chemotherapy in the TAS-102 group was 217.0 (range=136-337) days compared to 226.9 (range=122-335) days in the control group, with no significant difference in the duration of administration between the two groups. No significant difference in overall survival was identified between the two groups No serious adverse effects were encountered in either group. Conclusion: TAS-102 may be suitable as third-line chemotherapy for patients with mCRC.

P-1449

Impact of neoadjuvant chemotherapy on tumor infiltrating dendritic cell in esophageal squamous cell carcinoma

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Background: It has been reported that chemotherapeutic agents induce immunogenic cell death (ICD). Dendritic cell (DC) is the most important antigen presenting cell to induce anti-tumor immune response thorough proliferation of cytotoxic T lymphocytes. We previously reported that LAMP-3+ mature DCs infiltrated into tumor tissue after neoadjuvant chemotherapy and were associated with favorable prognosis of the patients with esophageal squamous cell carcinoma (ESCC). Purpose: The aim of this study was to examine the effect of chemotherapeutic agents on DCs.Material and Methods: We examined phenotype of DCs derived from peripheral blood with and without treatment with dying ESCC cancer cell line (T.T) treated with 5-FU and CDDP by flow cytometry. Results: We found the elevation of High-mobility group box 1 concentrations in the T.T supernatants when treated with 5-FU (30 μ M) or CDDP (30 μ M), indicating inducing ICD. We observed upregulation of CD80, CD86, HLA-DR and LAMP-3 by DCs derived from peripheral blood in the same condition. Conclusions: Our results suggested that neoadjuvant chemotherapy could induce ICD inducing mature phenotypes of DC in ESCC.

▲TOP

[P-1456] P18-2 [English/Japanese]
Chemosensitivity (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Yoshifumi Iwagami / Dept. Gastroenterological Surg., Grad. Sch. Med., Osaka Univ.

P-1456

Evaluation of cisplatin-resistant associated genes in hepatoblastoma cell lines

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Cisplatin(CDDP) is the keydrug for hepatoblastoma(HB), although 11% of HB patients have resistance for CDDP and their prognoses are poor. In this study, we investigated CDDP resistant associated genes in HB cell lines, HuH6 and HepG2.First, we established CDDP resistant HuH6 and HepG2 cell lines(HuH6CR and HepG2CR) by repeating CDDP treatment for 3 and 12 months, respectively. We then analyzed resistance to CDDP by MTS assay after exposure to CDDP for 48 hours.Next, we compared expression levels between the CDDP resistant cells and wild type cells using cDNA microarray analysis. In HuH6CR cells, 138 genes were upregulated and 104 genes were downregulated significantly. In HepG2CR cells, 2155 genes were upregulated and 1689 genes ware downregulated. Using a Venn diagram, we selected 19 upregulated genes and 15 downregulated genes in CDDP resistant cell lines. Three genes of them were found to be related to CDDP resistance in previous literatures.In conclusion, we selected 34 candidate genes related to CDDP resistance. They might play an important role in acquiring CDDP resistance and become prognostic markers for treatment outcome of chemotherapy in HB patients by further refining.

▲TOP

P-1457

Inhibition of tumor growth by polyenylpyrrole derivative on oral squamous cell carcinoma cells

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Conjugated polyenes, a class of polyketides metabolites, have been shown to antagonize microbial and tumor growth. Here, a class of synthesized polyenylpyrroles and analogs from a compound of soil microbe Gymnoascusreessii was utilized to assess effects on oral squamous cell carcinoma (OSCC) cells. In contrast to immortalized oral epithelial cells, cell viability of OSCC cells were significantly repressed by one of polyenylpyrrole derivatives, F236B. Moreover, cell proliferation, migration, invasion and tumorigenesis abilities of OSCC cells were also remarkably diminished under F236B treatment. Intriguingly, tumor-suppressive activity of F236B was corroborated using OSCC tumor-bearing mice and patient-derived xenograft (PDX). Via gene knock-down and pharmacogenomics analysis, mechanistically linking between anti-tumor activity of F236B, prostate apoptosis response-4 (Par-4) and IL-17 signaling pathway was further illustrated. Collectively, our data displayed an anti-tumor activity of polyenylpyrrole derivative, which might have therapeutic application for OSCC patients' treatment.

P-1458

Establishment of monoclonal antibody to detect ERCC1 overexpression, a possible biomarker for cisplatin resistance

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Cisplatin-based chemotherapy is one of the standard first-line treatments for unresectable and recurrent gastric cancer. Excision repair cross-complementing group 1 (ERCC1) has been reported as a candidate prognostic factor for gastric cancer patient, because overexpression of excision repair cross-complementing group 1 (ERCC1), that is involved in nucleotide excision repair, is suggested to cause resistance to cisplatin. However, ERCC1 antibodies capable of evaluating the expression levels of ERCC1 was not available. We have generated mouse monoclonal antibodies that can evaluate the ERCC1 expression in clinical specimen in this study. We selected four antibodies and further evaluated with the tumor specimen from gastric cancer patients. The one clone showed the most clear staining pattern. We further optimized the staining conditions for tumor specimen and characterized the antibody. The other co-authors: Emiko Udo, Tomonori Araki, Noriko Shibata.

P-1459

Ex vivo chemosensitivity assay using patient-derived spheroids of epithelial ovarian cancer

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In epithelial ovarian cancer (EOC), individual patients with the same histology often respond differently to the same chemotherapy due to interpatient heterogeneity. The aim of this study is to evaluate the clinical relevance of the chemosensitivity assay in EOC. We previously developed cancer tissue-originated spheroid (CTOS) method, a primary 3D culture system to prepare cancer cells from patient tumors. We conducted sensitivity assay for paclitaxel and carboplatin using CTOSs from EOC. We prepared 61 CTOSs from EOC. Characteristics of the original tumors were well preserved in CTOS. The overall success rate of CTOS formation was 100% (61/61 cases), and that of sensitivity assay was 87.8% (43/49 cases who received TC regimen). There were substantial differences in sensitivity among 43 CTOSs from different patients. The assay results were compared with clinical outcome. Clinically TC resistant cases showed higher IC50 levels than TC sensitive cases in suboptimal (>1cm residual disease) surgery group. Thus, the sensitivity assay using CTOS from EOC might reflect the heterogeneity in sensitivity to paclitaxel and carboplatin among patients, and be useful in precision medicine.

▲TOP

P-1460

A Chemosensitivity Study of Colorectal Cancer Using Xenografts of Patient-Derived Tumor Initiating Cells

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To predict chemosensitivity in each colorectal cancer patient, we developed an evaluation method using the primary tumor initiating cells xenografted in nude mice subcutaneously (patient-derived spheroid xenografts; PDSXs). Simultaneously, we also prepared the conventional patient-derived xenografts (PDXs) from the same patients' tumors, and compared the dosing results with those of PDSXs. We further compared the chemosensitivities of PDSXs with those of seven patients who had been given regimens such as FOLFOX and FOLFIRI to treat their metastatic lesions. As the results, the PDSX method provided much more precise and predictable tumor growth with less variance than conventional PDX. Likewise, drug dosing tests showed essentially the same results in PDXs and PDSXs, with stronger statistical power in PDSXs. Notably, the cancer chemosensitivity in each patient was precisely reflected in that of the PDSX mice along the clinical course. This "paraclinical" xenograft trials using PDSXs may help selection of chemotherapy regimens efficacious for each patient, and more importantly, avoiding inefficient ones by which the patient can lose precious time and QOL.

P-1461

Method to measure in vivo level of poly(ADP-ribose) for estimation of efficacy of PARP inhibitors

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PolyADP-ribosylation is a post-translational modification that play key roles in cellular physiological function and DNA damage responses. PolyADP-ribosylation is finely and dynamically regulated by various enzymes and factors involved in the synthesis and degradation of poly(ADP-ribose). Recent development of potent inhibitors of polyADP-ribosylation is expected to kill BRCA1/2 mutated breast cancer cells and ovarian cancer cells (synthetic lethality).
To know the efficacy of these inhibitors within cells or organs, it is necessary to develop highly sensitive and reproducible methods to measure in vivo level of poly(ADP-ribose). In addition, it is important to prepare samples without damaging the cell DNA as much as possible. We will present our data [1,2] and would like to discuss the methods thus far published by other researchers.
[1] An enzyme-linked immunosorbent assay-based system for determining the physiological level of poly(ADP-ribose) in cultured cells. Ida et al. Anal Biochem. 2016, 494:76-81.
[2] Effect of mild temperature shift on poly(ADP-ribose) and γ H2AX levels in cultured cells. Yamashita et al. Biochem Biophys Res Commun. 2016, 476(4):594-599.

▲TOP

[P-1467] P19 [English/Japanese]
Novel cancer diagnostic tools and treatments (2)

2018 / 9 / 27 (Thu) 17:15-18:00 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Takafumi Nakamura / Grad. Sch. of Med. Sci., Tottori Univ.

P-1467

Magnetic hyperthermia induces apoptosis in cancer cells and suppresses autophagy in skeletal muscle

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Necrosis-inducing anti-cancer drugs enhance release of high mobility group box 1 (HMGB1) at cell necrosis. HMGB1 induces autophagy in skeletal muscle to evoke muscle atrophy. Magnetic hyperthermia therapy (MHT) induced apoptosis by heating mouse subcutaneous tumors at 43°C using heat-control ling iron-aluminum (Fe-Al) milling alloy. In contrast, MHT using Fe line induced necrosis by heating with near 100°C. Moreover, MTH with Fe-Al milling alloy reduced stemness. MTH with Fe-Al milling alloy or Fe line, which provided the same levels of degeneration of skeletal muscle surrounding tumors induced pronounced autophagy, decrease of myosin heavy chain and high serum HMGB1 in using Fe line, whereas MTH using Fe-Al milling alloy induced heat shock protein 90, no autophagy and low serum HMGB1. These findings suggest MTH using Fe-Al milling alloy might be a good method for local treatment of tumors with reducing complication to skeletal muscle.

▲TOP

P-1468

Investigation of novel drug for nuclear medicine targeting to cancer specific amino acid transporter, LAT1

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Targeted alpha-therapy is receiving much attention in the field of theranostics because of its high cytotoxic effect to the targeting cancer cells. However, physiological uptakes in non-targeted organs are also observed in the targeted alpha-therapy, which might lead to the severe side effects. We should consider about both maximizing the treatment effect in the tumor and minimizing the side effects in the organs at risk. From this viewpoint, decision of targeting molecule was most important.We selected LAT1 as molecular target. LAT1 is one of the amino acid transporters. LAT1 has highly specificity to cancer tissues. We developed next-generation internal radiotherapy using chemicals targeting LAT1. First, we synthesized alpha-methyl-L-tyrosine labeled with ^{211}At. ^{211}At was produced by the cyclotron, and then quickly purified and combined to alpha-methyl-L-tyrosine (^{211}At-AAMT). Next, we performed cytotoxicity evaluation of that using PANC-1 cells. As a result, cell death and specificity were confirmed in ^{211}At-AAMT. We also found the anti-cancer effects in vivo study. In the immediate future, we will examine that the effects of ^{211}At-AAMT using several kinds of xenograft models.

P-1469

Novel theranostic chelate based on carbonic anhydrase-IX ligand combines imaging and therapy targeting tumor hypoxia

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Co-author：Masahiro Ono
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Tumor hypoxia is associated with a poor outcome regardless of the treatment approach, indicating that it might be one of the important cancer therapeutic targets. Theranostics is defined as a field that combines the modalities of therapy and diagnostic imaging with the aim of realizing the personalized medicine. In this study, we designed low-molecular-weight metallic chelates with a bivalent ureidosulfonamide (US) scaffold as the carbonic anhydrase-IX (CAIX)-binding moiety to conduct cancer theranostics targeting CAIX, which is overexpressed in many kinds of hypoxic cancer cells. An ^{111}In-chelate (^{111}In-US2) clearly visualized CAIX high-expressing (HT-29) tumor xenografts in mice with SPECT imaging. Ex vivo autoradiography demonstrated that ^{111}In-US2 distribution within the tumor was consistent with both CAIX and hypoxia localization. In addition, anti-tumor therapy by In-US2 with a higher concentration was conducted. Nonradioactive In-US2 administration (50 mg/kg) significantly inhibited HT-29 tumor growth in mice. These results suggest that metal-coordinated US2 could serve as the cancer theranostic agent, which combines diagnostic imaging and anti-tumor therapy.

P-1470

Development of contrast-enhanced four-dimensional dual-energy CT of hepatocellular carcinoma with PVTT in radiotherapy

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Purpose: To develop of the contrast-enhanced four-dimensional dual-energy CT (CE-4D-DECT) for hepatocellular carcinoma with portal vein tumor thrombosis (PVTT) delineation.Methods: The CE-4D-DECT acquisitions were performed for seven patients, and the optimal virtual monochromatic image (O-VMI), which provided the highest contrast to noise ratio (CNR) in quantitative spectral analysis (40-140 keV), was determined. The objective and subjective image qualities, expressed as CNR and five-point score, respectively, were compared between O-VMI and standard VMI (S-VMI, 77 keV).Results: The CNR was the highest in the VMI at 60 keV (4.7 ± 1.5), which was significantly higher ($p = 0.02$) than the corresponding value in the S-VMI (3.6 ± 1.1). The subjective image quality of the O-VMI determined by four radiation oncologists was significantly higher ($p = 0.02$) than that of the S-VMI (O-VMI vs S-VMI, 3.9 ± 0.5 vs 3.2 ± 0.4 and 3.9 ± 0.5 vs 3.0 ± 0.5 for overall image quality and tumor conspicuity, respectively).Conclusions: The O-VMI significantly improved image quality, and could provide a new imaging approach for PVTT delineation in radiotherapy.

▲TOP

P-1471

Comparison of biological characteristics of regrown tumors after repetitive irradiation

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Recurrence and relapse from the irradiated area is one of the big issues to be overcome for radiotherapy (RT). It has been reported that regrown tumor sometime acquired radiation resistance and malignancy. Therefore, it is important to understand unique properties of regrown tumor after irradiation (IR). In this study, we aim to clarify difference in properties of regrown tumor depending on radiation type and environment during IR. From mouse squamous cell carcinoma cell line NR-S1, we established 4 cell lines by repetitive IR. By repetitive IR in vitro, the photon irradiated regrown cells acquired remarkable radiation resistance. Repetitive C-ion IR also affected radiosensitivity of the regrown cells, but this change was not significant. On the other hand, both photon and C-ion IR in vivo did not influence radiosensitivity of regrown tumor. Surprisingly, enhancement of metastatic potential and growth rate was observed in the photon irradiated regrown tumor, but not in the C-ion irradiated regrown tumor. These results clearly indicated that radiation type and surrounding environment might have significant impact to change properties of the regrown tumor.

P-1472

Bystander effects in non-irradiated normal cells via secreted factor(s) to medium from carbon-ion irradiated tumor cells

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It should be important for developing radiotherapy to understand the communication between irradiated tumor and non-irradiated normal cells. This year, we examined biological effects in non-irradiated normal cells, focusing on the bystander effect via secreted factor(s) to culture medium from the irradiated tumor cells. Human glioblastoma cells (T98G) were irradiated with carbon-ion microbeams generated with the TIARA in QST (LET=103keV/μm), simulating the spot scanning irradiation system. The medium from the irradiated T98G cells was added to the flask inoculated on the normal human cells, and the cells were assayed for cell-killing effect and gene mutation. The medium transfer resulted in increasing both biological effects in normal human cells. Furthermore, the induced biological effects disappeared and returned to the control levels when using the ascorbic acid. There is clear evidence that the medium from the irradiated tumor cells enable to induce damage in the neighboring non-irradiated normal cells via secreted factor(s), which were scavenged by the ascorbic acid, mediated bystander effect. (This is the co-operative study with Drs. T.Funayama, M.Suzuki and Y.Kobayashi.)

▲TOP

[CS3-1] CS3 [English]
Regulation of tumor immunity and evolution of cancer treatments

2018 / 9 / 28 (Fri) 9:00-11:30 Room 1/5F Large Hall, Osaka International Convention Center Room 1

Yusuke Nakamura / Dept. Clin. Oncol, Univ. of Chicago、Yutaka Kawakami / Div. Cell. Signal., Inst. Adv. Med. Res., Keio Univ. Sch. Med.

Although cancer immunotherapies including immune checkpoint blockade have been shown to be effective for patients with various cancers, the efficacy is still limited. Further analyses on immunopathology of cancer and development of novel immune-modulating strategies are required. In this core symposium, recent topics in cancer immunology and immunotherapy will be discussed as follows; Mechanisms for fibrosis by newly discovered monocyte subsets, which may also be involved in cancer development, immunological subtypes of human cancer and their potential treatment strategies, metabolic dysfunction of tumor microenvironment which suppresses anti-tumor T cell responses and potential reversal by metabolic modulators, efficient generation of neo-antigen specific TCR transduced T cells for personalized adoptive cellular immunotherapy, potential use of induced stem cell memory like T cells converted from activated effector T cells, which have high in vivo proliferative activity, and roles of HLAs in immune checkpoint blockade. These new findings will lead not only to the further understanding of cancer immunology but also to development of novel immunotherapies for patients with cancers.

CS3-1
[Keynote] Towards understanding the mechanism of fibrosis

Shizuo Akira
IFReC, Osaka Univ.

Fibrosis is a life-threatening disorder that causes severe damage to the lung, kidneys, heart and other important tissues. Fibrosis is a common response of many organs to chronic injury and inflammation. However, the clear picture of mechanisms of fibrosis development and curative drugs are still unknown. Macrophages represent a diverse set of phagocytic cells distributed in the whole body. They play a central role in a variety of biological events including host defence against pathogens, tissue remodelling, chronic inflammation, fibrosis and cancer. Accumulating evidence indicates the existence of multiple and distinct subsets that exert different biological functions. Recently we have identified a new atypical monocyte subset critical for development of bleomycin-induced lung fibrosis. We named this monocyte subset SatM based on a unique nuclear shape and a hybrid character of monocyte and granulocyte. C/EBPβ deficiency results in a complete lack of SatM. Recently we have found that RBM7 protein is dramatically induced during the fibrotic phase and associated with apoptosis. I will discuss the role of SatM and RBM7 in bleomycin-induced lung fibrosis.

▲TOP

CS3-2

Immunological subtypes of human cancers and their modulations for combination immunotherapy

Yutaka Kawakami
Inst. for Advance Med. Res., Keio Univ.

Pretreatment immune status varies among cancer patients and correlates with prognosis and responses to various cancer therapies. Standardized scoring system of tumor infiltrating CD8+ T cells confirmed that high score predicts favorable postoperative prognosis in patients with early colon cancer even with MSS+ cancer. By gene expression analysis, immunological subtypes correlating with prognosis are classified. In advanced colon cancers, immune-status are changed with more immunosuppressive components. Similarly immunological subsets can be identified in other cancers. Some subtypes appear to be common and others are unique for cancer types. These results suggest that the molecules and cells related to the subtypes can be biomarkers and therapeutic targets for combination immunotherapy, and appropriate immune-interventions are needed for each subtype. We are screening various chemical libraries having an ability to modulate tumor immune-microenvironment using murine tumor models, and found compounds to enhance anti-tumor T cells by targeting various cells through a variety of mechanisms. These findings may lead to development of effective personalized combination immunotherapy.

CS3-3

Metabolic reprogramming of tumor microenvironment leads to immune-mediated tumor growth inhibition

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In the tumor microenvironment, glucose is consumed by tumors and not by immune cells, which allows tumors to grow. Effector memory CD8T lymphocytes (CD8TEM) requires glucose to fight against tumors, but in the absence of glucose, rapidly differentiates into central memory type (CD8TCM) whose ability of multiple cytokine production is hampered. Glucose deficiency allows expansion of Treg, MDSC and M2-like macrophages in the tumors, leading to strong inhibition of T cell-mediated anti-tumor immunity.
We recently observed that metformin, an anti-diabetic drug, as well as anti-PD-1 Ab activates CD8T lymphocytes to produce multiple cytokines, in a glucose transporter, Glut-1 dependent manner. Both metformin and anti-PD-1 Ab showed significant tumor growth inhibition in vivo. However, anti-oxidant blocks the effect of metformin but not of anti-PD-1 Ab and also abrogates combination effect of metformin and anti-PD-1 Ab, suggesting the involvement of reactive oxygen species (ROS) produced in mitochondria in the metformin-mediated anti-tumor immunity. We will discuss the mechanistic insight into metformin-induced anti-tumor immunity and its synergistic effect with PD-1 blockade.

CS3-4

Effective method to generate TCR-engineered neoantigen-specific cytotoxic T cells

Yusuke Nakamura
Dept. Med. Univ. Chicago

The success of immunotherapies has implicated neoantigens as major targets of anti-cancer cytotoxic T cells. Adoptive T cell therapy with neoantigen-specific T cell receptor (TCR)-engineered T cells would be an attractive new therapeutic option for advanced cancer patients. The most challenging part of this therapy is isolation of neoantigen-specific T cells and identification of neoantigen-specific TCRs. To establish the effective screening method for neoantigen-specific TCRs, we stimulated CD8 T cells with neoantigen epitopes for 10 days and a few to several hundred neoantigen-specific T cells were sorted out using antigen-specific oligomers. Then TCR sequencing was performed to determine unique TCRs recognizing neoantigens. This step from stimulation of T cells with neoantigen-loaded dendritic cells to the identification of neoantigen-specific TCRs required only two weeks. We confirmed functional activity of these identified TCRs by generating TCR-engineered T cells recognizing the corresponding neoantigens and showed cytotoxic activity in an antigen-dose-dependent manner. We also demonstrate the importance of careful validation to avoid severe immune-related adverse events.

▲TOP

CS3-5

Generation of human induced-stem cell memory T (iTscm) cells for cancer adoptive T cell immunotherapy

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Co-author：Taisuke Kondo、Makoto Ando
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Adoptive T cell therapy is an effective strategy for cancer immunotherapy. However, infused T cells frequently become exhausted, and consequently offer a poor prognosis after transplantation into patients. Stem cell memory T (Tscm) cells is expected to overcome this shortcoming since Tscm cells are highly proliferative, long-lived, and produce a large number of effector T cells in response to antigen stimulation. We show that activated effector T cells can be converted into Tscm-like cells (iTscm) by co-culturing with OP9 cells expressing Notch ligand, DL1. Here we optimized methodological parameters of human CD8+ iTscm cell generation. Regardless of the stimulation by anti-CD3/CD28 antibodies or by antigen-presenting cells, human iTscm cells were more efficiently induced from central memory type T cells as well as effector memory T cells. IL-7 and IL-15 (but not IL-2 or IL-21) most efficiently generate iTscm cells. EB virus-specific iTscm cells showed much stronger antitumor potentials than conventionally activated T cells did in humanized EB virus transformed-tumor model. Thus, adoptive T cell therapy with iTscm offers a promising therapeutic strategy for cancer immunotherapy.

CS3-Special_Remarks

The role of HLAs for immune-checkpoint blockade

Takehiko Sasazuki
Kyushu Univ., Inst. for Advanced Study

No Abstract

[SP3-1] SP3 [Japanese]
Development of Cancer Genomic Medicine Platform in Japan

2018 / 9 / 28 (Fri) 13:00-15:30 Room 1/5F Large Hall, Osaka International Convention Center Room 1

Hiroyuki Mano / Natl. Cancer Ctr. Res. Inst.、Yuichiro Doki / Dept. Gastroenterological Surg. Osaka Univ.、Koichi Goto / Natl. Cancer Ctr. Hosp. East, Dept. Thracic Oncol.

Japan has a unique national health insurance system, and, if Japan adapts cancer genomic medicine, this country has to prepare a platform that can be applicable to hundreds of thousands of patients. Last year, the Expert Meeting for Cancer Genomic Medicine Promotion Consortium submitted a blueprint for such platform to the Minster of Health, Labour and Welfare of Japan. Then, the Core Hospitals as well as Cooperative Hospitals for Cancer Genomic Medicine were designated, and the Center for Cancer Genomics and Advanced Therapeutics (C-CAT) was started which is expected to facilitate cancer genomic medicine through a tight cooperation with the hospitals above. C-CAT collects genomic and clinical information for cancer patients, makes Cancer Knowledge DataBase (CKDB) reports for the patients, and maximizes the opportunity for appropriate drug delivery. Furthermore, such large datasets shall be a foundation for the future development of cancer drugs and diagnostics. In this Symposium, the current status of the Hospitals, C-CAT and the Ministry is demonstrated and discussed.

SP3-1

A Proposal from the Expert Meeting for Cancer Genomic Medicine Promotion Consortium

Hiroyuki Mano
Ctr. Cancer Genomics Advanced Therap., Natl Cancer Ctr.

Japan is likely going to soon adapt gene-panel testing to optimize cancer treatments under national health insurance. To discuss a necessary platform to perform such cancer genomic medicine in Japan, The Expert Meeting for Cancer Genomic Medicine Promotion Consortium was held in the Spring of 2017 in The Ministry of Health, Labour and Welfare. The Expert Meeting recommends a step-wise adaptation of genomic medicine, i.e. such medicine should be first conducted only in designated hospitals, and the number of these hospitals shall be increased gradually. Another important proposal from the Expert Meeting is to set a central datacenter to aggregate genomic as well as clinical information of gene-panel tests. The Ministry has accordingly designated, in the Spring of this year, eleven Core Hospitals for Cancer Genomic Medicine and one hundred of Cooperative Hospitals for Cancer Genomic Medicine. The Ministry also established, in June 2018, The Center for Cancer Genomics and Advanced Therapeutics (C-CAT) to store genomic/clinical information. This entire platform is under construction to be ready for the approval of gene-panel tests, probably, in the next year.

▲TOP

SP3-2

Agenda and Status of C-CAT, the cancer genomic information management center

Teruhiko Yoshida
Ctr. for Cancer Adv. Therapeutics (C-CAT), Natl. Cancer Ctr.

Based on the Cancer Control Act implemented since April 2007, the 3rd term Basic Plan to Promote Cancer Control Program was decided by the Cabinet in March, 2018. The latest Plan has placed "Cancer Genomic Medicine" at the top of the agenda list in the diagnosis and treatment section. The Plan includes the development of the high-quality database by the government to accumulate genomic and clinical information generated in both the daily clinical practice and clinical research in cancer genomic medicine. Establishment of a "cancer genomic information management center" is also decided in the Plan to utilize the accumulated data for research and development including those of AI to analyze the big data. The Ministry of Health, Labour and Welfare called the "Expert meeting for Cancer Genomic Medicine Promotion Consortium", and its report released in June 2017 has laid the grand scheme of the entire system. The cancer genomic information management center is in the core and was launched on June 1, 2018 as C-CAT (Center for Cancer Advanced Therapeutics). C-CAT is still under construction in many key aspects, and the progress and prospects will be presented.

SP3-3

System maintenance of the core hospital for cancer genome medical care: action in the Tohoku University Hospital

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In the Tohoku University Hospital, the Personalized MEdicine Center (P-MEC) was established under the theme of "development of personalized medicine easy for a person and medical difference correction" in April, 2017. The organization maintenance in the hospital and the operative system were built until now so that a genome diagnosis and a biobank could be provided in patients with cancer. The divisions of clinical sequence and biobank are set up in the P-MEC, and we work on research and development of the cancer genomic medicine and the social implementation, cooperating with the Tohoku University Advanced Research Center for Innovations in Next-GEneration Medicine (InGEM) installed newly in the Tohoku Medical Megabank Organization (ToMMo). In February 2018, our hospital was appointed by MHLW as one of the 11 core hospitals for cancer genome medical care. In this symposium, our actions together with the 6 cooperation hospitals for the cancer genome medical care in the Tohoku area will be also introduced.

SP3-4

Development of Todai OncoPanel, a NGS-based multiplex gene assay, at the University of Tokyo Hospital

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Clinical sequencing for advanced solid tumors by Todai OncoPanel (TOP) is anticipated to receive approval as Advanced Medical Care from the Ministry of Health, Labor and Welfare. We are planning to analyze 200 cancer patients in cooperation with 14 institutions. Since February 2017, The University of Tokyo Hospital started TOP analysis as a project of AMED in > 200 patients. TOP includes DNA panel (mutations and copy number variations in 465 genes, as well as tumor mutation burden) and RNA panel (467 fusion genes, 125 genes' expression levels, and key genes' exon skipping). Germline mutations are currently analyzed in 23 genes, which can be commercially tested in Japan. There are various issues to be solved for establishment of the system. Firstly, the quality of the panel testing itself should be high enough with a sufficient and latest knowledge database (with reasonable cost). Secondly, a standard operating procedure should be developed, although it may be established nationwide. Thirdly, education to physicians, medical staffs, and patients is mandatory. In this symposium, the characteristics of TOP and our challenge as an Advanced Medical Care will be discussed.

▲TOP

SP3-5

Establishment of Designated Core Hospitals for Cancer Genomic Medicine-3: Osaka University Hospital

Shinichi Yachida
Dapt. Cancer Genome lnformatics, Grad. Sch. Med., Osaka Univ.

Detection of acquired variants in cancer is a paradigm of precision medicine. The Osaka University Hospital is one of Designated Core Hospitals for Cancer Genomic Medicine. At the Center for Cancer Genomics and Personalized Medicine in the Osaka University Hospital, we created the laboratory, which has been awarded an accreditation by the Commission on Laboratory Accreditation of the College of American Pathologists (CAP) to provide NGS (next-generation sequencing)-based oncology testing. We will perform targeted panel testing for genes involved in cancer, especially genes that become therapeutic targets as advanced medical equipment to aim the public health insurance redemption, and provide information to help anticancer drugs (especially molecular target drugs). The feature of our clinical NGS is a hospital-contained system, that is, all steps can be performed in the Osaka University Hospital. Our goal is to provide fast, reliable, broadly applicable and cost-effective targeted NGS testing.

SP3-6

Establishing and implementing cancer genomic medicine in Japan

Hideki Ueno
Health Service Bureau, Ministry of Health, Labour & Welfare

Co-author：Yosuke Mukai、Shoji Tanto、Masahiro Sasaki
Health Service Bureau, Ministry of Health, Labour & Welfare

Cancer has remained the leading cause of death since 1981 and remains a serious public health burden in Japan. Many multi-center collaborative national projects have been undertaken to promote cancer genomic medicine in developed countries. However, Japanese authorities have not yet implemented multi-center cancer genomic medicine projects. In 2017, the means of establishing systems for providing the latest cancer genomic medicine were discussed in the Expert Meeting for Cancer Genomic Medicine Promotion Consortium, and the report of this meeting was published.Based on these reports, the authorities designated 11 hospitals as Designated Core Hospitals for Cancer Genomic Medicine in February 2018, and identified 100 hospitals as Cooperative Hospitals for Cancer Genomic Medicine in order to provide quality-assured cancer genomic medicine nationwide in March 2018. In addition, a Center for Cancer Genomics and Advanced Therapeutics (C-CAT) has been established in the National Cancer Center in June 2018. The C-CAT plays vital roles in collection, management and operation of the national cancer genomic information. The authority plans to further promote cancer genomic medicine in Japan.

▲TOP

[SP4-1] SP4 [Japanese]

Joint symposium of the Japan Society of Human Genetics (JSHG), Japanese Society for Genetic Counseling (JSGC), and Japanese Society for Familial Tumors (JSFT): Genetic counseling

2018 / 9 / 28 (Fri) 15:30-18:00 Room 1/5F Large Hall, Osaka International Convention Center Room 1

Kaori Muto / IMS, the Univ. of Tokyo、Teruhiko Yoshida / Dept. Gen. Med. Serv., Natl. Cancer Ctr. Hosp.

The advent of the next generation sequencing technologies and a series of reference and knowledge databases to annotate/ curate detected variants has been transforming both medical research and practice in many disease fronts. Oncology takes the leading and unique position, because of the 1) high prevalence and mortality worldwide, 2) actionability in treatment and prevention, 3) necessity of both somatic and germline information in the understanding of the disease and care of the patients and their families. Such holistic approach obviously requires multidisciplinary interactions with appropriate parallel and joint works on several key agenda of the cancer genome medicine. This Special Program 4 would offer a probably unprecedented but timely and much-needed opportunity for JCA to meet 3 other Societies in the cancer genetics and genomics. Several other societies and study groups are also working in the oncology field, and we chairpersons apologize that we had to limit the 3 Societies this time for the time limitation. Together with the preceding sibling Special Programs 3, we expect an active and open, brain-storming type of discussions to reach the major goal of listing and identification of our tasks related to genetic counseling for cancer patients and families, where "the Somatic meets Germline".

SP4-1

The Japan Society of Human Genetics

Yoichi Matsubara
The Janpan Society of Human Genetics

The Japan Society of Human Genetics (JSHG) was established in 1956. The aim of JSHG is as follows, 1) contribution to the advancement of science through human genetics research, 2) promotion of clinical practice in the field of genetics, 3) contributing to healthcare and welfare system through research on diseases and health, and 4) spread of knowledge on human genetics for society through education and enlightenment. The society holds more than 4,800 members, including basic scientists, medical geneticists, and genetic counsellors. Annual meeting is held in autumn. The society has its own society journals: Journal of Human Genetics and Human Genome Variation, both of which have been published from Springer Nature. We, in cooperation with the Japanese Society for Genetic Counseling, have established the Japanese Board of Medical Genetics, Clinical Geneticist (1991), Clinical Cytogeneticist (1995), and Genetic Counselor (2005). JSHG is an official member of the East Asian Union of Human Genetic Societies and the International Federation of Human Genetics Societies. JSHG has successfully hosted the 13th International Congress of Human Genetics in 2016 in Kyoto.

▲TOP

SP4-2

The history and main activities of The Japanese Society for Familial Tumors

Naohiro Tomita
Div. Lower GI Surg., Hyogo College of Med.

In 1990, the Hereditary Colorectal Cancer Research Project was launched within the Japanese Society for Cancer of the Colon and Rectum (JSCCR), and it was independently reorganized as the Familial Tumor Society in 1994. Then, The Japanese Society for Familial Tumors was founded in 2005 and became a general incorporated association in 2016. Main activities are (1) Academic activities :The society holds an academic meeting annually and also issues the Journal of Familial Tumors, which was digitized in 2016, twice a year. (2) Educational activities:Since 1998, the society has held the Familial Tumor Counselor Training Seminar (currently, the Familial Tumor Seminar) once a year (currently twice a year) to help participants acquire knowledge and skills about management of familial tumors. (3) Titles and qualifications:In 2011, the society began to confer the title of familial tumor coordinator/counselor (the FCC system) on its members. Furthermore, a system to certify familial tumor medical specialists was launched in 2017. (4) Guidelines:The Guideline for Research and its Clinical Application on the Genetic Testing of Familial Tumors was released in 2000, and was revised in 2016.

SP4-3

Japanese Society of Genetic Couseling

Shinji Kosugi
Dept. Med. Ethics

Objective: Practice and research of integrated genomic medicine including genetic counseling for effective clinical application of markedly advanced genetic information and technology.History: Japanese Society of Genetic Counseling was established in 1977 as Collegium of Clinical Genetics (Rinshou Iden Kenkyukai). In 1986, it became Japanese Society of Clinical Genetics (Nihon Rinshou Iden Gakkai). Finally in 2001, The name of the society changed to the current name `Japanese Society of Genetic Counseling` after profound discussion of the role of the society.Number of members of the society is continuously increasing and is more than 1,400 in 2018.Major Activity:1. Annual scientific meeting2. Two kinds of seminars of genetic counseling (one is for generalized fields, the other is advanced course for specific fields) 3. Cultivation of Specialist Physician (Rinsho Iden Senmon-i) since 2002 in collaboration with Japanese Society of Human Genetics. The number is 1316. http://www.jbmg.jp/index.html4. Cultivation of Certified Genetic Counsellors (Nintei Iden Kaunserah) since 2005 in collaboration with Japanese Society of Human Genetics. The number is 226. http://plaza.umin.ac.jp/~GC/

SP4-4

Medical challenges for sequencing germline mutation in human disease

Yoshinori Murakami
Div. Mol. Pathol, Inst. of Med. Sci., Univ. Tokyo

Classic genetic medicine to identify germline mutation of single gene disorders and to translate the findings to their diagnosis and treatment has been established in the fields of obstetrics, pediatrics, neurology and familial cancer by the end of the 20th century. Since germline mutation is shared by not only patients but also members of pedigrees, medical care based on the germline mutation should be carried out with special consideration. Japan Society of Human Genetics has been involved in developing genetic medicine in such diseases and established a system of certified doctors and certified genetic counsellors to promote and control the quality of genetic medicine in Japan. On the other hand, thanks to recent technical advances, clinical application of genomic medicine to cancer is being established to choose the most effective treatment for each patient by sequencing somatic mutation of hundreds of candidate genes in individual tumors. In this symposium, I will discuss several critical issues to accomplish the genomic medicine in cancer successfully in Japan, paying special attention to clinical significance and problems of sequencing germline mutation in human disease.

▲TOP

SP4-5

Clinical Management of Familial (Hereditary) Cancer in the Era of Genomic Medicine

Kohji Tanakaya
Dept. Surg., Iwakuni Cln. Ctr., The Japanese Society for Familial Tumors

Co-author：Hideyuki Ishida[1]、Kazuo Tamura[2]、Naohiro Tomita[3]
[1]Dept. Digestive Tract

With the development of sequencing technologies, clinical practice concerning familial (hereditary) cancer has been rapidly changing. Conventionally, we have screened patients with a family history. However, nowadays, people with familial (hereditary) cancer may be found incidentally or secondarily using somatic multi-gene sequencing of cancer tissue. Furthermore, genetic testing as a companion diagnostic test for drug selection has been introduced. Despite several obstacles in Japan in the field of familial (hereditary) cancer, such as poor insurance coverage and an insufficient number of specialists, considering the genetic aspects of cancer is critical for providing a more appropriate diagnosis and effective treatment, along with enacting efficient preventative measures. We also need to discuss how to better facilitate genetic counseling using both somatic and germline genetic information.

SP4-6

Secondary findings in clinical cancer genome sequencing

Takahiro Yamada
Clin. Genetics Unit, Kyoto Univ. Hosp.

Co-author：Shinji Kosugi
Clin. Genetics Unit, Kyoto Univ. Hosp.

Recently, clinical next generation sequencing-based cancer testing has been highlighted. In clinical cancer genome sequencing targeting for somatic mutations, there is a potential for the recognition of germline mutations as secondary findings, which are results unrelated to the indication for ordering the sequencing but of medical value for healthcare of the patient and relatives. American College of Medical Genetics and Genomics released a policy statement of recommendations for reporting of secondary findings in clinical genome sequencing in 2013, and updated it in 2016. However, there is neither policy statements nor guidelines for this matter in Japan. In the research project: establishment of system for reporting secondary findings in clinical genomic medicine, the project team released "the the suggestion for the process to report the secondary findings in genomic medicine". We try to introduce the system and contents of this suggestion through the process of discussion in the research team and our experience in Kyoto university and Hokkaido university.

SP04-Debater

Debater

Hiroyuki Mano
Natl Cancer Ctr. Res Inst.

No Abstract

SP04-Debater

Debater

Hideki Ueno
Cancer & Disease Control Div., Health Service Bureau, Ministry of Heath, Labour & Welfare

No Abstract

▲TOP

[ML1] ML1 [Japanese]
Morning Lectures 1

2018 / 9 / 28 (Fri) 8:00-8:50 Room 2/5F Small Hall, Osaka International Convention Center Room 2

Yusaku Nakabeppu / Div. Neurofunc. Genomics, Med. Inst. Bioreg. Kyushu Univ.

ML1

Organoids-based Medicine: a new approach for understanding of cancer biology

Toshiro Sato
Dep. Gastro., Keio Univ. Sch. of Med.

Discussant：Tetsuji Takayama
Dept. Gastroenterol. Oncol, Inst. Biomed. Sci, Tokushima Grad. Sch.

Cancer is, in essence, caused by genomic aberration. Recent sequence technology has revealed genetic landscapes of cancers, however, its linkage to cancer phenotypes has yet to be unveiled owing to a lack of technological platform to understand biological traits of clinical cancers. In this circumstance, the latest technology, including organoids, patient-derived xenograft and CRISPR-Cas9, has garnered much attention as next-generation disease modelling tools for cancer research. Indeed, the combination of these technology has started to provide a multifaceted approach to characterize biological phenotype of clinical cancers. In this session, we focus on organoid technology and show how we combine and apply these state-of-the-art technologies towards understanding of carcinogenesis and development of new therapeutics for clinical cancers.

▲TOP

[IS5-1] IS5 [English]
The emerging role of exosome in carcinogenesis

2018 / 9 / 28 (Fri) 9:00-11:30 Room 2/5F Small Hall, Osaka International Convention Center Room 2

Takahiro Ochiya / Natl. Cancer Ctr. Res. Inst.、Tang-Long Shen / Natl. Taiwan Univ.

Exosomes are 50-150 nm size in diameter extracellular vesicles (EVs) secreted by multiple living cells into the extracellular space. They contain tissue or cell-specific bioactive materials, including DNA, mRNA, ncRNA, proteins, lipids, metabolites, etc. with their specific surface markers, such as, CD9, CD63, CD81, Alix, etc. Exosomes have been considered as information carriers in cell communication between cancer cells and non-cancer cells, which affect gene expressions and cellular signalling pathways of recipient cells by delivering their contents. Exosomes are promising tools for improving cancer care, but conversely may also contribute to tumor progression. Here, we highlight recently discovered roles of exosomes in modulating cancer microenvironment. We also discuss how exosomes could be exploited as biomarkers and delivery vehicles in cancer therapy.

IS5-1
Regulation on the glycosylation of exosomal integrins in cancer metastasis

Tang-Long Shen
Dept. Plant Pathol. & Microbiol., Natl. Taiwan Univ.

Metastasis is the most devastating outcome of cancer, accounting for the major cancer death. Although the expression of β4 integrin in both tumor cells and tumor-derived exosomes is crucial for malignant development and organotropic metastasis of cancer, the regulatory mechanism of β4 integrin expression in tumor cells and tumor-derived exosomes is still unclear. Here, we revealed that N-glycosylation of β4 integrin is mandatory for β4 integrin loading into exosomes. Mechanistically, oncogenic EGFR/Src signal is required for N- glycosylation of β4 integrin. Authentically, EGFR/Src signal-mediated N-glycosylation of tumor-derived exosomes facilitate tumor-derived exosomes uptake by normal human lung fibroblast cells, which in turn, promote the formation of cancer-associated fibroblasts. Moreover, cancer-associated fibroblasts contribute to the cancer malignant development. Our study uncovers a function of N-glycosylation of β4 integrin in tumor-derived exosomes, which is important for malignant development.

▲TOP

IS5-2

Development of novel cancer therapeutic methods by targeting extracellular vesicles

Yusuke Yoshioka
Dept. Mol. Cell. Med., Natl. Cancer Ctr. Res. Inst.

Co-author：Nobuyoshi Kosaka[1]、Takahiro Ochiya[2]
[1]Div. Mol. Cell Med., Natl. Cancer Ctr. Res. Inst., Dept. Translational Res. fir ExtraCell. Vesicles, Tokyo Med. Univ.、[2]Div. Mol. Cell Med., Natl. Cancer Ctr. Res. Inst., Inst. Med. Sci., Tokyo Med. Univ.

Extracellular vesicles (EVs) serve as versatile intercellular communication vehicles. Cell-to-cell communication via EV cargo contributes to cancer progression in the tumor microenvironment and pre-metastatic niche. Therefore, EVs and their components represent a novel class of therapeutic targets. For example, the molecules specific to EV production in cancer cells can be effectively targeted for cancer therapy. Recently, we identified miRNA, which suppress EV secretion, and its target gene in cancer cells, and found that the knockdown of the target gene decreased lung dissemination of breast cancer cells in an orthotopic mouse model. In addition to this other way for EV-targeting cancer treatment is the capture of circulating EVs from cancer cells. Recently, we reported that administration of antibody against EVs derived from cancer cells effectively suppressed EV-triggered metastasis in cancer and that the removal of EVs could be a novel strategy for cancer therapy. In this symposium, we will introduce our recent research on the development of EV-targeting cancer therapy and discuss on current challenges and future perspectives.

IS5-3

Exosome from senescent cells promotes tumorigenesis

Akiko Takahashi
Project for Cell. Senescence, Cancer Inst., JFCR, JST, PRESTO

Co-author：Ryo Okada[1]、Tze Mun Loo[2]、Kenichi Miyata[2]
[1]Project for Cell. Senescence, Cancer Inst., JFCR, Grad. Sch. Med. & Dent. Sci, TMDU, Res. Fellowship for Young Scientists (DC2), JSPS、[2]Project for Cell. Senescence, Cancer Inst., JFCR

Cellular senescence, a state of irreversible cell cycle arrest, prevents the proliferation of cells at risk for neoplastic transformation. Recently it has been proved that senescent cells increase the secretion of various pro-inflammatory proteins such as inflammatory cytokines, chemokines or growth factors, into the surrounding extracellular space. These novel senescent phenotypes, termed the senescence-associated secretory phenotype (SASP), reportedly contributes to tumorigenesis and various age-related pathologies through promoting chronic inflammation. In addition to pro-inflammatory proteins, we have reported that exosome secretion is significantly increased in senescent cells (Takahashi et al., Nature Communications, 2017). However, the biological roles of exosome secretion in exosome-secreting cells and exosomes secreted from senescent cells have remained largely unexplored. Therefore, we try to analyze the biological function of exosome derived from senescent cells. In conclusion, we have revealed a new role of exosomes derived from senescent cells promoting tumorigenesis as one of the SASP factors.

IS5-4

From Seeing to Believing: Visualization and Tracking of Extracellular Vesicles

Charles P. Lai
Inst. of Atomic & Mol. Sci., Academia Sinica

Extracellular vesicles (EVs) including exosomes and microvesicles are nanosized vesicles released in abundance by cancer cells. These EVs are capable of delivering a select subset of proteins and RNAs to promote tumor growth and metastasis. However, the exact time, location and cargo delivery capacity of EVs remained unknown due to a lack of available methods to detect them. Here we engineered bioluminescent and fluorescent reporter systems which allow EV visualization in real-time. The multimodal bioluminescent reporter labels EVs with a membrane-bound variant of Gaussia luciferase fused to a biotin acceptor peptide, thereby enabling whole-animal multimodal imaging and pharmacokinetics analysis of EVs. We next developed bifunctional fluorescent EV/EV-RNA reporters to study multiple EV populations with subcellular image resolution. By multiplexing the fluorescent and bioluminescent EV reporters, we elucidated the rapid dynamics of tumor EV uptake and translation of EV-mRNAs in cancer cells. These results revealed that EV mediates a dynamic and multidirectional cell-to-cell communication, thereby manipulating its recipient cells at neighboring and distant sites.

▲TOP

IS5-5

Progression and inhibition of cancer growth by extracellular vesicles derived from cancers and immune cells

Byeong-Cheol Ahn
Dept. Nuclear Med., Sch. Med., Kyungpook Natl. Univ.

The naturally produced biological nanoparticles, termed extracellular vesicles (EVs), are equipped to withstand lysis by body surveillance systems, and might carry out vital functions to surrounding tissues as well as peripheral cells by delivering various biological materials.Tumor derived EVs might promote a tumor-supporting microenvironment and tumor itself, in addition, benefit metastasis through their systemic circulation. And EVs derived from immune cells modulate diverse aspects of the immune system and can be used as immune theranostics for incurable cancers. EVs can be used as drug delivery vehicles, and drug loading to immune cell derived EVs might elevate tumoricidal effect of the EVs compared to naive EVs. In vivo molecular imaging of EV is one of essential technologies for developing immune theranostics using the EVs.In this presentation, biological effects of cancer derived EVs on the cancer and therapeutic effect of immune cell derived EVs to cancers will be discussed, and benefits of applying molecular imaging technologies for in vivo EV tracking will also be touched as well.

IS5-6

Ovarian cancer exosome promotes cancer invasion by affecting peritoneal mesothelial cells and can work as a biomarker

Kenjiro Sawada
Osaka Univ. Grad. Sch. Med., Dept. OB

Co-author：Masaki Kobayashi、Koji Nakamura、Akihiko Yoshimura、Tadashi Kimura
Osaka Univ. Grad. Sch. Med., Dept. OB

Exosomes mediate cell-cell communication through the transfer of proteins, microRNAs (miRNAs), etc. We intended to clarify the roles of ovarian cancer (OC)-derived exosomes. Exosomes were collected from OC cell lines and expressions of adhesion molecules and miRNAs were analyzed. CD44 was found to be enriched in exosomes. Upon exosome uptake, human peritoneal mesothelial cells (HPMCs) underwent a change in cellular morphology to a spindle phenotype. This increased CD44 expression promoted cancer invasion by inducing HPMCs to secrete MMP-9 and by increasing the clearance of mesothelial barrier. Microarray revealed that miR-99a is highly expressed in exosomes. Transduction of miR-99a promoted cancer invasion through the upregulation of vitronectin and fibronectin in HPMCs. Our data revealed the involvement of exosomes in the metastatic process. Furthermore, the potential of exosomal miRNAs was examined. Through miRNA RT-PCR assay using serum of OC patients, we revealed that miR-99a can work as a comprehensive biomarker of OC and miR-1290 can discriminate high grade serous carcinoma from other histological types, indicating the future utility of serum miRNA in clinical application.

IS5-7

HSP-enriched properties of extracellular vesicles involve survival of metastatic oral cancer cells

Kisho Ono
Dent Pharmacol, Okayama Univ., Oral Maxillofac Surg, Okayama Univ.

Co-author：Takanori Eguchi[1]、Chiharu Sogawa[2]、Akira Sasaki[3]
[1]Dent Pharmacol, Okayama Univ., ARCOCS, Grad. Sch, Okayama Univ.、[2]Dent Pharmacol, Grad. Sch, Okayama Univ.、[3]Oral Maxillofac Surg, Okayama Univ.

Cancer cells often secrete extracellular vesicles (EVs) that carry heat shock proteins (HSPs) with roles in tumor progression. Oral squamous cell carcinoma (OSCC) belongs to head and neck cancers (HNC) whose lymph-node-metastases often lead to poor prognosis. We have examined the EV proteome of OSCC cells and found abundant secretion of HSP90-enriched EVs in lymph-node-metastatic OSCC cells. Double knockdown of HSP90 α and HSP90 β, using small interfering RNA significantly reduced the survival of the metastatic OSCC cells, although single knockdown of each HSP90 was ineffective. Elevated expression of these HSP90 family members was found to correlate with poor prognosis of HNC cases. Thus, elevated HSP90 levels in secreted vesicles are potential prognostic biomarkers and therapeutic targets in metastatic OSCC.

▲TOP

[IS7-1] IS7 [English]
Beyond current immune-checkpoint inhibitors

2018 / 9 / 28 (Fri) 13:00-15:30 Room 2/5F Small Hall, Osaka International Convention Center Room 2

Toshihiko Torigoe / Dept. Path., Sapporo Med. Univ.、Junho Chung / Cancer Res. Inst., Seoul Natl. Univ.

Advances in immuno-oncology have been revolutionizing the modality of cancer treatment. However, many patients do not respond to current immunotherapy. In order to overcome the therapeutic resistance, basic mechanisms and rational biomarkers have to be elucidated. In this session, immune profiling of cancer tissues would be presented to identify biomarkers that predict response to immunotherapy. Proteogenomic approach to T-cell neo-epitopes would also open as yet unknown black box of natural epitopes and antigen processing machinery. In addition, we would discuss about rational combination therapies based on the recent discoveries on immunobiology.

IS7-1
Immune-Profiling of human B cell repertoire using next generation sequencing technology

Junho Chung
Cancer Res. Inst., Seoul Natl. Univ.

Co-author：DuckYoun Yoo[1]、Seungryul Lee[1]、Haejun Han[2]、Taehoon Ryu[2]、Jeungeun Kim[2]、Kihyun Kim[1]、Sangil Kim[1]、Hyori Kim[3]
[1]Cancer Res. Inst., Seoul Natl. Univ.、[2]Celemics、[3]Asan Inst. for Life Sci., Asan Med. Ctr.

Next-generation sequencing (NGS) has allowed a massive increase in capacity to sequence genomes at relatively low cost and in a short time frame. It has revolutionized multiple aspects of biological research and is actively being adopted into profiling human B cell receptor (BCR) repertoires. Several NGS platforms are currently available, with average read lengths of 75 bp to 8,500 bp and different error rates.Using NGS, we successfully constructed database of BCR repertoire from human volunteers.. Afterwards, we developed algorithms for analyzing the diversity, enrichment pattern, accumulation of somatic hyper-mutation in BCR repertoire. Through in silico analysis with machine learning technology we devised strategies to select clones of interest. Now we are actively applying this technology for the analysis of BCR repertoire in cancer patients.

▲TOP

IS7-2

Global characterization of T cells in non-small-cell lung cancer by single-cell sequencing

Xinyi Guo
BIOPIC, Sch. of Life Sci., Peking Univ., Beijing, China.

Co-author：Yuanyuan Zhang[1]、Liangtao Zheng[2]、Zemin Zhang[3]
[1]BIOPIC, Sch. of Life Sci., Peking Univ., Beijing, China.、[2]Peking-Tsinghua Ctr. for Life Sci., Peking Univ., Beijing, China.、[3]BIOPIC, Sch. of Life Sci., Peking Univ., Beijing, China., Peking-Tsinghua Ctr. for Life Sci., Peking Univ., Beijing, China., Beijing Advanced Innovation Ctr. for Genomics, Peking Univ., Beijing, China.

Cancer immunotherapies have shown sustained clinical responses in treating non-small-cell lung cancer (NSCLC), but efficacy varies and depends in part on the properties of tumor infiltrating lymphocytes (TILs). To depict the baseline landscape of TILs, we performed deep single-cell RNA sequencing for 12,346 T cells from 14 treatment-naive NSCLC patients. Combined expression and TCR-based lineage tracking revealed a significant proportion of inter-tissue effector T cells with a highly migratory nature. Besides tumor-infiltrating $CD8^+$ T cells undergoing exhaustion, we observed two clusters of cells exhibiting states preceding exhaustion, and high ratio of "pre-exhausted" to exhausted T cells was associated with better prognosis of lung adenocarcinoma (LUAD). Additionally, we observed further heterogeneity within the tumor regulatory T cells (Tregs), characterized by the bimodal distribution of TNFRSF9, an activation marker for antigen-specific Tregs. The gene signature of those activated tumor Tregs correlated with poor prognosis in LUAD. Our study will help further understand the functional states and dynamics of T cells in lung cancer.

IS7-3

Landscape of cancer antigens revealed by a proteogenomic approach

Takayuki Kanaseki
Dept. Path., Sapporo Med. Univ.

Immunecheckpoint inhibitors have brought about benefits for a wide variety of cancer patients. Neoantigens that arise form gene mutations are prioritized target antigens; however, T-cell immune surveillance, if not frequently, does occur even in patients with lower mutation burden, suggesting the presence of another type of cancer antigens irrelevant to mutations.We established a comprehensive method to analyze peptide landscape that are naturally presented by HLA of a variety of cancer types. The HLA ligandome analysis combines conventional proteomics using mass spectrometry with genomics data, thereby detecting novel types of cancer antigens. In fact, neoantigens were identified in mutation-rich cancer cells, and elicited considerably high cytotoxic CTL responses. Interestingly, we also noticed another type of cancer specific antigens encoded by lncRNAs. In general, lncRNAs do not yield stable proteins acting functionally, but our data suggest that at least some are translated into short protein fragments, enter HLA antigen processing pathway, and are consequently surveyed by T cells. We will discuss our findings on cancer antigen landscape from the viewpoint of immunotherapy.

IS7-4

Targeting CMTM6 to modulate cell surface PD-L1 expression and antitumour immune responses

Marian L. Burr
Peter MacCallum Cancer Ctr., Melbourne, Sir Peter MacCallum Dept. Oncology, Univ. of Melbourne, Cambridge Inst. for Med. Res., Univ. of Cambridge

Cancer cells exploit the expression of the programmed death-1 (PD-1) ligand 1 (PD-L1) to subvert T-cell mediated immunosurveillance. Therapies that disrupt PD-L1 mediated tumour immune evasion have achieved substantial clinical success, highlighting the need to understand the molecular regulation of PD-L1 expression. We recently identified CMTM6 to be a key regulator of both constitutive and cytokine-induced PD-L1 expression in a broad range of cancer cells. CMTM6 contains a conserved membrane-spanning MARVEL domain and associates with PD-L1 at the cell surface and in recycling endosomes, where it promotes PD-L1 recycling and protects it from lysosome-mediated degradation. CMTM6 depletion, via the reduction of PD-L1, significantly alleviates the suppression of tumour specific T-cell activity thereby highlighting the functional importance of CMTM6 in maintaining the PD-L1/PD-1 immune checkpoint. Through the study of CMTM6 we have gained novel insights into the biology of PD-L1 regulation that may open new therapeutic avenues to overcome immune evasion by cancer cells.

▲TOP

IS7-5

Combined blockade of IL-6 and PD-1/PD-L1 signals breaks mutual regulation of their immunosuppressive effects

Hirotake Tsukamoto
Dept. Immunol., Grad. Sch. Med. Sci., Kumamoto Univ.

Co-author：Koji Fujieda[1]、Azusa Miyashita[2]、Satoshi Fukushima[3]、Tokunori Ikeda[4]、Yosuke Kubo[3]、Satoru Senju[1]、Hironobu Ihn[2]、Yasuharu Nishimura[5]、Hiroyuki Oshiumi[6]
[1]Dept. Immunogenetics, Grad. Sch. Med. Sci., Kumamoto Univ.、[2]Dept. Dermatology

It is well known that pro-inflammatory cytokine IL-6 produced by tumor cells promotes their own survival, therefore is a poor prognostic factor in cancer patients. In terms of anti-tumor immune responses, we previously demonstrated that Th1 differentiation of tumor-specific CD4 T cells was attenuated in tumor-bearing animals in an IL-6-dependent fashion. We here extend our findings to further investigation of the immunosuppressive effects of IL-6 by focusing on the effectiveness of anti-PD-1/PD-L1 therapy. Considering the mechanistic linkage between IL-6 and PD-1/PD-L1 signals, we found that PD-L1 blockade prompted PD-1+macrophages to produce IL-6. Depletion of macrophages in melanoma-bearing mice revealed that macrophages functioned as a source of IL-6 during PD-L1 blockade, which was responsible for the defective Th1 response. Furthermore, combined blockade of IL-6 and PD-1/PD-L1 signals enhanced the infiltration of IFN- γ -producing CD4 T cells in tumor and exerted a synergistic anti-tumor effect. Collectively, these findings suggest that IL-6 is a rational immunosuppressive target for overcoming the narrow therapeutic window of anti-PD-1/PD-L1 therapy.

IS7-6

Bezafibrate enhances PD-1 blockade efficacy by activating mitochondrial biogenesis and effector function of CTLs

Alok Kumar
Dept. Immunol. & Genomic Med., Kyoto Univ.

Co-author：Partha S. Chowdhury、Kenji Chamoto、Tasuku Honjo
Dept. Immunol. & Genomic Med., Kyoto Univ.

PD-1 blockade therapy has revolutionized the cancer treatments through its high efficacy with limited side-effects. However, a significant fraction of patients still remains unresponsive. We earlier demonstrated that mitochondrial activation synergized with PD-1 blockade where up-regulation of PGC-1 α in CTLs is an integral part of such robust tumor inhibition. PGC-1 α is known to activate mitochondria through its partner transcription factors. In our current work, we found that the activation of PGC-1 α /PPAR pathway using bezafibrate have impressive synergism with PD-1 blockade through enhancing quality and quantity of CTLs. The CTLs have enhanced effector function as evidenced by higher T-bet expression and IFN γ production and were accompanied by high mitochondrial mass, membrane potential and reactive oxygen species bezafibrate combination therapy compared to PD-1 blockade alone. We also found upregulation of genes associated with mitochondrial biogenesis e.g. PGC-1 α , and TFAM. In conclusion, bezafibrate combination enhances the mitochondrial biogenesis and effector function of CTLs that lead to the effective immune response.

▲TOP

[SS2-1] SS2 [Japanese]
Women scientists in cancer research (WSCR symposia)

2018 / 9 / 28 (Fri) 15:30-18:00 Room 2/5F Small Hall, Osaka International Convention Center Room 2

Ai Kotani / Tokai Univ. Inst. Med. Sci. Dept. Hematol Malignancy/Dept. Hematol & Oncol., Tokai Univ. Sch. Med.、Sachiko Tsukita / Grad. Sch. of Front. Biosci., Grad. Sch. of Med., Univ. of Osaka

がん分野においても、研究者や医師の多様性を拡大し、がん分野研究者基礎・臨床人口のすそ野を広げることは喫緊の重要課題です。その中にあって、がん分野における女性がん研究者・医師の活躍が期待されています。
本シンポジウムは、日本癌学会が女性研究者・医師の活躍と増加を目指して、2014年から行われており、第一線で活躍中、あるいは、活躍が大いに期待される女性研究者・医師に最先端のがん研究を発表頂きます。発表演題は公募より選び、優れたご発表に WSCR シンポジウム 賞を授与します。
加えて、昨年からがん研究を志す女性研究者に目標を示すことを目的として、がん研究分野において優れた研究業績をあげた我が国の女性研究者に対しその功績を讃え、日本癌学会女性科学者賞を設けられました。今年は栄えある第一回受賞記念講演も行います。男女の役割分担にも多様性が増し、ライフイベントの影響は、もはや、女性だけには限りません。良き刺激と励みを求めて、どうぞ、男性、女性に限らずご来聴ください。
It has been a major issue that the number of women researchers, especially principal investigators, is low in Japan compared with other countries. Japanese Cancer Association (JCA) has been making efforts to expand woman researcher populations on cancer research and have hold a series of symposium “Women scientists in cancer research (WSCR)” since 2014. The purpose of the symposium is to introduce high quality of science carried by active woman researchers, enlightening next generation.For this 5th symposium, we have selected several speakers among the submitted abstracts. Moreover, JCA has established the new award to further promote women researchers last year and the 1st awardee will present the memorial speech at the symposium.

SS2-1

Analysis of the genes which showed synthetic lethal phenotype with BAP1 mutations in malignant mesothelioma cells

Yuko Murakami-Tonami
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Inhibiting genes that show synthetic lethal phenotype with a mutation of cancer-associated genes should exclusively kill cancer cells with the gene mutation but not normal cells. Thus, identification of such genes is important for new therapeutic strategies.
Inactivating mutations of the BRCA1-associated protein 1 (BAP1) tumor suppressor gene is frequently found in mesothelioma, uveal melanoma, and clear cell renal cell carcinoma. BAP1 encodes a deubiquitinating enzyme and is involved in the regulation of gene transcription, histone modification and DNA damage repair. To identify the genes which show synthetic lethal phenotype with BAP1 mutation, we performed genome-wide pooled lentivirus shRNA library screening. We found ubiquitin specific peptidase 1 (USP1) as one of the candidates. We confirmed that USP1 inhibitor ML323 also showed syntheic phenotype with BAP1 mutation. This phenotype was reproducible by soft agar assay. Furthermore, we tested whether BAP1 could deubiquitinate USP1 target proteins or not. We found overexpressed BAP1 could deubiquitinate FANCD2, but not PCNA.
These results suggested that FANCD2 could be a potential new target for BAP1-mediated deubiquitination.

▲TOP

SS2-2

Suppression of tumor metastasis through targeting the vascular integrity regulated by AM-RAMP2 system

Megumu Tanaka
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Co-author：Takayuki Sakurai、Akiko Kamiyoshi、Yuka Ichikawa-Shindo、Hisaka Kawate、Kazutaka Hirabayashi、Kun Dai、Nanqi Cui、Yangxuan Wei、Masaaki Tanaka、Haruka Tomiyama、Akihiro Yamauchi、Takayuki Shindo
Dept. Cardio. Res., Grad. Sch. Med., Shinshu. Univ.

Adrenomedullin (AM), together with its receptor-modulating protein RAMP2, plays critical roles in the regulation of vascular integrity. In this study, by using inducible vascular endothelial cell-specific RAMP2 knockout mice (DI-E-RAMP2-/-), we analyzed the roles of AM-RAMP2 system in tumor angiogenesis and metastasis. Subcutaneously transplanted melanoma cells showed less growth and angiogenesis in DI-E-RAMP2-/-, whereas spontaneous metastasis to the lung was enhanced. We found that endothelial AM-RAMP2 system regulates cortical actin formation, and that defect of this system caused disruption of actin formation, led to vascular hyperpermeability, and evoked endothelial-mesenchymal transition (EndMT). Within the lungs of DI-E-RAMP2-/-, endothelial cells were also deformed, and highly expressed S100A8/9 and SAA3, which mediate formation of pre-metastatic niche. These findings indicate that AM-RAMP2 system regulates vascular integrity, whereas disruption of this system promotes vascular permeability, EndMT, and formation of pre-metastatic niche. Vascular integrity regulated by AM-RAMP2 system could thus be a hopeful therapeutic target for suppressing tumor metastasis.

SS2-3

Development of a specificity-enhanced secondary biomarker for prostate cancer: PSA G-index

Yoshimi Haga
Cancer Proteomics Group, JFCR

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PSA is a powerful biomarker widely used for diagnosing prostate cancer (PCa), however, high false positive rate of PSA screening is a great issue to be resolved. Here we performed comprehensive and quantitative profiling of glycan structures on serum PSA using mass spectrometry to identify PSA glycoforms which would be specifically generated in PCa cells. PSA glycoforms were quantitatively evaluated using sera from 15 PCa patients or 15 benign prostate hyperplasia (BPH) patients whose PSA levels were at “gray zone” (4.0-10.0 ng/ml). A couple of PCa-specific glycoforms were statistically extracted, and subjected to establish a novel PCa-specific diagnostics based on logistic regression (PSA G-index). When diagnostic power was evaluated using a validation sample set (15 BPH and 15 PCa patients), the AUC of PSA G-index was 1.00, while those of total PSA or PSA f/T ratio were 0.50 and 0.60, respectively. Moreover, both PSA glycoforms showed significant correlation with Gleason scores. Thus PSA G-index could serve as not only the effective secondary screening method to preclude false positive diagnosis in PSA screening, but also the potential prostate cancer grade markers.

SS2-4

Blockage of the mevalonate pathway enhances the efficacy of MEK and mTOR inhibition via geranylgeranylation inhibition

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The mevalonate pathway is crucial for not only cholesterol synthesis but also cancer promotion by prenylation of small GTPases such as Rho, Rac and Ras. We here show that statins, common mevalonate pathway inhibitors, enhanced the efficacy of MEK inhibitors and mTOR inhibitors. First, we found that the combination of MEK inhibitors with statins resulted in a drastic increase of apoptotic cells in BRAF and KRAS mutated cancer cells. In these cell lines, MEK inhibition induced Akt activation, which was negated by statin-mediated inhibition of geranylgeranylation. Second, we found that the combination of mTOR inhibitors with statins led to G1 arrest against renal cell carcinoma (RCC) cells, whose mechanism was dependent on the inhibition of geranylgeranylation. This combinatorial efficacy was also confirmed in the RCC xenograft model. Furthermore, we retrospectively observed that statin use significantly improved the progressive-free survival of RCC patients prescribed with mTOR inhibitor everolimus. Taken together, co-targeting of the mevalonate pathway and the oncogenic signaling such as MEK and mTOR pathway could be expected as a feasible strategy in cancer treatment.

▲TOP

SS2-5

Sphingosine kinase 1 and tumor-associated immune cells in the HER2-positive breast cancer patients

Junko Tsuchida
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Co-author：Masayuki Nagahashi[1]、Kazuki Moro[2]、Mayuko Igarashi[2]、Masato Nakajima[2]、Kazuaki Takabe[3]、Toshifumi Wakai[1]
[1]Dig. Gen. Surg., Niigata Univ. Grad. Sch. Med. Dent. Sci.、[2]Div. Dig. & Gen. Surg., Niigata Univ.、[3]Roswell Park Cancer Inst.

Introduction: Sphingosine-1-phosphate (S1P), a bioactive lipid mediator, has been implicated as a key molecule in cancer progression. Although it has been indicated that S1P regulates immune system, the role of S1P in tumor-associated immune function has not been fully investigated in patients. Here, we test our hypothesis that S1P affects tumor-associated immune cells (TAICs) in HER2-positive breast cancer.
Methods: Utilizing The Cancer Genome Atlas database, we compared the expression of S1P-related and TAIC-related genes in HER2-positive breast cancer between sphingosine kinase 1 (SPHK1)-high and -low groups.
Results: There are significant differences in the levels of most of the S1P related genes between SPHK1-high and -low groups. Overall, the changes in these S1P related genes in SPHK1-high group were altered to promote S1P production. Interestingly, the high expression of SPHK1 was associated with not only high expressions of immune-stimulate genes, such as CD68 and IL-18, but also immune-suppressive genes, such as FOXP3 and PDCD1.
Conclusion: Our results implicated that S1P produced by SPHK1 plays important roles in immune reactions in HER2-positive breast cancer patients.

SS2-6

Metabolic reprogramming in cancer cells is regulated by tumor associated-antigen

Rina Takamiya
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Co-author：Tomoyoshi Soga[1]、Hiromu Suzuki[2]、Kazuaki Ohtsubo[3]
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Metabolic reprogramming is considered as a malignant phenotype of cancer cells, but the molecular mechanisms that regulate cancer metabolism remain to be elucidated. Sialyl-Tn antigen, which is synthesized by a glycosyltransferase ST6GalNAc- I and abnormally expressed in malignant types of cancers. Our recent study indicated that the pathogenic intratumoral hypoxia induces sialyl-Tn antigen expression that enhances metastatic potential of cancer cells. Moreover, sialyl-Tn expression on cancer cells enhanced Akt signaling and Nrf2 activation. Next, to elucidate whether sialyl-Tn antigen contribute to metabolic reprogramming, we performed metabolome analysis using capillary electrophoresis mass spectrometry (CE-MS). Sialyl-Tn antigen induced pentose phosphate pathway and nucleotide synthesis in H157 cells (lung cancer cell line). Sialyl-Tn antigen expressed on H157 cells also induced the production of oncometabolite, 2-hydroxyglutarate, but did not affects genome-wide alterations in DNA methylation. These findings indicate that sialyl-Tn antigen may be a key player of metabolic reprogramming in the processes of tumor progression.

SS2-7

miR-92a-3p promotes angiogenesis through the induction of partial endothelial-mesenchymal transition

Nami O. Yamada
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Co-author：Yukihiro Akao
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Extracellular vesicles (EVs) are emerging mediators of intercellular communication. We previously demonstrated that colorectal cancer cells secrete miR-92a-3p via EVs and promote angiogenesis. We also identified Dickkopf-3 (Dkk-3) as a direct target for miR-92a-3p regulation. However, pro-angiogenic function of miR-92a-3p is not only attributable to the downregulation of Dkk-3. In this study, we performed comprehensive analysis of gene sets affected by the ectopic expression of miR-92a-3p in endothelial cells. Modular enrichment analysis by using GeneCodis was also conducted to interpret the underlying biological processes. As results, the ectopic expression of miR-92a-3p upregulated cell cycle- and mitosis-related genes while it downregulated adhesion-related genes in endothelial cells. We also identified claudin-11 (CLDN11), an integral component of tight junction (TJ), as a novel target gene of miR-92a-3p. Our results suggest that EVs/miR-92a-3p promotes angiogenesis through the induction of partial endothelial-to-mesenchymal transition (EndoMT) in endothelial cells. EVs have a great potential to be therapeutic targets against cancer progression and metastasis.

▲TOP

SS2-8

The yin and yang role of cellular senescence in cancer development

Naoko Ohtani
Pathophysiol., Grad. Sch. Med., Osaka City Univ.

Cellular senescence is a state of irreversible cell proliferation arrest provoked by a variety of potentially oncogenic signals, and is known to functions as a fail-safe mechanism for tumor suppression. Recent studies, however, reveal that unlike apoptotic cells, senescent cells remain viable for a long time, and they secrete a series of inflammatory cytokines, chemokines and matrix-remodeling factors, a phenomenon termed senescence-associated secretory phenotype, or SASP. Some of the SASP factors reveal deleterious side effects such as inflammation and tumorigenesis. I will show in this symposium this novel feature of cellular senescence that we focus recently.

▲TOP

[ML2] ML2 [Japanese]
Morning Lectures 2

2018 / 9 / 28 (Fri) 8:00-8:50 Room 3/10F 1003, Osaka International Convention Center Room 3

Chikashi Ishioka / Dept. Clin. Oncol., IDAC, Tohoku Univ.

ML2

The latest progresses in immune checkpoint inhibitors

Hiroyoshi Nishikawa
Div. Can. Immunol., Res. Inst.

Discussant：Atsushi Aruga
TWIns, Tokyo Women's Med. Univ.

Harnessing the host's own defense mechanisms to attack cancer cells has demonstrated potential since William B. Coley had reported sporadic tumor shrinkage in cancer patients who experienced infection. The fields of immunology and oncology are now being linked, and cancer immunotherapy, particularly immune checkpoint blockade resurges the effector function of tumor-infiltrating T cells and provides clinical efficacy in various types of cancers. Upon the clinical application of immune checkpoint blockade in which treatment efficacy is dependent on the host immune system, it revealed that more than half of treated patients fail to respond to immune checkpoint blockade therapy, even in combination, uncovering a limited window of clinical responses. It is therefore required to develop more effective cancer immunotherapies and define biomarkers to properly evaluate immune responses in cancer patients for stratifying responders and non-responders with comprehensive cancer research including genome and immunology. In this session, we would like to discuss the current status and future possibility of immune checkpoint inhibitors, particularly from basic research side.

▲TOP

[AACR1-1] AACR1 [English]
Cancer Microbiome: exploring the roles and mechanisms of microbial communities in tumorigenesis

2018 / 9 / 28 (Fri) 9:00-11:30 Room 3/10F 1003, Osaka International Convention Center Room 3

Eiji Hara / Dept. Mol. Microbiol., Res. Inst. for Microbial Diseases, Osaka Univ.、Andrew T. Chan / Clin. & Translational Epidemiology Unit, Massachusetts General Hosp.

The human commensal microbiome provides a variety of benefits that contribute to proper functional activity in the host through the modulation of functional processes such as signal transduction, immunity and metabolism. The unbalance of this microbial profile, or dysbiosis, has been correlated with the development of several diseases such as cancers. However, the relationship between microbiome and cancer appear to be very complex. For instance, microbiome and cancer affect each other in a bidirectional manner which means that cancer development changes microbiome composition while such changes also contribute to cancer progression. In addition, it is still under debate if microbiome alterations can cause cancer or if they are merely associated with cancer. Despite these complexities, recent advance in metagenomic and metabolomic analyses started to reveal how microbiome affects host carcinogenesis. In this symposium, four distinguished speakers will present novel findings in the cutting-edge field of the microbiome and cancer. These findings will expand our understating of the molecular mechanisms underlying the cancer development and open up new possibilities for its control.

AACR1-1

Cellular senescence and cancer: a microbial connection

Eiji Hara
Dept. Mol. Microbiol., Res. Inst. for Microbial Diseases, Osaka Univ., Cancer Microbiome, Cancer Inst., Japanese Founation. for Cancer Res.

Multiple epidemiological studies have revealed that obesity is a major risk factor for not only diabetes and cardiovascular diseases but also cancer. Effective strategies for obesity prevention are therefore needed for cancer prevention. However, since the prevalence of excess bodyweight in most developed countries has been increasing markedly over the past several decades, alternative approaches are also required to conquer obesity-associated cancer. Although several phenomena have been proposed to explain how obesity increases cancer risk, the exact molecular mechanisms underlying these cancer have remained largely obscure. Recently, we have traced the association between obesity and increased cancer risk to microbiota communities that provoke cellular senescence. The analyses also revealed the role of cellular senescence in obesity-associated cancer. In this symposium, I will provide an overview of our recent work on cellular senescence in obesity-associated cancer and discuss the next steps, focusing on the potential clinical implications of these findings.

▲TOP

AACR1-2

Diet, the gut microbiome, and colorectal cancer

Andrew T. Chan
Clinical and Translational Epidemiology Unit, Massachusetts General Hospital

Recent experimental data demonstrate that Fusobacterium nucleatum may contribute to colorectal carcinogenesis through modulation of host immunity and activation of pathways associated with cellular proliferation. Prudent dietary patterns – rich in fruits, vegetables, and whole grains – have been associated with a lower risk of colorectal cancer and adenoma. In contrast, Western dietary patterns – dominated by red and processed meats – have been linked with colorectal carcinogenesis. Although mechanisms underlying these diet-cancer associations remain unclear, it is postulated that the gut microbiota may play a mediating role. Evidence also suggests that diet influences intestinal F. nucleatum. In this session, I will discuss work from our group supporting the hypothesis that the possible cancer-preventative effects of prudent diets rich in whole grains and dietary fiber may be mediated by modulation of F. nucleatum in local colonic tissue. I will also present ongoing work demonstrating the development of a large, prospective cohort study to examine the complex intersection of diet, the gut microbiome, and carcinogenesis.

AACR1-3

The impact of gut microbiota-derived metabolites in tumorigenesis

Shinji Fukuda
Inst. Adv. Biosci., Keio Univ., JST PRESTO, KISTEC-KAST, Metabologenomics

The gut microbiota form a highly complex ecological community together with host intestinal cells. The so-called gut ecosystem has a profound influence on human physiology and immunology. It has been reported that imbalance in the structure of gut ecosystem could be a risk factor in human disorders including not merely gut-associated disorders, but also systemic diseases. However, the molecular basis of the host-microbial crosstalk remain obscure. To this end, we firstly established a highly integrated omics-based approach, and found that lactate derived from dietary fiber fermentation by lactic acid bacteria accelerates colonic epithelial cell turnover and development of aberrant crypt foci after carcinogen treatment. In a large cohort study of colorectal cancer patients together with fecal metagenomic and metabolomic analyses, we showed that fecal metagenomic and metabolomic profiles shift from precursor polyps to colorectal carcinoma. Furthermore, we found that specific fecal microbes and metabolites were correlated with the stage of colorectal carcinoma. Taken together, gut microbiota-derived metabolites are considered to be an important factor for understanding tumorigenesis.

AACR1-4

The role of microbiome-derived metabolism in cancer prevention and therapy

Scott J. Bultman
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Co-author：Darcy Holley[1]、Aadra Bhatt[2]、Matthew Redinbo[2]
[1]Dept. Genetics, Univ. of North Carolina at Chapel Hill、[2]Dept. Chemistry, Univ. of North Carolina at Chapel Hill

Gut microbiota have prodigious metabolic capacities that influence cancer prevention and treatment. Prevention: Utilizing gnotobiotic mouse models of CRC colonized with wild-type and mutant bacterial strains, we have demonstrated that dietary fiber protects against CRC in a microbiota- and butyrate-dependent manner. Fiber is fermented by colonic microbiota into butyrate, which is a SCFA with metabolic and energetic properties and is tumor suppressive because it accumulates in tumor cells due to the Warburg effect. Butyrate functions as an HDAC inhibitor, and ChIP-seq studies are aimed at understanding how specificity might be conferred. Treatment: Many chemotherapy drugs cause GI toxicity, which can limit the dose or duration of treatment. One mechanism for these adverse effects is the metabolic activity of gut microbiota that reactivate drugs following their inactivation in the liver. Utilizing a mouse model of triple-negative breast cancer, we demonstrate that the efficacy of irinotecan is improved by co-administration of an inhibitor of bacterial glucuronidase (GUS) enzymes. The GUSi prevents reactivation of the drug, which is a topoisomerase II inhibitor, to diminish diarrhea.

▲TOP

[LS14] LS14 [English]
Digital Genomics applied to Liquid Biopsies

2018 / 9 / 28 (Fri) 11:50-12:40 Room 3/10F 1003, Osaka International Convention Center Room 3
：Sysmex Corporation

Shinzaburou Noguchi / Department of Breast and Endocrine Surgery, Osaka University Graduate School of Medicine)

LS14

Digital Genomics applied to Liquid Biopsies

Kenneth W. Kinzler
Ludwig Center at Johns Hopkins University, Johns Hopkins Kimmel Cancer Center, USA

No Abstract

▲TOP

[S11-1] S11 [English]
Molecular target therapy under precision medicine

2018 / 9 / 28 (Fri) 13:00-15:30 Room 3/10F 1003, Osaka International Convention Center Room 3

Yoshihiko Maehara / Kyushu Central Hosp. of the Mutual Aid Association of Public Sch. Teachers、Takashi Kohno / Div. Genome Biol, Natl Cancer Ctr Res Inst.

Precision medicine, which selects and provides the optimal treatment for individual cancer patients based on genetic changes, has been conducted at cancer care hospitals in Japan. Genes and molecules that serve as biomarkers are diverse and the biomarkers and corresponding targeting agents differ by cancer types. There are multiple choices, in treatment lines, of molecular targeted agents, such as kinase inhibitors and immune checkpoint inhibitors. Novel findings and new ideas of therapies have been generated every day. So, it is not easy to carry out precision medicine in a true sense for each cancer patient. In this symposium, speakers who are active at the cutting edge of precision medicine research in colorectal, stomach, liver, breast, and lung cancers will talk about the latest findings, problems at the present, and the prospection for the future of precision medicine. From the next year, gene panel-based diagnoses using next generation sequencers will be included in insurance, and precision cancer medicine will become full-scale in near future. Here, we will also discuss about how to solve variants of unknown significance (VUS), one of the biggest hurdles. I hope many audiences will participate in this symposium and discuss together.

S11-1

Molecular alterations and biomarkers in colorectal cancer; current concepts and future directions

Eiji Oki
Dept. Surg. & Sci., Grad, Sch. of Kyushu Univ.

Co-author：Koji Ando[1]、Qingjiang Hu[2]、Yasuo Tsuda[1]、Yuichiro Nakashima[1]、Hiroshi Saeki[1]
[1]Dept. Surg. & Sci., Grad, Sch. of Kyushu Univ.、[2]Dept. Surg. & Sci. Grad. Sch. Med. Sci. Kyushu Univ.

Chemotherapy for colorectal cancer (CRC) has developed significantly in the last decade. A major breakthrough was the establishment of RAS mutations as biomarkers for resistance against anti-epidermal growth factor receptor antibodies. From 2018, BRAF mutations and microsatellite instability will also be used as predictive markers in treatment decisions for CRC, which will become standard clinical practice in Japan. As different biomarkers are being discovered, consensus molecular subtypes were introduced as an intrinsic molecular classification system. These molecular alterations are considered potential CRC biomarkers because they can provide diagnostic, prognostic, and treatment response information, and will be of great benefit for future clinical research since it enables uniform categorization of CRC across different regions. For patients with resected colon cancer, a multigene expression assay, such as the Oncotype DX colon cancer assay, can help inform the discussion regarding the role of adjuvant chemotherapy. Clinical use of these biomarkers in the future should be carefully studied, with respect to ethical and economic considerations.

▲TOP

S11-2

Challenges of precision medicine for gastric cancer

Kohei Shitara
Natl. Cancer Ctr. Hosp. East

Until now, more than 20 phase 3 trials for metastatic gastric cancer have been conducted to investigate new agents. However, only 5 trials showed significant improvement of survival, with Anti-HER2-, VEGFR2- and PD-1 pathway. Efficacies are still not sufficient and more efficient strategy might be necessary based on its molecular profiles. We have conducted the Nationwide Cancer Genome Screening Project using Next Generation Sequencing in advanced gastrointestinal cancer, called as the SCRUM-Japan GI-SCREEN. Approximately 5000 patients have been screened and more than 80 patients have been enrolled for clinical trials base on targetable genomic alteration. Circulating Tumor DNA analysis is also incorporated as an additional project, which may potentially overcome intra tumor heterogeneity or clonal evolution. Possible predictive biomarkers of PD1 blockade in several types of cancers have been identified so far and these factors should be analyzed in gastric cancer trials, to elucidate predictive and resistance mechanisms. Precision therapy stratifying patients based on genomic or immune profile into right combinations might be necessary to improve outcomes of GI cancers.

S11-3

Molecular target therapy for liver cancer under precision medicine

Shinji Tanaka
Dept. Mol. Oncol., Sch. Med., Tokyo Med. & Dent. Univ.

Heterogeneity is one of the essential hallmarks of malignancies. As inter-patient heterogeneity, a striking variability differs in biological characteristics including the stemness feature, cell-cell interaction, metastatic tendency and even response to treatment, resulting in patient prognosis. Whole genome sequencing revealed mutational landscape underlying the phenotype diversity of cancers. Based on the integrated genomic, epigenomic, transcriptomic, metabolic, proteomic and phenomic analyses on surgical tissue samples, the subtype stratification has been achieved in various malignancies including liver cancer. Identification of each molecular subtype is expected to realize the precise medicine targeting the subtype-specific molecules; however, there are obstacle limitations to determine whether matching druggable targets or synthetic lethal interactions. Current breakthroughs in genome editing technology can provide us with unprecedented opportunity to recapitulate the subtype-specific pathophysiology in vitro and in vivo. Given a great potential, on-demand editing system can design the actionable strategy and revolutionize precision cancer medicine based on surgical oncology.

S11-4

Molecular target therapy for breast cancer in the precision medicine era

Eriko Tokunaga
Dept. Breast Oncol., NHO Kyushu Cancer Ctr.

Breast cancer (BC) is highly heterogeneous and could be divided into different molecular subgroups; luminal A, luminal B, HER2-enriched and basal-like. Clinical subtypes evaluated by available biomarkers such as ER, PgR, HER2 are used practically in order to determine the appropriate therapy. Endocrine therapy is the most important therapy for ER-positive BC. PI3K/Akt/mTOR and CCND1/CDK4/6-Rb pathway are associated with endocrine resistance. CDK4/6 inhibitors (palbociclib, abemaciclib) and mTOR inhibitor (evelorimus) in combination with endocrine therapy have revealed high efficacy for metastatic BC. Anti-HER2 therapies like trastuzumab, pertuzumab, and T-DM1 have dramatically improved the prognosis of the patients with HER2+ BC. New anti-HER2 therapies are now in development. The efficacy of the PARP inhibitor (PARPi), which induces cell death through synthetic lethality, has been shown for the patients with BRCA1/2-mutated BC. Immune checkpoint inhibitors, anti-PD1 antibodies and anti-PD-L1 antibodies are now developed, especially for triple negative breast cancer. Thus, new target therapies for BC have been developed based on their efficacy in subgroup populations.

▲TOP

S11-5

Molecular targeted therapy for lung cancer

Takashi Nakaoku
Div. Genome Biol., Natl. Cancer Ctr. Res

Co-author：Takashi Kohno
Div. Genome Biol., Natl. Cancer Ctr. Res, Div. Translational Genomics, EPOC, Natl. Cancer Ctr.

Driver oncogene alterations is an effective target for lung cancer treatment as exemplified by EGFR and BRAF mutations; and ALK and ROS1 fusions. Decision-making based on genetic alterations using NGS-based gene panel tests is becoming reality in lung cancer clinic. The success of such a genome-based medicine needs more druggable alterations as well as annotation methods for them. We and others identified oncogenic fusion of the NRG1 gene encoding neuregulin as a novel therapeutic target in lung and other cancers (Nakaoku et al., Clin Cancer Res, 2014). NRG1 fusion conferred cancer stem cell-like properties through a IGF2 autocrine/paracrine circuit (Murakami, Nakaoku et al, Cancer Res, 2015). In addition, we annotated a secondary mutation which were detected in a case carrying CCDC6-RET fusion through functional genomics analysis. The mutation was revealed to have an allosteric effect by combined biological, biochemical and molecular dynamics simulation studies (Nakaoku et al., Nat Comm, 2018). Importance of functional genomics studies for precision lung cancer medicine will be discussed.

S11-6

Molecular targeted therapy for cancer with variant of unknown significance

Shinji Kohsaka
Div. Cell. Signaling, Natl. Cancer Ctr. Res. Inst.

Co-author：Masaaki Nagano[1]、Hiroyuki Mano[2]
[1]Dept. Thoracic Surg., Grad. Sch. Med.、[2]Div. Cell. Signaling, Natl. Cancer Ctr. Res. Inst.

Numerous variants of unknown significance (VUS) have been identified through large-scale cancer genome projects, although their functional relevance remains uninvestigated. We developed a mixed-all-nominated-mutants-in-one (MANO) method to evaluate the transforming potential and drug sensitivity of oncogene VUS in a high-throughput manner and applied this method to 101 nonsynonymous epidermal growth factor receptor (EGFR) mutants. We discovered a number of mutations conferring resistance to EGFR tyrosine kinase inhibitors (TKIs), including gefitinib- and erlotinib-insensitive missense mutations within exon 19 and other gefitinib-resistant mutations such as L833V, A839T, V851I, A871T, and G873E. About 13% of L858R-positive tumors were found to harbor compound mutations primarily in the cis allele, which decreased gefitinib sensitivity of these tumors. Thus, these data support the importance of examining the clinical relevance of uncommon mutations within EGFR and of evaluating the functions of compound mutations. This method may become a foundation for the in vitro and in vivo assessment of variants of cancer-related genes and help customize cancer therapy for individual patients.

S11-Special_Remarks

Special Remarks

Hirotoshi Akita
Dept. Med. Oncol., Faculty of Med. & Grad. Sch. Med., Hokkaido Univ.

No Abstract

▲TOP

[SP05-1] SP5 [Japanese]
Meet your good collaborators

2018 / 9 / 28 (Fri) 15:30-17:00 Room 3/10F 1003, Osaka International Convention Center Room 3

Masamitsu Konno / Dept. CFS Grad. Sch. Med. Osaka Univ.、Hiroyasu Kidoya / Dept. Signal Transduction, RIMD, Osaka Univ.

若手研究者の研究が加速するためのパートナーに出会うことを目的とした、癌学会初の企画です。ネット掲示板を活用しながら、自らの研究に必要なパートナー(共同研究者、ポスドク、自分はこんな技術があるから雇って欲しい! など) を見つけ出せるように討論も交えながら進めていきます。この企画の演者は会場の聴衆の皆さまです。聴衆が動かなければ何も動きません。若手企画1のパネラーも参加しますので、ぜひ積極的に討論に参加して、自分の研究を推進し、自らの研究フィールドを作り上げるためのパートナーを見つけてください。

SP05-1

No Abstract

▲TOP

[ML3] ML3 [Japanese]
Morning Lectures 3

2018 / 9 / 28 (Fri) 8:00-8:50 Room 4/10F 1001, Osaka International Convention Center Room 4

Takashi Kanematsu / Nagasaki City Hosp. Organization

ML3

Highly accurate cancer detection using C. elegans olfaction

Takaaki Hirotsu
Hirotsu Bio Sci. Inc.

Discussant：Yasuhiro Koh
3rd Dept. Int. Med. Wakayama Med. Univ., Sch. Med.

To increase cancer checkup rate, it is critical to develop the 1st cancer screening system which is economical, non-invasive and highly accurate. We noticed cancer-specific smells and *C. elegans* which has fine olfactory systems. We demonstrated *C. elegans* displayed attractive chemotaxis towards urine from cancer patients but avoided control urine. We tested 242 samples to measure the performance of the cancer examination using *C. elegans* (N-NOSE), and found the sensitivity was 95.8%. This is markedly higher than that of other existing tumor markers. This test was able to diagnose various cancer types tested at the early stage.
The N-NOSE has many advantages as noninvasive, convenient, highly accurate, comprehensive and low costs. The biggest difference between this method and the other established ones is an only word to describe 'Biological diagnosis'. It becomes possible to provide high-sensitivity and low-cost examination with using the olfaction which especially excels in animal's sense and choosing the low-cost organism. We established a company aiming practical use of N-NOSE. We introduce our recent progress of research and development of N-NOSE.

▲TOP

[E-2001] E7 [English]
Genomic analysis

2018 / 9 / 28 (Fri) 9:00-10:15 Room 4/10F 1001, Osaka International Convention Center Room 4

Masahito Kawazu / Div. Cell. Sig., Natl. Cancer Ctr. Res. Inst.

E-2001

Mutational landscape of Cancer-Related Genes in Colorectal Cancer in Hong Kong

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Identification of somatic mutations helps to understand the molecular mechanism of colorectal cancer (CRC) and the development of novel therapeutics. Next-generation sequencing (NGS) was performed with a targeted panel of 107 cancer related genes for 208 CRC samples.A total of 2070 non-silent somatic mutations were found. The mutation rate of 208 tumors ranged from 3.67 to 187.44 with a median of 16.53 (per 10^6 bases). All tumors with low mutation burden (n=194) were microsatellite stable (MSS). Of the 14 tumors with high mutation burden, 13 (93%) were MSI-H and one tumor harbored the P286R POLE mutation, suggesting ultra-mutator phenotype. Among these non-silent mutations, 15 significantly mutated genes were identified. In addition to the most frequently altered genes -APC (79%), TP53 (68%), KRAS (50%), frequent mutations were found in RUNX1, PIK3CA, SOX9, BRAF, FBXW7, GNAS, MSH3, NRAS, ACVR2A, SMAD4, TCF7L2, RNF43. Significantly mutated genes mainly involved in WNT, MAPK, PI3K, TGF-b and p53 pathways.Targeted sequencing provides comprehensive genetic alteration data and has led to identify novel significantly mutated genes.

▲TOP

E-2002

Cell-free DNA exome sequencing of pancreatic juice from intraductal papillary mucinous tumors of pancreas

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Intraductal papillary mucinous neoplasm (IPMN), despite being an indolent neoplasm of pancreas, has high risk to develop to invasive cancer or co-occur with malignant lesion. Hence, it is important to assess its malignant risk by non-invasive way. In order to do so we performed deep exome sequencing analysis of cell-free DNAs obtained from pancreatic juice and blood from patients with IPMN with or without malignant lesion. After filtering low-quality sample data, we detected somatic mutations of KRAS, GNAS, TP53, and RNF43 among others and analyzed copy number alterations (CNAs) in order to find significantly amplified or deleted regions across the dataset, finding significant amplification in the regions 7q21.12 (P = 0.012) and 8q24.22 (P = 0.011). These findings indicate that the use of cell-free sequencing analysis of PJ for detecting SNVs and CNAs of driver genes could have a high potential to assess the malignant progression risk of IPMNs.

E-2003

Analysis of noncoding indels in the surfactant-encoding genes in lung cancer

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Aim: A recent study identified indel mutations in the noncoding region of surfactant genes in certain patients with lung adenocarcinoma (Cell, 2017). Methods: The subjects were 86 patients with lung cancer who underwent surgery in our department. The cancer panel was designed in our institution for analyzing noncoding regions. DNA was extracted from FFPE samples and targeted sequencing was performed. Results: Among the subjects, indels in the 3 ′ UTR of surfactant-encoding genes were identified in patients with adenocarcinoma (28.1%), squamous cell carcinoma (21.4%), and other histological types (12.5%). In patients with multiple cancers, the indels can be used to determine whether the tumor was primary or metastatic. In two patients with mediastinal lymph node cancer, lung cancer was determined as the primary site because of the presence of the indels. Discussions: Indels in the 3'UTR of surfactant-encoding genes represent the precise cell of origin for the lung cancer, irrespective of histological type and disease stage. In clinical practice, the indels can be used as clonal markers in patients with multiple cancers and for determining the origin of cancer of unknown primary.

E-2004

Fusion kinases identified by genomic analyses of microsatellite instability-high colorectal cancers

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Microsatellite instability-high colorectal cancers (MSI-H CRCs) have been heretofore conventionally divided into hereditary (Lynch syndrome, LS) and sporadic categories.
We performed MSI testing on approximately 2,800 resected CRCs; whole-exome sequencing, transcriptome sequencing, and methylation analyses were done on 149 out of 162 tumors showing MSI in BAT25 and BAT26 loci.
Sporadic MSI-H CRCs are further classifiable into somatic mismatch repair gene mutation (Lynch-like, LL) or MLH1 methylated (MM) types. There were significant differences between LS/LL and MM groups in age, tumor location, number of insertions/deletions, and recurrent mutations of KRAS/PIK3CA/APC and BRAF/RNF43. Eleven fusion kinases were detected only in MM MSI-H CRCs lacking oncogenic KRAS/BRAF missense mutations and were associated with worse post-relapse prognosis. In another cohort, we validated a cost-effective strategy for detecting MM tumors and fusion kinases. These findings enable us to screen patients who can be treated with kinase inhibitors.

▲TOP

E-2005

Genomic insights into immune suppression in liver cancer

Masashi Fujita
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The clinical success of immune checkpoint inhibitors urges further understanding of immune suppression in tumors. We analyzed RNA-Seq of 430 tumorous and non-tumorous liver tissues along with their whole genome sequences. Cytolytic activity (CYT) was computed as a quantitative measure of cell-mediated immunity. CYT in tumors was associated with better overall survival, whereas CYT in adjacent liver tissues was associated with poor disease-free survival. Tumor CYT was significantly lower than liver CYT, indicating immune suppression in tumors. Tumor CYT was negatively associated with somatic mutation of ARID2 but was not correlated with liver CYT, etiology and the number of neoantigens. Expression levels of various immune genes, including PD-1, PD-L1 and CTLA4, were positively correlated with CYT. Differential gene expression with correction for CYT demonstrated that tumors were abundant in regulatory T cells and antigen presentation machinery, whereas livers were more biased toward humoral immunity and showed signs of T cell exhaustion. Genomic analysis serves as valuable tool for understanding anti-tumor immunity and identifying potential immuno-therapeutic targets.

E-2006

Clonality and loss of heterozygosity are associated with prognosis and subtypes in high grade serous ovarian cancer

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[1]Dept. OB

High grade serous ovarian cancer (HGSOC) comprises four gene expression subtypes, of which the Mesenchymal type has the worst prognosis. High degree of loss of heterozygosity (LOH) indicates homologous recombination deficiency, a characteristic associated with sensitivity to platinum and PARP inhibitors. This study aimed to clarify genetic features of HGSOC by using The Cancer Genome Atlas data (n=573). Clonality Index (CI), indicating intratumor heterogeneity, was determined as we previously reported (PMID;28734796). We found high CI (≥3) cases exhibited short progression-free survival (PFS) (p=0.016), whereas high LOH score was associated with prolonged PFS and overall survival (OS) (p=0.020, p<0.0001, respectively). The CI and the LOH scores were significantly different among the four gene expression subtypes (p<0.0001, respectively), of which the highest CI and the lowest LOH scores were observed in the Mesenchymal type. In conclusion, this study revealed the relationship between gene expression subtype and genetic alteration in HGSOC, suggesting the poor prognosis of the Mesenchymal type is caused by increased intratumor heterogeneity and decreased platinum sensitivity.

▲TOP

[E-2007] E3-1 [English]
Virus, bacteria infection, inflammation and cancer (1)

2018 / 9 / 28 (Fri) 10:15-11:30 Room 4/10F 1001, Osaka International Convention Center Room 4

Masashi Fukayama / Dept. Pathol. Grad. Sch. of Med. Univ. of Tokyo

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E-2007

EB-virus promotes metastatic potential by remodeling Stim1-mediated Ca 2+ signaling in nasopharyngeal carcinoma cells

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Nasopharyngeal carcinoma (NPC) is a unique EBV-associated head and neck cancer. Our earlier works showed that EBV empowers NPC cells to acquire various capacities that required for metastasis by enhancing cytosolic Ca^{2+} responses. However, the pathway through which EBV manipulates Ca^{2+} signaling still remains unappreciated. Here we demonstrated that EBV amplified EGFR activation-launched Ca^{2+} signaling through the intracellular aggregation of Stim1, which serves as a Ca^{2+} sensor in the endoplasmic reticulum to stimulate store-operated Ca^{2+} entry. In addition, Stim1 was upregulated in NPC tissues compared with normal nasopharyngeal epithelium, and expression level of Stim1 positively correlated with the severity of cervical lymph metastasis. Silencing of Stim1 effectively interrupted EGF-induced EMT and reduced invasiveness in EBV-infected NPC cells. Utilizing both zebrafish and mouse models, we validated that knockdown of Stim1 in EBV-infected NPC cells inhibited hematogenous and lymphatic metastases *in vivo*, respectively. Taken together, EBV amplifies the Stim1-mediated Ca^{2+} signaling, which regulates EMT, and thereby contributing to the highly metastatic potential in NPC cells.

▲TOP

E-2008

EBV-associated histone modifications resulting in cisplatin resistance in nasopharyngeal carcinoma

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Nasopharygeal carcinoma (NPC), the endemic malignancy, is highly associated with Epstein-Barr Virus (EBV) infection. However, the exact role of EBV contributing to NPC development is still poorly understood. As we previously identified that hypermethylation in NPC is very frequent, in this study, the possible roles of EBV in histone modifications via H3K4me3 in the host cells were elucidated. Two pairs of EBV+/- nasopharyngeal epithelial (NPE) cell lines were used for chromatin immunoprecipitation sequencing (ChIP-Seq) by next-generation sequencing approach to identify EBV-regulating genes in the host cells. A total of 18 DNA damage repair members, from base excision repair (BER), homologous recombination, non-homologous end-joining, and the mismatch repair (MMR) pathways were significantly enriched with reduction of H3K4me3 in the EBV-infected NPE cells. Down-regulation of seven BER members and MLH1 from MMR were further validated in the NPE cell lines and NPC biopsies. Furthermore, in vitroand in vivoassays showed that MLHknockout in MLH1-expressing NPC cells are more resistant to cisplatin, while MLH1re-expression in MLH1-deficient NPC cells are more sensitive to cisplatin.

E-2009

The presence of defective Epstein-Barr virus (EBV) infection in EBV-associated hematological malignancies

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Background
Epstein-Barr virus (EBV) infects >90% of humans. Despite this high prevalence, only a small subset develops EBV-associated neoplasms. The reasons for this are very poorly understood.
Methods
We analyzed the EBV genome in 132 patients with various EBV-associated diseases.
Results
We identified frequent intragenic EBV deletions in patients with EBV-positive diffuse large B-cell lymphoma (10/14, 71%), extranodal NK/T-cell lymphoma (10/23, 43%), and chronic active EBV infection (27/77, 35%). These deletions were not identified in patients having infectious mononucleosis (0/4) or other non-neoplastic diseases (0/14). These deletions most frequently affected viral microRNAs which are known to suppress viral transcription factors that are required for the lytic reactivation of EBV. The deletions also affected several genes that are essential for viral production. These deletions observed in our study are thought to upregulate lytic cycle-associated genes, some of which benefit neoplasms by inducing genomic instability and immune escape, and protect infected cells from death caused by the lytic cycle.
Conclusion
Our findings link intragenic EBV deletions and neoplastic proliferations.

E-2010

Promoter-mediated nuclear retention of HBZ RNA is involved in proliferation of ATL cells

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HTLV-1 encodes an antisense gene named HBZ in the negative strand of the provirus. HBZ is constantly detected in ATL cells, suggesting its crucial role in viral pathogenesis. Interestingly, HBZ RNA has noncoding functions in addition to encoding the HBZ protein. It is considered that HBZ RNA functions as a regulatory RNA, since it modulates promoter activity of cellular genes and deregulates their expression. In this study, we employed the single-molecule RNA fluorescent in situ hybridization (FISH) and found that HBZ RNA is localized dominantly in the nucleus in ATL cells. HBZ RNA was found less polyadenylated than HTLV-1 sense RNAs. It has been known that promoter potency is associated with the efficiency of polyadenylation. HTLV-1 3' LTR was involved in the nuclear retention and less polyadenylation of HBZ RNA. Furthermore, 3' LTRs of other retroviruses also seem to retain the transcribed RNAs, indicating that there may be a conserved mechanism. We also found that an HBZ expression vector, which enables more nuclear distribution of the HBZ RNA, exhibited enhanced cell proliferation., suggesting that nuclear retention of HBZ RNA is important for proliferation of ATL cells.

▲TOP

E-2011

Dynamic changes of chromatin structure and transcriptome by transient expression of HTLV-1 Tax

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HTLV-1 Tax is transiently expressed in some ATL cell lines, which induces drastic changes in host transcriptome. In this study, we analyzed structural change of host chromatin accompanied by transient Tax expression to clarify the transcriptional regulation of host genes in ATL cells. ATL cell lines (MT-1 and KK-1), which express EGFP under the control of Tax, were used to sort Tax-expressing and -non-expressing cells, and each population was subjected to H3K27ac ChIP-seq and ATAC-seq. There were more H3K27ac-marked active enhancers in Tax(+) than Tax(-) cells. ATAC-seq results showed that motifs of NF-κB, and AP-1 family (e.g., JunB, Fra1/2) were significantly enriched in Tax(+) cells compared to Tax(-) cells, which is consistent with the results of pathway analyses of RNA-seq. In contrast, motifs of CTCF and BORIS were enriched in Tax(-) cells. Importantly, mRNA levels of NF-κB and AP-1 transcription factors were significantly higher in Tax(+) than Tax(-) cells. These findings suggest that structural changes of NF-κB and AP-1 family recognition sites, and activation of pathways related to these factors could trigger global transcriptional changes by transient Tax expression.

E-2012

Impaired T-cell responses in natural infection of STLV-1 as a primate model of immune suppression in HTLV-1 infection

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HTLV-1-specific CTL response is an important anti-tumor host defense mechanism, which is impaired in patients with adult T-cell leukemia/lymphoma (ATL) and a small proportion of asymptomatic HTLV-1 carriers. We previously reported the anti-ATL therapeutic potential of vaccines activating Tax-specific CTL, and one of such vaccines is currently under a clinical trial. To further extend this strategy to prevention of ATL development, a suitable animal model is required, which reproduces impaired T-cell responses in a high-risk group of HTLV-1 carriers. Here, we analyzed T-cell responses in Japanese macaques that had been naturally infected with simian T-lymphotropic virus type 1 (STLV-1), closely related to HTLV-1 sharing some similarities in the viral genome. We found that STLV-1-specific T-cell responses varied among individuals, and that an impaired T-cell response was associated with a poor control of STLV-1. These findings indicate that a Japanese macaque with an impaired STLV-1-specific T-cell response can be a suitable animal model for the vaccine studies. (Ms. Annabelle Chung, an exchange-program student from Imperial College partly performed immunological analysis).

▲TOP

[LS15] LS15 [Japanese]
Clinical implication for whole-genome sequencing in human cancers

2018 / 9 / 28 (Fri) 11:50-12:40 Room 4/10F 1001, Osaka International Convention Center Room 4
：Illumina K.K.

Mitsuo Shimada / Department of Surgery, Tokushima University

LS15

Clinical implication for whole-genome sequencing in human cancers

Keisuke Kataoka
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No Abstract

▲TOP

[E-2049] E3-2 [English]
Virus, bacteria infection, inflammation and cancer (2)

2018 / 9 / 28 (Fri) 13:00-14:15 Room 4/10F 1001, Osaka International Convention Center Room 4

Akinori Takaoka / Div. Signaling in Cancer & Immunol., Inst. for Genet. Med., Hokkaido Univ.

E-2049

Oxidative Stress and Immune Responses During Hepatitis C Virus Infection in Tupaia belangeri

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Co-author：Michinori Kohara[1]、Kyoko Kohara[2]
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To address the molecular basis of HCV pathogenesis using tupaias (Tupaia belangeri), we evaluated host responses upon HCV infection. Adult tupaias were infected with HCV genotypes 1a, 1b, 2a, or 4a. Viral RNA, alanine aminotransferase, anti-HCV core and anti-nonstructural protein NS3 antibody titres, reactive oxygen species (ROS), and anti-3β-hydroxysterol-δ24reductase (DHCR24) antibody levels were measured at 2-week intervals from 0 to 41 weeks postinfection. All HCV genotypes established infections and showed intermittent HCV propagation, and produced anti-core and anti-NS3 antibodies. ROS levels in sera and livers were significantly increased, and induced DHCR24 antibody production. Lymphocytic infiltration, disturbance of hepatic cords, and initiation of fibrosis were also observed in livers from HCV-infected tupaias. Intrahepatic levels of Toll-like receptors 3, 7, and 8 were significantly increased in all HCV-infected tupaias. Thus, our findings showed that humoral and innate immune responses to HCV infection, ROS induction, and subsequent increases in DHCR24 auto-antibody production occurred in our tupaia model, providing novel insights into understanding HCV pathogenesis.

▲TOP

E-2050

APOBEC signature mutagenesis in the genome of human papillomavirus and its relevance to cervical carcinogenesis

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To explore within-host genetic diversity of HPV and its relevance for cervical carcinogenesis, whole-genome sequences of HPV16/52/58 were amplified by type-specific PCR from total cellular DNA of cervical exfoliated cells collected from patients with cervical intraepithelial neoplasia and invasive cervical cancer, and were deep-sequenced. Nucleotide positions showing changes with more than 0.5% frequencies compared to the consensus viral sequence were determined for individual samples. A total of 1,052 positions of nucleotide variations were detected in HPV genomes from 151 samples. Overall, C-to-T and C-to-A substitutions were the dominant changes observed across all histological grades. Analysis of the trinucleotide context for substituted bases revealed that TpCpN, a preferred target sequence for cellular APOBEC cytosine deaminases, was a primary site for C-to-T substitutions in the HPV genome. Interestingly, APOBEC signature mutations detected in the long control region resulted in the activation of the viral early promoter responsible for E6/E7 oncogenes expression in reporter assays, suggesting a potential role for APOBEC-mediated mutagenesis in cancer development.

E-2051

Amelioration of metaplasia and re-emergence of normal gastric lineages after MEK inhibitor to H. pylori infected gerbils

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Gastric metaplasia induced by H. pylori is known as the precancerous lesion. The effects of eradication therapy to this lesion is not sufficient for recovery. The metaplasia in humans and rodents is reported to show elevated pERK, and selumetinib reduces metaplasia in KRas(G12D) over-expressing mice. The purpose is to know the efficacy of selumetinib in H. pylori infected animals. Methods: Mongolian gerbils, infected with H. pylori for a year, were randomly assigned to placebo group (n=13) or selumetinib group (n=12). After 4 weeks treatment, their stomach were examined histologically and immunohistochemically. Results: The placebo group showed oxyntic atrophy and extensive metaplasia. The selumetinib group showed restitution of parietal cells, and marked decrease of TFF2, GSII lectin positive SPEM and TFF3 positive intestinal metaplasia was observed. The selumetinib group showed decrease and relocation of pERK and Ki67 positive cells, meaning the successful delivery of MEK inhibitor. Conclusions: Selumetinib ameliorated metaplasia and reconstructed normal linages in H. pylori infected gerbils. These findings suggest that selumetinib could prevent gastric cancer after eradication.

E-2052

Characterization of metaplastic lineage in the gastric mucosa of Mongolian Gerbils with Helicobacter pylori infection

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Intestinal metaplasia and Spasmolytic polypeptide-expressing metaplasia (SPEM) are considered precursor lesions of gastric adenocarcinoma in humans. Loss of parietal cells and chronic inflammation cause the development of SPEM. However, how metaplastic lesions progress to dysplasia is still unknown. Therefore, we characterize metaplastic lineage in Mongolian gerbil gastric mucosa with Helicobacter pylori (H. pylori) strain 7.13 infection. Six weeks following H. pylori infection, SPEM developed in the base of oxyntic glands associated with parietal cell loss and inflammation. During chronic severe inflammation, SPEM glands changed to aberrant phenotypes, including branched lesions, dilated lesions and penetrating invasive glands. Clusterin was expressed in the tips of branched and dilated lesions and throughout regions of invasive glands. Intriguingly, clusterin-positive regions in these lesions expressed Ki67 and MMP-7, suggesting that clusterin-positive regions in progressive phenotypes of SPEM have invasive characteristics. Taken together, SPEM was generated after H. pylori infection and then changed to neoplastic phenotypes.

▲TOP

E-2053

Inflammatory and mitogenic signals drive IL23A secretion in intestinal epithelial cells

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The heterodimeric cytokine interleukin 23 (IL23A/IL12B) is produced by dendritic cells and macrophages to promote the activities of Th17 cells and innate lymphoid cells. Here, we report a strong induction of IL23A expression by TNF/NF-κB and MAPK signals in intestinal epithelial cells. Their activities were enhanced by the tumor suppressor RUNX3. Moreover, a strong crosstalk between the NF-κB and MEK pathways was observed. We confirm the secretion of endogenous IL23A by immunoprecipitation from activated CRC culture supernatants. Interestingly, the secreted IL23A could not be detected by ELISA specific for heterodimeric IL-23, owing to the absence of IL12B expression in this cell type. Subsequently, we evaluated the efficacy of NF-κB and MEK inhibitors in attenuating IL23A expression, especially in the context of MAPK pathway driver mutations in CRC cells. Accordingly, trametinib (MAPK inhibitor) and BAY 11-7082 (IKKα/IκB inhibitors) displayed effectiveness in human CRC lines with mutant KRAS or $BRAF^{V600E}$. Together, these data demonstrate the regulation of IL23A by proinflammatory and mitogenic signals and its secretion, which could be targeted in cancer therapy.

E-2054

Stress response protein RBM3 promotes the development of colitis-associated cancer

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Colitis-associated cancer (CAC) is caused by chronic intestinal inflammation and often results from refractory inflammatory bowel disease (IBD). Stress response proteins Cirp and HSPA4 are involved in the refractory clinical course and development of CAC. RNA-binding motif protein 3 (RBM3) is induced in response to various stresses. We assessed RBM3 function in 263 human samples from IBD patients and in Rbm3-deficient mice.Expression of RBM3 was correlated with the expression of Cirp, HSPA4, HSP27, Bcl-xL, and stem cell markers in the colonic mucosa of IBD patients. RBM3 expression increased and significantly correlated with R-spondin expression in the colonic mucosa of patients with refractory IBD, a condition associated with increased cancer risk, and RBM3 was overexpressed in human CACs. In the murine CAC model, Rbm3 deficiency decreased R-spondin and Bcl-xL expression and increased apoptotic cell number in the colonic mucosa, leading to reduced tumor multiplicity. Transplantation of wild-type and Rbm3 deficient bone marrow did not alter tumor burden. RBM3 could be a predictive biomarker of CAC risk and a new therapeutic target for cancer prevention in patients with IBD.

▲TOP

[E-2055] E15-1 [English]
New biomarker for digestive cancers

2018 / 9 / 28 (Fri) 14:15-15:30 Room 4/10F 1001, Osaka International Convention Center Room 4

Kikuya Kato / Lab. Med. Genomics, Nara Inst. Sci. Tech.

E-2055

Circulating microRNA classifiers to distinguish digestive cancers

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Many reports show that circulating miRNA profiles can denote the presence/absence of malignant disease. However, it is unclear whether different types of cancer can be discriminated based on circulating miRNAs. Here, we used microarray analysis to comprehensively analyze the serum miRNA profiles of every patients with esophageal squamous cell carcinoma (ESCC), gastric cancer (GC), colorectal cancer (CRC), hepatocellular carcinoma (HCC), pancreatic cancer (PC), or cholangiocarcinoma (CCA), along with those of individuals with no cancer (n=347 per group). Analyzed samples were randomly assigned (2:1) to discovery and validation sets. In the discovery set, we constructed a 55-miRNA classifier to discriminate between four groups based on diagnostic methods: ESCC+GC (esophagogastroduodenoscopy), CRC (colonoscopy), HCC+PC+CCA (abdominal ultrasound), and non-cancer. In the validation set, diagnostic sensitivities were 71% for ESCC+GC, 38% for CRC, and 81% for HCC+PC+CCA; specificity was 96%. These results show that analysis of circulating miRNA can be used to screen for digestive cancers. However, additional strategies, such as machine learning, are needed to discriminate CRC accurately.

▲TOP

E-2056

Serum miRNA in pre- and post-treatment gastric cancer patients compared to Singapore cohort by the new sensitive assay

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Serum miRNA is one of the liquid biopsy that provides information about cancer. We developed a unique 3-primer assay technology and it enables more sensitive, specific and speedy analysis for serum miRNA. 133 miRNAs, discovered through Singaporean cohort compared between gastric cancer patients and control subjects, were analyzed for 67 gastric cancer patients, pre- and post-ESD, 20 patients, pre- and post-surgery, and 100 non-cancer subjects in Japan. Serum of 18 Singaporean patients, pre- and post-surgery was also analyzed. 37 miRNA was up-regulated and 25 miRNA was down-regulated in cancer patients compared to normal subjects. 87 miRNA in ESD patients, 47 miRNA in Japan surgery patients, and 33 miRNA in Singapore surgery patients were significantly changed by treatment. Among them, 10 upregulated miRNA and 6 downregulated miRNA by treatment were overlapped. Inter-cohort correlation between Japan surgery patients and Japan ESD patients, and between Japan surgery patients and Singapore surgery patients correlated very well. This new sensitive method for miRNA detection is feasible and these overlapped miRNA are the new candidates for biomarkers of gastric cancer.

E-2057

Usefulness of plasma exosomal microRNA as biomarker for recurrence and prognosis in each tumor stage of gastric cancer

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Background: We clarified the usefulness of plasma exosomal microRNA as a diagnostic and prognostic biomarker in gastric cancer (GC) patients at each tumor stage. Methods: We first selected recurrence specific exosomal microRNA (miRNA) by microarray. Subsequently, we validated the usefulness of selected miRNA at each tumor stage of 232 GC patients. Results: In the miRNA microarray analysis, microRNA-23b (miR-23b) of the recurrence group displayed the most marked change compared to that of the healthy control and non-recurrence group. A significant association of miR-23b was revealed between the plasma exosomes and tumor tissues. miR-23b demonstrated a significant association with tumor size, depth of invasion, liver metastasis and TNM stage. The overall survival (OS) and disease-free survival (DFS) rates of low-miR-23b patients were significantly worse than those of high-miR-23b patients at stage I, II, III or IV. Cox multivariate analysis indicated that exosomal miR-23b is an independent prognostic factor for OS and DFS at each tumor stage. Conclusions: Plasma exosomal miR-23b has potential as predictive biomarker for the recurrence and prognosis of all stages of GC patients.

E-2058

Clinical significance of PD-1, PD-L1 and CD8 gene expression levels in gastric cancer

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Background: Anti-PD-1 therapy has shown a promising clinical outcome in gastric cancer (GC). We focused on the mRNA expression levels of immune-related genes of tumor tissue to identify predictive biomarkers for prognosis. Methods: mRNA expression levels of PD-1, PD-L1 and CD8 were evaluated by quantitative RT-PCR using tumor and adjacent normal tissues of 163 GC patients who underwent surgery. Results: PD-1, PD-L1 and CD8 mRNA levels in tumor tissue were significantly lower than those in normal tissue: 0.35-, 0.76- and 0.4-fold changes, respectively ($P<0.0001$, $P<0.05$ and $P<0.0001$). GC patients with low PD-L1 mRNA levels had significantly poorer overall survival (OS) than those with high PD-L1 mRNA levels ($P<0.05$), especially in GC patients with undifferentiated adenocarcinoma ($P<0.001$). Multivariate analysis showed that low PD-L1 mRNA expression levels were independent poor prognostic factors for OS (OR 2.29, 95%CI 1.20-4.29, $P<0.05$). Conclusions: Low PD-1, PD-L1 and CD8 mRNA levels may reflect immune suppression in GC, and the low PD-L1 mRNA levels were predicting biomarker for poor prognosis in GC patients.

▲TOP

E-2059

A New Biomarker for Peritoneal Lavage Using Digital PCR in Patients with Pancreatic Ductal Adenocarcinoma

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Aims: The significance of the peritoneal washing cytology (CY) in patients with pancreatic ductal adenocarcinoma (PDAC) is controversial in Japan. Our aim was to evaluate a new biomarker using intra-operative peritoneal lavage to predict the prognosis in PDAC. Methods: Peritoneal lavage samples were collected from 89 patients with PDAC undergoing pancreatectomy. Digital PCR targeting KRAS was employed to detect tumor-derived DNAs in the samples. Results: KRAS mutations were detected in 41 (46%) patients, including all in nine CY positive patients and 32 (40%) in 80 CY negative patients. When the optimal cut-off value of the KRAS allele frequency (AF) was determined, high AF group was significantly associated with poor overall survival (p=0.007) and disease-free survival (p=0.014). Multivariable analysis for overall survival identified high AF as an independent prognostic factor (HR, 2.27, p=0.037). The cumulative incidence of peritoneal recurrence was significantly higher in high AF group (42% vs. 11%, p=0.007). Conclusions: This new biomarker for peritoneal lavage may be promising for predicting PDAC prognosis, particularly for peritoneal recurrence.

E-2060

Development of liquid biopsy diagnostics for colorectal cancer by proteomic profiling of cultured tissue-derived exosome

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Early detection of colorectal cancer (CRC) is essential for improvement of prognosis by enabling therapeutic intervention at early stage. To identify early detection biomarkers for CRC, we performed comprehensive proteome analysis of tissue-exudative extracellular vesicles (Te-EVs), which were obtained from culture media of freshly resected viable CRC tissue or adjacent normal mucosa (n = 17). Among 6,149 identified Te-EV proteins, 393 proteins were significantly overexpressed (paired t-test, $p < 0.05$, fold change > 5.0) in EVs from CRC tissues compared to paired normal mucosa.We especially focused on GAM ($p = 8.2 \times 10^{-3}$, fold change = 5.5) as a novel biomarker candidate showing significant overexpression in CRC cells by IHC staining analysis. The expression level on plasma EVs from CRC patients was also higher than that from healthy donors in EV-sandwich ELISA assay. Moreover, the uptake of GAM^{++} EVs significantly enhanced vascular endothelial cell growth and angiogenesis via constitutive activation of a critical metabolic cycle in vascular endothelial cells. Thus EV-GAM might have great potential as a target for both CRC diagnosis and therapy.

▲TOP

[E-2061] E15-2 [English]
New biomarker / liquid biopsy

2018 / 9 / 28 (Fri) 15:30-16:45 Room 4/10F 1001, Osaka International Convention Center Room 4

Hiroshi Inoue / Dept. Surg., Eikoh Hosp.

E-2061

Circulating tumor DNA predicts relapse after allogeneic hematopoietic stem cell transplantation in AML and MDS

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Relapse remained the major problem in patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) undergoing allogeneic hematopoietic stem cell transplantation (alloSCT). In this context, the tumor-specific genomic component in serum, known as circulating tumor DNA (ctDNA), might be a suitable biomarker for predicting relapse. To address this issue, we retrospectively investigated the impact of the residual ctDNA status post-alloSCT on the outcome of 51 patients with AML and MDS undergoing myeloablative alloSCT. Eight patients were in complete remission at alloSCT. We identified driver mutations in 51 of 53 patients by next-generation sequencing. Serum ctDNA analysis was performed by the droplet digital PCR. Of evaluable patients, ctDNA was detected in 20 of 47 cases at 1 month and 14 of 44 cases at 3 months post-alloSCT. During a median follow up of 32 months, 14 patients relapsed at a median of 11 months post-alloSCT. ctDNA detected status at 1 and 3 months post-alloSCT had a significantly higher relapse rates and inferior overall survival rates. These results demonstrate that residual ctDNA status is a powerful predictor of relapse post-alloSCT in AML and MDS.

▲TOP

E-2062

Can the monitoring of translocation in the serum serves as a potential biomarker for Ewing's sarcoma?

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Background: Circulating cell-free DNA (cfDNA) has the potential to be used to monitor tumor burden in real time, although little has been reported for the detection of gene fusion. The objective of this study was to identify whether gene fusion in cfDNA can be used as a marker of tumor burden in patients with Ewing's sarcoma. Methods: Genomic breakpoint of each patient was detected by multiplex long-range genomic PCR, and probes for digital PCR were designed. cfDNA was extracted from the serum which was drawn at multiple time-points (pre-treatment, completion of the treatment, and relapse). cfDNA quantification was performed by digital PCR. Results: Patient-specific gene fusions were detected in 6 (75%) of 8 patients. Relative fusion copy ratio (RFCR: gene fusion copy number / wild KRAS copy number) was highest in pre-treatment samples and decreased as the treatment progressed. RFCR was elevated in the serum of relapsed patients in advance of clinical manifestation, whereas did not in the patients with disease-free. Conclusion: Gene fusion in cfDNA strongly correlated with the treatment stage and would be useful as an early biomarker for Ewing's sarcoma patients.

E-2063

The establishment and optimization of customized circulating tumor DNA cancer screening panel

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Circulating tumor DNA (ctDNA) derived from primary and metastatic tumor site(s) is a target for liquid biopsy, which provides a non-invasive approach to evaluate tumor mutation profiles from plasma samples. This study aims to establish a ctDNA cancer screening panel to detect mutations focusing on the 10 most common cancers in Japan. A total of 129 amplicons from 29 genes were shortlisted after analyzing cancer genome datasets from TGCA and COSMIC databases. This panel incorporated unique molecular indices (UMI) on individual DNA molecule before target enrichment to reduce the chances of PCR/sequencing errors. This panel is predicted to detect >80% of colon, lung and pancreatic cancers. The limit of detection, sensitivity and specificity were evaluated by using ctDNA reference standard and tumor DNA with known mutations. To optimize the methodology, the number of UMI was evaluated by increasing the ctDNA input, increasing targeted panel concentration, or reducing the number of samples that were pooled for next-generation sequencing. This proof-of-concept study suggested the feasibility of customizing ctDNA panel to fit the study design considering the limited ctDNA in blood.

E-2064

Emerging role of microRNA-based liquid biopsy biomarkers and anticancer treatments in digestive system cancers

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The development of minimally invasive biomarkers in body fluids, so-called 'liquid biopsy' have been required. Since the first report in gastric cancer in 2010, we have identified more than 20 candidate microRNAs for liquid biopsy in plasma of patients with digestive tract cancers, which could be useful in early cancer detection, monitoring disease status, and predicting malignant potential, prognosis and treatment sensitivity. During these processes, we also indentified that a low level of some tumor suppressor microRNAs in plasma relates to tumor progression and poor outcomes of esophageal cancer in miR-375 (Expert Opin Biol Ther 2012) and miR-655 (In press 2018), pancreatic cancer in miR-107 (Sci Rep 2017), and gastric cancer in miR-101 (Oncotarget 2017) and miR-148a (In press 2018). Moreover, we demonstrated that the restoration and maintenance of these plasma microRNA levels may be a novel therapy for inhibiting tumor and metastatic progression in the in vivo analyses using BALB/c mice. In this conference, we will present the possibility and issues of clinical applications of these microRNAs as a liquid biopsy and anti-cancer treatments from viewpoints of surgical oncologists.

▲TOP

E-2065

EGFR Hotspot Mutations Detection in Cell-Free DNA in Lung Adenocarcinoma Patients

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Cell-free DNA (cfDNA) as liquid biopsy is recently used as noninvasive tool for cancer diagnosis. Here we evaluated next-generation sequencing (NGS) for multiple target genes and droplet digital PCR (ddPCR) for EGFR hotspot mutations detection in cfDNA. cfDNA was extracted from 36 serum samples of lung adenocarcinoma patients with EGFR mutated tumor. Targeted-sequencing was done by Ion AmpliSeq PGM and Colon and Lung Cancer Research Panel. Then, ddPCR was tested on a subset of cfDNA from patients with EGFR hotspot mutations detected in tumor DNA. We also performed serial dilution of EGFR mutated cell lines and tumor DNA analysis by ddPCR. Mean coverage of cfDNA sequenced was 2966 reads. NGS found alterations in EGFR (27.8%), NOTCH1 (22.2%), PTEN (11.1%), STK11 (11.1%), MET (5.6%), KRAS (5.6%), PIK3CA (2.8%) and TP53 (2.8%). Detection limit of ddPCR were 0.026% for L858R and 0.0164% for E746-A750del. Hotspot mutations in cfDNA were detected in 5 of 15 patients (33.3%) by NGS and 10 of 13 patients (76.9%) by ddPCR. Our study shows that NGS enable multiple gene alteration detections in cfDNA. However, ddPCR displays higher sensitivity for EGFR hotspot mutations detection in cfDNA.

E-2066

Monitoring of soluble PD-L1 levels in sera in non-small-cell lung cancer

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In this study, we examined whether the level of soluble programmed death-ligand 1 (PD-L1) in sera would reflect the PD-L1expression level on tumor cells in non-small-cell lung cancer (NSCLC). We semi-quantitatively scored PD-L1 expression on tumor cells (0-300) by immunohistochemistry of 25 NSCLC cases that had received radical surgery. Levels of soluble PD-L1 in sera obtained during perioperative periods were analyzed by ELISA. Median PD-L1 expression score of tumor was 45.0 (0.0-252.9), and median preoperative serum PD-L1 level was 65.9 ng/ml (43.1-603.2). Soluble PD-L1 levels in sera was not associated with PD-L1 expression intensity of tumor cells. However, the reduction rate of serum PD-L1 levels one month after surgery compared with baseline levels before surgery showed a trend of positive correlation with PD-L1 expression score of tumor. Further study in larger sets of samples would be warranted, however not only PD-L1 expression on tumor cells but some interactions, such as tumor-immune cells reaction, would affect the levels of soluble PD-L1 in sera.

▲TOP

[ML4] ML4 [Japanese]
Morning Lectures 4

2018 / 9 / 28 (Fri) 8:00-8:50 Room 5/10F 1002, Osaka International Convention Center Room 5

Keiichi Nakayama / Dept. Mol. Cell. Biol., Med. Inst. Bioreg., Kyushu Univ.

ML4

Writing skills to publish attractive papers in English

Masahide Takahashi
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Writing attractive papers in which many scientists are very much interested is a dream for people belonging to the academic community. However, it is not easy to write English papers particularly for young Japanese scientists. The majority of young scientists struggle to write manuscripts in English which is not their native language. Both writing skills and logical thinking are necessary for them to succeed in publishing their findings in international journals. Also, these abilities are essential in order to step up in their academic careers. This morning lecture is meant to provide basic knowledge for young scientists such as graduate students, postdoctoral fellows and assistant professors, to present their findings in English in an attractive way. The lecture will be given in Japanese with powerpoint slides also in Japanese so that the participants can easily understand the points. I hope this lecture will be able to provide hints to get your manuscript accepted by highly regarded international journals.

▲TOP

[E-2013] E14-5 [English]
Brain tumor

2018 / 9 / 28 (Fri) 9:00-10:15 Room 5/10F 1002, Osaka International Convention Center Room 5

Motomasa Furuse / Dept. NeuroSurg., Osaka Med. College

E-2013

POLD2, a subunit of DNA polymerase &delta in glioblastoma tumor malignancy

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POLD2, a component of DNA polymerase delta complexes, plays a critical role in DNA replication, repair and contributes to genomic stability. We studied the expression and function of POLD2 in glioma cells and specimens. POLD2 expression significantly correlate with poor outcome. POLD2 knockdown inhibits glioma cell proliferation, invasion, cell cycle progression and sensitizes the glioma cells to the chemo-/radiation-therapy. Conversely, over-expression of POLD2 leads to induce cell proliferation, invasion, colony formation and resistance to the chemo-/radiation-therapy. Moreover, we found that POLD2 expression/function correlates with the expression of stem cell markers Sox2 and CD133, and self-renewal ability in GBM stem cell. Importantly, We found that POLD2 expression is induced by EGFR signaling and regulates EGFR functions. Inhibition of POLD2 in GBM cells strongly inhibited in vivo glioma xenograft growth. Together, these findings represent the first comprehensive analysis of the roles of POLD2 in gliomas. We demonstrate that the expression of POLD2 is associated with glioma malignancy.The results also suggest that POLD2 could be a potential therapeutic target for glioma.

▲TOP

E-2014

Integrated phospho-glyco-proteogenomics identified the potential clinical target signals against glioma stem cells

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To study molecular mechanisms and develop clinical targets against malignant glioma, we established glioma stem clones (GSC) from glioma patient's tissues, having potential of differentiation/promotion to glioblastoma, and subjected to a unique integrated phospho-glyco- proteogenomics. Using original GSC-iPEACH data integration/extraction revealed that membrane adhesion molecules including integrin aV/ECMs followed by RAS-MAPK signalings were significantly up-regulated to form a specific differentiation niche, meanwhile, SOX2, CD133, and proteoglycans such as CSPGs/CS synthetic-enzymes/metabolic pathways were down-regulated during the GSC differentiation. Their knockdown significantly effects GSC differentiation, and CS-degradation enzyme dramatically induces the GSC differentiation with GFAP and ERK upregulation, suggesting the presence of specific GSC glyco-niche. CSPG interactomes including integrin-aV/signal factors regulate GSC differentiation and their inhibitors such as RGDs/CS increased the GSC chemosensitivity leading longer survivals of mouse GSC xenograft-models. These results suggest that GSC glyco-niche could be a new clinical target against malignant glioma.

E-2015

Withdrawn

No Abstract

E-2016

Boron neutron capture therapy (BNCT) for the patients with recurrent malignant glioma using nuclear reactor

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We have applied tumor-selective particle radiation boron neutron capture therapy (BNCT) to malignant brain tumors. Standard treatment for recurrent MG has not yet been established. In most cases, a full course of radiotherapy has been applied after primary diagnosis; therefore, application of re-irradiation has to be applied with caution.We have treated a group of >150 patients with high-grade gliomas/meningiomas with BNCT. Here we introduce our clinical result using reactor-based BNCT with borono-phenylalanine (BPA) for recurrent MG.Twenty-three patients were treated by BNCT with reactor epithermal neutron. The minimum tumor dose was calculated as 40 Gy-Eq and maximum brain dose was 10.5 Gy-Eq. Median survival was 11.6 (95%CI: 7.6-16.5) months after BNCT with 6- and 12- month survival of 74 and 42%, respectively.With recent technical advancement, radiation therapies can deliver high local doses as an effective salvage treatment with low rates of side effects. However, even if the radiographically remaining, progressed tumor could be targeted with higher irradiation doses, there is still remaining problem to be solved. BNCT will overcome this problem with acceptable toxicity.

▲TOP

E-2017

Critical role of PIK3 pathway gene alterations for malignant transformation in oligodendroglial tumors

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Oligodendroglioma (OD) is a subtype of diffuse astrocytic and oligodendroglial tumors. Although prognosis in OD tumors are relatively favorable, majority of OD develop malignant transformation. Therefore, understanding of molecular mechanism is crucial to identify therapeutic target. However, there are few available patient-derived OD xenograft model, which diminish preclinical investigations. Here, we present novel anaplastic oligodendroglioma (AOD) xenograft models. We confirmed OD phenotype and the presence of IDH1 mutation and 1p/19q co-deletion in xenograft tissue. Through genomic analysis, we found the critical role of PIK3CA mutation to develop malignant phenotype as well as xenograft formation in AOD patient. We also implanted patient-derived AOD cells with or without endogenous PIK3CA or PIK3R1 mutation. Of note, PIK3CA or PIK3Rl mutant tumor formed, whereas no tertiary mutant tumor did not form xenograft. Also, we found such mutant tumor cells were vulnerable to alkylating agents and PIK/AKT/mTOR pathway inhibitors. In summary, we established novel patient-derived OD xenograft models, which harbor PI3K gene mutations, for future preclinical investigations.

E-2018

Response to seizure and tumor-progression by perampanel in uncontrollable epilepsy with gliomas

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Background: Excessive extracellular glutamate activates AMPA-type glutamate receptors (AMPA receptors) and induces seizures. Antagonistic activation of AMPA receptors inhibits epilepsy and glioma growth in in vitro and in vivo studies. Patients and Methods: We tested perampanel (PER), a novel AMPA receptor antagonist, in twelve glioma patients with uncontrollable epilepsy. Seizure response, PER concentration, and tumor volume were assessed. Results: Obvious seizure responses control were observed in 10 analyzed patients (100%) with 6 patients (60%) of seizure-freedom. Median plasma concentrations of PER were 296 ng/ml in those with 4 mg/day PER treatment and 518 ng/ml in those with 8 mg/day PER treatment. High-intensity lesions of MRI-FLAIR images were volumetrically assessed to analyze the tumor size. The volume reduction was detected for the 6 months in correlation with increased plasma levels of PER. Conclusion: PER treatment was effective in uncontrollable epilepsy with gliomas. MR images showed the inhibition of tumor growth.

▲TOP

[E-2019] E14-6 [English]
Genetic analysis and new treatment of hematological malignancies

2018 / 9 / 28 (Fri) 10:15-11:30 Room 5/10F 1002, Osaka International Convention Center Room 5

Mineo Kurokawa / Dept. Hematol. Oncol., Grad. Sch. Med., Univ. Tokyo

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E-2019

Characterization of pediatric T-cell acute lymphoblastic leukemia based on integrated DNA methylation analysis

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To unveil the epigenetic profiles of T-cell acute lymphoblastic leukemia (T-ALL), we performed EPIC methylation array analysis for 79 pediatric T-ALL cases, combined with mutation, expression, and clinical data.Unsupervised consensus clustering identified 4 distinct methylation clusters (M1, M2, M3 and M4). In M1 cluster (n=39), PI3K-AKT pathway mutations were enriched with *TAL1* fusions. In M2 (n=20) and M3 (n=11) clusters, epigenetic and JAK-STAT pathways were frequently mutated with ETP and *TLX* expression pattern, regardless of different methylation status. M4 cluster (n=9) was characterized by *SPI1* fusions with high *SPI1* and RAS pathway expression leading to enrichment in RAS and myeloid cell pathways, showing dismal outcome (Log-rank $p=4.4\times10^{-7}$). Immunophenotype of M1 cluster exhibited a late cortical thymocyte profile after T-cell receptor rearrangement (CD4+CD8+TCRαβ+) contrast to M3 cluster (CD4+CD8+TCRαβ-). M2 and M4 clusters exhibited an uncommitted double negative profile (CD4-CD8-) with more immature stage in M2 cluster.Our results, the biological phenotype of T-ALL is mediated by both genetic and epigenetic regulations, might be helpful for a new therapeutic strategy.

▲TOP

E-2020

Loss of TET2 and TET3 alleles accentuate development of hematological malignancies

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Background: Somatic mutations in Tet2 have been identified in myeloid malignancies. Tet3 expression has been found to decline with age. We hypothesized that somatic mutations of Tet2 and age–related downregulation of Tet3 lead to development of hematological malignancies. Methods: $Tet2^{FL}$ and $Tet3^{FL}$ mice were crossed with Mx1–Cre transgenic mice. Results: All $Tet2^{FL/FL}$ $Tet3^{FL/FL}$ Mx1–Cre^{+} (homo/homo) mice developed acute myeloid leukemia(AML) and died. $Tet2^{FL/WT}$ $Tet3^{FL/FL}$ Mx1-Cre^{+} (hetero/homo) and $Tet2^{FL/FL}$ $Tet3^{FL/WT}$ Mx1-Cre^{+} (homo/hetero) mice mainly died due to AML. Median survival age was 8.4 weeks and 27 weeks respectively. Multiplex PCR and exome sequencing showed additional loss of wild type Tet2 allele in all three hetero/homo mice with AML and one of two homo/hetero mice with AML. Conclusion: Number of allele deficiency determined the latency of development of hematological malignancies in our mouse model.

E-2021

Comprehensive analysis for genetic factors predictive of azacitidine treatment for MDS

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We investigated the effect of gene mutations on response to hypomethylating agent, azacitidine (AZA), in which retrospective samples collected from a total of 219 AZA-treated MDS patients before treatment were analyzed for mutations in 89 genes commonly mutated in myeloid malignancies using targeted-capture sequencing. In 110 patients, post-treatment samples were also analyzed to evaluate the response in terms of the reduction in clone size. Mutations were strongly biased by high frequency of high-risk patients accounting for 170 (78%) of 219 cases, most frequently affecting TP53 (33%). Notably, 13 of the 17 patients who achieved CR had TP53 mutations, showing almost complete disappearance of mutated clones. All 13 cases had biallelic alterations and accompanied no additional driver mutations, while none of the 14 cases with additional driver mutations achieved CR. No other mutations were significantly associated with AZA response, except for SRSF2 mutations; 8 of 17 SRSF2-mutated patients achieved marrow CR, compared to 20 of 116 patients without SRSF2 mutations (P=0.009). Confirmation of the positive associations is warranted in future studies.

E-2022

Midostaurin reduces Regulatory T cells markers in Acute Myeloid Leukemia

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Allogeneic stem cell transplant (HSCT) is the only curative approach for patients with high-risk acute myeloid leukemia (AML). Donor T-cells targeting leukemic clones play a significant role in HSCT success. FLT3-ITD, a common mutation in AML, is associated with poor prognosis. In addition to their multikinase inhibition effect on T-cell signaling, FLT3-inhibitors (FLT3-INHs) induce apoptosis of malignant cells and enhanced antigen presentation to activate T-cells. Considering the increased clinical use yet the limited clinical benefit of FLT3-INHs in AML, understanding how they affect T-cell population and function is needed to improve their effectiveness. We examined the effect of four FLT3-INHs (midostaurin (Mido), sorafenib, tandutinib and quizartenib) on T cells of healthy donors and of patients with AML. Mido significantly decreased CD4+CD25+ T cell population and FOXP3 mRNA in healthy and AML cells. Mido treated patients showed a decrease in Tregs markers post-treatment. Mido also reduced IFN γ TNF α and IL-10 levels. Mido was recently FDA approved for pre-transplant patients with AML, these finding provides strong rationale to evaluate its use in post-transplant patients

▲TOP

E-2023

DOT1L inhibition blocks multiple myeloma cell proliferation by suppressing IRF4-MYC signaling

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Epigenetic alterations play an important role in the pathogenesis in multiple myeloma (MM), but their biological and clinical relevance is not fully understood. Here, we show that DOT1L, which catalyzes methylation of histone H3 lysine 79, is required for MM cell survival. DOT1L expression levels were higher in monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SmMM) than in normal plasma cells. Treatment with DOT1L inhibitors induced cell cycle arrest and apoptosis in MM cells, and strongly suppressed cell proliferation in vitro. The anti-myeloma effect of DOT1L inhibition was confirmed in a mouse xenograft model. ChIP-seq and microarray analyses revealed that DOT1L inhibition downregulated H3K79 dimethylation (H3K79me2) and expression of IRF4-MYC signaling genes in MM cells, suggesting that repression of IRF4-MYC axis contributed to the anti-myeloma effect. In addition, DOT1L inhibition upregulated genes associated with immune responses and interferon signaling. Our data suggest that DOT1L plays an essential role in the development of MM and that DOT1L inhibition may provide new therapies for MM treatment.

E-2024

Calcium/calmodulin dependent protein kinase 2 is identified as a potential therapeutic target of myelofibrosis

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Myelofibrosis (MF) is an intractable myeloproliferative neoplasm and limited number of humanized disease-models are available to develop a therapeutic strategy. JAK2 inhibitors including ruxolitinib have been developed but cannot satisfactorily control MF. Previously we have reported that induced pluripotent stem cells (iPSCs) from patients are one of the humanized disease-models of MF (Hosoi et al. 2014).We established iPSCs from three MF patients. Screening of 192 compounds showed that KN93, calcium/calmodulin dependent protein kinase (CAMK) 2 inhibitor, inhibited the viability of MF-hematopoietic progenitor cells (HPCs), compared to normal-HPCs.To address the efficacy of CAMK2 inhibition in models mimicking MF, we used Ba/F3 cell line ectopically expressing MPL W515L mutant (Ba/F3_MPLmu_S), along with ruxolitinib-resistant Ba/F3_MPLmu (Ba/F3_MPLmu_R) cells. KN93 and Trifluoperazine, another CAMK inhibitor induced apoptosis, and suppressed the phosphorylation of Stat5 in Ba/F3_MPLmu_S and _R cells. Moreover, CAMK2 inhibitors and ruxolitinib exhibited cooperative inhibition in Ba/F3_MPL cells and primary MF samples. Taken together, CAMK2 is a potential therapeutic target of MF.

▲TOP

[LS16] LS16 [Japanese]
Capture and Recovery of Circulating Tumor Cells Using Biocompatible Materials

2018 / 9 / 28 (Fri) 11:50-12:40 Room 5/10F 1002, Osaka International Convention Center Room 5
：Sumitomo Rubber Industries, Ltd.

Yuko Kitagawa / Department of Surgery, Keio University, School of Medicine,

LS16
Capture and Recovery of Circulating Tumor Cells Using Biocompatible Materials

Tanaka Masaru
Kyushu University Institute for Materials Chemistry and Engineering

No Abstract

[E-2067] E14-9 [English]
Breast cancer

2018 / 9 / 28 (Fri) 13:00-14:15 Room 5/10F 1002, Osaka International Convention Center Room 5

Hirotaka Iwase / Dept. Breast & Endocrine Surg. Kumamoto Univ.

E-2067

Identification of breast luminal stem/progenitor cells as an origin of precancerous lesion

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Stem/progenitor cell are known as major source of various cancer, and presence of adult stem cell in mammary gland is still not clear. We previously found that 270 bp Runx1 enhancer element, named eR1, marks mouse hematopoietic and stomach stem cell. Recently, we found this eR1 marks a subset of luminal epithelial cells in mammary gland. Six month of lineage tracing by eR1-CreERT2 mice showed that eR1+ luminal cells regenerated other luminal epithelial cells in puberty mice and milk producing alveolar cells in lactating mice. Regeneration of basal cells were rarely observed. However, in vitro organoid lineage tracing showed generation of luminal and basal cells from eR1+ cells. These results indicated that eR1+ cells possess stem cell activity. We, then, investigated role of Runx1 and Runx3 in eR1+ luminal stem cells. Conditional knockout of Runx1 and/or Runx3 genes by eR1-CreERT2 showed growth promotion of mammary epithelial cells with robust expression of ERα, and their histology were similar to precancerous lesion Ductal Carcinoma In Situ. We also observed These observations indicated that Runx proteins regulate mammary luminal stem/progenitor cell growth via expression of ERα.

▲TOP

E-2068

The lncRNA NR2F1-AS1 as a fine-tuner of Breast Cancer Recurrence

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The tenacity of recurrence in breast cancer is still a major clinical issue. Accumulative evidence involves long non-coding RNAs (lncRNAs) in cancer. This prompted us to question whether lncRNAs might be also related to recurrence. Hence, we performed a whole transcriptome analysis of 24 non-treated clinical samples from primary breast tumors (10:24 displayed recurrence years after treatment). The results unveiled a gain of expression for oncogenes in recurrence and an increased expression of lncRNAs, including NR2F1-AS1 as the most significant differentially expressed. The decay of ERalpha correlates with an inverse expression of NR2F1-AS1 suggesting a negative regulation. ChIP assay for the occupancy of ERalpha and PRG on the NR2F1-AS1 promoter region shown enrichment for both hormone receptors, with an evident gain for PRG. The overexpression of NR2F1-AS1 arrested cell proliferation and induced a dormant phenotype. Cells which skipped dormancy displayed raised levels of metastasis-initiating genes SOX2 and SOX9. Consistently, NR2F1-AS1 may turn into an impact prognostic marker and a potential therapeutic target.

E-2069

Critical role of O-glycosylation of estrogen receptor alpha by GALNT6 in breast cancer cells

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Molecular roles of the alteration of protein O-glycosylation in various human cancers including breast cancer have not been fully understood. We reported GALNT6 (polypeptide N-acetylgalactosaminyltransferase 6, GalNAc-T6) upregulation in a great majority of breast cancers. Here we further report O-glycosylation of estrogen receptor alpha by GALNT6 and the significant role of its nuclear localization in breast cancer cells. Knockdown of GALNT6 by siRNA could attenuate expression of ER-&alpha at transcriptional and protein levels. We confirmed GALNT6-dependent ER-&alpha O-glycosylation in F domain of ER-&alpha through LC-MS/MS analysis. Furthermore, we designed cell-membrane permeable peptides including the O-glycosylation site and found that the growth suppressive effect of breast cancer cells depended on the ER-&alpha and GALNT6 expression. Our study suggests that targeting the GALNT6 enzymatic activity as well as the GALNT6-ER-&alpha interaction could be a promising therapeutic approach to ER-&alpha positive breast cancer patients.

E-2070

Estrogen-inducible lncRNA facilitates estrogen receptor signaling and contributes to breast cancer tumorigenesis

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Estrogen is a primary hormone that regulates the biology of hormone-naive breast cancer through activating estrogen receptor (ER) signaling. Recent advance in high-throughput sequencing has revealed that long non-coding RNAs (lncRNAs) are also involved in tumorigenesis. In the present study, we screened estrogen-inducible lncRNAs by performing strand-specific RNA-sequencing for ER-positive MCF-7 cells. Among hormone-dependent antisense lncRNAs, we focused on an lncRNA designated as BClnc-Y. siRNA-mediated knockdown of BClnc-Y repressed the viability of breast cancer cells and modulated the expression of genes related to estrogen signaling and cell proliferation. Intriguingly, BClnc-Y knockdown destabilizes ESR1 mRNA. siRNA screening study showed that BClnc-Y-interacting proteins could modulate the expression of ESR1 and cancer-related genes. In situ hybridization study for clinical breast cancer specimens demonstrates that BClnc-Y is a poor prognosis factor of the disease. Taken together, BClnc-Y is a functional lncRNA that facilitates estrogen signaling and contributes to breast cancer tumorigenesis.

▲TOP

E-2071

Integrative analysis of long noncoding RNAs with competing endogenous RNA network in triple negative breast cancer

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Triple negative breast cancer is a particular subtype of breast cancer with the poorer prognosis than other molecular subtypes. Therefore, this research integrated expression profiles, including data on mRNAs, lncRNAs and miRNAs from TCGA. The differentially expressed mRNAs, miRNAs and lncRNAs were obtained. Hereafter, weighted gene co-expression network analysis was performed to identify the hub mRNAs (22) and the expression characteristics. Eleven key dysregulated DEmiRNAs were identified that were significantly associated with the hub mRNAs. Moreover, we found that 14 key DElncRNAs could interact with the key DEmiRNAs. Then the ceRNA network of TNBC was constructed in Cytoscape. We analyzed and described the potential characteristics about biological functional and pathological roles of the TNBC ceRNA network, moreover, the survival analysis was performed for each molecular eventually. From these findings, the revealed ceRNA crosstalk network could play an important role in the development and progression for TNBC. In addition, some of the identified molecules in ceRNA network that possess clinical correlation and prognosis.

E-2072

Circulating tumour cell analysis to predict efficacy of Eribulin for metastatic breast cancer patients

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Circulating tumour cells (CTCs) in breast cancers treated with Eribulin, which reportedly suppresses epithelial mesenchymal transition (EMT) as a mechanism of tumour suppression, were investigated to test the possibility of this method serving as a tool for predicting Eribulin efficacy. Twenty-two patients with metastatic/Stage Ⅳ breast cancer were enrolled and peripheral blood samples were collected before Eribulin-based treatment. CTCs were examined using a Microfluidic Chip device at Nihon Gene Research Laboratories. CTCs positive for pan-cytokeratin and vimentin were defined as epithelial (E-CTC) and mesenchymal CTCs (M-CTC), respectively. CTCs were detected in 21 patients. The median number of M-CTCs was higher in triple negative than in luminal (3.0 and 0.5 per 10ml, respectively) tumours. Progression-free-survival was significantly shorter in patients having high numbers of total CTCs than in those with fewer CTCs (p=0.02), and the same trend was observed for M-CTCs (p=0.04). However, there was no difference according to the number of E-CTCs. Our data suggest that E-CTC and M-CTC determinations might both serve as good tools for predicting Eribulin responsiveness.

▲TOP

[E-2073] E14-10 [English]
Molecular characteristics of gynecologic cancer; from carcinogenesis to immune circumstances

2018 / 9 / 28 (Fri) 14:15-15:30 Room 5/10F 1002, Osaka International Convention Center Room 5

Kiyoshi Yoshino / Dept. Ob&Gyn. Univ. of Occupational & Environmental Health

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E-2073

Comprehensive modeling for high-grade serous ovarian carcinoma with murine fallopian tube organoids

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High grade serous carcinoma (HGSC) is the most common type of ovarian cancer. *TP53* is inactivated in most cases, while the RB1 and PI3K/RAS pathways are deregulated in about half cases. Many lines of evidence suggest that HGSC originates from fallopian tube (FT) cells, which subsequently turn into serous tubal intraepithelial carcinoma, and eventually progress to HGSC. On the other hand, we previously succeed in recapitulating multistep carcinogenesis in subcutaneous of nude mice by *in vitro* lentiviral transduction of organoids derived from various organs. Here we report establishment of FT an organoid-based model to quickly and comprehensively evaluate relevant genetic interactions for HGSC development. While single reconstitution of genetic alteration was insufficient for tumorigenesis of FT organoids, certain combinations induced tumors like carcinoma in situ, HGSC, and carcinosarcoma within 2 months. Considering that fewer number of genetic alterations was able to induce tumor development in shorter period of time, this approach might be a powerful tool to validate tumorigenicity of a variety of genetic alterations in FT cells.

▲TOP

E-2074

Investigation of epigenetic regulation in the high-grade serous ovarian carcinogenesis

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Ovarian cancer is the most lethal gynecologic malignancy. To realize early detection and new therapeutic approaches for reducing mortality of ovarian cancer, we have to understand its origin and pathogenesis further. It has currently been proposed that high-grade serous ovarian cancer originates in fallopian tube secretory epithelial cells (FTSECs), whereas the molecular mechanisms underlying its multistage tumorigenesis have not been clearly determined through comprehensive genetic analyses. Therefore, to investigate the epigenetic regulation associated with ovarian tumorigenesis, we performed the integrative analysis of ATAC-seq and RNA-seq on the established model which demonstrated that overexpression of AKT or c-Myc, along with dominant-negative p53 and KRAS, induced tumorigenesis in immortalized FTSECs. ATAC-seq is a powerful method to map open chromatin sites, predict transcription factor binding sites, and determine nucleosome positions. Comparing ATAC-seq peaks and RNA profiling on a genome-wide scale among the cell lines, the epigenetic signatures correlated with gene expression could be identified and have potential for drug targets and biomarkers.

E-2075

Tumor suppressive roles of MARK3 in high-grade serous ovarian carcinomas

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High-grade serous ovarian carcinoma (HGSOC) is the most aggressive histological type, causing approximately 70% of death by ovarian cancer. The purpose of this study is to identify novel candidate molecular targets and to clarify their functions in HGSOC. We analyzed publicly available microarray datasets to identify genes, which are dysregulated and associated with prognosis in HGSOC. We then found that a microtubule affinity-regulating kinase 3 (MARK3) was significantly downregulated in HGSOC, and downregulation of MARK3 was associated with poor progression-free survival ($p<0.0001$). In silico analysis revealed that one of the mechanisms to repress MARK3 expression in HGSOC was copy number deletion. MARK3 overexpression significantly inhibited tumor proliferation in vitro. Transcriptome analysis suggested that MARK3 activated Hippo signaling and repressed cell cycle. MARK3 directly phosphorylated CDC25B at Ser 323, which inhibited nuclear localization of CDC25B. Overall, MARK3 appears to be a novel tumor suppressive checkpoint kinase. Dysregulation of Hippo signaling and CDC25s axis caused by repression of MARK3 may be a potential therapeutic target against HGSOC.

E-2076

Establishment of an immunocompetent mouse endometrial cancer model of Uterine Serous Carcinoma (USC)

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[Objective] USC has the worst prognosis among endometrial cancer, but there is no apt in vivo USC model to investigate new immunotherapies. The aim of this study is to evaluate microenvironmental features of USC and to develop an immuno-competent mouse USC model. [Methods] Tumor infiltrating CD8+ cells (TILs) and CD33+ cells (MDSC) of 42 USC clinical samples were counted and MDSC/TILs ratio was calculated. A mouse USC cell line (myc^+EC) was established by introducing cmyc in a mouse endometrial cancer cell line with conditional Pten/Trp53 deletion (myc-EC). RNA sequencing analysis was performed on myc^+EC and myc-EC tumors. [Results] TILs were significantly enriched in the tumor without recurrence ($p=0.03$). Overall survival was better in cases with lower MDSC/TILs ratio ($p=0.02$). The myc^+EC tumor histologically showed frequent mitosis and nuclear atypia, whereas TILs were limited. The myc^+EC tumor expressed genes associated with epithelial mesenchymal transition as well as cell proliferation. [Conclusion] TILs and MDSCs are prognostic factors in human USC. Our new immuno-competent mouse model mimics human USC. This model may be suitable for assessing tumor microenvironment of USC.

▲TOP

E-2077

Ectopic synthesis of CD69 is important for intra-peritoneal survival of ovarian clear cell carcinoma cells

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Ovarian clear cell carcinoma (CCC) is poor prognositic and frequent in Japan. Thus, exploration of novel therapeutic target molecules of this disease is essential. CD69 protein is a cell surface glycoprotein predominantly expressed in immune cells and may regulate adequate immune response. We unexpectedly found that CD69 is highly synthesized in CCC cells in response to serum starvation and hypoxia (SSH). It was demonstrated that CD69 enhances CCC cell-fibronectin interaction through activation of cell surface β1-integrin, thereby increasing cell motility and invasiveness under SSH. Here we report expression pattern of CD69 in surgically resected ovarian cancer tissues with various histological subtypes. We demonstrated that CD69 is predominantly expressed in CCC tissues. We further tested the effect of CD69 on intra-peritoneal survival of CCC cells in mouse xenograft model. We found that a CD69-positive CCC cell line OVISE can grow within peritoneal cavity although OVISE cells in which CD69 is silenced by RNA interference could not survive. These results suggest that CD69 synthesis potentially under SSH is a key mechanism for CCC cell survival under peritoneal environments.

E-2078

A subgroup with a T- cell inflamed phenotype in homologous recombination proficient high-grade serous ovarian carcinoma

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The benefit of PARP inhibitors has shown in patients with high-grade serous ovarian carcinoma (HGSC) who have homologous recombination deficient (HRD) tumors. There is a need to develop a new treatment for HGSC patients with HR proficient (HRP) tumors. The aim of this study is to investigate the immunological characters of HRP-HGSC. A total of 80 cases of HGSC were analyzed in the study. Exome, RNA sequencing and methylation arrays were performed. Neoantigen load, antigen presentation machinery, and local immune profile were investigated. Thirty-four and 46 patients were classified as having HRD and HRP tumors, respectively. Increased numbers of neoantigens were observed in HRD tumors. However, 39% of the patients with HRP tumors had high neoantigen load. Patients with both high neoantigen load and high HLA-class I expression had an improved survival in HRP tumors. Gene set enrichment analysis showed that the gene sets for T cells, Th1, and interferon response were enriched in patients with high neoantigen load and high HLA-class I expression in HRP tumors, suggesting a T cell-inflamed phenotype. This subgroup of cases might be appropriate candidates for the immunotherapy

▲TOP

[E-2079] E14-11 [English]
Colorectal cancer

2018 / 9 / 28 (Fri) 15:30-16:45 Room 5/10F 1002, Osaka International Convention Center Room 5

Satoshi Nagayama / Dept. Gastroenterological Surg., Cancer Inst. Hosp., JFCR

E-2079

The role of oral genotoxic bacteria in the development of colon cancer

Sho Kitamoto
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The gut microbiota is known to play a role in the development of colorectal cancer (CRC). In this context, certain members of the Enterobacteriaceae family, which harbor genotoxic activity, have been identified as tumor-associated bacteria in CRC. However, the origin of these bacteria remains unknown. In this study, we discovered that 1) oral inflammation results in the expansion of genotoxic Enterobacteriaceae (e.g., Enterobacter, Klebsiella, E. coli) in the oral cavity. 2) These genotoxic bacteria are ingested and ectopically colonize the intestine when colonization resistance by the gut resident bacteria is perturbed (e.g., by intestinal inflammation). 3) Ectopic colonization of these bacteria induces DNA damage in the colonic epithelium and potentiates tumorigenesis in murine CRC models. These results indicate that the inflamed oral cavity acts as a reservoir of genotoxic bacteria whose ectopic colonization in the intestine increases the risk of CRC. Thus, optimal oral care can be a novel prevention and management strategy for CRC. This work was performed at the University of Michigan (Principal investigator: Nobuhiko Kamada).

▲TOP

E-2080

Visualization of epithelial-mesenchymal transition in inflammatory microenvironment-colorectal cancer crosstalk

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Epithelial-mesenchymal transition (EMT) is a biological process, by which epithelial cells acquire mesenchymal characteristics. In malignant tumors, EMT program is crucial for acquiring mesenchymal phenotype with invasive and metastatic properties, leading to tumor progression. Inflammatory microenvironment is expected to be responsible for the development and progression of colorectal cancer (CRC); however, the precise role of inflammatory microenvironment in the EMT-related CRC progression remains unclear. Here we show the spatiotemporal visualization of EMT-undergoing CRC cells using a fluorescence-guided EMT imaging system, in which mesenchymal vimentin promoter derives red fluorescent protein (RFP). Inflammatory microenvironment including TNF-alpha, IL-1beta, and cytokine-secreting inflammatory macrophages, induced the RFP expression in association with EMT phenotype in CRC cells. In vivo experiments further demonstrated the distribution of RFP-positive CRC cells in the rectal and metastatic liver tumors. Our data suggest that EMT imaging system is a powerful tool for monitoring the EMT state in the inflammatory microenvironment-CRC crosstalk.

E-2081

CANCER-DERIVED EXOSOMES SUPPRESS AUTOPHAGY through FIBROBLAST ACTIVATION in COLON CANCER

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Exosomes are small vesicles and play important role in cell to cell communication. Autophagy is a catabolic process to degrade of unnecessary or dysfunctional cellular components. The relationship between autophagy and cancer derived exosomes (CDEs) in tumor microenvironment is unclear. Here, we investigated the relationship of CDEs and autophagy of fibroblasts within colon cancer. We used a non-contact co-culture system between fibroblasts and colon cancer cells in vitro and investigate autophagy in fibroblasts. Alpha smooth muscle actin, one of the features in cancer-associated fibroblasts, elevated after co-culture with colon cancer cells. Simultaneously, autophagy was suppressed. Activated fibroblasts in which autophagy was suppressed and promoted proliferation of colon cancer cells compared with normal fibroblasts. To reveal the mechanism of fibroblast activation by co-existence of cancer cells, we extracted CDEs and added to fibroblasts. After CDEs addition, autophagy in fibroblasts was suppressed compared with fibroblasts cultured without exosomes. Thus, our results suggest that CDEs activate fibroblast through suppression of autophagy in colon cancer.

E-2082

Obstruction is associated with perineural invasion in T3/T4 colon cancer

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Perineural invasion (PNI) is a risk factor for recurrence and metastasis, and consequently leads to decreased survival in patients with various malignancy. It was reported that stent placement in obstructive colon cancer increases the frequency of PNI. We hypothesized that mechanical stress including obstruction itself may be associated with PNI. We reviewed 496 patients with T3 or T4 colon cancer who did not received preoperative treatment. The relationships between PNI and other clinicopathological factors were analyzed using univariate and multivariate analyses. PNI was observed in 239 patients (48%). Obstruction was more frequent in PNI-positive cancer (39%) than in PNI-negative cancer (24%, p=0.0003). Multivariate analyses identified obstruction as one of the significant factors associated with PNI (odds ratio 1.68, p=0.028). In 414 patients with stage 2/3 who underwent R0 resection, PNI was independently associated with poor recurrence-free survival (RFS; hazard ratio 2.35, p=0.003). The co-existence of PNI and obstruction resulted in greater decreases in RFS. It was suggested that obstruction is associated with PNI, and contributes to an increased recurrence in colon cancer.

▲TOP

E-2083

Characterization of tumor subclonal heterogeneity in colorectal cancer using cancer tissue-originated spheroid method

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Cancer is characterized by extensive intratumor heterogeneity, which evolves over time and comprise several tumor subclones. However, the development of new approaches to evaluate the subclonal dynamics through disease progression remains a challenge. We previously established the Cancer Tissue-Originated Spheroid (CTOS) culture method for primary tumor cells. A CTOS is a multicellular spheroid and may consist of different subclones. CTOSs of colorectal cancer were dissociated into single-cells and each cell was seeded in a well. The sphere formation from single cell was evaluated, which reflects the ability of self-renewal and proliferation. We found wide range of growth within the spheroids. The proliferative potential of each subclone remains preserved over several rounds of sphere formation assay and after xenotransplantation, indicating the existence of phenotypically varied tumor-initiating cells. The subclones with different growth capacity were interchangeable after forming xenografts, demonstrating the plasticity of these tumor-initiating cells. These results highlight the usefulness of CTOS method for modeling the tumor subclonal heterogeneity.

E-2084

In vitro human tumor model for predicting therapeutic effect of anti-cancer drugs

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Primary culture of cancer cells derived from an individual patient's tumor provides important information regarding the tumor character. We established a novel culture method for primary colorectal cancer (CRC) with phenotypic heterogeneity as a human tumor model, named isolated tumor-derived cancer cells (iCCs). The success rate of iCC growth in vitro was 100%, passage was 90%, and establishment of a xenograft model from iCC was 80%. We examined the sensitivities of iCCs isolated from 10 patients to anti-cancer drugs. The in vitro sensitivity was compared to clinical outcomes (RECIST criteria) in four patients that all had distant metastases. The concentration of anti-cancer drugs was set as estimated concentration in human tissue. In the examination using 5-FU and oxaliplatin, the survival rates of iCCs were more than 85% in one PD patient. The survival rates of iCCs were less than 83% in three SD patients (median, 70%). The survival rates were also less than 83% in two patients who underwent adjuvant chemotherapy with no recurrence (median 70%). In conclusion, our primary culture model may be a novel tool for the prediction of anti-cancer drugs in the clinical courses.

▲TOP

[ML5] ML5 [Japanese]
Morning Lectures 5

2018 / 9 / 28 (Fri) 8:00-8:50 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Toshiyoshi Fujiwara / Dept. Gastroenterol. Surg., Okayama Univ. Grad. Sch. Med.

ML5

The Exosome Biology in Cancer: Current Topics and Perspectives

Yusuke Yamamoto
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Discussant：Eishi Baba
Dept. Clin. Oncol, Fac. Med., Kyushu Univ.

Exosomes are small, biologically active extracellular vesicles secreted by most cell types, and were originally considered a sort of a trash bin working to sweep out disused biomolecules in cells. However, the finding which exosomes carried miRNA and mRNA opened up a new research avenue into cancer biology. Accumulating evidence has suggested that exosomes are involved in intercellular crosstalk between cancer cells and the surrounding microenvironment, and are critical for creating favorable environment in cancer development. Functional transfers of cancer components such as miRNAs, mRNA, DNAs, proteins, and metabolites by exosomes facilitate local and distal cell-to-cell communications, leading to pre-metastatic niche formation, metabolic reprogramming in stroma and controlling immune system. Furthermore, the circulating exosomes have also been an attractive target for liquid biopsies as biomarkers in cancer surveillance and screening, because exosomes in body fluids provide valuable information to monitor cancer status. In this session, we provide an overview of current research on exosomes in cancer biology as well as therapeutic strategies targeting cancer-specific exosomes.

▲TOP

[E-2025] E11-3 [English]
Cancer stem cell (1)

2018 / 9 / 28 (Fri) 9:00-10:15 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Kazuhito Naka / Dept. Stem Cell Biol., Res Ins Radiation Biol & Med., Hiroshima Univ.

E-2025

Tumor suppressors network repress pluripotency through proteasomal degradation of the core reprogramming proteins

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Proteasomal degradation of the core reprogramming proteins determines the stem cell decision whether to proliferate or differentiate. Little is known about how the core reprogramming proteins are identified and recruited for degradation. Here, we demonstrate that mutual functions of the retinoblastoma (RB) and the ataxia telangiectasia mutated (ATM) repress the pluripotency and self-renewal ability of the stem cell-like populations included in genetically modified mouse embryonic fibroblasts (MEFs) and A-T human adult fibroblasts (A-T HAFs) through acetylation-driven ubiquitination and subsequent proteasomal degradation of the core reprogramming proteins Oct3/4, Sox2, Klf4, Nanog and c-Myc (OSKNM). We discovered that RB recruits lysine acetyltransferase-3b (Kat3b) and inhibits the transcription of histone deacetylase-5 (Hdac5) whereas, ATM shuttles Hdac5 into the nucleus and serve as adaptor protein, which identify and assemble the acetylated-OSKNM proteins into ubiquitination complexes with the E3 ubiquitin ligase Uhrf1 or Fbxw7. These novel findings have important implications in regenerative medicine, neurodegenerative diseases and cancer.

▲TOP

E-2026

ROCK inhibitors suppress tumorigenesis by inducing terminal adipocyte differentiation in stem-like osteosarcoma cells

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We established a mouse osteosarcoma (OS) model through overexpression of c-MYC in bone marrow stromal cells derived from Ink4a/Arf (-/-) mice. These OS-initiating cells were composed of two distinctly different clones: highly tumorigenic cells (termed AX cells), similar to bipotent-committed osteochondral progenitor cells, and low tumorigenic tripotent cells (termed AO cells), similar to mesenchymal stem cells. AO cells were highly resistant to chemotherapeutic agents such as Adriamycin unlike AX cells. Here we show that ROCK inhibitors induce terminal adipocyte differentiation of chemoresistant stem-like AO cells through regulation of the transcriptional coactivator MKL1 (megakaryoblastic leukemia 1) by actin cytoskeleton dynamics. We found that ROCK inhibitors significantly suppressed in vitro growth and in vivo tumorigenesis of AO cells. Furthermore, in parental OS-initiating cells, combination of ROCK inhibitors and Adriamycin destroyed AX-type cells and triggered terminal adipogenesis of AO-type cells. Our findings suggest that trans-terminal-differentiation approach of cancer stem cells by regulating actin dynamics is a potential approach for some tumor types.

E-2027

Generation of Hepatocellular Carcinoma Cancer Stem Cell from induced Pluripotent Stem Cells

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Hepatocellular carcinoma (HCC) is the prominent Primary liver cancer. Every type of tumors including HCC contains CSCs, which provide cancer cell population in tumor tissue. The signalling mechanism regulating the development of CSCs, which provides HCC remains unknown.
In this study we tried to establish a model of HCC CSC from miPS by the conditioned medium (CM) of HCC cell line.
First of all, CM was collected from Huh7 cells. Then, iPSCs cells were cultured in the presence of 50% CM for 4 weeks. miPSCs cultured in the complete medium with LIF were used as a control. The survived cells (5x105 cells) were injected into the liver of BALB/c nude mice. After 25 days malignant tumor was formed in the liver while benign teratoma was formed from iPSCs injection.
Immunohistochemical analysis with GFP antibody showed that malignant tumor sustained GFP expression while teratoma from miPSCs did not. The primary cells from the malignant tumor are rich in CSC-like cells with high expression of GPC3 and CK19, these results paves the way to establish a model of HCC CSC, which will help in understanding the molecular mechanisms necessary to maintain HCC CSC.

E-2028

DNA Hypomethylation and overexpression of Class IB PI3K genes in the Oncogenic Conversion of iPSCs into CSCs

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We have succeded in converting mouse iPSCs (imiPSCs) into CSC-Like Cells (miPS-LLCcm) by treating the miPSCs with conditioned medium (CM) of Lewis Lung Carcinoma (LLC) cells. miPS-LLCcm cells developed highly angiogenic and malignant adenocarcinoma as well as lung metastasis when subcutaneously translplanted into nude mice. We traced the development of CSC phenotype by the change of DNA methylation levels, which would provide the difference between the miPSCs and drived miPS-LLCcm cells. Significant overall DNA hypomethylation was found occoured in the miPS-CSCs and considered to result in the activation of certain proto-oncogene. Among the hypomethylated genes, the expression of candidate genes involved in PI3K-Akt signaling pathway were found up-regulated in miPS-LLCcm cells. As the result, the hypomethylation of Pik3r5 gene was thought responsible for the up-regulation and closely related to the activation/phosporylation of Akt, which represent the malignant conversion by epigenetic regulation even without any mutations. By using our CSCs model, we successfully demonstrated the DNA hypomethylation responsible for the early stage of CSC development.

▲TOP

E-2029

Elucidation of a linkage of cancer metabolism and epigenetic control in cancer stem cells for drug discovery

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Recently, an involvement of cancer metabolism has emerged, while epigenetic control is a promising target against therapy resistant cancer cells, including cancer stem cells (CSCs). Here we studied a central metabolic mechanism of CSCs, and demonstrated the role of polyamine (PA) flux, which associated with the epigenetic control of the methylation at lysine 4 of histone H3 (H3K4), an activating mark for transcription. The results indicated that the PA metabolism plays a critical role in CSC control, and that PA modulates H3K4 demethylation enzymes, KDM1A/LSD1 and KDM5B/JARID1B; the data indicated that the former controlled a stemness gene ID1 at enhancer, while the latter regulated promoter of p16INK4a gene in slowly cycling cells, both important mechanisms in CSCs. Given that the data indicated that the unique target KDM5B induced cellular senescence and reduced CSCs in vivo, we have performed the high throughput screening and chemical synthesis of candidate compounds. The present study elucidated that the underlined mechanism of therapy resistant CSCs, and opened an avenue to the development of the next generation drugs.

E-2030

Functional significance of long non-coding RNA NEAT1 in liver cancer stem cells via CD44

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Aims: NEAT1 was reported as a cancer-related long non-coding RNA. However, its role is controversial. To gain insights into the clinical and biological significance of NEAT1 in hepatocellular carcinoma (HCC), we generated two HCC cell lines in which NEAT gene is overexpressed or knocked out, and investigated their phenotypic changes, in particular, with regard to cancer stemness.
Methods: Human HCC cell lines, HuH7 (H7) and HepG2 (G2) cells were used. A NEAT1-expressing plasmid vector was stably transfected into H7 and G2. The NEAT1 gene in H7 and G2 was knocked out by the CRISPR/Cas9 system (H7KO, G2KO).
Results: Overexpression of NEAT1 resulted in increased spheroid formation and drug resistance. Consistently, H7KO and G2KO showed decreased spheroid formation and drug resistance. The CSC marker CD44 was significantly upregulated by NEAT1 overexpression while downregulated in H7KO and G2KO.
Discussion: These observations suggest that NEAT1 is required for maintaining the phenotypes of cancer stem cells (CSCs) in HCC, and that CD44 might be involved in the mechanism.
Conclusion: NEAT1 is a potential therapeutic target for HCC, especially containing CD44-posive CSCs.

▲TOP

[E-2031] E11-4 [English]
Cancer stem cell (2)

2018 / 9 / 28 (Fri) 10:15-11:30 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Yoshihiro Kawasaki / IQB., Tokyo Univ.

E-2031

Glioma cells at the tumor border acquire chemo-radioresisitant ability from the special microenvironments

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Glioblastoma (GBM) usually develops in adult brain white matter and shows rapid growth and invasion. Even after complete resection, GBM recurs around the tumor removal cavity, where GBM cells acquire chemo-radioresistance and survive. Characterization of the tumor border microenvironment is critical for improving prognosis in patients with GBM. Here, we compared microRNA (miRNA) expression in samples from the tumor, tumor border, and peripheral region far from tumor mass by miRNA microarray. The top three miRNAs showing higher expression in the tumor border were related to oligodendrocyte differentiation, and pathological oligodendrocyte lineage cells increased in the border, where numbers of macrophages and microglia also colocalized. Medium cultured with oligodendrocyte progenitor cells (OPCs) and macrophages induced stemness and chemo-radioresistance in GBM cells, similar to that produced by FGF1, EGF and HB-EGF, IL-1β, corresponding to OPCs and macrophages, respectively. Thus, OPCs and macrophages/microglia may form a glioma stem cell niche at the tumor border, representing a novel, promising target for prevention of recurrence.

▲TOP

E-2032

Sox2 gene endows colon cancer cells with cancer stem like property

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Sox2 is a transcription factor that induces pluripotent stem cells. Recent studies have shown that Sox2 may provide cancer stemness in skin and other types of human malignancies. In this study we estimated the impact of Sox2 expression on prognosis of colorectal cancer (CRC) patients and explored a possibility of Sox2 positive cells as being cancer stem cells of CRC. RT PCR assay for Sox2 mRNA indicated that high Sox2 gene expression was an independent prognostic factor. Because CSC population is usually rare, siRNA approach may have limitation to target CSC population alone. We therefore made up a lentivirus-mediated construct by which Sox2-positive cells emit red fluorescence signal in response to Sox2 promoter activation. Red fluorescent and Sox2-positive cells were 2.48% and 0.48% in HCT116 and HT29, respectively. We found that these red cells had high expression of CSC markers such as CD44v9 and NANOG, and they displayed significantly low proliferative activity and increased chemo-resistance to 5-FU and oxaliplatin. Taken together, these findings suggest that Sox2 may be a novel CSC marker in colon cancer.

E-2033

RAB3B gene was identified as a gene involved in chemoresistance of induced cancer stem-like sphere cells

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Background: In the previous study, we have successfully induced cancer stem-like sphere cells (CSLCs) from hepatoma cell lines using a unique medium. The obtained CSLCs showed increased metastatic potentials and resistance to anti-cancer drugs. In this study, we identified a responsible gene for the characteristics of CSLCs.
Methods: The human hepatoma cell line SK-HEP-1 was used. RNA-seq analysis was performed with cell line derivatives and clinical specimens. To assess the chemoresistance, the MTS assay was performed. The knock-down and knock-out experiments were accomplished by using siRNAs and CRISPR-Cas9 genome-editing, respectively.
Findings: As a result of integrated analysis with RNA-seq, a RAB3B encoding one of small GTPase was identified as an up-regulated gene in both CSLCs and poor prognostic HCCs. Knock-down of RAB3B promoted conversion from sphere cells to adherent cells. Furthermore, cells harboring mono-allelic RAB3B knock-out showed altered sphere formation, a cell cycle distribution, ABCG2 expression, and significantly lower chemoresistance than that of parental cells.
Conclusion: It was suggested that the up-regulation of RAB3B plays important roles in CSLCs.

E-2034

Preexisting Drug Resistant subpopulation in Luminal subtype of Breast Cancer Cells as Revealed by Single-cell Analysis

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Despite the continuous progresses in breast cancer treatment (BC), a subset of patients presents drug resistance, highlighting the need of further studies. In addition, BC presents high intratumor heterogeneity, hindering the identification of individual cells with drug resistance ability. To address this issue, we performed an extensive single-cell gene expression profiling in luminal A-type BC cell line, and several derivatives resistant to docetaxel. We identified 27 genes relevant for drug resistance, containing EMT, stemness and cell cycle regulators genes. Those were highly correlated among them at single-cell level. Furthermore, these genes were translated to protein and its expression was confirmed by immunostaining in vitro and tissue samples. Interestingly, the gene pattern found in the resistant cell line, was also found in a small subset of parental cells, implying that the untreated primary cells already contained a rare subpopulation of stem-like cells showing an inherent predisposition to docetaxel resistance. Our data suggests that during chemotherapy, this population might be positively selected and become the major cell population, causing treatment failure.

▲TOP

E-2035

Addressing tumor resistance by combining CSC-targeting strategies and high-LET radiation therapy

Guillaume Vares
OIST

Co-author：Hong Huat Hoh、Tadashi Yamamoto
OIST

Treatment resistance and relapse in challenging cancer models might be related to the presence of a small population of radio-resistant cancer stem cells. We have tested an approach combining charged particle therapy and CSC-targeted therapy, based on the modulation of micro-RNA expression by specific oligonucleotides (micro-RNA mimics) and molecular inhibitors (IDH and mTOR inhibitors), in three experimental models (pancreatic cancer, chondrosarcoma and triple-negative breast cancer). We have identified several treatments capable of improving carbon-ion therapy efficiency by targeting and reversing radioresistant CSC phenotype in those models. The role of miR-34 in regulating stemness and resistance to stress in chondrosarcoma was studied. Administration of a miR-34 mimic treatment in a human chondrosarcoma xenograft model in mice resulted in an inhibition of tumor growth. Combined treatments might be more efficient for controlling or reversing resistant tumors, relapse and metastasis.

E-2036

15-PGDH inhibition causes Kras-driven tumor expansion through PGE2-ALDH1 signaling in the pancreas

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Arachidonate cascade is a major inflammatory pathway that produces prostaglandin E2 (PGE2). Although inhibition of 15-hydroxyprostaglandin dehydrogenase (15-PGDH) is reported to lead to PGE2 accumulation and expand tissue stem cell fraction, the role for pancreatic tumor progression is still unknown. The aim of this study is to elucidate the role of 15-PGDH for cancer stem-like cell expansion in pancreas. We found that 15-PGDH expression is frequently down-regulated in pancreatic ductal adenocarcinoma (PDAC) cells and inversely correlated with ALDH1 expression in PDAC tissues. Pharmacological blockade of 15-PGDH led to PGE2 accumulation, and promoted growth and sphere formation through the expansion of ALDH1-positive cells. We also elucidated the molecular mechanism that PGE2 accumulation by 15-PGDH inhibition increases CYP26A1 expression and subsequently depletes all-trans retinoic acid, and results in ALDH1 up-regulation. Finally, genetic ablation of 15-Pgdh promoted tumorigenesis in KrasLSL-G12D; Ptf1aCre/+ mice through the expansion of ALDH1-positive cells. Our findings highlight the role and significance of PGE2 degradation pathway for PDAC progression.

▲TOP

[LS17] LS17 [Japanese]
Early detection of cancer by liquid biopsy

2018 / 9 / 28 (Fri) 11:50-12:40 Room 6/10F 1004+1005, Osaka International Convention Center Room 6
：Toray Industries, Inc.

Kenichi Matsubara / Professor Emeritus, Osaka University

LS17

Early detection of cancer by liquid biopsy

Takahiro Ochiya
National Cancer Center Research Institute, Division of Molecular and Cellular Medicine

No Abstract

[E-2085] E2-1 [English]
Gene-manipulated animal models

2018 / 9 / 28 (Fri) 13:00-14:15 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Hiroshi Seno / Dept. Gastroenterol. & Hepatol., Kyoto Univ. Grad. Sch. Med.

E-2085

Functional loss of p53 cooperates with the in vivo microenvironment to promote malignant progression of gastric cancers

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p53 mutations are frequently detected in malignant gastric cancers. However, the mechanisms by which loss of p53 function promotes gastric cancer are not clear. We utilized Gan mice (K19-Wnt1/C2mE), which have functional p53 and develop intestinal-type gastric tumors, to investigate the role of p53 in gastric cancer progression by knocking out p53. We found that gastric epithelial cells acquire tumorigenicity in the subcutis of C57/BL6 mice as a result of Wnt activation, COX-2 activation and p53 deficiency. With repeated allograft transfers, these gastric epithelial cells gradually acquired the properties of malignant gastric cancer. Loss of p53 conferred cell stemness and induced epithelial to mesenchymal transition (EMT) in gastric epithelial cells, and these properties were further enhanced by the in vivo microenvironment, ultimately leading to gastric cancer formation and metastasis. We also found that the in vivo microenvironment enhanced activation of the COX-2 pathway, which further contributed to cancer progression. With this system, we have succeeded in recapitulating the development of malignant gastric cancer from gastric epithelial cells in a normal immune environment.

▲TOP

E-2086

CXCR4 has critical role on desmoplastic reaction of PDAC

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Many functions of CXCL12/CXCR4 signaling has been reported in various tumors using inhibitors or knocking down of CXCR4. However, there is no report of CXCR4 depletion in cancer model. Here, we investigated the function of CXCR4 in the formation of pancreatic ductal adenocarcinoma (PDAC) by crossing Pdx1-Cre; LSL-KrasG12D; LSL-p53R175H (KPC WT) with CXCR4 flox/flox mice (KPC f/f). Although the incidence of PDAC had no changes, the tumor features in KPC f/f mice were dramatically changed compared with KPC WT. The tumor was composed of numerous pleomorphic cells with significantly scant fibroblasts and collagen fibers in KPC f/f mice. Immunohistochemically, the expression of vimentin was markedly high whereas that of cytokeratin was low in KPC f/f mice. Applying to human cancer, KPC f/f tumors imitated the undifferentiated carcinoma which was stained with vimentin and had little fibrosis. In addition, human undifferentiated carcinomas were not stained with CXCR4 while well differentiated PDACs were stained with CXCR4.These data show CXCR4 has critical role on desmoplastic reaction and suggested that CXCR4 depletion may transform well differentiated PDACs to undifferentiated tumors.

E-2087

Effective of Dasatinib in a T-cell lymphoma mouse model

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Both the G17V RHOA mutation and the loss-of-function TET2 mutation have been identified in 70% of Angioimmunoblastic T-cell lymphoma (AITL). G17V mutant RHOA augmented T-cell receptor (TCR) signaling. However, it remains elusive whether inhibition of TCR pathway by the inhibitor such as dasatinib serves as an effective treatment in AITL. [Methods] Cells derived from tumors developed in $Tet2^{-/-}$G17VRHOA mice were transplanted into nude mice Dasatinib was orally given to transplanted nude mice at 5mg/kg/day. [Results] Approximately 70% of $Tet2^{-/-}$G17VRHOA mice developed AITL-like phenotype and died around 40 w.o. The serum concentrations of Il-2, Il6, and TNF&alpha in $Tet2^{-/-}$G17VRHOA mice were higher than those of the controls ($p<0.05$). Nude mice transplanted with tumor-derived cells showed splenomegaly and lymphadenopathy preceded by elevation of the serum concentrations of Il-2, Il-6 and TNF&alpha . Mice given with dasatinib showed higher overall survival and lower serum concentrations of Il-2, Il-6, and TNF&alpha compared to those of the vehicle controls ($p<0.05$). Conclusion: Dasatinib is effective in treatment AITL-like mice model mimicking the genetic disorder in human AITL.

E-2088

Spontaneous development of intratumoral heterogeneity in a transposon-induced mouse model of glioma

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Glioma is the most common form of malignant brain cancer in adults. The Sleeping Beauty transposon-based glioma mouse model allows for effective in vivo analysis of candidate genes. In this study, we developed a transposon vector encoding the triple combination of PDGFA, and shRNAs against Nf1 and Trp53 (shNf1/shp53). Transduction of the vector into neural progenitor and stem cells (NPCs) in the subventricular zone of the neonatal brain induced highly penetrant malignant gliomas within 2 to 4 months. Two transposon vectors, encoding either PDGFA or shNf1/shp53 were co-electroporated into NPCs. Cells expressing PDGFA or shNf1/shp53 were labeled with unique fluorescent proteins allowing visualization of the spatial distribution of cells with different genetic alterations within the same tumor. Tumor cells located at the center of tumors expressed PDGFA at higher levels than those located at the periphery. Moreover, tumor cells located at the periphery and the center of tumors showed phenotypic differences, indicating that intratumoral heterogeneity spontaneously developed within the same tumor. Our method is useful for reconstituting genetically heterogeneous cells in the same tumor.

▲TOP

E-2089

Establishment of mice conditionally expressing the Helicobacter pylori CagA oncoprotein

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Chronic infection with cagA-positive H. pylori plays a key role in the development of gastric cancer. CagA protein, a cagA gene product, is delivered into gastric epithelial cells and undergoes tyrosine-phosphorylation. The tyrosine-phosphorylated (pY) CagA disturbs intracellular signaling pathways via aberrant activation of SHP2 oncoprotein and inhibition of PAR1 kinases, the deregulations of which are crucial for the malignant transformation of epithelial cells. Here, we report the development of ROSA26-targetted cagA knock-in mice ($R26^{LSL\text{-}cagA}$), which conditionally express CagA protein under the control of the Cre-loxP system. Immunoblot analysis of the lysates prepared from the gastric epithelia or MEF cells of $R26^{LSL\text{-}cagA}$; CAG-$creER^{T2}$ compound mice showed inducible expression of pY CagA in a tamoxifen/4-OHT dependent manner. We also found that CagA expression exhibited morphological abnormality in MEF as well as lethality to compound mice during embryogenesis, both of which may be due to the SHP2 deregulation. The newly established CagA-inducible mice will provide to be a powerful tool in elucidating the spatio-temporal role of H. pylori CagA in in vivo gastric carcinogenesis.

E-2090

Ral-NLRP3 inflammasome pathway promotes colitis-associated cancer

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Background and Aims: Small GTPase Ral regulates tumorigenesis and invasion/metastasis in some cancers, however, the role of Ral in colitis-associated cancer (CAC) has not been investigated. We aimed to elucidate the role of Ral in the mechanism of CAC.
Methods: We used RalGTPase-activating protein α 2 (RalGAP α 2) knockout (KO) mice that could activate Ral. CAC was induced in wild-type (WT) mice and RalGAP α 2 KO mice by intraperitoneal injection of azoxymethane following adding of dextran sulfate sodium. Colon26 cell were transfected with RalGAP α 2 siRNA to examine the effect of Ral activation on the migratory and invasion capacity.
Results: RalGAP α 2 KO mice had a significantly larger number and size of tumors with higher proportion of tumors invading the submucosa than WT mice. Colon26 cells transfected with RalGAP α 2 siRNA had the increased migratory and invasive capacity. In addition, the expressions of IL-1 β , NLRP3, ASC, and Caspase-1 were significantly elevated in tumors of RalGAP α 2 KO mice in comparison with those of WT mice.
Conclusion: Ral activation is involved in the mechanism of CAC development through Ral-NLRP3 inflammasome pathway.

▲TOP

[E-2091] E2-2 [English]
Animal models for cancer (1)

2018 / 9 / 28 (Fri) 14:15-15:30 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Satoshi Nishizuka / Div. Biomed. R&D., Inst. Biomed. Sci., Iwate Med. Univ.

E-2091

Genetically engineered mouse models of prostate cancer: from man to mouse and back

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Genetically engineered mice (GEM), developed to mimic human pathologies, have been invaluable tools to study and characterize human cancers. GEMs develop tumors *de novo* in a natural immunocompetent tumor microenvironment. Tumors from the mice share many features of their human counterparts including pathologic and molecular heterogeneity, and a natural progression including disease dissemination. As such, GEM models have proven to be superior to traditional cell-based xenograft models for preclinical efficacy drug evaluation. Here, we demonstrate the utility of GEM models of *Pten/Trp53*-deficient prostate cancer to develop targeted molecular therapeutic strategies for castration-sensitive and castration-resistant prostate cancer. We first show the efficacy of targeting the androgen receptor (AR) and PI3K/AKT signaling pathways, since these are essential for the survival and progression of prostate tumors. We further show that targeting these pathways leads to the activation of alternate/compensatory signaling that drive therapeutic resistance and provide evidence to support the development of rational pharmacological combination therapy for the management of human prostate cancer.

▲TOP

E-2092

Persistent hepatocyte apoptosis accelerates diethylnitrosamine(DEN)-induced liver tumor formation

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Background and Aim: Hepatocyte apoptosis is frequently observed in chronic hepatitis. The aim of this study is to clarify the impact of persistent hepatocyte apoptosis on liver tumorigenesis.Methods: Wild-type(WT) or hepatocyte-specific Mcl-1 knockout (KO) mice, which demonstrate persistent hepatocyte apoptosis with ALT elevation, were injected with DEN or PBS at age of 2 weeks.Results: At age of 6months, any of PBS-treated WT or KO mice did not develop macroscopic liver tumors, and only 8 (2/26) % DEN-treated WT mice developed them. In contrast, all DEN-treated KO mice (15/15) developed them. At 2 weeks, immunohistochemistry of γ-H2AX revealed that DEN induced significantly high levels of DNA damage both in WT and KO mice. At 6 weeks, while γ-H2AX-positive hepatocytes in DEN-treated WT mice disappeared, those in DEN-treated KO mice remained at high levels. DEN-treated KO mice also showed significantly high ratio of hepatocyte regeneration with increasing TNF-α and Mcp-1 expressions compared with DEN-treated WT mice.Conclusion: Persistent hepatocyte apoptosis hold DNA damage for a long time, which might contribute to liver tumorigenesis.

E-2093

A novel cancer syndrome caused by KCNQ1-deficiency in the golden Syrian hamster

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The golden Syrian hamster is an emerging model organism for human diseases. However, until recently a significant challenge for the use of hamsters was the inability to generate genetically engineered hamsters. This barrier has recently been surmounted by our group, resulting in gene knockouts and knockins in the hamster by employing CRISPR/Cas9-mediated gene targeting, PiggyBac-mediated transgenesis and pronuclear injection. One of the first genes that we investigated is KCNQ1 which encodes for the KCNQ1 potassium channel. We generated eight KCNQ1 homozygous knockout hamsters and investigated the effects of KCNQ1-deficiency on tumorigenesis. By 70 days of age seven of the eight homozygous KCNQ1 knockouts used in this study began showing signs of distress and upon necropsy six of the seven ill hamsters had visible cancers, including synchronous T-cell lymphomas, plasma cell tumors, hemangiosarcomas, and suspect myeloid leukemias. None of the hamsters in our colony that were wildtype or heterozygous for KCNQ1 mutations developed cancers indicating that the cancer phenotype is linked to KCNQ1-deficiency. This study is also the first evidence linking KCNQ1-deficiency to blood cancers

E-2094

Cancer proteomics for patient-derived sarcoma model: proteomic profile changes during model establishment

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Patient-derived model is an emerging preferred platform for pre-clinical cancer research. However, patient-derived models such as cell lines and patient-derived xenografts (PDXs) may not recapitulate the spectrum of tumor heterogeneity in patients. To identify preserved proteomic fractions, we compared proteomic profiles between original primary tumors, their PDXs and cell lines by mass spectroscopy. We focused on sarcomas, and examined the original tumor tissues, PDX tissues, and cell lines. Using LC-MS/MS, we observed more than 3,000 proteins in individual samples. The identified proteins were classified according to their possible functions, and molecular pathways which they belong to. The results were summarized by a treemap format. We found that proteome profiles were changing during the model establishment. The proteome of original primary tumor tissues was firstly changed, when they were inoculated in the mice skin. Then, the proteome profile further changed when the cell lines were established. These observations may suggest the limited utility of patient-derived cancer models, and the importance of omics study to make best use of the models.

▲TOP

E-2095

Patient-derived xenograft as preclinical model for small bowel adenocarcinoma

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Background: Combination of oxaliplatin and capecitabine is recommended for treatment for advanced small bowel adenocarcinoma (SBA) without randomized clinical trials and preclinical studies. Methods: Patient-derived xenograft (PDX) was established from the primary tumor and peritoneal metastasis of the patient with SBA. Drug sensitivities of oxaliplatin (OHP), irinotecan (CPT-11), 5-fluorouracil (5-FU), and combination of OHP and 5-FU or CPT-11 and 5-FU were assessed by tumor growth inhibition rate (TGI). Pathological and genetic features with drug sensitivity of the patient were compared to those of PDX. Results: TGI after completion of drug treatment was 0.23, 0.31, and 0.52 by monotherapy of OHP, irinotecan, and 5-FU, respectively. By combination therapy of OHP and 5-FU, TGI after completion of treatment was 0.84 from primary tumor and 0.83 from peritoneal metastasis, respectively. By combination therapy of irinotecan and 5-FU, TGI after completion of treatment was 0.46. These results corresponded to the clinical response. Pathological and genetic features of PDX resembled to those of the patient. Conclusion: PDX model was suitable for preclinical model of SBA.

E-2096

Establishment of highly intrahepatic metastatic cell lines of HCC by in vivo selection and investigation of mechanism

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[Background] The prognosis of hepatocellular carcinoma(HCC) is poor due to the high incidence of intrahepatic metastasis. However, the mechanism of intrahepatic metastasis is not fully elucidated. [Aims] The aim of this study is to investigate the mechanism of intrahepatic metastasis in HCC. [Methods] Highly intrahepatic metastatic HCC cell line (HuH-7) was established by in vivo selection using trans-portal vein metastatic model. After tumor dissociation and expansion in culture, the resulting cell populations were subjected to the next cycle. After 4 cycles, highly intrahepatic metastatic cell and parent cell were compared in vitro and in vivo. Subsequently, we performed integrated microarray analysis to identify the target molecules. [Results] Highly metastatic intrahepatic cell line was established. Proliferative potential was increased and apoptosis was suppressed. In addition, tumorigenesis was exacerbated. Integrated expression profiling of miRNA and mRNA was performed, and several candidate molecules were indicated. [Conclusions] Highly intrahepatic metastatic cell was established by in vivo selection, and several candidates were indicated by integrated microarray analysis.

▲TOP

[E-2097] E5-1 [English]
Cancer specific signal transduction (1)

2018 / 9 / 28 (Fri) 15:30-16:45 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Naoto Tsuchiya / Lab. Mol. Carcinogenesis, Natl. Cancer Ctr. Res. Inst.

E-2097

A novel cancer therapy to stimulate oncogenic ERK signaling by ACA-28, a novel compound inducing ERK-dependent apoptosis

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The ERK MAPK pathway is frequently hyperactivated in approximately, one-third of all human cancers such as melanoma. Here, we identified ACA-28, a compound targeting the ERK hyperactivation. We demonstrated that ACA-28 selectively inhibited the growth of three types of melanoma cancer cells wherein ERK MAPK signaling is hyperactivated, whereas the growth of normal human epidermal melanocytes was less affected by ACA-28. Moreover, ACA-28 specifically induced apoptosis in NIH/3T3 cells oncogenically transformed with HER2/ErbB2 or oncogenic Ras, but not in the parental NIH/3T3 cells. We investigated the mechanisms of action of ACA-28 to selectively kill cancer cells. Surprisingly, ACA-28 further stimulated ERK phosphorylation thereby inducing ERK-dependent apoptosis in various cancer cells wherein ERK is hyperactivated. To our knowledge, this is the first demonstration of a small molecule compound which selectively kills cancer cells by inducing ERK-dependent apoptosis. ACA-28 may be effective in various cancer cells wherein ERK signaling is hyper-activated. Our data propose a novel cancer therapy to stimulate oncogenic ERK signaling and induce ERK-dependent apoptosis.

▲TOP

E-2098

Stemness Is Enhanced in Gastric Cancer by a SET/PP2A/E2F1 Axis

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The cancer stem cells are critical for initiation, maintenance, metastasis, and relapse of cancers; however, the molecular mechanisms supporting cancer stemness remain largely unknown. Increased kinase and decreased phosphatase activity are hallmarks of oncogenic signaling. Protein phosphatase 2A (PP2A) functions as a tumor suppressor enzyme and elevated levels of SET/I2PP2A, an endogenous PP2A protein inhibitor, are correlated with poor prognosis of several human cancers. Here it was determined that SET expression was positively correlated with poor survival of human gastric cancer patients. Mechanistically, SET knockdown decreased E2F1 levels and suppressed the stemness of cancer cell lines. Immunoprecipitations show SET associated with the PP2A-B56 complex, and the B56 subunit interacted with the E2F1 transcription factor. Treatment of gastric cancer cells with the SET-targeting drug OP449 increased PP2A activity, decreased E2F1 protein levels, and suppressed stemness of cancer cells. These data indicate that a SET/PP2A/E2F1 axis regulates cancer cell stemness and is a potential target for gastric cancer therapy. (Mol Cancer Res. 2018, 16(3):554-563)

E-2099

ROR1-CAVIN3 interaction required for caveolae-dependent endocytosis and pro-survival signaling in lung adenocarcinoma

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The receptor tyrosine kinase-like orphan receptor 1 (ROR1), a transcriptional target of the lineage-survival oncogene NKX2-1/TTF-1, sustains pro-survival signaling from multiple receptor tyrosine kinases (RTKs) in lung adenocarcinomas. In addition to its kinase-dependent role, ROR1 functions as a scaffold protein to facilitate interaction between CAV1 and CAVIN1, and consequently maintains caveolae formation. Here, we report that ROR1 possesses a novel scaffold function indispensable for efficient caveolae-dependent endocytosis. CAVIN3 was found to bind with ROR1 at a site distinct from those for CAV1 and CAVIN1, a novel function required for proper CAVIN3 subcellular localization and caveolae-dependent endocytosis, but not caveolae formation itself. Furthermore, evidence for a mechanistic link of ROR1-CAVIN3 interaction as well as consequential caveolae trafficking with RTK-mediated pro-survival signaling towards AKT in early endosomes was found in lung adenocarcinoma cells. The present findings warrant future study to develop novel therapeutic strategies for inhibiting the multifaceted scaffold functions of ROR1.

E-2100

Interaction of Akt with VRK2 at the lysosome controls induction of autophagy

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The role of lysosomal Akt is currently unknown. To characterize the molecular function of activated Akt at the lysosomes in the process of autophagy, we identified VRK2 interact with Akt at the lysosomes after induction of autophagy by TOF/MS analysis. VRK2 interacts with Akt1 and Akt2, but not with Akt3; the C terminus of Akt and the N terminus of VRK2 facilitate the interaction of Akt and VRK2. Kinase-dead VRK2A (KD VRK2A) failed to interact with Akt. BiFC experiments showed that at the lysosomes, Akt interacted with VRK2A, but not with VRK2B or KD VRK2A. Immunofluorescent assays revealed that VRK2 and phosphorylated Akt accumulated at the lysosomes after autophagy induction. WT VRK2A, but not KD VRK2A or VRK2B, facilitated accumulation of phosphorylated Akt at the lysosomes. Downregulation of VRK2 abrogated the lysosomal accumulation of phosphorylated Akt and impaired nuclear localization of TFEB which coincided to inhibition of autophagy induction. The VRK2-Akt complex is required for control of lysosomal size, acidification, bacterial degradation, and for viral replication. The study suggested the role of VRK2-Akt complexes at the lysosomes for the modulation of autophagy.

▲TOP

E-2101

Regulation of ERK activity dynamics in the intestinal epithelium

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The extracellular signal-regulated kinase (ERK) signaling pathway regulates a variety of biological processes including proliferation, differentiation, and tumorigenesis. Among many growth factor receptors that can activate ERK, epidermal growth factor receptor (EGFR) plays a central role in normal and cancer cells. Recent studies have revealed that EGFR signaling can generate complex spatiotemporal ERK activity at the single cell level, however its signaling dynamics and regulatory mechanisms remain elusive in the intestinal epithelial cells. By using highly sensitive Forster resonance energy transfer (FRET) biosensor for ERK activity, we uncover the ERK activity dynamics in the intestine. In vivo imaging demonstrates the presence of two distinct modes of ERK signaling activity, sustained basal activity and transient pulse-like activity. Further analyses with intestinal organoids reveal that the two modes of ERK activity are generated by distinct upstream EGFR family receptors. Moreover, we show that Wnt signaling activation alters the ERK signaling dynamics underlying the enhanced responsiveness of tumour cells to EGFR inhibition.

E-2102

Identification of potential regulatory mutations using multi-omics analysis and haplotyping of LUAD cell lines

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Sarun Sereewattanawoot, Ayako Suzuki, Masahide Seki, Yoshitaka Sakamoto, Takashi Kohno, Sumio Sugano, Katsuya Tsuchihara, Yutaka Suzuki
The functional relevancy of mutations occurring in the regulatory regions in cancers remains elusive. Here, we identified regulatory mutations having transcriptional consequences in LUAD-derived cell lines. We phased the mutations in the regulatory regions to the downstream heterozygous SNPs in the coding regions and examined whether the ChIP-Seq variant tags of the regulatory SNVs and the RNA-Seq variant tags of their target transcripts showed biased frequency between the mutant and reference alleles. We identified 137 potential regulatory mutations affecting the transcriptional regulation of 146 RefSeq transcripts with at least 84 SNVs that create and/or disrupt potential transcription factor binding sites. For example, in the regulatory region of NFATC1, a novel and active binding site for the ETS transcription factor family was created and experimentally validated. Further examination revealed that 31 of these disruptions were presented in clinical lung adenocarcinoma samples and were associated with prognosis of patients.

▲TOP

[ML6] ML6 [Japanese]
Morning Lectures 6

2018 / 9 / 28 (Fri) 8:00-8:50 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Hiroshi Yokozaki / Div. Pathol., Dept. Pathol., Kobe Univ. Grad. Sch. Med.

ML6

New primary culture method and the prediction model for clinical treatment

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Discussant：Soji Ozawa
Dept. Gastroenterol Surg., Tokai Univ., Sch. Med.

Primary culture of cancer cells derived from each patient's tumor can provide important information of the "individual tumor." It is general to use cell lines in basic research filed. However, that of cell lines is quite different from clinical cancers. The primary culture method of clinical cancer is optimized in some gastrointestinal tumor. We have developed a unique 2D/3D-culture method for primary tumor. We obtained clinical samples from surgically resected gastrointestinal cancers. They were mechanically and enzymatically digested and collected using customized filters. And we cultured the obtained cells on a matrix-coated plate with chemically defined stem-cell culture medium. All these primary cultured cells grew and about 90% of them were successfully passaged. The morphology and gene expressions were similar to each parental clinical tumor. We examined the culture medium, and multi-drug sensitivity assay including anti-cancer drugs commonly used was performed. In this lecture, I will introduce our primary culture method including other 2D/3D-culture method, PDX model, leading to the personalized medicine.

▲TOP

[J-2001] J5-1 [Japanese]
Cancer specific signal transduction (2)

2018 / 9 / 28 (Fri) 9:00-10:15 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Takashi Matozaki / Div. Mol. Cell. Sig. Kobe. Univ. Grad. Sch. Med.

J-2001

Chronic TGF-β exposure drives stabilized and mTOR-dependent EMT and tumor stemness

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Tumors comprise cancer stem cells (CSCs) and their heterogeneous progeny within a stromal microenvironment. In response to TGF-β, epithelial and carcinoma cells undergo partial or complete epithelial-mesenchymal transition (EMT), which contributes to cancer progression. This process is seen as reversible, since cells revert to an epithelial phenotype upon TGF-β removal. We found that prolonged TGF-β exposure, mimicking the state of in vivo carcinomas, promotes stable EMT in mammary epithelial and carcinoma cells, in contrast to reversible EMT induced by shorter exposure. The stabilized EMT was accompanied by stably enhanced stem cell generation and anticancer drug resistance. Furthermore, prolonged TGF-β exposure enhanced mTOR signaling. A bitopic mTOR inhibitor repressed CSC generation, anchorage-independence, cell survival and chemoresistance, and efficiently inhibited tumorigenesis in mice. These results reveal a role for mTOR in the stabilization of stemness and drug resistance of breast cancer cells, and position mTOR inhibition as a treatment strategy to target CSCs.

▲TOP

J-2002

ROCK-dependent phosphorylation of NUP62 regulates p63 nuclear transport and squamous cell carcinoma proliferation

Masaharu Hazawa
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p63, more specifically its ΔNp63α isoform, plays essential roles in squamous cell carcinomas (SCCs), yet the mechanisms controlling its nuclear transport remain unknown. Nucleoporins (NUPs) are a family of proteins building nuclear pore complexes (NPC) and mediating nuclear transport across the nuclear envelope. Recent evidence suggests a cell type-specific function for certain NUPs; however, the significance of NUPs in SCC biology remains unknown. In this study, we show that nucleoporin 62 (NUP62) is highly expressed in stratified squamous epithelia and is further elevated in SCCs. Depletion of NUP62 inhibits proliferation and augments differentiation of SCC cells. The impaired ability to maintain the undifferentiated status is associated with defects in ΔNp63α nuclear transport. We further find that differentiation-inducible Rho kinase reduces the interaction between NUP62 and ΔNp63α by phosphorylation of phenylalanine-glycine regions of NUP62, attenuating ΔNp63α nuclear import. Our results characterize NUP62 as a gatekeeper for ΔNp63α and uncover its role in the control of cell fate through regulation of ΔNp63α nuclear transport in SCC.

J-2003

Analysis of the molecular mechanism of transcription factor Nrf2

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Because effects of anti-cancer agents against lung cancer are still insufficient, an effective anti-cancer drug development has been expected. We have recently found that the transcription factor Nrf2 is constitutively activated at a frequency of 30% in lung cancer. The transcription factor Nrf2 binds to the antioxidant-responsive element to activate transcription of cytoprotective genes. Therefore, up-regulation of these enzymes by constitutive activation of Nrf2 in lung cancer cells led to anti-cancer drug resistances. This observation strongly supports that inhibition of Nrf2 may provide new therapeutic approaches in lung cancers with activation of Nrf2. Therefore to find weak points of Nrf2, we are trying to elucidate the molecular mechanism of Nrf2 activity. To explore interacting proteins with Nrf2, we purified the Nrf2 protein complex from transiently Halo-tag-Nrf2 expressing HEK293T cells using Halo-tag-based affinity purification. The liquid chromatography-tandem mass spectrometry identified transcription factors, protein-modifying enzymes, and protein degradation enzymes as components of the Nrf2 protein complex. We will analyze how these proteins affect Nrf2 activity.

J-2004

Mutation profiling by a highly sensitive detection system for PIK3CA mutations in patients with breast cancer

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PIK3CA mutations are the common activated mutations in breast cancer and sensitive detection methods are required. We established a novel detection method using the quenching probe (QP) system to identify PIK3CA mutations, using 309 DNA samples from breast cancer tissue. In a developmental cohort, we determined the optimal detection threshold of QP system with 119 freshly frozen samples. In a validation cohort, we evaluated whether that mutation threshold obtained from developmental cohort was applicable on FFPE derived DNA. Independent researchers, who were blinded to the results of QP system, performed direct sequencing (DS) and droplet digital polymerase chain reaction (ddPCR) with the 30 DNA samples among 190 samples, which had been examined with QP system. The sensitivity and specificity based on the ddPCR result was 100% and 100% (QP system) and 60% and 100% (DS), respectively. We analyzed the relationship between clinical outcome and the PIK3CA mutational status of the 309 samples. PIK3CA mutations, especially H1047R, were favorable prognostic factors of relapse-free survival. Our novel system for detecting PIK3CA mutations may be useful with higher sensitivity than DS.

▲TOP

J-2005

Identification and functional analysis of FGFR2 binding proteins in scirrhous gastric cancer

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Dept. Biochem., Kitasato Univ. Med.

Scirrhous gastric cancer (SGC) is highly invasive subtype of gastric adenocarcinomas and frequently exhibit scattered peritoneal metastasis. Previous studies have shown that genes of receptor tyrosine kinases (RTKs) such as FGFR2 or Met are amplified in SGC cell lines with high frequency. Hence, these cells exhibit oncogene addiction to sustained activity of these RTKs for maintenance of malignant phenotype. In order to gain novel insight in the downstream signaling pathway of SGC-specific RTKs, phosphoproteomic analysis was performed. Phosphotyrosine-containing proteins associated with RTKs were purified through two sequential rounds of immunoprecipitation from lysates of RTK-amplified SGC cell lines. Here we will show the initial functional data of transferrin receptor (TFRC), one of the identified proteins by MS analysis. In the SCG cells with FGFR2 amplification, TFRC proteins were found to bind FGFR2 and were phosphorylated at Tyr20. Moreover, shRNA knockdown of TFRC as well as inhibitor of FGFR2 revealed effective impairment in cell proliferation. These results suggest that TFRC has essential roles in malignancy of FGFR2-dependent SGC.

J-2006

Pharmacoproteomic analysis targeting multiple post-translational modifications for systems biology in drug sensitivity

Yuichi Abe
NIBIOHN, Proteome

Co-author：Takeshi Tomonaga、Jun Adachi
NIBIOHN, Proteome

It is widely known that abnormality in post-translational modification (PTM is closely related to oncogenesis. Thus, understanding of global PTM status significantly contribute to cancer biology and drug discovery against cancer. So far, several PTM proteomics such as phosphotyrosine modification were conducted by using immunoprecipitation with pan-PTM antibodies.To increase their identifications, we optimized enrichment step of phosphotyrosine peptides with immunoprecipitation, and achieved highly sensitive detection (more than 1,000 phosphotyrosine sites) from samples without any stimulation.In this study, we examined optimization for enrichment step of peptides containing other PTM sites. Additionally, we applied fractionation of enriched peptides for increasing identification of PTM sites. Newly developed protocol is commonly applicable for enrichment of several types of PTMs. We successfully measured more than 4,000 phosphotyrosine site, and modulation of PTM status by treatment of several inhibitors.This technique would lead to understand complexed crosstalk between multiple PTM signalings and find novel therapeutic targets.

▲TOP

[J-2007] J5-2 [Japanese]
MicroRNAs in cancer progression (1)

2018 / 9 / 28 (Fri) 10:15-11:30 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Masahiko Kuroda / Dept. Mol. Pathol. Tokyo Med. Univ.

J-2007

Dysregulation of miRNA in chronic hepatitis B is associated with HCC risk after nucleos(t)ide analogue treatment

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Hepatitis B virus (HBV) infection is a major cause of hepatocellular carcinoma (HCC). Nucleos(t)ide analogue (NA) therapy reduces the incidence of HCC, but it doesn't completely prevent the disease. Here, we show that dysregulation of microRNAs (miRNAs) are involved in post-NA HCC development. We divided chronic hepatitis B (CHB) patients who received NA therapy into two groups: those who did not develop HCC during the follow-up period after NA therapy (no-HCC group) and those who did (HCC group). miRNA expression profiles were significantly altered in CHB tissues as compared to normal liver, and the HCC group showed greater alteration than the no-HCC group. NA treatment restored the miRNA expression profiles to near-normal in the no-HCC group, but it was less effective in the HCC group. A number of miRNAs implicated in HCC, including miR-101, miR-140, miR-152, miR-199a-3p and let-7g, were downregulated in CHB. Moreover, we identified CDK7 and TACC2 as novel target genes of miR-199a-3p. Our results suggest that altered miRNA expression in CHB contributes to HCC development, and that improvement of miRNA expression after NA treatment is associated with reduced HCC risk.

▲TOP

J-2008

MicroRNA-126-3p contributes to suppress migration/invasion by regulating PI3K/AKT pathway in cervical cancer

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Previously, we reported that miR-126-3p was significantly up-regulated in squamous cell carcinoma and adenocarcinoma of the cervix compared with normal, and their expression levels correlated with disease severity and high-risk human papillomavirus infection. However, its cancer-relevant biological effects and molecular mechanisms by miR-126-3p still remains unknown in cervical cancer. Thus, we transfected with miR-126-3p mimic or negative control (NC) mimic in cervical cancer. Cell proliferation, migration and invasion analyzed by CCK-8, wound-healing and transwell assays. Furthermore, the underlying molecular mechanisms of the effect of miR-126-3p were explored using western blot analysis. Compared with NC mimic, overexpression of miR-126-3p suppressed proliferation and migration/invasion. Moreover, overexpressed miR-126-3p decrease the protein expression of p110α, phosphorylated PDK-1, phosphorylated AKT, phosphorylated PTEN, phosphorylated P70S6, phosphorylated GSK3β, and CyclinD1. These data suggest that miR-126-3p has the tumor suppressive function by regulating PI3K/AKT pathway. miR-126-3p may be a promising candidate for the miRNA-based therapy in cervical cancer.

J-2009

KHSRP is involved in esophageal squamous cell carcinoma progression by inducing the expression of oncogenic miRNAs

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KH-type splicing regulatory protein (KHSRP) is a multifunctional RNA-binding protein, which is involved in several post-transcriptional aspects of RNA metabolism, including microRNA (miRNA) biogenesis. It affects distinct cell functions in different tissues and can have an impact on various pathological conditions. Here, we investigated the oncogenic functions of KHSRP and their underlying mechanisms in the pathogenesis of esophageal squamous cell carcinoma (ESCC). KHSRP expression levels were elevated in ESCC tumors when compared with those in non-tumorous tissues by immunohistochemistry, and cytoplasmic KHSRP overexpression was found to be an independent prognosticator for worse overall survival. KHSRP knockdown inhibited growth, migration, and invasion of ESCC cells. KHSRP knockdown also inhibited the maturation of cancer-associated miRNAs, such as miR-21, miR-130b, and miR-301, and induced the expression of their target mRNAs, resulting in the inhibition of epithelial-to-mesenchymal transition. Our findings uncover a novel oncogenic function of KHSRP in esophageal tumorigenesis and implicate its use as a putative therapeutic target in ESCC.

J-2010

High metastatic tumor exosome-miRNA promotes metastasis via alteration of endothelial cells

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Cancer cells secrete exosomes to create a suitable environment for themselves. We identified miR-1246 that is more abundant in high metastatic melanoma exosomes compared with in low metastatic melanoma exosomes. The purpose of this study is to elucidate the involvement of exosome miR-1246 in cancer metastasis. We analyzed the effect of exosome miR-1246 on the adhesion between endothelial cells and between endothelial cells and cancer cells. Overexpression of miR-1246 decreased the expression of VE-Cadherin and increased that of ICAM-1 in endothelial cells. High metastatic tumor exosome treatment enhanced endothelial permeability and increased the adhesion of cancer cells to endothelial cells. In vivo experiments, miR-1246 knockdown decreased lung metastasis in tumor xenograft model. Intravenous administration of high metastatic tumor exosomes increased the adhesion of cancer cells to the lung, resulting in promoted lung metastasis. Thus, it was suggested that exosome miR-1246 promotes lung metastasis by destroying the endothelial cell barrier and inducing the adhesion of cancer cells to endothelial cells.

▲TOP

J-2011

Evaluation of prediction system in treatment effect and prognosis of esophageal cancer based on Radiogenomics theory

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[Introductio] Integrated analysis of epigenomic information (Epigenomics) and image information (Radiomics) as Ragioepigenomics [Methods] 1, Expression analysis of miR, evaluation as prognostic predictor, 2, Image information analysis: a, Normalization of contrast CT images, feature quantities by 3D gray level co-occurrence matrix Extraction. b, Normalization of SUV of FDG-PET image. Extraction of feature quantity by 3D gray level co-occurrence matrix. c, Image analysis of features. 3, Integrated analysis of Epigenomics and Radiomics information: We analyzed the relationship between miR expression level and variables obtained from image analysis. [Results] As a result of Cox regression by the selection method using elastic net, three variables were selected from CT, and the results of both were consistent. Furthermore, when the survival curve and the log rank test were performed for the cluster shade high value group and the low value group, a significant difference was observed in the 2 groups. [Conclusion] A multimodal approach so called RadioEpigenomics, which is a system for analyzing molecular information from image analysis will be applied to future Precision Medicine.

J-2012

The KRAS/PAX3-FOXO1 networks control the cell proliferation in rhabdomyosarcoma cells

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Rhabdomyosarcoma (RMS) is a soft tissue sarcoma which is the most frequent in children. In RMS, there are two major subtypes which are embryonal RMS (ERMS) and alveolar RMS (ARMS). ARMS is exclusively worse prognosis caused by forming the chimeric PAX3-FOXO1 gene. Recently, it was reported that 40% of clinical RMS samples have mutations in FGFR4/RAS/AKT pathway. On the other hand, we found that the ectopic expression of synthetic miR-143 (miR-143#12) induces a significant tumor suppression through targeting KRAS, AKT and ERK in colorectal cancer cells (Akao et al., 2018). Then, we investigated the tumor suppressive effects and regulation of the chimeric gene using miR-143#12 in ARMS cells. Ectopic expression of miR-143#12 in ARMS cells exhibited a significant tumor suppressive effect through the induction of apoptosis and autophagic cell death. Interestingly, we found that miR-143#12 inhibited the expression of chimeric PAX3-FOXO1, and that KRAS regulates the expression of PAX3-FOXO1. These findings suggested that miR-143#12 can perturb the KRAS/PAX3-FOXO1 networks in ARMS.

▲TOP

[LS18] LS18 [Japanese]
Expectation for new technology and Premonition of the cancer immunotherapy

2018 / 9 / 28 (Fri) 11:50-12:40 Room 7/10F 1006+1007, Osaka International Convention Center Room 7
：Fluidigm K.K.

Yoshihiro Kakeji / Division of Gastrointestinal Surgery, Department of Surgery, Graduate School of Medicine, Kobe University

LS18

Expectation for new technology and Premonition of the cancer immunotherapy

Kiyoshi Yoshimura
Department of Clinical Immuno Oncology, Clinial Research Institute of Clinical Pharmacology and Therapeutics, Showa University

No Abstract

▲TOP

[J-2049] J3 [Japanese]
Virus, bacteria infection, inflammation and cancer (3)

2018 / 9 / 28 (Fri) 13:00-14:15 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Tetsuya Tsukamoto / Dept. Diag. Path., Fujita Health Univ. Sch. Med.

J-2049

Helicobacter pylori CagA-induced secretory phenotype creates a tumorigenic microenvironment

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Co-author：Naoko Kamiya、Masanori Hatakeyama
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The cytotoxin-associated gene A (CagA) protein of *Helicobacter pylori* is delivered into gastric epithelial cells via the bacterial type IV secretion system, where it perturbs host signaling pathways. It has been demonstrated that persistent presence of CagA in human gastric epithelial cells induces premature cell senescence through accumulation of the CDK inhibitor p21. Given this, we hypothesized that CagA provokes senescence-associated secretory phenotype (SASP), thereby establishing a cancer-predisposing microenvironment. To test the idea, GES-1 human gastric epithelial cells expressing CagA were cultured for 6 days and the culture supernatant was applied to the parental GES-1 cell culture. As a result, the supernatant significantly enhanced proliferation of GES-1 cells. The candidate molecules responsible for this mitogenic effect were screened by DNA microarray analysis and their growth-stimulating activities on GES-1 cell were confirmed. These observations indicate that CagA induces secretory phenotype to create microenvironment that supports proliferation of cancer-precursor cells. The present study provides new insight into the role of CagA in gastric carcinogenesis.

▲TOP

J-2050

CADM1 enhances extravasation of adult T-cell leukemia cells

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Co-author：Yuki Kumagai、Yoshinori Murakami
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Adult T-cell leukemia (ATL) is a highly tissue-infiltrative leukemia derived from $CD4^+$ T cells triggered by infection of HTLV-I. CADM1, an immunoglobulin superfamily cell adhesion molecule, is ectopically expressed in ATL cells and established as a diagnostic cell-surface marker for ATL. CADM1 promotes multiple organ infiltration of ATL cells in mouse model but its underlying mechanism remains unclear. Here, we showed knockdown of CADM1 in MT-2, an HTLV-I-transformed T-cell line, suppressed trans-endothelial migration in vitro and extravasation in the liver tissue of immune-deficient mice after tail vein injection, which resulted in reduced tumor nodule formation on the liver surface and infiltration into the liver as well as lung and spleen tissues. On the other hand, overexpression of CADM1 in EL4 mouse T-cell lymphoma cells increased tumor nodule formation on the liver of C57BL/6 mice but not on the liver of $Cadm1^{-/-}$ mice. In addition, ATN-1 cells showed spread morphology when incubated on CADM1-Fc-coated glasses. These results suggest that homophilic interaction of CADM1 between ATL cells and host endothelial cells may enhance organ infiltration of ATL cells.

J-2051

ycophenolic acid enhances the cytotoxic effect of Abacavir on adult T-cell leukemia

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Adult T-cell leukemia (ATL) is an aggressive T-cell malignancy caused by human T-cell leukemia virus type 1. We recently reported that abacavir (ABC), an anti-HIV-1 drug, selectively kills ATL cells. ABC is a GTP analog and induces DNA double-strand breaks in ATL cells. Mycophenolic acid (MPA) inhibits inosine monophosphate dehydrogenase and leads to the depletion of intracellular GTP pools, especially in lymphocytes. In this study, we investigated a synergistic effect between MPA and ABC for ATL cells. MPA was significantly cytotoxic to the ATL cell lines and enhanced the lethality of ABC on p53-deficient ATL cell lines, but not on p53-intact ATL cell lines. We examined the effect of MPA on cell cycle in ATL cells and found that MPA induced accumulation of S-phase in p53-deficient ATL cells, but G1 arrest in p53-intact ATL cells. In addition, we confirmed that Chk1 activation by MPA induced p53 expression, leading to G1 arrest in ATL cells. It suggests that p53 expression through ATR-Chk1 pathway may rescue ATL cells from MPA and ABC treatment. Collectively, our results suggest that MPA enhances the cytotoxicity of ABC in p53-deficient ATL cell lines.

J-2052

Involvement of Enterococcus faecalis in the pathogenesis of pancreatic cancer

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Bacterial infection is concerned with the pathogenesis of many human diseases. Previously, we found that the existence of Enterococcus faecalis (Ent.f.) in pancreatic juice as well as pancreatic tissue and that Ent.f. can survive in pancreatic juice with co-culture experiment with bile. In the present study, we investigated the antibacterial activity of pancreatic juice and the involvement of Ent.f. in the progression of pancreatic cancer. On close inspection of bacterial presence in many bile samples, we detected Ent. f. and Enterobacter in most bile samples by 16S ribosomal RNA gene PCR and direct sequencing. In contrast, the antibacterial activity against Ent.f. was different in each pancreatic juice. Alkaline pH was one of the most important factors of the antibacterial activity in pancreatic juice. Furthermore, we identified dermcidin, known as antibacterial peptide, in a pancreatic juice. Addition of Ent.f. to pancreatic cancer cell lines increased the expression of inflammatory cytokines. In conclusion, we found a possibility that the infection of Ent.f. is involved in the progression of chronic pancreatic diseases, and pancreas have a defense mechanism against bacteria.

▲TOP

J-2053

3D imaging analysis of the promotion process from β-catenin accumulated crypts to colonic adenomas in mice model

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Early process of adenoma formation is not fully understood. We examined cell proliferation status of neoplastic cells and microvascular structures during the early adenoma from BCAC. DSS was given to ApcMin/+ mice at 5 weeks of age in drinking water for 7 days to induce colonic inflammation and adenoma formation. Serial sections of whole BCAC were stained for Ki-67 and podocalyxin, and reconstructed the 3D images using VG studio Max 2.0. Ki-67 positive and negative cell numbers were quantitatively analyzed during the early adenoma. ApcMin/+mice has multiple BCACs but BCACs rarely develop to colon adenoma. DSS initiated adenoma formation and visible adenomas with multiple branching structures were developed within 3-4 weeks. Increase of Ki-67 positive cells in BCACs was observed as early as 5th day during DSS treatment and reached to 90 % cell numbers in 3-4 weeks. But, Ki-67 negative cell was downward trend rise at 5 weeks and remain the same to about 10% cell number in 1-4weeks. DSS treatment induced developmental transition of BCAC to adenomas. During this process, Ki-67 positive and negative cell in BCACs was increase in a geometrical ratio from at 1 to 4weeks after DSS.

J-2054

Antithrombin prevents the susceptibility to hepatocarcinogenesis through suppressing inflammation

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Background and Aims: We previously reported that plasma antithrombin (AT) level is the prognostic factor for hepatocellular carcinoma (HCC) after hepatectomy. We aimed to investigate whether AT is associated with development of HCC using mice model. Methods: HCC were developed in AT-insufficient (AT+/−) mice and wild-type (AT+/+) mice treated with DEN and CCl4. The development of HCC were compared between AT-insufficient and wild-type mice. AT was administered to AT-insufficient mice treated with DEN and CCl4. Results: The tumor size and the number of DEN and CCl4-induced HCC were significantly enhanced in AT-insufficient mice compared with wild-type mice. Administration of AT significantly reduced DEN and CCl4-induced HCC in AT-insufficient mice. Serum IL-6 and TNF-α were significantly elevated in AT-insufficient mice compared with wild-type mice. AT administration significantly reduced serum IL-6 and TNF-α in AT-insufficient mice treated with DEN and CCl4. Conclusion: AT insufficiency led to the increased susceptibility to hepatocarcinogenesis through amplifying inflammation. Modulation of plasma AT level may provide a novel strategy for suppression of HCC recurrence.

▲TOP

[J-2055] J11-3 [Japanese]
Cancer stem cell (3)

2018 / 9 / 28 (Fri) 14:15-15:30 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Tomoaki Tanaka / Dept. of Mol. Diag., Chiba Ionic., Grad. Sch. Med.

J-2055

The recycling endosomal CD133 functions as an inhibitor of autophagy at the pericentrosomal region

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CD133 is a transmembranous protein that mainly localizes to the plasma membrane in hematopoietic and neural stem cells as well as cancer stem cells. Although CD133 also localizes to the cytoplasm, the mechanism of action and function of cytoplasmic CD133 currently remain unknown. We herein demonstrated that when Src-family kinase activity is low, membranous CD133 interacts with HDAC6 and is transported to the pericentrosomal region after internalization and endosome formation via the dynein-based traffic system. Pericentrosomal CD133 is then recycled to the plasma membrane via recycling endosomes. At the pericentrosomal region, endosomal CD133 captures GABARAP, an initiator of autophagy, and inhibits GABARAP-mediated ULK1 activation and the subsequent initiation of autophagy. In addition, pericentrosomal CD133 suppresses cell differentiation such as primary cilium formation and neurite outgrowth by inhibiting autophagy. Thus, our results provide evidence to suggest that pericentrosomal CD133 plays an essential role in maintaining the undifferentiation status of cancer cells.

▲TOP

J-2056

NOX1 induces mTORC1 activation and proliferation of colon cancer stem cells via interaction with Ca2+-binding proteins

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Elucidating the essential pathways for the maintenance and proliferation of cancer stem cells (CSCs) is crucial to devise an innovative anti-cancer therapy. Here, we investigated CSC spheroids derived from surgical specimen of colon cancer to identify a unique pathway for their proliferation. Spheroid proliferation and their stem cell-related properties were dependent on mTORC1 kinase activity, which was dependent on reactive oxygen species (ROS) produced by an NADPH oxidase 1 (NOX1). Cells that expressed NOX1 were restricted in Lgr5-positive CSCs in tumor xenografts, and NOX1 expression was markedly induced during colon carcinogenesis in mouse organoid models. Mass spectrometry analyses revealed that the mTORC1 was bound to calcium binding proteins, in a ROS-dependent manner. One of calcium binding proteins was oxidized by NOX1-produced ROS and required for the mTORC1 activation and CSC spheroid proliferation.

J-2057

Reprofiling of Antimalarial Drug is a Novel Therapeutic Target for Colon Cancer Stem Cell

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Introduction; Cancer stem cells (CSCs) are deeply involved in resistance to treatment and are the most important target cells in cancer treatment. We focused on autophagy and lysosomal activity of CSCs and colon CSC targeting by drug repositioning.Methods; Autophagy and lysosome were labeled by LC3B imaging vector and Lysotracker respectively. Sphere formation assay and limiting dilution assay were performed. Additionally, anti-tumor effect was assessed.Results; Both LC3B+ and Lysotracker+ cells were characteristically existed in CD44V9+/ CD133+ CSC fraction as a small cell subpopulation. Lysotracker+/CD44v9+ cells showed high sphere formation and tumorigenic activities. During the process of screening of antimalarial drugs, anti-lysosomal effect of mefloquine was identified. Mefloquine treatment significantly decreased CD44v9+/ CD133+ cell number. Mefloquine demonstrated the synergic effect on oxaliplatin. In the patient-derived xenografted (PDX) mice, combined treatment of mefloquine with oxaliplatin drastically abrogated the tumorigenic activity of cancer cells. Conclusions; Repositioning of mefloquine to colon cancer will be a promising strategy for cure of colon cancer.

J-2058

CDX1 regulates stemness through MYC pathway modulation and reprogramming gene activation in neuroblastoma

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Tumor sphere formation ability is a property of cancer stem cells (CSCs). We previously reported that transcription factor CDX1 expressed specifically in sphere-forming neuroblastoma (NB) cells. We conducted microarray-based expression analysis of NBs; Pathway analysis of microarray data indicated that genes in pluripotent stem cells-related pathway were induced both in CDX1-expressing NB cells and NB spheres. Cell proliferation related gene set, MYC module was significantly downregulated in CDX1-expressing NB cells or NB spheres. Of note, MYC-coactivator MAX was suppressed at protein and mRNA levels by CDX1 high-expression. MAX was downregulated by sphere formation in NB cells and upregulated by neural crest differentiation from iPS cells. In addition, direct binding of CDX1 to MAX promoter region was confirmed by qChIP analysis. These results indicated that CDX1 not only induced stem cell-related genes transcription, but also reduced cell proliferation thru MAX transcriptional suppression. In conclusion, CDX1 plays an important role in NB stemness by suppression of MYCN-related cell cycle progression and makes CSCs dormant having undifferentiated properties.

▲TOP

J-2059

BEX2 induces dormant status in cholangiocarcinoma cells

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Dormant cancer stem cell (CSC) is refractory to treatment and believed to be a good therapeutic target, but precise mechanisms of maintaining dormant CSC is largely unknown. We previously reported that dormant CSC is enriched in the $CD274^{low}$ (also known as PD-L1) subpopulation in cholangiocarcinoma. In this study, we tried to elucidate the mechanisms of maintaining dormant CSC in cholangiocarcinoma. We focused on BEX2 gene, upregulated in CD274 knock down cells in microarray analysis. BEX2-knock down in HuCCT1 (a human cholangiocarcionoma cell line) cells decreased tumorigenicity, ALDH activity, and subpopulation of G_0 phase, while overexpression of BEX2 increased G_0 subpopulation. We found that BEX2 bound E3 complex and was degraded by proteasome. Furthermore, BEX2 bound mitochondrial protein, and knock down of BEX2 increased mitochondrial oxygen consumption. These data suggested that BEX2 suppresses oxydative phosphorylation and induces dormant status in chonalgicarcinoma cells.

J-2060

Suppression of mTOR pathway-induced autophagy maintains leukemia stem cell in murine AML model

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Leukemia stem cell (LSC) is associated with relapse and resistance to chemotherapy. We recently revealed that inactivation of autophagy reduced LSCs. Therefore, we aimed to clarify the molecular mechanisms of activating autophagy in LSCs. Firstly, we confirmed lower LC3 protein expression in LSCs than non-LSCs using mouse AML model, suggesting that LSCs have higher autophagic activity. Actually, mRNA levels of autophagy machinery genes were up-regulated in LSCs compared to non-LSCs. In addition, we found that Pten mRNA level was upregulated in LSCs. Since Pten is a major suppressor of mTOR pathway signaling, which is a negative regulator of autophagy, we compared the activity of mTOR pathway signaling between LSCs and non-LSCs. Western blot analysis showed lower expression levels of phosphorylated Akt and mTOR in LSCs, suggesting that activated autophagy in LSCs is in part induced by suppression of mTOR pathway. Interestingly, induction of autophagy by rapamycin, mTOR inhibitor, resulted in increase of immunophenotypic LSCs in vivo. These findings indicated that activation of Akt/mTOR signaling could be a potent therapeutic target against LSCs through autophagy inhibition.

▲TOP

[J-2061] J11-4 [Japanese]
Cancer stem cell (4)

2018 / 9 / 28 (Fri) 15:30-16:45 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Yohei Shimono / Dept. Biochem., Fujita Health Univ.

J-2061

Identification of quiescent cancer stem cells in esophageal squamous cell carcinoma

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Background: The low affinity neurotrophin receptor (p75NTR) has been reported to be expressed in a candidate cancer stem cell (CSC) population in esophageal squamous cell carcinoma (ESCC).Aim: We isolated and characterized quiescent CSCs population in ESCC. Methods: p75NTR-positive KYSE cells were fractionated into quiescent and proliferating cells by flow cytometry using a fluorescent DNA-staining dye to assess their CSC phenotype. Results: Isolated p75NTR-positive cells in the G0-G1 phase (p75NTR-positive/G0-1 cells) but not in the S-G2-M phase (p75NTR-positive/S-G2-M cells) showed strong expression of stem cell-related genes Nanog, BMI-1, and p63; high colony formation ability; high tumorigenicity in a mouse xenograft model; and strong chemoresistance against cisplatin along with the expression of drug resistance genes ABCG2 and ERCC1. Conclusion: Our results suggest that p75NTR-positive/G0-1 cells represent quiescent CSCs in ESCC, providing us with targets to investigate molecular processes regulating CSC phenotype and to develop novel therapeutic strategies.

▲TOP

J-2062

Growth inhibition of colorectal cancer stem-like cells by tankyrase inhibitors and its mode-of-action

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Cancer stem cells are highly tumorigenic and drug resistant, causing relapse of cancer. Therefore, targeting cancer stem cells would be important for cancer eradication. Tankyrase, a member of the poly(ADP-ribose) polymerase family, destabilizes Axin, a β-catenin repressor, and promotes β-catenin signaling. Using colorectal cancer cells that contain the cancer stem-like CD44(+) subpopulation, we have shown that tankyrase inhibitors exert a higher growth inhibitory effect on CD44(+) cells than CD44(-) cells. However, the underlying mechanism remains elusive because tankyrase inhibitors downregulate β-catenin signaling comparably in both cells. To address this question, here we performed transcriptome analysis and found that tankyrase inhibitors downregulated a set of stem cell-related genes that were overexpressed in CD44(+) cells. Among those genes, we identified a kinase whose expression was CD44(+) selective and required for CD44(+) cell maintenance. Tankyrase inhibitors downregulated its expression at mRNA and protein levels. These data suggest that tankyrase inhibitors can target colorectal cancer stem cells by repressing the expression of stem cell-related factors.

J-2063

Differential functions of mTORC1 and mTORC2 in the maintenance of stem-like properties of pancreatic cancer cells

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Background: The $CD133^+$ pancreatic cancer cells exhibit cancer stem cell (CSC)-like properties. Rapamycin, a mechanistic/mammalian target of rapamycin (mTOR) inhibitor, reduced the CSC-like properties of pancreatic cancer cells. The aim of this study is to evaluate the functions of mTOR complex 1 (mTORC1) and 2 (mTORC2) in the maintenance of CSC-like properties.
Methods: We established a $CD133^+$ cell rich subline from Capan-1 cells. Using this subline as a model of pancreatic CSCs, we examined the effect of mTORC1/mTORC2 dual inhibitor KU-0063794 and the results were compared to mTORC1 inhibitor rapamycin.
Results: In the sphere formation assay, an index of self-renewal capacity of CSCs, KU-0063794 decreased sphere number less than 10% at high concentration, showing a contrast to the inhibition by rapamycin which reaches plateau around 60%. The analysis of phosphorylation status of Akt/mTOR signaling pathway detected different signaling output after the treatment with two inhibitors, suggesting the differential functions of two complexes.
Conclusions: The signaling downstream mTORC1 correlates with stem-like properties, while mTORC2 seems to modulate this signaling by activating Akt.

J-2064

Functional analysis of transcribed-ultraconserved regions in cancer stem cell using colorectal cancer organoids

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Transcribed-ultraconserved regions (T-UCRs) are a novel class of long non-coding RNAs transcribed from UCRs that are completely conserved among orthologous regions of the vertebrates. It has been reported that T-UCRs have distinct signatures in human cancers. However, little has been known about the correlation between T-UCRs and tissue/cancer stem cells so far. In this study, we aimed to clarify the involvement of T-UCRs in cancer stem cell biology of colorectal cancer (CRC) and validate their functional role.We focused on 61 T-UCRs that were reported as the dysregulated T-UCRs in CRC by microarray analysis. We examined them using 3 pairs of CRC and non-neoplastic organoids by qRT-PCR. Through the validation, 4 T-UCRs were picked out according to the specific upregulation in CRC organoids compared to those in normal organoids. Among these T-UCRs, Uc.266+A only showed significant correlations between its expression and drug resistance against several chemo-attractants as well as advanced clinico-pathological factors.In order to clarify the solid evidences, especially the direct effect of T-UCRs onto the stem cells features such as dormancy, further in vitro studies are needed.

▲TOP

J-2065

In silico screening for agents targeting drug-tolerant CD44v-positive cells in patient-derived gastric cancer

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Tumors consist of heterogeneous cell populations, among which drug-resistant subpopulations of cancer cells preexist even before chemotherapy and cause relapse. We have shown, in gastric cancer patient-derived cell models, that CD44 variant-positive [CD44v(+)] cancer cells, a reported cancer stem cell fraction, were enriched in residual cells after treatment with cytotoxic anticancer agents, suggesting the involvement of the cell fraction in drug resistance. Here we searched for agents that target the CD44v(+) cells in silico with JFCR_LinCAGE, our chemotherapeutic agent and gene expression database. By transcriptome analysis of patient-derived cells, we obtained CD44v(+) cell-selective gene set and compared it with gene expression changes caused by the agents in the database. As a result, we identified agents that repress the expression of CD44v(+) cell-selective genes. These agents decreased the CD44v(+) cell population in gastric cancer cells, and preferentially inhibited the colony formation of CD44v(+) cells. Thus, our in silico screening would be a powerful approach to identify agents that target the minimal residual disease of tumors. Collaborator: Naomi Kawata[1,4]

J-2066

A model of quiescent cancer stem cell through condensation of ODC degron+ cells

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Cancer stem cell（CSC）is a rare population that causes tumor relapse. Ornithine decarboxylase (ODC) degron-fluorescent ZsGreen system enables visualization of CSCs in certain cancer cell types based on the low proteasome activity. Although ZsGreen(+) cell is generally infrequent (<0.1%), we attempted to produce 100% ZsGreen(+) cells by repeat FACS sorting. Using colon cancer HCT116 cell line, ODC-degron-ZsGreen was transduced by retoviral infection. After selection of G418, the cells were expanded followed by FACS sorting followed by repeat cell expansion and FACS sorting more 3 to 4 times, we could obtained stablely100% ZsGreen positive cells (ZsGreen(100)). ZsGreen(100) cells showed high Lgr5 and Bmi1 mRNA expression, and increased chemoresistance to 5-FUand L-OHP as compared to ZsGreen(-)or even ZsGreen(+) cells. Moreover, ZsGreen(100) cells had a decrease in CK20 epithelial cell marker, and showed a long BrdU retaining in mouse tumors, which is a hallmark as a CSC in vivo. Interestingly ZsGreen(100) cells lost high tumorigenicity. Thus, we postulate that it is suggested that this model may mimic quiescent CSC that is positioned top in the hierarchy of CSC.

▲TOP

[ML7] ML7 [Japanese]
Morning Lectures 7

2018 / 9 / 28 (Fri) 8:00-8:50 Room 8/10F 1008, Osaka International Convention Center Room 8

Hideki Wanibuchi / Mol. Path., Grad. Sch. Med., Osaka City Univ.

ML7

Applications of tissue clearing technology in cancer research

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Discussant：Shigehira Saji
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Tissue clearing technology has been mainly applied to neuroscience research, but not to cancer biology. Therefore, we are trying to establish a new imaging modality for monitoring cancer metastasis with high resolution. CUBIC (Clear, Unobstructed Brain/Body Imaging Cocktails) is a combination of hydrophilic chemicals and allows the whole mouse body or organ tissue clearing with high quality. After mouse organs became transparent, we successfully visualized micro-metastasis in deeper tissues. In addition, using this technology, we could monitor the cancer metastasis by 3D imaging, and found that patterns of metastasis appeared to be dependent upon each cancer cell line or organ. This system also enabled us to quantify the number of metastasis with single-cell resolution and we showed that epithelial-mesenchymal transition (EMT) contributed to the extravasation process and anti-apoptotic ability of the A549 lung cancer cells in experimental lung metastasis model. Currently, we are trying to visualize host stromal cells, including blood and lymphatic vessels, by the tissue clearing technology, to understand the mechanism of cancer metastasis.

▲TOP

[J-2013] J15-1 [Japanese]
New biomarker (1)

2018 / 9 / 28 (Fri) 9:00-10:15 Room 8/10F 1008, Osaka International Convention Center Room 8

Yasushi Shintani / Dept. Gen. Thoracic. Surg., Osaka Med. Univ., Sch. Med.

J-2013

Clinical implications of CEA in serum exosomal fraction of patients with colorectal cancer

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The association of a fraction of various serum proteins such as carcinoembryonic antigen (CEA) with circulating exosomes has been debated. The use of enzyme-linked immunosorbent assays (ELISAs) to measure serum exosomal molecules is rare in research laboratories and totally absent in clinical settings. In this study, we optimized a method for assessment of serum exosomal molecules. Whole blood samples were collected from patients with colorectal cancer. Exosomes were isolated using the ExoQuick reagent, solubilized in an assay buffer and subjected to CEA detection by ELISA. A five-fold increase in the concentration of the exosomes in the assay buffer and the addition of bovine serum albumin (BSA) resulted in more accurate measurements of the serum exosomal CEA. The thawing temperature of frozen serum samples before exosome extraction was also optimized. A validation study that included one hundred sixteen patients with colorectal cancer demonstrated that serum exosomal CEA from samples thawed at 25℃ exhibited a better AUC value, sensitivity, and specificity as well as a more correct classification than serum CEA.

▲TOP

J-2014

A study for development of a novel screening kit of colorectal cancer with analysis of gut microbiome

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<Background>Because the incidence and mortality of colorectal cancer (CRC) have been increasing in Japan, development of a novel screening method for CRC is urgently needed. Now, the high-throughput technique of sequencing bacterial gene has enabled us to grasp the gut microbiota composition of individuals. Besides, there have been increasing reports indicating that a specific gut microbe may have causal roles in CRC development. We therefore aimed to develop a novel CRC screening kit by analyzing gut microbiota. <Method>We have collected stool samples from 451 CRC patients and 971 healthy people. We analyzed gut microbiota in each sample by meta-sequencing analysis of 16S rRNA gene. We developed a diagnostic model using a set of bacterial species with significant difference of entity between CRC patients and healthy people. <Result>We developed a diagnostic model for early CRC and that for advanced CRC separately. The average values of area under the curve (AUC) of the receiver operating characteristic curve were 0.82 and 0.89 respectively. <Conclusion>It would be possible that we could detect CRC patients more accurately by the diagnostic model with analysis of gut microbiome.

J-2015

Circulating pre-microRNA-488 in peripheral blood is a potential biomarker for predicting recurrence in breast cancer

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Purpose: Circulating microRNAs (miRs) in blood have been highlighted as diagnostic, prognostic and predictive biomarkers for "Precision medicine". The aim of this study was to explore the possibility of using circulating precursor miRs (pre-miRs) as clinical biomarkers of recurrence in breast cancer (BC).Methods: We performed miR microarray analyses of miRs in serum exosome from patients with or without recurrence of BC, and identified miR-488-5p as a recurrence-related miR. Then, we examined the relationship between pre-miR-488 and miR-488-5p expression in blood by quantitative RT-PCR. Finally, we assessed the clinicopathological and prognostic significance of pre-miR-488 expression in blood of 330 BC patients.Results: A positive correlation was found between pre-miR-488 and miR-488-5p expression in blood ($p<0.05$). Pre-miR-488 was present mainly in the plasma and was downregulated in either tumor tissues or blood. Multivariate analysis revealed that high expression of pre-miR-488 was an independent poor prognostic factor for recurrence free survival ($p<0.05$).Conclusions: pre-miR-488 expression in blood is a novel prognostic biomarker for predicting recurrence in BC patients.

J-2016

Evaluation of cfDNA mutation spectrum in metastatic breast cancer

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Evaluation of mutations from circulating tumor DNA (ctDNA) provide a non-invasive method to monitor cancer progression from blood samples. Mutations in ESR1 reported to be a cause for aromatase inhibitors (AI) resistance in hormone receptor positive metastatic breast cancer. This study aims to assess ctDNA mutation spectrum of metastatic breast cancer by using targeted next generation sequencing (NGS). Sixty-nine patients were enrolled in this study. Oncomine Breast cfDNA assay that incorporates with molecular barcodes to reduce sequencing error were utilized for NGS. This assay covers 10 breast cancer related genes. As a result, up to 71% of samples have detectable ctDNA mutations. Among them, 52.1% mutations were derived from TP53, 24.6% from PIK3CA, 14.4% from ESR1. All the ESR1 mutations are located at the ligand-binding domain. Sequencing results were validated with droplet digital PCR and the results from both assays are highly concordance with $R2>0.9$. This study has demonstrated the feasibility of utilizing NGS to evaluate multiple mutation targets and validated the detection of ESR1 mutations in resistance to aromatase inhibitor from ctDNA for breast cancer patients.

▲TOP

J-2017

Patient-specific circulating tumor DNA monitoring using digital PCR in esophageal squamous cell cancer patients

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This study aimed to monitor therapeutic response of esophageal squamous cell carcinoma (ESCC) using circulating tumor DNA (ctDNA) by digital PCR (dPCR) with individual ESCC tumor-specific mutations. A mutation screening of primary tumors from 27 ESCC patients (Stage I/II/III/IV: 5/3/12/7) was performed by amplicon sequencing using the ESCC panel targeting 31 genes. First-line therapies included surgery, chemoradiotherapy, and chemotherapy. With the median follow-up was 459 days, 288 blood samples were examined for ctDNA. Among 45 mutations identified from primary tumors were analyzed in pre-treatment blood, 34 (83%) were detectable as a ctDNA. In 35 mutations from Stage II or higher cases, ctDNA was detectable in 97% (34/35). The Mutation allele frequency (MAF) of good responders could decrease to 0 after one cycle of chemotherapy, whereas the MAF of non-responders stayed high levels. A patient who had a recurrence after resection for stage II ESCC showed that ctDNA had been detected six months before the recurrence was noticeable by CT scan. The patient-specific ctDNA monitoring readily indicates the disease Stage and possibly facilitates an early recurrence detection of ESCC.

J-2018

ClinicoPathological Analysis of HSPA6 expression in esophageal squamous cell carcinoma

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[background]Heat shock proteins (HSPs) are induced by heat shock or other stressors. HSPs are known to play important roles in cancer development and metastasis. HSPA6, a member of 70 kDa HSPs, has shown controversial effects to cancers. HSPA6 high expression reported to be associated with recurrence of HCC, and to inhibit the abilities of migration and invasion in bladder cancer EJ cells. We investigated the expression of HSPA6 in esophageal squamous cell carcinoma (ESCC). [method]107 patients with ESCC who underwent curative surgery were evaluated. The expression of HSPA6 in surgical specimens was immunohistochemically assessed and used in the analysis of clinicopathological features and Disease-free survival (DFS). [result]80 patients reveal high expression. No correlation was found with clinicopathological features in all stage cancers. There is a significant relationship between high expression and better DFS in Stage I or II cases (n=64). Furthermore, multivariate analysis shows that HSPA6 expression is an independent prognostic factor for Stage I or II ESCC. [conclusion]The high expression of HSPA6 can be the novel bio-marker of the recurrence risk in Stage I or II of ESCC.

▲TOP

[J-2019] J15-2 [Japanese]
New biomarker (2)

2018 / 9 / 28 (Fri) 10:15-11:30 Room 8/10F 1008, Osaka International Convention Center Room 8

Shingo Dan / Div. Mol. Pharmacol, Cancer Chemother. Ctr. of JFCR

J-2019

Pilot study on the CTC cytology for colon, lung and breast cancer patients using a 3D metal filter-based platform

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Detection of CTC in the blood of cancer patients provides a potential diagnostic and prognostic marker and also source for liquid biopsy. To date, CTCs have been identified based on the immunofluorescence criteria; keratin+/EpCAM+/CD45-/DAPI+ under dark field. However, this approach provides insufficient morphological and cytological information on the CTCs. Recently, we developed a new cytology-based platform involving enrichment of CTCs from whole blood by filtration with 3D metal filter, followed by efficient transfer of CTCs from the filter to glass slides, immunocytochemistry and Papanicolaou staining of the CTC slides, and subsequent light microscopic observation. Using this platform, we carried out pilot study of the CTC from both peripheral blood and drainage vein blood of breast, colorectal and lung cancer patients. From the results obtained above studies, we proposed the cytological criteria for CTCs. This new cytology-based CTC platform could therefore be a useful tool for investigating the significance of CTCs, and as a liquid biopsy in clinical studies.

▲TOP

J-2020

C4BPA identified as a novel biomarker is expressed in the stroma of pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is known as the highest mortality disease. To improve the outcome, a highly sensitive and specific biomarker for early detection of PDAC is urgently required. Using tandem mass tag (TMT) labeling and LC-MS/MS, we performed comparative analyses of pre- and postoperative sera from PDAC patients and identified C4b-binding protein α-chain (C4BPA) as a potential biomarker. Serum C4BPA level was significantly higher in the preoperative PDAC patients ($p<0.008$), and the levels in patients with PDAC were significantly higher than those in healthy controls as well as in patients with pancreatitis and other malignancies including biliary tract cancers ($p<0.001$). The respective area under the receiver operator characteristics curve was 0.912 for C4BPA, 0.737 for CA19-9 in Stage I and II of PDAC. Among 245 pancreatic tumors including 165 PDAC samples, we evaluated serum C4BPA levels using our own established ELISA system. Furthermore, we found that C4BPA were mainly expressed in the stroma surrounding cancer cells in immunohistochemistry. Further study will elucidate the functional roles of C4BPA in PDAC initiation and progression.

J-2021

The expression level of miR-1246 in body fluids in pancreatic cancer patients

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[Introduction]Biomarker discovery is important to detect pancreatic cancer and evaluate its progress.Certain circulating microRNAs are also abundant in cancer patients. The miR-1246 has been reported to be associated with malignant tumors.The aim of this study was to evaluate whether expression of miR-1246 in body fluid such as blood, urine and saliva is an indicator of pancreatic cancer diagnosis.[Materials and Methods]The miR-1246 were collected from the plasma, urine and saliva samples of pancreatic cancer patients (n=5), and healthy subjects (n=13). The miRNA expression of body fluids was analyzed by qRT-PCR. [Results]The miR-1246 levels of plasma and urine were significantly elevated in pancreatic cancer patients as compared to healthy subjects. ($p=0.041$ and $p=0.047$, respectively)In addition, there was a correlation between miR-1246 expression level in serum and urine ($p=0.01$).There was no significant difference in miR-1246 level in saliva compared to healthy subjects and and there was no correlation with miR-1246 level in serum or urine.[Conclusion]In conclusion, the miR-1246 in serum and urine are potentially useful as a biomarker for pancreatic cancer.

J-2022

Haptoglobin phenotype is a critical factor for evaluating serum fucosylated haptoglobin as a cancer biomarker

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[Background and Aim]
We previously found that the serum level of fucosylated haptoglobin (Fuc-Hpt) was increased in pancreatic cancer patients with lectin-antibody ELISA. To develop more convenient detection method, we have recently generated 10-7G monoclonal antibody (mAb) specific for Fuc-Hpt. It is known that haptoglobin (Hpt) has three phenotypes. In this study, we investigated a relationship between Hpt phenotype and serum Fuc-Hpt levels in the above two methods.
[Methods]
Fuc-Hpt levels were assayed with AAL lectin or 10-7G mAb ELISAs, as reported previously. Haptoglobin phenotype was determined with Western blot analysis.
[Results]
Fuc-Hpt levels were much lower in Hpt1-1 phenotype, compared to Hpt2-1 and Hpt2-2 phenotypes both in healthy volunteers and pancreatic cancer patients. However, significant increase in Fuc-Hpt levels was observed in each phenotype. Area Under Curve value was slightly changed as a result from ROC analysis for diagnosis of pancreatic cancer.
[Conclusion]
Haptoglobin phenotype is a critical factor for evaluating Fuc-Hpt. Hpt1-1 phenotype should be excluded in the examination of Fuc-Hpt like Lewis negative people for CA 19-9 assay.

▲TOP

J-2023

Clinical significance of monitoring KRAS in tissue and plasma of pancreatic cancer patients

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Background: KRAS monitoring provides valuable information for early diagnosis and prediction of treatment outcome in colorectal cancer. In this study, we elucidated the clinical significance of KRAS monitoring in pancreatic cancer patients. Methods: KRAS in tumor tissues and plasma was analyzed for mutations by RASKET or droplet digital PCR. Results: KRAS mutation in tumor tissues was detected in 74 of 83 patients (89.2%). These 74 patients showed significantly poorer prognosis (MST; 32) than the 9 patients without mutation, whose MST were 193. Monitoring of KRAS in plasma revealed KRAS mutation in 35 of 88 patients (39.8%). In patients who underwent the chemotherapy (N=33), 2years OS of patients who detected KRAS mutation in plasma (N=23) was 16.4% and them which not detected it (N=10) was 53.3% (p=0.18). But in the curative resection group (N=45), 3years OS of patients who detected KRAS mutation in plasma (N=12) was 16.7% and them which not detected it (N=33) was 68.2% (p=0.00). Conclusions: KRAS mutation in tissue and plasma could be a valuable predictive and prognostic biomarker in pancreatic cancer patients.

J-2024

Practice of Genome Diagnosis in Pancreatic Tumor

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Background & Aim: Over 90% of PDACs harbor KRAS mutation, cause therapeutic difficulty. The aims of this study were to evaluate the KRAS mutation screening on codon 12/13 using ddPCR (KMS) in the diagnosis, and explore the possibility of launching precision medicine using NGS. Method: KMS was performed to tissue DNA from 66 FNA samples (PDAC: 40, NET: 10, mass-forming pancreatitis: 16) and ctDNA from 30 serum samples of PDAC. The NGS on Cancer Hotspot Panel V.2 was performed to 29 cases of PDAC. Results: In the tissue analysis, 38 cases were KMS positive (PDAC: 38/40, non-PDAC: 0/26, cutoff: 1.0%) and 7 cases of PDAC were pathologically diagnosed as non-PDAC. So the sensitivity of KMS and pathological diagnosis was 95.0% and 82.5%, respectively. In the ctDNA analysis, KRAS mutation were detected in 14 cases (liver or lung metastasis: 10/11, peritoneal metastasis: 2/9, locally advanced: 2/10). NGS analysis has revealed 27/29 cases harbored KRAS mutation, and those of which harbored mutation of TP53 in 13/27, CDKN2A in 4/27, and SMAD4 in 3/27. 1 case of KRAS wild PDAC harbored NRAS Q61R. Conclusion: KMS and NGS may be useful in PDAC diagnosis and launching precision medicine.

▲TOP

[LS19] LS19 [Japanese]
Cancer metabolic disorder diagnosed by photodynamic technology

2018 / 9 / 28 (Fri) 11:50-12:40 Room 8/10F 1008, Osaka International Convention Center Room 8
：SBI Pharmaceuticals Co.,Ltd

Kazuhiro Yoshida / Department of Surgical Oncology, Gifu University, Graduate School of Medicine

LS19

1) Utility of fluorescence cytology with aminolevulinic acid in diagnosis of pancreatic cancer

Tsukasa Ikeura
The Third Department of Internal Medicine, Kansai Medical University

No Abstract

▲TOP

LS19

2) Paradigm shift of the decision making using photodynaminc diagnosis in bladder cancer

Hideyasu Matsuyama
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No Abstract

▲TOP

[J-2067] J14-7 [Japanese]
Pancreatic cancer (4)

2018 / 9 / 28 (Fri) 13:00-14:15 Room 8/10F 1008, Osaka International Convention Center Room 8

Tomoo Kosuge / Sangenjaya Dai Ichi Hosp.

J-2067

Alteration of gene profiles in anchorage-dependent multicellular aggregates formed by PDAC cells

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Aggressive features in pancreatic ductal adenocarcinoma (PDAC) cells have been well studied, but those exhibited by collective cell behavior in PDAC cells are largely unknown. We found that, when CD44v3-10^{high}/CD44s^{low} PDAC cells were cocultured with HEK293T cells, they formed anchorage-dependent multicellular aggregates (Ad-MCAs) and acquired further intractable properties. The transcriptomes of PDAC cells forming Ad-MCAs were very rapidly and largely changed by coculture with HEK293T cells. To validate the clinical relevancy of the gene profile obtained from PDAC cells cocultured with HEK293T cells, we employed data sets from pancreatic cancer patients obtained from publicly available microarray data on NCBI Gene Expression Omnibus (GEO), and compared with genes downregulated by the coculture. Highly downregulated genes in PDAC cells forming Ad-MCAs by coculture with HEK293T cells were significantly upregulated in malignant lesions from pancreatic cancer patients. These results suggest that PDAC cells forming Ad-MCAs partially return to a normal tissue gene profile.

▲TOP

J-2068

Three-dimensional cancer tissue using patient-derived pancreatic cancer cells recapitulate cancer ecosystem

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Cancer ecosystem is composed not only of cancer cells but also various stromal cells such as mesenchymal cells and vascular endothelial cells. Patients with pancreatic cancers have poor prognoses and tumors become resistant to chemotherapeutic drugs. The tumor stromal microenvironment is thought to impact drug resistance and cancer cell behavior. Previously, we succeeded in generating iPSC derived liver tissue with stroma in vitro (Nature 2013, 2017).By applying this technology to cancer research, we established a novel stroma-rich pancreatic cancer organoid culture system that recapitulates multilineage cellular interactions between cancer, endothelial and mesenchymal cells. The stroma-rich cancer organoids exhibit anti-cancer drug resistance when compared to stroma-deficient organoids in vitro. Cancer xenograft derived from stroma-rich organoid reproduce the proportion of stroma observed in the pancreatic cancer tissue and exhibit drug resistance in in vivo xenograft model. Comprehensive transcriptome and proteome analysis identified several genes considered to be involved in cancer cell- stromal interaction and responsible for drug resistance observed in stroma-rich organoid.

J-2069

Usefulness of exosomeal microRNA-451a as a biomarker for recurrence and prognosis in pancreatic ductal adenocarcinoma

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Background:We investigated the predictive and prognostic value of plasma exosomal microRNA-451a (miR-451a) in patients with pancreatic ductal adenocarcinoma (PDAC). Methods:Microarray-based expression profiling of miRNAs derived from exosomes in the plasma of 6 PDAC patients with UICC stage Ⅱ was employed to identify a biomarker to distinguish between patients with and without recurrence. For validation analysis, plasma exosome samples of other 50 PDAC patients were measured by Taqman microRNA assays. Results:In the miRNA microarray analyses, miR-451a showed the highest up-regulation in the stage Ⅱ patients who showed recurrence after surgery. In the relationship to pathological factors, exosomal miR-451a showed a significant association with tumor size and stage. The overall survival (OS) and disease-free survival rates (DFS) of the high exosomal miR-451a patients were significantly worse than those of the low miR-451a patients. In Cox proportional hazards model analysis, exsomal miR-451a showed significance to OS and DFS. Conclusions:Plasma exosomal miR-451a is a useful biomarker for the prediction of recurrence and prognosis in PDAC patients.

J-2070

BM-derived cells recruited to the pancreas compose the tumor microenvironment and promote invasion of pancreatic cancer

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Cancer malignancy increase due to tumor-stroma interactions. In breast cancer, a subset of stromal cells was descended from bone marrow (BM)-derived cells. While BM-derived cells seem to be involved in remodeling of microenvironment and pancreatic cancer progression, this mechanism remains unknown. We aimed to investigate an association between pancreatic cancer progression and BM-derived cells.BM-derived GFP^+ cells were intravenously transplanted into KPC mice after sublethal irradiation. GFP^+ cells consisting of a few T cells, a few NK cells, or macrophages were engrafted in recipients' peripheral blood, BM, pancreas, and liver. In recipients' pancreas, GFP^+, $F4/80^+$, and $CD163^+$ cells were accumulated around ADM/PanIN and at invasive front, and a few GFP^+ α SMA^+ cells were detected. Invasive capacity of pancreatic cancer cells (PCCs) co-cultured with BM-derived macrophages significantly increased. Some BM-derived macrophages treated with PCCs supernatant expressed pancreatic stellate cell (PSC) marker. The present data suggest that BM-derived macrophages are involved in infiltration of PCCs, and also that some BM-derived macrophages transformed PSCs-like by interaction with PCCs.

▲TOP

J-2071

Thymidine Kinase-1 is potential target for tumor marker and therapy of pancreatic cancer

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Clinical outcome of pancreatic ductal adenocarcinoma (PDAC) has not been improved in the last three decades due to the lack of early detectable ideal tumor marker and effective molecular-targeted drugs. To identify diagnostic and therapeutic target for PDAC, we have performed genome-wide microarray analysis and found that Thymidine Kinase-1 (TK-1) was up-regulated in the vast majority of PDAC. TK-1 has already been reported as potential tumor marker in several cancers such as gastric, breast, prostate and liver cancer. However, there was no data about PDAC patients. In our study, overexpression of TK-1 m-RNA was confirmed by RT-PCR in pancreatic cancer cell line (4/5 cell line) and PDAC patients (11/12 cases). And also overexpression of TK-1 protein was confirmed by pancreatic cancer cell line (5/5 cell line) and immunohistochemical analysis in patients with PDAC (45%; 36/80 cases). Serum TK-1 was elevated in approximately 50% of cases of UICC Stage II PDAC patients (6/13 cases) using ELISA analysis. These results suggest that TK-1 could be a potential target for tumor marker and therapy for patients with PDAC.

J-2072

Antitumor effect of KR12, alkylating agent targeting KRAS mutation in Pancreatic cancer

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KRAS-activating mutations are found in over 90% of pancreatic ductal adenocarcinoma(PDAC). However, direct pharmacological targeting of activated KRAS protein has been unsuccessful for clinical use. We previously reported that an DNA-alkylating Pyrrole-Imidazole polyamide, KR12, which is designed to recognize KRAS G12D/V mutation, showed the antitumor effect in a spontaneous PDAC mouse model, PKF mouse (LSL-KrasG12d/+, Ptf 1aCre/+, Tgfbr2flox/flox). In this study, we have further investigated the antitumor effect of KR12 in human PDAC cells and xenograft models of transplantation of human PDAC cells. WST assays demonstrated that KP4 (G12D) and CAPAN-1(G12V) cells harboring KRAS mutation were sensitive to KR12 with IC50 of 2.1 and 3.6 nM, respectively. KP4 cells treated with KR12 showed reduced expression of KRAS at mRNA and protein levels. In the xenograft model using KP4 cells, KR12 treatment significantly suppressed tumor growth, consistent with PKF mouse model. FITC-labeled KR12 treatment demonstrated its localization in PKF tumor tissues 24 hours after injection. These data suggest that KR12 is a promising drug candidate for pancreatic cancer with KRAS G12D/V mutations.

▲TOP

[J-2073] J14-8 [Japanese]
Liver cancer

2018 / 9 / 28 (Fri) 14:15-15:30 Room 8/10F 1008, Osaka International Convention Center Room 8

Tomoharu Yoshizumi / Dept. Surg. & Sci., Kyushu Univ.

J-2073

Identification of novel regulators of telomerase reverse transcriptase expression in hepatocellular carcinoma

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Background. The promoter mutation in the telomerase reverse transcriptase (TERT) gene is the most common genetic alteration in hepatocellular carcinoma (HCC), suggesting that TERT upregulation is a critical event in hepatocarcinogenesis. Thus, regulators for TERT expression would be a promising target for HCC prevention and treatment. Here, we conducted a functional screening of TERT regulators to find novel therapeutic targets. **Methods.** A genome-wide short-hairpin RNA library was used for the screening. The activity of the TERT promoter was measured by a luciferase assay. **Results.** C15orf55 and C7orf43 were identified to regulate TERT expression, possibly via the SP1 axis and Hippo pathway, respectively. The expression of both genes was higher in tumor and its adjacent non-tumor tissues of HCC patients than in normal liver tissues with benign disease. Survival data from the TCGA database showed that high expression of these genes was related to poor long-term outcomes of HCC patients. **Conclusion.** C15orf55 and C7orf43 as well as their-related pathways deepen our understanding of the regulatory mechanism of TERT expression and open new avenues to prevent and combat HCC.

▲TOP

J-2074

Prognostic impact of Kinesin superfamily 15, an intracellular transport gene expression in HCC

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Background: Kinesin superfamily 15(KIF15) is a motor protein expressed during mitosis to maintain spindle apparatus. Recent study reported its important role in cancer cell proliferation in HCC.Material and method: We assessed clinical significance of KIF15 expression in HCC using public database (TCGA, GEO). The mRNA expression of KIF15 was measured in 59 surgically resected HCC in our hospital by RT-qPCR, and its localization was identified by immunohistochemical staining. Result: The high expression of KIF15 was significantly associated with poor prognosis in our samples and public datasets. The expression of KIF15 was significantly higher in the tumor tissues. KIF15 was mainly localized to inflammatory cells including lymphocytes round the cancer cells. Conclusion: High expression of KIF15 in lymphocytes could be a poor prognostic biomarker in HCC, suggesting KIF15-expressed lymphocytes established a microenvironment exacerbating cancer progression. We now investigate how KIF15-expressed lymphocytes accelerate HCC progression from the viewpoint of tumor-host interaction.

J-2075

Interleukin 33, released with hepatectomy, facilitated recurrent disease of cholangiocarcinoma

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Background and Purpose: Interleukin 33 (IL-33) has been shown to facilitate the development of cholangiocarcinoma (CCA) in a murine model, and IL-33, which binds to nuclear chromatin stably, is an alarmin released during tissue injury. Based on this information, we reported that the high expression of hepatic IL-33 was a risk factor for CCA recurrence following surgery and IL-33 was released during liver surgery for CCA. We postulated that anti-IL-33 antibody administration during hepatic surgery may inhibit CCA development. Methods: To examine the effects of the inhibition of released-IL-33 after hepatic resection, the syngraft transplantation with using murine CCA cell lines was performed with or without anti-IL-33 antibody administration. Results: The syngraft murine model of CCA showed aggressive tumor progression in the remnant liver after hepatic resection, whereas the blockage of IL-33 during hepatic surgery significantly inhibited the tumor progression. Conclusion: Perioperative anti-IL-33 antibody administration ameliorated CCA development in the remnant liver after hepatectomy.

J-2076

Expression of mucin 1 reflects the malignancy of hepatocellular carcinoma

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Mucin 1 (MUC1) is a glycoprotein and is expressed on the apical membrane of epithelial cells. In the liver, MUC1 is detected in cholangiocytes but not in hepatocytes. We performed immunohistochemical detection of MUC1 in resected specimens of hepatocellular carcinoma (HCC) using monoclonal antibodies against total MUC1 and glycosylated MUC1. As a result, MUC1 was detected in tumor cells in about half of HCC cases, while subcellular localization of MUC1 was heterogenous among MUC1-positive cases. MUC1 staining in the circumferential membrane or the cytoplasm of tumor cells significantly correlated with hepatitis B virus infection, high-level serum alpha-fetoprotein, and poorly differentiated histology. Moreover, patients showing such staining had shorter time to recurrence and unfavorable outcomes. These results suggest that the circumferential membrane/cytoplasmic localization of MUC1 is associated with the malignant progression of HCC.

▲TOP

J-2077

Modified ubenimex targets aminopeptidase N and exerts an antitumor effect in hepatocellular carcinoma

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Background: Ubenimex inhibits aminopeptidaseN (APN)/CD13 activity that acts as a scavenger in the survival of cancer stem cells of hepatocellular carcinoma (HCC) by reducing reactive oxygen species (ROS). But anti-tumor effect of ubenimex is too weak to clinical application, so we made modified ubenimex using the drug delivery system (DDS) and examined it compared with conventional ubenimex.Methods & Results: Comparison of the anti-tumor effect of improved ubenimex and conventional ubenimex in MTT assay revealed that at low concentration of 25 μg/ml, it showed a strong anti-tumor effect in improved ubenimex. Comparison of the enzyme activity after 4 hours of exposure to the modified ubenimex and the conventional ubenimex, it was found that the modified ubenimex significantly inhibited the enzyme activity than the untreated and the conventional ubenimex. In addition, the modified ubenimex increased ROS more strongly than the conventional ubenimex and induced apoptosis. In vivo experiments, the modified ubenimex significantly inhibited the tumor progression compared to the conventional ubenimex.Conclusions: In future, the modified ubenimex might be a novel therapeutic agents in HCC.

J-2078

Host genetic factors affecting NAFLD/NASH-related HCC in the Japanese population

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The SNP in PLPNA3 is the most famous and repeatedly validated genetic factor which confers susceptibility to nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). Fatty Acid Binding Protein 1 (FABP1) has also been considered as one of candidate genes of NAFLD/NASH susceptibility. There are many papers which investigated the functional role of FABP1 T94A in lipid metabolism.
In this study, we investigated the associations between some candidate single nucleotide polymorphisms (SNPs) for NAFLD/NASH and NAFLD/NASH-related hepatocellular carcinoma (HCC). We genotyped SNPs of PNPLA3, SAMM50, PARVB, GCKR, TM6SF2, and FABP1 by PCR-based Invader assay using 548 Japanese NAFLD/NASH patients.
As a results, SNPs of PNPLA3 and neighbor genes showed significant associations (Odds ratio [OR] = 2.8 ~ 3.4). The FABP1 SNP showed a marginal association (P = 0.054, OR = 1.70). After adjustment for age and gender by multiple logistic regression analysis, the association of FABP1 SNP became significant (P = 0.040).
In conclusion, our results suggest that the SNP rs2241883 which causes T94A substitution in FABP1 affect NASH/NAFLD-related HCC.

▲TOP

[E-2103] E14-12 [English]
Biliary tract cancer

2018 / 9 / 28 (Fri) 15:30-16:45 Room 8/10F 1008, Osaka International Convention Center Room 8

Shogo Kobayashi / Dept. Gastroenterol. Surg., Osaka Univ.

E-2103

Whole-Genome and Epigenomic Landscapes of Etiologically Distinct Subtypes of Cholangiocarcinoma

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Cholangiocarcinoma (CCA) is a hepatobiliary malignancy with wide geographical variation. We analysed 489 CCAs from 10 countries, combining whole-genome sequencing, targeted/exome sequencing, copy-number, gene expression, and DNA methylation. Integrative clustering defined four CCA clusters, revealing radically different molecular landscapes between Fluke-Positive and Fluke-Negative CCAs. Fluke-Positive CCAs, confined to Clusters 1 and 2, are enriched in *ERBB2* amplifications and *TP53* mutations. Conversely, Fluke-Negative CCAs (Clusters 3 and 4) exhibit high copy-number alterations and *PD-1*/*PD-L2* expression (Cluster 3), or epigenetic mutations (*IDH1/2*, *BAP1*) and *FGFR/PRKA*-related gene rearrangements (Cluster 4), with whole-genome analysis highlighting *FGFR2* 3'UTR deletion as an additional mechanism for *FGFR2* upregulation. Two clusters (1; Fluke-Positive and 4; Fluke-Negative) exhibit strikingly distinct patterns of DNA hypermethylation, targeting different genomic regions and mutation signature. Our results exemplify how genetics, epigenetics and environmental carcinogens may interplay across different geographical regions to generate distinct molecular subtypes of cancer.

▲TOP

E-2104

The importance of aromatase, an estrogen biosynthesis enzyme, in cholangiocarcinoma progression

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Aromatase is an estrogen biosynthesis enzyme which converts androgen to estrogen. Elevated serum estrogen level and high expressions of estrogen-related proteins are often found in patients with cholangiocarcinoma (CCA), a cancer of cholangiocyte cells. However, the expression and function of aromatase in CCA has never been explored. Our immunohistochemical analysis in CCA tissues (n=74) showed that aromatase was overexpressed in the cancer cells compared with the adjacent normal cells. High aromatase expression in CCA tissues was positive correlated with metastasis status of the patients. Moreover, CCA cell migration and proliferation activities was significantly reduced after aromatase gene silencing by siRNA. Additionally, aromatase inhibitors (exemestane and letrozole) sensitivities of CCA lines were related to the basal level of aromatase expression. Thus, aromatase promotes CCA progression and is associated with aggressive clinical outcomes via increase of cancer cell migration and proliferation activities. Therefore, aromatase can be a potential chemotherapeutic target for CCA treatment.

E-2105

Novel Murine Genetic Model of Cholangiocarcinoma

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Cholangiocarcinoma (CCA) is a lethal neoplasm originating from the biliary apparatus. Our aim was to generate a mouse model of CCA mimicking the human disease. IL-33 has been shown to be a biliary mitogen. Ectopic oncogene expression in the biliary tract was accomplished by the Sleeping Beauty transposon transfection system with transduction of AKT and YAP. Intrabiliary instillation of the transposon-transposase complex was coupled with lobar bile duct ligation in CL57/BL6 mice, followed by administration of IL-33 for three consecutive days. Tumors developed in 72% of the male mice receiving both oncogenes plus IL-33 by 10 weeks, but in only 20% of the male mice transduced with the oncogenes alone. Tumors expressed SOX9 and pancytokeratin but were negative for HepPar1. RNA profiling revealed substantive overlap with human CCA specimens, indicating that the novel murine model developed a murine CCA with morphological and biochemical features of the human disease. Moreover, 7 clones were established from the murine CCA, and the syngraft transplantation model using the cell lines were stably succeeded. Using this model and cell lines would be a great help for further experiments.

E-2106

Effect of xanthohumol in combination with praziquantel on oxidative stress-induced cholangiocarcinogenesis

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Chronic inflammation induced by liver fluke (Opisthorchis viverrini; Ov) infection contributed to oxidative stress that is the major risk factor for cholangiocarcinoma (CCA). The objective of this study is to investigate whether xanthohumol (XN), an anti-oxidant and anti-inflammatory natural compound, could inhibit Ov-induced CCA genesis. The effects of XN were examined in four groups of hamsters: group I, Ov and N-dinitrosomethylamine (NDMA) administration (ON); group II, Ov and NDMA administration and praziquantel (PZ) treatment (ONP); group III, similar to ON group but received XN (XON) and group IV, received XN plus PZ (XONP). The immunohistochemical analysis showed the increased expression of 8-oxodG (a DNA damage marker), CD44v8-10 (a cell surface in ROS defense system), transferrin receptor-1, and iron accumulation whereas expression of phosphop38^{MAPK} (a major ROS target) was decreased during CCA progression. The highest level of fibrosis was found on day 180 and all the markers were effectively reduced in the groups of XN with or without PZ. Our results suggest that XN plus PZ could efficiently prevent and provide potential chemopreventive benefits in Ov-induced CCA.

▲TOP

E-2107

Crizotinib as a new therapeutic approach for Cholangiocarcinoma

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Cholangiocarcinoma (CCA) is a notorious cancer of the bile duct with limited therapeutic options. CCA is highly prevalent in Thailand and the etiology is strongly associated with Opisthorchis viverrini infection. ALK, c-Met and ROS1 are receptor tyrosine kinases, the mutations and gene amplification of which are involved in tumorigenesis. Aberrant ALK, c-Met and ROS1 expressions have been documented in some CCA. Preliminary immunohistochemistry analysis of CCA tissue samples revealed high ALK and c-Met expression in CCA cells compared to the surrounding stroma. CCA cell lines, HuCCA-1, KKU-100, RBE and TFK-1 express c-Met as determined by Western-blot, and treatment with Crizotinib, a potent inhibitor of ALK, c-Met and ROS1, significantly reduced the cell viability. Moreover, Crizotinib was found to be effective in combination with standard chemotherapeutic agent Gemcitabine alone or Gem/Cis combination but not with Cisplatin alone. Further experiments will be performed to determine the mechanism of Crizotinib sensitivity in CCA cells and possible synergistic interaction with standard chemotherapy in CCA.

E-2108

ER stress-induced AGR2 expression enhances the metastasis of cholangiocarcinoma

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Anterior gradient 2 (AGR2) is an endoplasmic reticulum (ER) resident protein that responses during ER stress. Previous reported showed that, AGR2 was overexpressed in high metastatic cholangiocarcinoma CCA cells and promoted the metastatic phenotypes. In this study, we aimed to investigate the molecular mechanism of AGR2 induced metastasis of CCA. Tunicamycin was used for ER stress induction. The results showed that expression of ER stress response markers, BIP, PERK and CHOP, were significantly increased after tunicamycin treatment. Under this condition, AGR2 expression was increased by dose- and time-dependent manners. Furthermore, the EMT activity was enhanced by increasing of vimentin expression in the ER stress inducing KKU-213L5 cells and subsequently promoting cell migration. In contrast, these results were not occurred in the AGR2 stable knockdown cells. Therefore, it is suggesting that ER stress was an important event to induce AGR2 expression which is exert the enhancing of EMT activity leading to cell migration in CCA cell lines. Our data provide the understanding of AGR2 plays role in CCA metastasis and may offer a molecule target for CCA treatment.

▲TOP

[JWSA] JWSA [Japanese]
JCA Women Scientists Award

2018 / 9 / 28 (Fri) 8:00-8:30 Room 9/10F 1009, Osaka International Convention Center Room 9

Mari Kannagi / Dept. ImmunoTherap., Tokyo Med. & Dent Univ.

JWSA

Molecular pathological approach to epigenome mechanisms of multistage human carcinogenesis

Yae Kanai
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No Abstract

[IS6-1] IS6 [English]
Emerging roles of RUNX genes

2018 / 9 / 28 (Fri) 9:00-11:30 Room 9/10F 1009, Osaka International Convention Center Room 9

Kinuko Mitani / Dept. Hematology & Oncology, Dokkyo Med. Univ.、Suk-Chul Bae / Dept. Biochem., Chungbuk Natl. Univ.

IS6-1

Runx1 enhancer element, eR1, identifies tissue stem cells in multiple organs

Yoshiaki Ito
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Between two promoters of Runx1 gene, there are enhancer elements. One of them, which we call eR1, is 270 bp and specifically drives the expression of Runx1 in hematoipoietic stem cells. When eR1 was linked to heterologous promoter and GFP and transgenic mouse was made, GFP was expressed precisely in the cells that are known to have Runx1 expression when hematopoietic stem cells emerge from endothelial cells. Interestingly, this transgenic mouse also labeled adult tissue stem cells in multiple organs by GFP. We reported that eR1 targets rapidly multiplying stem cells in the isthmus of stomach (Gastroenterol, 2017). Subsequently, we found that these rapidly proliferating cells express a protein, we tentatively call Isthmus Factor, that drives rapid proliferation of stem cells. This Isthmus Factor is associated with GTP-bound form of Ras and mediates strong growth signal to downstream targets. Therefore isthmus stem cells are new type of stem cells, distinct from Lgr5+ stem cells that are Wnt regulated and present in the base of gland unit in the corpus as reserve stem cells (Leushacke et al, 2017). I will describe the properties of isthmus stem cells and Isthmus Factor.

▲TOP

IS6-2

Roles of RUNX1 in T-cell acute lymphoblastic leukemia: the core regulatory circuit and super-enhancer

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RUNX1 has long been recognized as a tumor suppressor in myeloid malignancies. This gene is also known as a critical regulator of hematopoiesis. The enhancer of RUNX1 gene (eR1) is active in normal hematopoietic stem cells (HSCs) and other tissue stem cells, implicating its roles in normal development. Interestingly, recent studies have suggested that RUNX1 serves as an oncogenic factor in the context of T-cell acute lymphoblastic leukemia (T-ALL). The ChIP-seq analysis demonstrated that the eR1 locus is highly activated as evidenced by the presence of a large super-enhancer. RUNX1 collaborates with the oncoprotein TAL1 and its regulatory partners in T-ALL cells by forming a positive auto-regulatory loop (core regulatory circuit). RUNX1 frequently co-occupies the regulatory elements with TAL1 and coordinately regulates their expressions in T-ALL cells, many of which are associated with super-enhancers. Importantly, RUNX1 protein is required for T-ALL cell growth, and other groups have also shown that a loss of Runx1 inhibits T-ALL development in murine models. Those studies provide a novel evidence showing the oncogenic roles of RUNX1 as a master transcription factor in T-ALL.

IS6-3

Runx3 defends against endogenous oncogenic K-Ras-induced lung tumorigenesis

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Early work showed that oncogenic Kras induced tumorigenesis is effectively suppressed by the p53 pathway in primary mouse embryonic fibroblasts (MEFs). However, the Kras LA strain, in which expression of Kras(G12D) can be spontaneously activated by random recombination, develops lung adenomas (ADs) and adenocarcinomas (ADCs). Furthermore, restoration of p53 activity in Kras(G12D) driven mouse lung cancer models results in regression of ADCs, but does not affect ADs that are likely to develop into ADCs. These results raise the fundamental question of whether mammals have an effective defense mechanisms against endogenous level of oncogene activation that is sufficient to induce tumors. In this study, we targeted Runx3, p53 and/or Kras in a very small number of cells. The results revealed that Kras(LSL G12D) and p53f/f;Kras(LSL G12D) mice were completely tumor free at 1 year after birth. However, all Runx3f/f;Kras(LSL G12D) mice rapidly developed severe lung adenocarcinoma within 85 days of birth. These observations demonstrate that mammals can effectively defend against an endogenous level of oncogenic Kras and the defense mechanism is abrogated by Runx3 inactivation.

IS6-4

RUNX3 is oncogenic in natural killer/T-cell lymphoma and is transcriptionally regulated by MYC

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RUNX3 is a master regulator of gene expression in major developmental pathways. It acts as a tumor suppressor in many cancers but is oncogenic in certain tumors. We observed upregulation of RUNX3 mRNA and protein expression in nasal-type extranodal natural killer (NK)/T-cell lymphoma (NKTL) patient samples and NKTL cell lines compared to normal NK cells. RUNX3 silenced NKTL cells showed increased apoptosis and reduced cell proliferation. Potential binding sites for MYC were identified in the RUNX3 enhancer region. Chromatin immunoprecipitation-quantitative PCR revealed binding activity between MYC and RUNX3. Co-transfection of the MYC expression vector with RUNX3 enhancer reporter plasmid resulted in activation of RUNX3 enhancer indicating that MYC positively regulates RUNX3 transcription in NKTL cell lines. Treatment with a small-molecule MYC inhibitor (JQ1) caused significant downregulation of MYC and RUNX3, leading to apoptosis in NKTL cells. The growth inhibition resulting from depletion of MYC by JQ1 was rescued by ectopic MYC expression. In summary, our study identified RUNX3 overexpression in NKTL with functional oncogenic properties.

▲TOP

IS6-5

Oncogenic Runx3 in osteosarcoma development

Kosei Ito
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Inactivation of p53 is frequently reported in sporadic osteosarcoma (OS) in human. In mouse, an osteoblast-specific p53-knockout line (p53f/f Sp7/Osx-Cre; "OS mouse") has the high incidence of OS, and the pathological characteristics closely resemble human OS. We found that RUNX3/Runx3 is markedly upregulated in human and mouse OS. Osteoblast-specific Runx3-knockout reduced OS development of the OS mice. We identified a series of potent oncogenes upregulated by Runx3 in OS. Genomic mutations in the RUNX consensus sites of the target gene introduced by CRISPR/Cas9 system abolished the aberrant gene-upregulation by Runx3 and tumorigenicity of OS cells in nude mice. The binding affinity of Runx3 to the Runx consensus sites was found higher than those of Runx1 and Runx2. Furthermore, p53 protein physically and specifically interacted with Runx3, and attenuated the Runx3-DNA binding. These results reveal a novel oncogenic Runx3 function indispensable for p53-deficient osteosarcomagenesis.

IS6-6

RUNX3 controls a metastatic switch in pancreas cancer

Sunil R. Hingorani
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Pancreatic ductal adenocarcinoma (PDA) is an extremely lethal disease with a high rate of metastasis. Median survival of patients with resected disease is less than two years, reflecting that even when diagnosed early and surgically removed, distant micrometastatic deposits eventually advance to kill patients. We have recently identified a role for the runt-related transcription factor RUNX3 in establishing a program for dissemination of pancreas cancer cells. RUNX3 expression is tightly constrained to immune cells in the normal pancreas, however, in genetically engineered mouse models (GEMM) of PDA and human specimens, RUNX3 expression can become activated in tumor epithelia after acquisition of cardinal genomic alterations. RUNX3 expression is linked to TP53 point mutation and loss of heterozygosity, and also exhibits biphasic dependence on levels of SMAD4 protein. Activated RUNX3 expression in PDA correlates with higher rates of metastasis, facilitated by RUNX3-mediated remodeling of the metastatic niche, and poor prognosis. Targeted deletion of Runx3 in GEMMs is being used to explore its influence on the balance between primary and metastatic disease burden in PDA.

▲TOP

[LS20] LS20 [Japanese]
Clinical question of the systemic chemotherapy for colorectal cancer

2018 / 9 / 28 (Fri) 11:50-12:40 Room 9/10F 1009, Osaka International Convention Center Room 9
：Eli Lilly Japan K.K.

Hideo Baba / Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University

LS20

Clinical question of the systemic chemotherapy for colorectal cancer

Tetsuya Hamaguchi
Department of Gastroenterological Medical Oncology, Saitama Medical University, International Medical Center

No Abstract

[IS8-1] IS8 [English]
Application of epidemiological knowledges into personalized medicine for cancer prevention in Asia

2018 / 9 / 28 (Fri) 13:00-15:30 Room 9/10F 1009, Osaka International Convention Center Room 9

Keitaro Matsuo / Div. Mol. & Clin. Epidemiology, Aichi Cancer Ctr. Res. Inst.、Youlin Qiao / Dept. Cancer Epidemiology, Cancer Inst. & Hosp., Chinese Academy of Med. Sci., Peking Union Med. Sci.

Long standing efforts on seeking on causes of cancer all over the world have enabled us to utilize evidence in cancer prevention. Evidences covering through environmental to genetic further enabled us to use them in personalized cancer prevention. This international session is organized to introduce research activities focusing on application of epidemiological knowledges into personalized cancer prevention from researchers from Asian countries.

IS8-1

Precise primary prevention of HPV infection and related cancer in China

Youlin Qiao
Cancer Institute

BACKGROUND:HPV-associated cancer is a complex disease involving different HPV genotypes infection followed by the activity of HPV E6/E7 oncogenes and environmental factors. After 10 years delay, 3 HPV prophylactical vaccines are in Chinese markets now. Which kind of vaccine is using for what population is still remaining questions.OBJECTIVE:To discuss HPV predominance genotype distribution and the precise primary prevention of HPV infection and related cancer in Chinese women.FINDINGS:HPV infected Women had 150 times higher risk for CIN2+ than HPV- with attributable risk of 99%. About 15% women infected oncogenic HPV and the unique double peak age distributions of HPV infection. HPV prevalence and type-distribution in invasive cervical cancer (ICC) and precancers in different geographical regions of China. HPV 16, 18, 31, 52 and 58 were identified as predominate HPV types in ICC, among which HPV16 and 18 accounted for 84.5% of the ICC without remarkable geographic variations. CONCLUSION:Precise prevention of cervical cancer by using HPV type specific vaccination and HPV DNA screening + E6 protein triage and move program from rural areas to cities and whole Chinese women.

▲TOP

IS8-2

Risk modeling of breast cancer and its application to personalized cancer prevention in Japan

Hidemi Ito
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Co-author：Keitaro Matsuo
Div. Cancer Epidemiology & Prevention, Aichi Cancer Ctr. Res. Inst.

More than 100 common breast cancer susceptibility loci have been identified in genome-wide association studies (GWAS) world-wide. In the post-GWAS era, the potential for practical application of GWAS-identified risk loci is widened in breast cancer prevention. To apply them to breast cancer prevention, we have developed a risk prediction model using 22 GWAS-identified risk loci among Japanese population. It would help identifying women at high risk of breast cancer in personalized prevention. In addition, based on our risk prediction model and combined with obesity, one of risk factor of breast cancer, we estimated the cumulative risk of developing breast cancer because risk communication by absolute risk might be more effective to promote preventive behavior. We are now ongoing a randomized controlled trial to evaluate whether genetic breast cancer risk feedback is effective for women promoting their prevention behavior of breast cancer. In this session, we will introduce our research activity for development of personalized cancer prevention.

IS8-3

Germline pathogenic variants of 11 hereditary breast cancer genes in Japanese

Yukihide Momozawa
Lab. for Genotyping Development, IMS, RIKEN

Pathogenic variants in highly penetrant genes are useful for the diagnosis, therapy, and surveillance for hereditary breast cancer. However, large-scale studies of breast cancer genes are needed to inform future testing and variant classification processes in non-European populations. We performed a case control association study for variants in coding regions of 11 hereditary breast cancer genes in 7,051 unselected breast cancer patients and 11,241 female controls of Japanese ancestry. Clinical significance of each germline variant was categorized according to the ACMG guidelines. Association of pathogenic variants with clinical manifestations was further examined. We identified 244 germline pathogenic variants, of which 131 were novel. Pathogenic variants were found in 5.7% of unselected breast cancer patients, ranging from 15% in women diagnosed younger than 40 years to 3.2% in patients older than 80 years. Patients with pathogenic variants in BRCA1/2 or PTEN had significantly younger age at diagnosis compared to patients who had no pathogenic variants in all 11 genes. These important data will guide genetic testing for breast cancer susceptibility genes in Asian populations.

IS8-4

Risk stratified screening and management for cervical cancer

Fanghui Zhao
Natl. Cancer Ctr., Cancer Hosp., Chinese Academy of Med. Sci. & Peking Union Med. College

Background: Several test modalities could be adopted by China for cervical screening due to the diversities in economics and availabilities of these technologies. Appropriate and consistent management of various screening positives is becoming a critical issue in China.
Methods: We evaluated the immediate risk of cervical cancer and precancer for different screening results through a pooled analysis of 17 cross-sectional population-based cervical cancer screening studies. The immediate absolute risks of CIN3+ for each independent test modality and for their combinations were calculated by using a Risk Stratification Model.
Results: The absolute risks of CIN3+ were 1.5% in ASC-US, 5.4% in LSIL, 37.4% in HSIL+, 10.1% in VIA Positives and 10.5% in HPV Positives. Adding HPV test to cytology, the risk of ASC-US, LSIL and HSIL+ among HPV Positives increased to 3.9%, 6.3% and 39.8%, respectively. A very low risk (0.01%) was estimated among the triple negatives, but largely due to HPV negative.
Conclusions: All tests have their values in the screening, which can be optimized as pragmatic strategies in different resource settings. CIN 3+ Risk for various test scenarios could serve as the basis of Cervical Screening and Management Guidelines among Chinese Population at this moment.

▲TOP

IS8-5

Genetic risk score to stratify high risk group for cancer in the population level

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Risk prediction models incorporate established environmental and behavioral risk factors to estimate individual risk, and numerous models have been developed for cancer. Recently, these models have additionally included biologic or genetic risk factors to access cancer risk more accurately. Among the various types of genetic variants, single-nucleotide polymorphisms (SNPs) that are prevalent in the general population may be useful in risk stratification to identify at-risk populations. The polygenic risk score (PRS) combines the effects of multiple SNPs that are associated with disease and prevalent in the general population, and its discrimination ability was estimated both alone or when used in combination with conventional risk prediction models. These prediction models for cancer mostly target breast cancer, prostate cancer, or colorectal cancer, which are prevalent in Western countries. However, the SNPs associated with cancer, especially breast cancer, in European populations were not well replicated in Asian population. Thus, investigating Asian-specific genetic markers, in corporation with them into PRS should be considered for benefit from more precise risk estimations.

IS8-6

Knowledge and Awareness of Early Detection Methods, Symptoms and Risk Factors towards Breast & Cervical Cancer

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In developing countries the global incidence of breast and cervical cancer is rising and the increase is occurring at a faster rate. A total of 1004 adult female students of age 18 to 26 years were randomly included in the study using a multistage cluster sampling technique. A questionnaire included socio-demographic characteristics and information related to their knowledge of breast and cervical cancer. Of the 61.3% respondents who were undergraduates, only 37.5% were aware of breast cancer risk, 25.7% of detection methods for breast cancer and only 21.1% of cervical cancer risk factors. About 87.4% of respondents did not have any family history of cancer, while 3.5% participants had a sister or mother with breast tumor and 22.1% with more than one close relative with cancer. Of the participants, 2.5% drank alcohol, 24.7% girls wore tight brassieres and 4.1% have benign breast diseases. Participants with better knowledge scores were 8.1% times more likely to practice breast self-exam while only 6.3% were examined at clinical breast examinations. A broad-based study on a larger population with sound methodology is required to determine the situation in Bangladesh.

▲TOP

[J-2079] J12-3 [Japanese]
Antitumor effector cells

2018 / 9 / 28 (Fri) 15:30-16:45 Room 9/10F 1009, Osaka International Convention Center Room 9

Kiyoshi Yoshimura / Showa Univ. Dev. of Immuno Oncology

J-2079

Bladder cancer-associated antigens-derived long peptides activate both CTLs and Th1-cells expressing converged TCRs

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We previously identified two novel tumor-associated antigens (TAAs), DEPDC1 and MPHOSPH1 frequently overexpressed in bladder cancer by using genome-wide cDNA microarray analyses. We also identified these TAAs-derived short peptides (SPs) that can activate tumor-reactive CTLs, and immunization of bladder cancer patients with these SPs activated specific CTLs *in vivo* to prolong OS of patients in the phase I/II clinical trial. In this study we identified these TAAs-derived long peptides (LPs) that can induce both Th1 cells restricted by common and promiscuous HLA class II molecules, and tumor-reactive CTLs specific to SPs included in LPs by cross-presentation in both human *in vitro* culture system and HLA-class I transgenic mice *in vivo*. The bulk Th1 cell lines generated by stimulation with those LPs *in vitro* responded to DC pulsed with TAA proteins suggesting that these LPs were naturally processed from TAA proteins and presented by DC. Furthermore these bulk Th1 cells expressed only one pair of TCR-α and -β genes. These TAA-LPs-specific Th1 cells were also observed in bladder cancer patients suggesting the usefulness of these TAA-SPs and -LPs for cancer immunotherapy.

▲TOP

J-2080

Promising use of gene-modified γδ T cells for Cancer Immunotherapy

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Adoptive transfer of gene-modified T cells such as CAR-T cells targeting CD19 molecule has already been recognized to be a potent immunotherapy for cancer patients. In most cases, αβ T cells have been used as the source of T cells for CAR-T therapy. In this study, we explored the possibility of γδ T cells as the source of T cells. We applied the newly synthesized bisphosphonate PTA (tetrakis-pivaloyloxymethyl 2- (thiazole-2-ylamino) ethylidene-1,1-bisphosphonate) instead of Zoledronate to stimulate and expand Vγ2Vδ2 T cells from peripheral bloods. Indeed, we observed that PTA efficiently expanded Vγ2Vδ2 T cells (>5,000-fold increase) with high purity (>98%) from a small amount of blood. In particular, we successfully transduced CAR genes to Vγ2Vδ2 T cells expanded by PTA in vitro. Furthermore, we confirmed these gene-modified Vγ2Vδ2 T cells were able to secrete a variety of cytokines and lyse tumor cells in an antigen-dependent manner. Considering that γδ T cells potently recognize and damage tumor cells independent of MHC expression, we envisage that Vγ2Vδ2 T cells could be a promising source of adoptive CAR-T therapies.

J-2081

Immunological Effect of hydrogen gas-Hydrogen gas restores exhausted CD8+ T cells to improve prognosis-

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In cancer patients, the affected microenvironment causes metabolic insufficiency of terminal $CD8^+$ T cells, characterized by persistent loss of mitochondrial function and mass, following progressive loss of PGC–1α, which become exhausted $CD8^+$ T cells expressing PD–1 molecule. Hydrogen gas (HG) is reported to activate PGC–1α, thereby potentially restoring the exhausted $CD8^+$ T cells and improving clinical prognosis. In the present study, HG reduced the number of terminal PD–1^+CD8^+ T cells and increased terminal PD–1^-CD8^+ T cells in the peripheral blood of 55 patients with stage Ⅳ colorectal carcinoma. Multivariate analyses demonstrated that the ratio of terminal PD–1^+CD8^+ T cells before and after HG (terminal PD–1^+ $CD8^+$ T cell ratio) was an independent predictor of shorter PFS and OS, while the high terminal PD–1^- $CD8^+$ T cell ratio corresponded to longer survival rates than the lower ratio. Further, the patients treated with nivolumab+HG showed a significantly longer OS than those treated with nivolumab only. These results suggest that HG restored terminal PD–1^+CD8^+ T cells possibly by enhancing mitochondrial function, resulting in improvement of the survival rates.

J-2082

Development of mRNA nano-carriers based on environment-responsive materials and for the application to cancer vaccines

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mRNA-based vaccines have been prospected as alternatives to conventional vaccine approaches because of effective protein expression and no risk of oncogenesis. We previously reported that lipid nanoparticles modified with KALA peptide, an α-helical pH-responsible cationic peptide, delivered encapsulated pDNA effectively to mouse bone-marrow derived dendritic cells (BMDCs) and significantly induce cytokine production. In this study, we design new mRNA carrier which is consisted for environment-sensitive materials with vitamin E scaffold, ssPalmE. As an inducer of cellular uptake to BMDCs, KALA peptide was further modified an application for DC-based cancer vaccine. As a result, transfection with mRNA using ssPalmE-KALA conferred the significant gene expression accompanied with induction of cytokine production. Furthermore, experiment using BMDCs transfected ovalbumin-coding mRNA by ssPalmE-KALA showed prophylactic anti-tumor effect against OVA-expressing tumor. In addition to the application to the DC-based vaccine, development of the directly injectable RNA vaccine is now ongoing, taking an advantage of immuno-stimulatory activity of mRNA-encapsulate ssPalmE particles.

▲TOP

J-2083

Anti-tumor activity of CAR-T cells targeting the intracellular oncoprotein WT1 can be enhanced by vaccination

Yasushi Akahori
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The Wilms tumor 1 (WT1) is an intracellular oncogenic transcription factor that is an attractive target for cancer immunotherapy because of its over-expression in a wide range of leukemias and solid tumors, and a low level of expression in normal adult tissues. In the present study, using a phage-display library, we have isolated a mAb specific to WT1235-243/HLA-A*2402 complex. Furthermore, we demonstrate that the therapeutic efficacy of T cells transduced with this scFv in a xenograft model, which is further enhanced by vaccination with DCs loaded with the corresponding antigen. This enhanced efficacy appeared to be mediated, at least partly, by the expansion and activation of CAR-T cells. CAR-T cells shown in the present study not only demonstrate the potential to expands the range of targets available to CAR-T cells, but also provide a proof-of-concept that efficacy of CAR-T cells targeting peptide/MHC can be boosted by vaccination.

J-2084

anti-tumor immune cell dynamics during immunotherapy with anti-PD-1 antibody and IL-18

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We previously found that the anti-tumor activity of immune checkpoint blockade (ICB) was augmented by IL-18 with the induction of pre-mNK cells. However, the detailed mechanisms are not clear. In this study, we examined the anti-tumor effector cell dynamics during immunotherapy with anti-PD-1 antibody (αPD-1) and IL-18. In a peritoneal dissemination model of CT-26, a combination treatment of mice with αPD-1 and IL-18 led to early accumulation of pre-mNK cells in the peritoneal cavity, which was followed by CD8+ T cell populations. These pre-mNK cells and T cells were found to express CXCR3. The combination treatment also induced the expression of CXCR3 ligands (CXCL9, 10) in PECs, and the $CD103^+$ $XCR1^+$ dendritic cells (cDC1) strongly expressed CXCL9. Of note is that the pre-mNK cells expressed an XCR1 ligand, XCL1, and that NK-depletion reduced recruitment of cDC1 in the peritoneal cavity. These observations together suggest that the combination treatment with αPD-1 and IL-18 elicits sequential immune cell mobilization which involves 1) pre-mNK cell-mediated mobilization of cDC1s via XCL1-XCR1 axis and 2) cDC1-mediated recruitment of effector T cells via CXCL9/10-CXCR3 axis.

▲TOP

[ML8] ML8 [Japanese]
Morning Lectures 8

2018 / 9 / 28 (Fri) 8:00-8:50 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Shinji Tanaka / Dept. Mol. Oncol., Tokyo Med. Dent. Univ.

ML8

The Single Cell Multiomics Dissects Cell Identity And Its Application To Oncology And Regenerative Medicine

Akira Watanabe
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Discussant：Yasuhito Terui
Div. Chemother., Cancer Chemother. Ctr., JFCR

Each of the billions of the cells in our body exhibits their identity with unique gene expression profile. Recent advances in single cell transcriptomics enable to conduct cell taxonomy identifying new cell types and to re-arrange cells in order of pseudo-time course describing differentiation status of each cell. Even though the cost is still high, the single cell transcriptome analysis now becomes one of the conventional assays. However, we and the other experts of single cell genomics experienced failures, due to cell viability, low RNA content or so on. And also the single cell RNA-seq requires specific pipelines for in silico analysis. We built up original workflow from sample preparation to data analysis, including single allele-level gene expression analysis and single cell DNA copy number analysis in combination with single cell whole genome analysis. We found that heterogenous allelic expression and DNA copy number by single cell analysis. I will talk about the success to describe how the undifferentiated cells like iPS cells differentiate with defined factors, and also provide tips to select the technologies of single cell genomics, for easy access.

▲TOP

[J-2025] J4-1 [Japanese]
Cancer related genes

2018 / 9 / 28 (Fri) 9:00-10:15 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Keishi Yamashita / Kitasato Univ., Sch. Med.

J-2025

Transcriptional coactivator TAZ negatively regulates tumor-suppressor p53 activity and cellular senescence

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Co-author：Hidetoshi Hayashi
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Transcriptional coactivator with PDZ-binding motif (TAZ) is one of the mammalian orthologs of Drosophila Yorkie, which is the transcriptional coactivator of Hippo pathway. TAZ is suggested to act as a regulator that modulates the expressions of cell proliferations and anti-apoptotic genes, to stimulate cell proliferation. Interestingly, TAZ is reported to associate with the poor prognosis in breast cancer. However, the physiological roles of TAZ in the process of tumorigenesis have not been clarified. In this study, we report that TAZ negatively regulates the activity of tumor suppressor p53. We found that overexpression of TAZ resulted in the down-regulation of p53 transcriptional activity and its downstream genes expression. Conversely, knockdown of TAZ upregulated the p21 expression induced by p53 activation. Simvastatin, which inhibits the nuclear localization of YAP/TAZ, induced hyperactivation of p53. Moreover, TAZ knockdown induced cellular senescence in a p53-dependent manner. These results suggest that TAZ negatively regulates tumor-suppressor fucntions of p53 and attenuated p53-mediated cellular senescence.

▲TOP

J-2026

Identification of Tumor Suppressor RBM4a as a Repressor of Cancer-Specific Mature mRNA Re-splicing

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Pre-mRNA splicing is a precisely regulated process, which is essential for biological functions. Dysregulation of pre-mRNA splicing potentially produce deleterious proteins, and often ends up serious diseases and cancers. The human TSG101 Δ 190-1090 (TSG101 Δ) mRNA is a well-documented major aberrantly spliced product detected in various cancers. We previously demonstrated that TSG101 Δ mRNA is generated by re-splicing of mature TSG101 mRNA.The mRNA re-splicing in various cancer cells implies an important mechanism that prevents deleterious extra re-splicing in normal cells. The control of the re-splicing could be promoted by unknown activator upregulated and/or repressor downregulated in cancer cells. We screened siRNA library (including 156 kinds of human nuclear proteins), and identified RBM4a as a mRNA re-splicing repressor. RBM4a, downregulated in cancers, is known as a tumor suppressor via controlling apoptosis, proliferation and migration. We are currently investigating whether the loss of RBM4a leads to mRNA re-splicing of TSG101. We postulate that global prevention of aberrant mRNA re-splicing by key factors, including RBM4a, is critical for the consequent tumor suppression.

J-2027

Tumor-suppressive effect of LRIG1 in non-small cell lung cancer harboring mutant EGFR

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Mutations of the EGFR gene are commonly found as oncogenic driver mutations in non-small cell lung cancer (NSCLC). Leucine-rich repeat and immunoglobulin-like domain protein-1 (LRIG1) is a cell-surface protein that is known as a negative regulator of the ErbB family (EGFR, HER2-4). In this study, we first confirmed that the expression levels of LRIG1 were much lower in NSCLC than in non-malignant cells or tissues. Next, we established clones stably overexpressing LRIG1, using EGFR-mutant cells. Transfection of LRIG1 was associated with a decrease in the expression and phosphorylation levels of EGFR in the EGFR-mutant cells. It was also associated with strong suppression of the cell proliferative, invasive, migratory and tumorigenic potential of the EGFR-mutant cells. In addition, LRIG1 also downregulated the expression and phosphorylation levels of other tyrosine kinase receptors, such as HER2, HER3, MET and IGF-1R, and prevented the epithelial-to-mesenchymal transition induced by TGF- β . These findings suggest that LRIG1 exerts important tumor-suppressive effects in EGFR-mutant NSCLC, and has the potential to become a novel therapeutic target for EGFR-mutant NSCLC.

J-2028

DYRK2 contributes to the tumor cell proliferation through CDK14 in breast cancer cells

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Tumor progression is the main cause of death in patients with breast cancer. Accumulating evidence suggests that dual-specificity tyrosine-regulated kinase 2 (DYRK2) functions as a tumor suppressor by regulating cell survival, differentiation, proliferation and apoptosis. However, little is known about the mechanisms of transcriptional regulation by DYRK2 in cancer progression, particularly with respect to cancer proliferation. Here, using a comprehensive expression profiling approach, we show that cyclin-dependent kinase 14 (CDK14) is a target of DYRK2.
We established stable DYRK2-depleted cells and both stable DYRK2- and CDK14-depleted cells. The depleted cells were compared with the control cells by various assays.
We found that reduced DYRK2 expression increases CDK14 expression, which promotes cancer cell proliferation in vitro, in addition to tumorigenicity in vivo.
We further identified androgen receptor (AR) as a candidate of DYRK2-dependent transcription factors regulating CDK14.
Taken together, our findings suggest a mechanism for cancer cell proliferation through the DYRK2-AR-CDK14 axis.

▲TOP

J-2029

UCHL1 has prognostic relevance and is a therapeutic target in high-grade neuroendocrine lung cancers

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Co-author：Tatuso Ohira、Norihiko lkeda
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We found that ubiquitin carboxy-terminal hydrolase L1 (UCHL1) was widely shown in mesenchymal NSCLC and was responsible for promoting metastasis. Little is known about the biologic role of UCHL1 and its therapeutic potential in high-grade neuroendocrine lung cancers (HGNEC). Here we show preclinical efficacy by targeting UCHL1 relevant to prognosis in HGNEC. We assessed the protein levels of UCHL1 in lung cancer cell lines, additive chemotherapeutic effects by using the combination of cisplatin/etoposide (PE) or cisplatin/irinotecan (PI) with UCHL1 inhibitors, prognostic significance of the immunostaining of UCHL1 in HGNEC patients, and the detectability of exosomal UCHL1 mRNA in patients. UCHL1 was overexpressed in all the SCLC cell lines and combined the target of UCHL1 by the selective inhibitors improved the response of PE/PI therapies. Immunohistochemistry of 72 HGNEC patient's samples demonstrated that UCHL1 expression correlated with prognosis. Exosomes-derived UCHL1 mRNA level was increased in serum from SCLC patients while no expression was observed in that from adenocarcinoma patients. UCHL1 can be a potential prognostic marker and a new therapeutic target in HGNEC.

J-2030

Arginine methylation of HSP90A by protein arginine methyltransferase PRMT5 promotes development of adult T-cell leukemia

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Adult T-cell leukemia (ATL) is an aggressive type of T-cell malignancy caused by HTLV-1 infection. We previously reported that loss of N-myc downstream-regulated gene 2 (NDRG2) plays an important role in the development of ATL and other cancers through the aberrant activation of signalling pathways. In addition, we identified NDRG2 as a novel tumor suppressor to dephosphorylate PTEN through the recruitment of PP2A, resulting in the suppression of PI3K/AKT and NF-κB signalling pathways (Nat Commun 2014, Cancer Res 2017). Here, we identified protein arginine methyltransferase 5 (PRMT5) as a NDRG2/PP2A binding partner that inhibits the phosphorylation of PRMT5 at S335. High-phosphorylated PRMT5 through loss of NDRG2 was localized in cytoplasm and methylated arginine residues of HSP90A (R345 and R386) for the maintenance of oncogenic client proteins. Since knockdown of PRMT5 expression or treatment with PRMT5 inhibitor induced apoptosis and suppressed tumorigenesis through the degradation of client proteins with loss of HSP90A arginine methylation, interfering with PRMT5 becomes a feasible and effective strategy in NDRG2-deficient ATL through the inhibition of HSP90A function.

▲TOP

[J-2031] J4-2 [Japanese]
Oncogenes and tumor-suppressor genes (1)

2018 / 9 / 28 (Fri) 10:15-11:30 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Issei Imoto / Risk Assessment Ctr., Aichi Cancer Ctr. Hosp.

J-2031

Introduction of mutant HRAS and Myc into p53-deficient hepatocytes induces combined hepatocellular-cholangiocarcinoma

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The cellular origin of combined hepatocellular-cholangiocarcinoma (cHCC-CC) has been a subject of debate. Although p53 is often inactivated in liver cancers, its role in the determination of tumor phenotypes has not yet been elucidated. Here, we examined whether the phenotype of HRAS- or HRAS/Myc-induced tumors might be affected by hepatocyte-specific p53 knockout (KO). To generate hepatocyte-specific homozygous p53-KO, p53 (fl/fl) mice were infected with Cre-expressing AAV8. Liver tumors were induced by hydrodynamic tail vein injection of HRAS and Myc-expressing transposon cassette plasmids, along with a transposase-expressing vector, into hepatocytes. In contrast to hepatocellular carcinoma induced by HRAS or HRAS/Myc in wild type mice, the tumors induced in p53-KO mice demonstrated partial bile duct differentiation, reminiscent of cHCC-CC. HRAS/Myc-induced tumors in p53-KO also expressed various hepatoblast markers, such as IGF2, H19, AFP, and Dlk1, and more aggressive. The dedifferentiating effect of p53-KO was associated with the augmentation of Myc expression. Our results suggest that cHCC-CC with the dedifferentiated phenotype can be originated from adult hepatocytes.

▲TOP

J-2032

A broken tumor suppressor RNF43 is repaired by phosphorylation

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Dysregulation in the Wnt pathway leads to various types of cancer. RNF43 and ZNRF3 promote the degradation of Frizzled. Both RNF43 and ZNRF3 function as tumour suppressors and are frequently mutated in many cancer types, which is indicative of their importance in tumourigenesis.
Here we show that the function of RNF43 is completely dependent on the phosphorylation of multiple serines, also conserved in ZNRF3. Blocking CK1-mediated phosphorylation abolished RNF43 function whilst a phospho-mimetic mutant retains its full activity, showing an importance of the phospho-regulation in Wnt signaling. Three naturally occurring cancer-associated mutations were found in four serines to ablate the phosphorylation sites and so abolish RNF43 activity on Wnt signalling. Conversely, an oncogenic RNF43 mutant reverts to a functional tumour suppressor with the phospho-mimetic mutation and strongly suppresses Wnt-Ras cooperation-induced tumourigenesis. Phosphorylation of RNF43 is therefore an essential switch to activate the function as a tumor suppressor. Our findings provide a novel mechanistic insight for tumourigenesis and a target for the cancer therapy.

J-2033

Chromatin remodeling by the Ewing sarcoma fusion protein EWS-FLI1

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Dev. Carcinogenesis, Cancer Inst., JFCR

Co-author：Miwa Tanaka、Yukari Yamazaki、Mizuki Homme、Takuro Nakamura
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Ewing sarcoma (ES) is a highly aggressive bones or soft tissue neoplasm with small round cell morophology affecting child and young adults. Though the incidence of ES is different among the human races, its molecular basis remains unclear. EWS-FLI1(EF) plays a key role in development and progression of ES. It is reported that EF preferentially binds to GGAA microsatellites (GM) resulting in dynamic chromatin remodeling and upregulation of target genes. The length of GM is highly polymorphic and it is suggested that there might be correlation between ES incidence and GM polymorphism. We have developed an ex vivo mouse model for ES by introducing EF into embryonic chondrogenic progenitors (eSZ cells) of Balb/c background. Recipient mice invariably develop ES whereas no tumor has developed when eSZ cells of C57BL/6 background were used. ChIP-seq analysis revealed that association of EF and GM in our mouse model, and GM lengths of EF binding loci are different between Balb/c and C57BL/6. Although genomic distribution of GM is not conserved between human and mouse, the data suggest that racial and strain differences of GM polymorphism might be crucial for EF function and ES development.

J-2034

MOZ is critical for leukemic cell proliferation and immortalization through repression of p16Ink4a gene

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Monocytic leukemia Zinc finger protein (MOZ), a histone acetyltransferase, is involved in chromosome translocations associated with acute myeloid leukemia (AML). To explore the roles of MOZ in AML development, we introduced AML-associated various fusion genes or Myc oncogenes into MOZ deficient hematopoietic/stem progenitor cells (HSPCs), and then performed colony replating assays to evaluate proliferation and immortalization ability. As a result, deletion of MOZ reduced proliferation and colony forming activity in cell expressing fusion genes. Especially in cells expressing c-Myc or N-Myc, immortalization ability was completely lost. Gene expression analysis revealed that expression of p16Ink4a tumor suppressor gene were significantly elevated in MOZ deficient leukemic cells. Expression of p19Arf and p15Ink4b were also increased in MOZ deficient ones. Deficiency of p16Ink4a promoted the proliferation of MOZ deficient Myc-leukemic cells. These results suggest that MOZ is important for leukemic cell proliferation and immortalization to suppress p16Ink4a gene expression.

▲TOP

J-2035

Induction of macropinocytic cell death by oncogenic RAS in human epithelial cells

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Oncogenic mutations of RAS genes, found in about 30% of human cancers, are considered to play important roles in cancer development. However, in some cell lines, oncogenic RAS has been reported to induce non-apoptotic cell death. Here, we investigated effects of oncogenic RAS expression in several types of normal human epithelial cells. Oncogenic RAS but not wild-type RAS stimulated macropinocytosis with accumulation of large phase-lucent vacuoles in the cytoplasm, subsequently leading to cell death. Overexpression of MYC attenuated oncogenic RAS-induced such accumulation, cell cycle arrest and cell death. MYC suppression in some cancer cell lines harboring oncogenic mutations in RAS genes induced cell death with accumulation of macropinosomes. These results suggest that macropinocytic cell death is a tumor suppressing mechanism acting against oncogenic RAS mutations in normal human epithelial cells, which can be overcome by MYC overexpression, raising the possibility that its induction might be a novel approach to treatment of RAS mutated human cancers.

J-2036

Significance of additional genetic hit(s) in MLL-AF4 fusion-positive acute lymphoblastic leukemia

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MLL-AF4 is characteristically and commonly observed fusion in infant acute lymphoblastic leukemia. As genetic data from patients' samples and animal models showed conflicting results, the necessity of 2nd hit mutations in leukemogenesis with MLL-AF4 is still under debate.The MLL-AF4 fusion gene was introduced into mouse ES cells to obtain ES cell clones with stable expression of MLL-AF4. These ES cells were conditioned to differentiate into hematopoietic lineages and retroviral insertional mutagenesis was used to introduce additional genetic defect(s) as candidates for tumor-promoting 2nd hit mutations.ES cells with MLL-AF4 expression showed skewed differentiation toward non-hematopoietic lineage. Additional genetic change in Tie2-positive cells accelerated tumorigenesis of MLL-AF4 expressing cells, causing hematopoietic malignancies in injected immunodeficient mice. Viral integration often caused overexpression of gene(s) located near the integration site.Clonal expansion of MLL-AF4-positive cells after introduction of retroviral insertional mutagenesis suggests the necessity of additional genetic change(s) that activate certain target gene(s) in leukemogenesis with MLL-AF4.

▲TOP

[LS21] LS21 [English]
Clinical Utility and Outcome of Guardant360 in Cancer Patients in Asia

2018 / 9 / 28 (Fri) 11:50-12:40 Room 10/11F 1101+1102, Osaka International Convention Center Room 10
：Guardant Health Japan K. K.

Hisahiro Matsubara / Chiba University Graduate School of Medicine, Department of Frontier Surgery

LS21

Clinical Utility and Outcome of Guardant360 in Cancer Patients in Asia

Herbert H F Loong
The Chinese Univresity of Hong Kong

No Abstract

[J-2085] J14-9 [Japanese]
genetic abnormalities of hematological malignancies

2018 / 9 / 28 (Fri) 13:00-14:15 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Akifumi Takaori-Kondo / Dept. Hematol. /Oncol., Grad. Sch. of Med., Kyoto Univ.

J-2085

The molecular mechanism of cytokine receptor activation by mutant molecular chaperon

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In a subset of patients with myeloproliferative neoplasms, frameshift mutations on the gene called calreticulin (CALR) that encodes an endoplasmic reticulum-localized molecular chaperone were found. We have previously shown that mutant CALR preferentially binds and activates thrombopoietin receptor MPL, and thus transforms cells. However, the molecular mechanism how mutant CALR activates MPL was not clearly understood. Here, we have found that mutant but not wild-type CALR forms a homomultimeric complex, and demonstrated that the interaction between mutant CALR is required for the binding and activation of MPL. This study proposed a novel molecular mechanism for the cellular transformation in which a homomultimer of mutant molecular chaperone simultaneously interacts with two molecules of type-I cytokine receptor that forms a homodimer for the activation.

▲TOP

J-2086

Aberrant Histone Acetylation by HBO1-fusion Generates Clinically Relevant CMML Pathogenesis

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Chronic myelomonocytic leukemia (CMML) is an incurable hematopoietic malignancy. While many mutations have been identified, the mechanisms of how these mutations cause CMML remain unclear. HBO1 is a histone acetyltransferases (HAT) belonging to MYST family. Recently, we identified a new NUP98-HBO1 fusion containing intact MYST domain in a patient with CMML. Overexpression of NUP98-HBO1 in hematopoietic cells recapitulated CMML phenotypes in mice. Gene expression array analysis revealed that NUP98-HBO1 regulates CMML-specific gene signature, including HOXA9, through aberrant histone acetylation. Genetic inhibition of the HAT activity blocked NUP98-HBO1-mediated leukemogenesis in human cells and mice.Given that NUP98-HBO1 generates phenotypically and genetically relevant CMML pathogenesis, our model will be a useful preclinical tool for dissecting the CMML pathogenesis and testing therapeutic options.

J-2087

Molecular profiling of blastic transformation in chronic myeloid leukemia

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A proportion of chronic phase (CP) chronic myeloid leukemia (CML) patients fail to respond to tyrosine kinase inhibitor (TKI) therapies and progress to blast crisis (BC). However, molecular mechanisms of blastic transformation in CML are poorly understood. Here, we performed whole-exome sequencing of 53 CML-BC samples as well as corresponding CP controls to investigate acquired mutations during disease progression from CP to BC. We also performed targeted-capture sequencing of driver genes in an additional 15 CML-BC samples. Mutations frequently involved known driver genes, such as ASXL1, RUNX1, ABL1, TP53, BCOR/BCORL1, and WT1. In addition, we identified novel targets of recurrent mutations, including UBE2A, NBEAL2 and KLC2. Copy number abnormalities (CNAs) were newly acquired in 31 (58%) cases with CML-BC, 18 of which had abnormalities mimicking complex karyotype. When mutations and CNAs were combined, most BC cases had at least one driver alteration, which might be involved in blastic transformation of CML. Because few or no driver mutations were detected in CP, our results highlight a role of additional driver events during the clonal evolution to BC.

J-2088

Functional analysis of DDX41 germline and somatic mutations in myeloid neoplasms

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DDX41 is a newly identified leukemia predisposition gene encoding an RNA helicase, whose germline mutations are tightly associated with late-onset myeloid malignancies. In typical cases, a germline loss-of-function allele is compounded by a somatic missense mutation affecting the helicase domain in the remaining allele (p.R525H). To clarify the role of these DDX41 alleles, we generated CRISPR/Cas9-mediated Ddx41 heterozygous knock-out mice, as well as mice carrying the conditional knock-out and/or R525H missense allele. Homozygous deletion of Ddx41 in hematopoietic systems resulted in embryonic lethality, suggesting that Ddx41 is indispensable for normal hematopoiesis. While the recipient mice transplanted with Ddx41 R525H bone marrow (BM) cells showed significant and progressive leukopenia in noncompetitive transplantation experiments, Ddx41 R525H-mutated BM cells exhibited no significant changes in competitive BM reconstitution compared to controls. Our results suggest that Ddx41 is required for normal hematopoiesis, whereas somatic hotspot Ddx41 R525H mutation by itself is not sufficient for the development of myeloid malignancies.

▲TOP

J-2089

Highly prevalence of the Ph-like signature in acute lymphoblastic leukemia in children with Down syndrome

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Background
Children with Down syndrome (DS) are predisposed to develop acute lymphoblastic leukemia (ALL). DS-ALL displays unique genetic alterations; however, molecular basis of DS-ALL is still incomplete.

Methods
We applied whole transcriptome sequencing to 25 DS-ALL and 123 non-DS-ALL samples. We also performed mutational analysis of JAK2 and RAS pathway genes and copy number analysis n 25 DS-ALL samples.

Results
Our hierarchical clustering, using the previously reported gene set, revealed seven DS-ALL samples had the Ph-like signature. All DS-ALL samples with JAK2 mutations and CRLF2 fusions had the Ph-like signature in expression analysis, which was similar to non-DS-ALL. Using genes set with a significantly high expression in already detected Ph-like samples revealed two additional DS-ALL samples with a similar gene expression profile. In total, nine out of 25 DS-ALL samples had the Ph-like signature.

Conclusion
Our integrated genetic analysis disclosed that unlike non DS-ALL, Ph-like signature was relatively common feature in DS-ALL. Molecular targeting agents may be also promising for treatment of DS-ALL with Ph-like signature.

J-2090

Investigation of myeloid differentiation suppression effect on normal hematopoietic cells in low risk MDS

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In low risk myelodysplastic syndromes (MDS), cytopenia is frequent even though bone marrow is not filled with highly proliferating blasts, and especially neutropenia is often a fatal problem. In this study, we analyzed the effect of MDS cells on normal hematopoiesis using mice models. We used c-kit+ Sca1+ lineage- (KSL) cells from Nup98-Hoxd13 (NHD) transgenic (Tg) mice and immortalized wild type (WT) KSL cells with retroviral transduction of NHD (NHD cells) as a disease model. In competitive transplantation assay, normal cells transplanted with NHD Tg cells showed highly suppressed myeloid differentiation. Normal KSL cells cocultured with NHD cells in vitro also showed impaired myeloid differentiation, while coculture under noncontact condition didn't show myeloid differentiation arrest. To investigate the molecular basis, we extracted candidate genes using gene expression datasets from our in vivo analysis and public database. We silenced these genes in NHD cells and evaluated whether myeloid differentiation arrest was mitigated. Our study suggested the previously unnoticed role of MDS cells in suppressing myeloid differentiation of normal cells possibly through direct contact.

▲TOP

[J-2091] J14-10 [Japanese]
Hematological malignancies: pathogenesis, treatment and drug resistance

2018 / 9 / 28 (Fri) 14:15-15:30 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Masashi Sanada / Dept. Advanced Diagnosis, Clin. Res. Ctr., Nagoya Med. Ctr.

J-2091

HDAC and AKT inhibitors enhance anti-myeloma effects of daratumumab

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Daratumumab (DARA), an anti-CD38 monoclonal antibody, is a promising agent, showing high activity in relapsed/refractory multiple myeloma. However, some patients become resistant to DARA, partly due to reduced CD38 expression. It was shown that panobinostat, a pan-HDAC inhibitor, upregulates CD38 and ADCC. In addition, increased expression of NKG2D ligand promotes ADCC. CD55 and CD59 are thought to weaken the complement activation and suppress CDC. We examined whether HDAC inhibitors, including panobinostat, romidepsin, ACY-1215, and ACY-241, could modify the expression of these antigens. Although CD38 increase induced by HDAC inhibitors was slight, HDAC inhibitors strongly upregulated NKG2D ligand epigenetically. On the other hand, CD55 and CD59 were downregulated. As a result, enhancement of ADCC and CDC by the HDAC inhibitors was observed. Furthermore, afuresertib, an AKT inhibitor, downregulated CD55 and CD59 in MM cells and activated CDC. In addition, AKT inhibitors, combined with ACY-1215 or ACY-241, showed higher cytotoxicity than that of each single agent in a proliferation assay. These results suggest the possible usefulness of addition of HDAC and AKT inhibitors to DARA.

▲TOP

J-2092

Nationwide survey of chemotherapy for CAEBV in Japan

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Chronic active Epstein-Barr virus infection (CAEBV) is classified into T- or NK-cell neoplasms in the new WHO classification revised in 2017. We performed a nationwide survey to clarify the current state of treatment in Japan and effects of chemotherapy for CAEBV. We defined complete response (CR) as the disappearance of all disease activity and defined viral CR as CR with a disappearance of EBV DNA in the peripheral blood. One hundred cases aged 1-75 years (median, 21 years) were evaluated. The main chemotherapies employed were a combination of cyclosporine A, steroids, and etoposide (cooling therapy), 36 cases and CHOP, 23 cases. The rate of CR was as follows: cooling therapy, 56% and CHOP, 39%. Viral CR was not observed. Three-year overall survival was 0% in the patients treated with only chemotherapy (n=20) and 69% (95% confidence interval, 54%-79%) in the patients who received allogeneic hematopoietic stem cell transplantation (n=59). In conclusion, chemotherapy is currently insufficient to resolve the disease activity and eradicate the infected cells in CAEBV. The development of an effective treatment is an urgent issue.

J-2093

Development of the treatment for an aggressive subgroup of DLBCL: results of the primary analysis in a phase Ⅱ study

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DLBCL is a heterogeneous group of lymphomas. CD5+ DLBCL is characterized by short survival and frequent CNS relapse. To explore a more effective treatment, we are conducting a phase Ⅱ study of DA-EPOCH-R/HD-MTX in patients (pts) with newly diagnosed stage Ⅱ-Ⅳ CD5+ DLBCL. The primary endpoint was 2-year (yr) PFS. From Aug 2012 to Nov 2015, 47 pts were enrolled in the study and exhibited the following features: median age, 62 yrs; stage Ⅲ/Ⅳ, 53%; and IPI HI/H, 47%. In analysis of cell-of-origin of DLBCL by means of NanoString analysis system (n = 46), 39 pts (85%) had ABC type DLBCL. The median follow-up time was 3.1 yrs (range, 2.0-4.9). The 2-yr PFS was 79% (95% CI, 64-88%), which met the primary endpoint (H0 = 51%). The 2-yr OS rate was 89%. The 2-yr CNS relapse rate was 9% (n = 4). Among the 4 pts, 1 pt had primary testicular DLBCL and 2 of the remaining 3 pts had high-grade B-cell lymphoma, NOS with MYC rearrangement. In conclusion, DA-EPOCH-R/HD-MTX is an effective treatment for newly diagnosed stage Ⅱ-Ⅳ CD5+ DLBCL. Long-term efficacy and toxicity will be evaluated in a 5-yr follow-up in Nov 2021.

J-2094

Therapeutic effects of newly established anti-CD10 mAb(NEP1) on Lymphoma and other cancer cell lines

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CD10 (CALLA) Neutral endopeptidase molecules are cell membrane metallopeptidase widely distributed in hematopoietic cells and their neoplasms. Recently, it is reported that CD10 associated with therapeutic resistance and cancer stem cells-like properties of tumor. We raised mouse against human CD10 monoclonal antibody for the purpose of establishing therapeutic devices for Burkitt lymphoma. We tested whether the newly established mAb inhibited the proliferation of the Burkitt lymphoma cell lines by thymidine uptake test. The treatment of our mAb inhibited the proliferation of Burkitt lymphoma cell line, Raji and Daudi as a function of the dose. The proliferation was reduced by at least 60 and 70% by 0.3 u/ml of mAb. In the same time of measurement of the cells proliferation rate, the cells were prepared for cell cycle to confirm proliferation rate result. The fixed cells were stained with PI, cell cycle phase was assayed by flow cytometry. in proliferation analysis. The rate of G0/G1 phase was increased and the rate of G2-M was increased.We concluded that CD10 may serve as a target molecule in the treatment of several malignancy.

▲TOP

J-2095

mTORC2-mediated metabolic processes contributes drug resistance in leukemia

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Long-term treatment often develops drug-resistance in leukemia patients. Recent studies demonstrated that unique metabolic features of leukemic stem cells may contribute to drug-resistance. To understand molecular mechanisms govern drug-resistance in leukemia, we focused on mammalian target of rapamaycin (mTOR) signaling pathway that regulates cell metabolism by forming two complexes, mTORC1 and mTORC2. We generated mTORC2-deficient human leukemia cell line, and found that mTORC2-deficient leukemia shown reduction of colony formation under treatment with chemotherapeutic agent. In addition, xenograft experiments suggested that combination with mTORC2 deficiency and chemotherapy efficiently suppresses leukemic development. To identify downstream molecules of mTORC2, we performed gene expression profiling, and CRISPR-based functional screening. From 1,000 candidate genes, we found that some metabolic pathways are critical for leukemic cell survival under chemotherapeutic condition. These data suggested that mTORC2 regulates drug-resistance by regulation of metabolic processes, and our study may contribute to finding of novel target of metabolic process for leukemia therapy.

J-2096

Significance of exosomes secreted from cancer-associated fibroblasts in lymphoma microenvironment

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Cancer associated fibroblasts (CAFs) are known to be one of important components of the tumor microenvironment. Exosomes secreted from tumor bystander cells have also emerged as a key player in tumorigenesis; however the role of them in lymphoma has not been fully understood. Here we uncovered the significance of exosomes secreted from CAFs in the lymphoma microenvironment. The amount of exosomes from CAFs differs according to the survival support effect to lymphoma cells. The support of exosomes from CAFs with strong supports was in a dose-dependent manner. While, higher dose of exosomes from CAFs with few survival supports did not display support effects, which indicated that not only the quantity but also the quality of exosomes were associated with the support effects. Using siRNA for Rab27B involved in the exosome regulation, the amount of exosomes secretion from CAFs was suppressed resulting in the reduced survival support. Intriguingly, exosomes from CAFs elicited resistance to gemcitabine in lymphoma cells in vitro and in vivo. Taken together these data indicate that exosomes secreted from CAFs play a pivotal role in the lymphoma microenvironment.

▲TOP

[J-2097] J14-11 [Japanese]
Molecular analysis for development and survival mechanisms of gynecologic malignancies

2018 / 9 / 28 (Fri) 15:30-16:45 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

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J-2097

Genomic Alteration Profiles of Patients with Cervical Cancer in a Japanese Population

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Cervical cancer is the second most common cause of cancer death in young adult women worldwide, and there is only a few reports that focus on the genomic alteration profiles of patients with cervical cancer in Asian populations. To estimate the rates of actionable genetic alterations in cervical cancer of Japanese, we performed a target sequencing for 124 cervical cancer genomes. A total of 124 patients with cervical cancer who underwent potentially curative resection between 2008 and 2017 at the National Cancer Center Hospital was enrolled. Genomic DNAs from formalin-fixed and paraffin-embedded tumor tissues were subjected to the analysis using an Ion AmpliSeqTM Cancer Hotspot Panel v2 to detect the hotspot mutations in 50 cancer-related genes. In addition, copy number alterations of *PIK3CA*, *ERBB2*, *PTEN* and *STK11* were examined by quantitative genomic PCR. Clinical significance of genetic variants was assessed by information deposited in the TCGA and ClinVar databases. Notably, activating aberrations of PIK3CA were frequently detected in the study cases (33/124; 27%). These cases might benefit from therapy using PI3K/mTOR inhibitors.

▲TOP

J-2098

Two distinct tumorigenic processes of endometrial endometrioid carcinoma

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Endometrial endometrioid carcinoma (EEC) has been conventionally considered to be a single disease entity and developed from hyperplasia as a precursor lesion (conventional EEC: group1). We previously reported another EEC subtype that arises directly from normal endometrium (de novo EEC: group2) and shows distinct clinical features from those of conventional EECs. To seek biological relevance for these two different pathways in EEC development, we performed genomic and epigenomic analyses with group1 hyperplasia and carcinoma as well as group2 carcinoma. Transcriptional profiling detected activation of estrogen signaling pathways in group1 and DNA damage pathways in group 2 carcinomas. Exome sequencing revealed POLE mutated, microsatellite instable and copy number high EECs are predominantly enriched in group2, while most of carcinomas in group1 exhibit copy number low character. Methylation analyses showed hypermethylation was observed in group 2. In group1 cases, driver mutations and methylation were mostly shared in pairs of concomitant hyperplasia and carcinoma. This study demonstrates biological relevance of the differential tumorigenic process in EECs.

J-2099

STAT1 Phosphorylation May Confer Cisplatin Resistance in Uterine Serous Carcinoma

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Uterine serous carcinoma (USC) is the most aggressive endometrial cancer with high metastatic and chemoresistant features. Our previous findings showed high STAT1 was positively correlated with tumor progression, further investigation was conducted to reveal that high STAT1 is involved in chemoresistance. shSTAT1 sensitized USC cells to increase cisplatin mediated apoptosis. Cisplatin exposure led to a significantly nucleus phosphorylation of STAT1 on serine (nSer-pSTAT1) but not on tyrosine. Induction of dominant-negative nSer-pSTAT1 can enhance cisplatin-inducing apoptosis. When a specific CK2 (Casein kinase) inhibitor was applied, the nSer-pSTAT1 was reduced to cause DNA damag. Furthermore, nSer-pSTAT1 mediated chemo-resistance by suppressing CTR1, resulting in less intracellular cisplatin accumulation. In vivo xenograft model exhibited nSer-pSTAT1 inhibition using either CK2 inhibitor or dominant-negative alteration prior to cisplatin treatment significantly suppressed tumor growth. These findings indicate that nSer-pSTAT1 may play a key role in platinum resistance as well as tumor progression and targeting STAT1 pathway by CK2 inhibitor could enhance chemosensitivity of USC.

J-2100

Drug sensitivity test with a panel of patient-derived spheroids of small cell neuroendocrine carcinoma of uterine cervix

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Small cell neuroendocrine carcinoma (SCNEC) of uterine cervix is a rare disease, representing about 1-2% of all uterine cervical cancers. Patients with SCNEC have an extremely poor prognosis than other histologic cell types for which no standard treatment is established. We previously developed the cancer tissue-originated spheroid (CTOS) method, a primary culture method for cancer cells. To date, we established a panel of 12 SCNEC CTOS lines with the success rate of 100%. Using the CTOS panel, we performed ex vivo drug sensitivity test with 7 anticancer drugs; paclitaxel, carboplatin, irinotecan, SN-38, cisplatin, etoposide and gemcitabine. All drugs had substantial variations in sensitivity among the lines. Cerv54 which is one of the SCNEC CTOS lines showed most sensitive to irinotecan and intermediately sensitive to SN-38 among the lines. Cerv54 had high levels of carboxylesterase (CES) which converts irinotecan to SN38. The sensitivity test using CTOS might be useful to select sensitive drugs in each patient.

▲TOP

J-2101

FRZB is induced by RAS-MAPK signaling and counteracts transformation

Ichiro Onoyama
OB

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[1]OB

RAS-MAPK signaling is aberrantly activated in gynecologic cancers. BRAF or KRAS activation induces DNA methylation and demethylation, and this epigenetic changes include silencing of tumor suppressor genes, such as SFRP2, negative regulator of Wnt signaling, however, BRAF/KRAS mutation alone cannot transform normal cells. To understand impacts induced by BRAF/KRAS mutation, we performed RNA-sequencing and RRBS analysis using MEFs isolated from BrafV600E knock-in mice. We got many genes which expressions were increased or decreased after BrafV600E activation. Among them, we focused on Frzb gene, a member of SFRPs family proteins. RRBS showed hypomethylation of the upstream regions of Frzb, and Frzb is induced after oncogenic Braf and Kras activation. Braf or Kras activated cells with Frzb knockdown showed increased focus formation ability, indicating that Frzb induced by Braf/Kras activation would function as a tumor suppressor gene. Also, atypical endometrial hyperplasia showed higher expression of FRZB compared with normal endometrium and endometrioid carcinomas. These results indicate that FRZB might suppresses transformation at early stage of carcinogenesis.

J-2102

Myeloid derived suppressor cells (MDSC) increase cancer stem cells (CSC) and tumor PD-L1 expression in ovarian cancer

Naoko Komura
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Objective: To investigate the role of myeloid derived suppressor cells (MDSC) in the induction of cancer stem cells (CSC) and programmed cell death 1 ligand 1 (PD-L1) expression in ovarian cancer. Methods: CSC were defined as tumor cells expressing high levels of aldehyde dehydrogenase 1. 1) We inoculated G-CSF-expressing or Mock-expressing ovarian cancer cells into nude mice and the frequencies of MDSC and CSC in tumors of these models were examined and compared by flow cytometry. 2) To directly demonstrate the role of MDSC in the induction of CSC, we performed in vitro co-culture experiment. 3) Finally, we examined the role of MDSC in the expression of PD-L1 by real-time RT-PCR. Results: 1) Increased MDSC and CSC were observed in G-CSF-expressing ovarian cancer cells-derived tumors. 2) The number of CSC was significantly increased when ovarian cancer cells were co-cultured with MDSC. 3) PD-L1 was overexpressed in CSC compared with non-CSC. MDSC increased tumor PD-L1 expression by inducing CSC in G-CSF-expressing ovarian cancer cells-derived tumors. Conclusion: Myeloid derived suppressor cells (MDSC) increase the stem cell properties and tumor PD-L1 expression of ovarian cancer.

▲TOP

[ML9] ML9 [Japanese]
Morning Lectures 9

2018 / 9 / 28 (Fri) 8:00-8:50 Room 11/12F Conference Hall, Osaka International Convention Center Room 11

Hiroyuki Aburatani / Genome Sci., Res. Ctr. Adv. Sci. Tech., the Univ. of Tokyo

ML9
Clinical Sequencing

Shinichi Yachida
Dapt. Cancer Genome lnformatics, Grad. Sch. Med., Osaka Univ.

Clinical testing for somatic variants in cancer specimens is a common modality of precision medicine. Detection of somatic variants in cancer specimens can be used for a wide range of clinical purposes-to assist with diagnosis, determination of prognosis, and the selection and monitoring of therapy. With the rapid adoption of next-generation sequencing (NGS) in the clinical laboratory, many laboratory will shift from testing a single gene to panel-based NGS testing. Some Designated Core Hospitals for Cancer Genomic Medicine started a panel-based NGS testing as advanced medical equipment to aim the insurance redemption. Targeted panel testing, which varies between hospitals, may be broad for both solid cancer and sarcoma, or may be more focused for a type of malignancy. The use for NGS in clinical practice unlike that in research should be performed under laboratory quality systems. The clinical application of NGS will increase as the technology, bioinformatics, and resources evolve to address the limitations and improve quality of results. The challenge for clinical laboratories is to ensure testing is clinically relevant, cost-effective, and can be integrated into clinical care.

▲TOP

[E-2037] E14-7 [English]
Pancreatic cancer (2)

2018 / 9 / 28 (Fri) 9:00-10:15 Room 11/12F Conference Hall, Osaka International Convention Center Room 11

Masahiro Tanemura / Pept. Surg., Osaka Police Hosp.

E-2037

Accelerated hyaluronan processing phenotype (AHPP) as a novel therapeutic target in pancreatic ductal adenocarcinoma

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[Background] The aggressive nature of pancreatic ductal adenocarcinoma (PDAC) depends on its interactions with extracellular matrix, especially hyaluronan (HA). We analyzed the expression and functional significance of multiple molecules related to HA processing in PDAC.[Methods] Using a panel of PDAC cell lines, we analyzed the expression and functional significance of HA–synthesizing molecules (HAS), HA–degrading molecules (HYAL and KIAA1199/CEMIP), and HA–binding molecules (hyaluronan–binding protein 2: HABP2). The molecules overexpressed in PDAC cell lines were analyzed for their function using a specific inhibitor, siRNA, CRISPR–Cas9 system, and/or forced gene transduction.[Results] Of the molecules analyzed, HAS2, HYAL1, KIAA1199, and HABP2 were independently or simultaneously overexpressed in PDAC, suggesting the presence of accelerated hyaluronan processing phenotype (AHPP). All of these HA–related molecules overexpressed in PDAC promoted the aggressive behavior by mainly increasing the migratory ability. [Conclusions] We propose the presence of AHPP in a subset of PDAC, suggesting that targeting HA processing pathway could be a novel therapeutic target.

▲TOP

E-2038

Detection of KRAS mutations of cfDNA in intention-to-resect pancreatic cancer undergoing preoperative chemotherapy

Tatsuo Hata
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【Backgrouod】 KRAS gene is the most frequently mutated in pancreatic cancer (PC). We assessed KRAS mutations in plasma cell-free (cfDNA) from intension-to-resect PC patients. 【Methods】 Droplet digital PCR was used to detect KRAS mutant alleles at codons 12/13. Mutant allele frequencies (MAF) were evaluated in each subgroup classified by the resectability (based on the Japan Pancreas Society). 【Results】 A total of 47 samples from 28 PC patients were analyzed. KRAS mutations were identified 4 of 13 (30.8%) R-PC, 2 of 10 (20.0%) BR-PC, 3 of 5 (60.0%) UR-PC. Two R-PC patients with positive peritoneal cytology harbored KRAS mutant alleles in cfDNA (MAF, 2.2% and 0.5%). One BR-PV case with undetectable KRAS mutations had received chemotherapy and the therapeutic effect was progressive accompanied by KRAS mutation emergence (MAF, 0.4%). After the pancreatectomy, the mutations were undetectable again. One UR-LA case with KRAS mutations (MAF, 1.4%) received chemoradiotherapy. However, the treatment was failed as multiple liver metastases with high abundance KRAS mutant alleles (MAF, 39.5%). 【Conclusion】 KRAS mutant allele might be a therapeutic marker for intention-to-resect PC.

E-2039

The functional role of Eset in exocrine pancreatic regeneration and pancreatic cancer initiation

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One group of epigenetic regulators known to be altered in cancer development is DNA methylation. Eset, a histone methyltransferase that methylates histone H3 on lysine 9 (H3K9) has been implicated in human cancers. In this study, we investigated the functional role of Eset in exocrine pancreatic regeneration and pancreatic tumorigenesis. Eset was expressed in pancreatic ductal cells and a small subset of pancreatic acinar cells. Pancreatic deletion of Eset resulted in pancreatic atrophy due to increased apoptosis of acinar cells with increased expression of p53 after caerulein-induced pancreatitis. Pancreatic deletion of Eset in the context of oncogenic Kras dramatically accelerated spontaneous development of acinar to ductal metaplasia (ADM) and pancreatic intraepithelial neoplasia (PanIN), the most common precursor lesion of pancreatic ductal adenocarcinoma (PDA). Acinar cell culture experiments revealed that Eset-deleted acinar cells were induced ADM in a cell autonomous manner. Finally, Eset was absent in a subset of human PDA. Thus, Eset blocks apoptosis of acinar cells during exocrine pancreatic regeneration and inhibits Kras-driven pancreatic tumorigenesis in mice.

E-2040

Deferasirox, a novel iron chelator, with gemcitabine inhibits pancreatic cancer cell growth in vitro and in vivo

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Objectives: Iron is an essential element for cell proliferation and growth processes. This study aimed to elucidate the effects of combination of gemcitabine (GEM), standard chemotherapy for pancreatic cancer, and deferasirox (DFX), an oral iron chelator, in vitro and in vivo.
Methods: BxPC-3 was used in all experiments. Cellular proliferation rate was measured using MTS assay. Apoptosis was evaluated by flow cytometry and by measuring caspase 3/7 activity. In the tumor xenografts in nude mice models, when five weeks after engraftment, drug administration began (day 0). After treatment for 21 days, the mice were sacrificed and the tumors were excised. Protein levels of ribonucleotide reductase (RR) subunit 1 (RRM1) and RR subunit 2 (RRM2) were assessed by western blot in vitro.
Results: GEM+DFX showed antiproliferative activity and induced apoptosis in vitro. GEM+DFX suppressed xenograft tumor growth compared with control, GEM, and DFX. RRM1 and RRM2 protein levels were substantially reduced by DFX in vitro.
Conclusion: GEM+DFX has significant anticancer effects on pancreatic cancer cell through RR activity suppression.

▲TOP

E-2041

A high CEA level in the pancreatic juice associated with invasive intraductal papillary mucinous carcinoma

Seiko Hirono
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[1]Second Dept. Surg., Wakayama Med. Univ.、[2]2nd. Dept. Surg., Wakayama Med. Univ.、[3]2nd Dept. Surg., Wakayama Med. Univ.

Introduction: Invasive intraductal papillary mucinous carcinoma (IPMC) may have distant or lymph node metastasis, leading to poor survival. To identify the predictors of invasive IPMC. Methods: This study included 286 consecutive patients undergoing resection for IPMN. We compared clinical features between invasive and noninvasive IPMNs. Results: High mural nodule size was an independent predictor of invasive IPMC in all types (BD-IPMN; P=0.01, OR, 1.992; mixed-IPMN; P=0.042, OR, 1.178; MD-IPMN; P=0.01, OR, 1.443). Its cutoff values, determined by a receiver operating characteristic were 9 mm in BD-IPMN and 6 mm in mixed- and MD-IPMNs. A high carcinoembryonic antigen (CEA) level in the pancreatic juice was an independent predictor of mixed- and MD-invasive IPMCs (mixed-IPMN; P=0.011, OR, 1.002, MD-IPMN; P=0.048, OR, 1.002) and the cutoff values were determined to be 150 and 300 ng/ml, respectively. The accuracy for these predictors was 86.0% for differentiation between invasive and noninvasive IPMN. Conclusions: The measurement of mural nodule size in all types and the CEA level in the pancreatic juice in mixed- and MD-IPMNs might play important roles in predicting invasive IPMC.

E-2042

Oncolytic adenovirus-mediated p53 transactivation induces profound immunogenic cell death in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is the most lethal cancer, and immunotherapy is less sensitive to PDAC because of non-immunogenic cold tumors. Recently, immunogenic antitumor therapies, including chemotherapy, radiotherapy, and virotherapy, have been emerged to improve the antitumor immune response and the immunotherapeutic effect via induction of immunogenic cell death (ICD). In this study, we explored whether transactivation of the tumor suppressor p53 synergizes with oncolysis for inducing the ICD in human PDAC cells. We used tumor-specific replication-competent oncolytic adenoviruses OBP-301 and OBP-702, in which the hTERT promoter drives the expression of the viral E1A and E1B genes for tumor-specific viral replication. OBP-702 induces the Egr1 promoter-driven wild-type p53 expression. Virus-mediated ICD induction state was assessed by analyzing the level of extracellular ATP and high-mobility group box protein B1 (HMGB1). OBP-702 significantly induced the higher amount of ATP and HMGB1 in human PDAC cells compared to OBP-301. Our data suggest that oncolytic adenovirus-mediated p53 transactivation induces profound ICD for boosting the antitumor immune response in PDAC.

▲TOP

[J-2037] J14-5 [Japanese]
Pancreatic cancer (3)

2018 / 9 / 28 (Fri) 10:15-11:30 Room 11/12F Conference Hall, Osaka International Convention Center Room 11

Toru Kitagawa / Kyowakai Med. Corporation

J-2037

Clinicopathological significance of mutations in BRCA1, BRCA2, and PALB2 in pancreatic ductal adenocarcinoma

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Mutations in genes of the breast cancer susceptibility gene (BRCA) pathway, namely, *BRCA1*, *BRCA2*, and *PALB2*, can provide useful information for the efficacy of platinum-based anti-cancer agents or poly ADP-ribose polymerase inhibitors. Pancreatic ductal adenocarcinoma (PDAC) is an important target for them. We analyzed mutations in the entire coding regions of the BRCA pathway genes, expression of BRCA2 protein, and mutations in hotspots of 50 cancer-associated genes in 42 surgically resected PDACs, and evaluated their associations with clinicopathological features. We identified 13 rare germline mutations in the BRCA pathway genes; 68 somatic mutations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A*, *GNAS*, *SMARCB1*, and *RB1*; and 2 germline variations in *MLH1*. Patients harboring conceivable damaging mutations in the BRCA pathway genes showed significantly better prognosis than those with benign mutations or no mutation. These results indicate that rare germline variations in the BRCA pathway genes could be found more frequently than previously anticipated and, more importantly, damaging mutations of them could be a favorable prognostic factor in patients with apparently sporadic PDACs.

▲TOP

J-2038

Genomic analysis using EUS-FNA samples in patients with unresectable pancreatic cancer

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Next-generation sequencing (NGS) based genomic profiling has revealed a mutational landscape of pancreatic cancer (PC). However, few studies have addressed genomic analysis in “unresectable” stages because of the difficulty in sample acquisition suitable for genomic analysis in low-cellularity tumors such as PC. Here, we report genomic analysis using EUS-FNA samples obtained from primary tumors in patients with unresectbale PC (n=107). We conducted whole-exome sequencing (WES, n=20) using NGS and genome-wide copy number analysis using array-based comparative genomic hybridization (n=101). We detected somatic mutations in known cancer related genes (KRAS, TP53, CDKN2A, SMAD4, ARID1A and MLL2), and focal amplifications (GATA6, MYC and KRAS) and deletions (CDKN2A, SMAD4 and TGFBR2). Importantly, a subset of tumors had genetic alterations which can be potentially therapeutic targets. A high mutation burden (42/Mb) was identified in one tumor, suggesting a subset of tumors as a potential candidate for the immune checkpoint inhibitors. We also identified druggable focal amplifications in one-fourth of tumors (FGFR1, FGFR4, ERBB2, CCND1, CCNE1, CDK4 and CDK6).

J-2039

Suppressor effect of pancreatic adenocarcinoma in passenger strands of microRNA

Tetsuya Idichi
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【BACKGROUND】 Pancreatic adenocarcinoma (PDAC) is the most malignant tumor with poor prognosis. In our department, we analyzed to focus on abnormal expression and function analysis on "passenger-strand" microRNA with PDAC, which has been reported to decay and to lose functionality. However, in recently reports on “passenger-strand” cancer have also been approved. 【MATERIAL AND METHOD】 "passenger-strand" microRNA is analyzed with PDAC cells, we reveal the effect of "passenger-strand" microRNA on functional analysis. Furthermore, we identify microRNA-regulated oncogenes by microarray analysis. 【RESULT】 We made an expression profile by microRNA analysis using the Next Generation Sequencer. It included passenger strands that were previously considered non-functional, such as miR-216a-3p, miR-216b-3p, miR-148a-5p and miR-129-1-3p. Each microRNA. In functional analysis by PDAC cells, it was suggested that each microRNA acts as tumor-suppressing microRNA and regulated target oncogenes. 【CONCLUSION】 Even the "passenger-strand" microRNA, which was considered to have no function until recently, we proved to act as a tumor suppressor microRNA.

J-2040

Vasohibin-2 plays essential role in invasion and metastasis of pancreatic ductal adenocarcinoma

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Our laboratory has isolated anti-angiogenic Vasohibin-1 (VASH1) and its homologue Vasohibin-2 (VASH2). VASH2 is overexpressed in various cancer cells, and promote not only tumor angiogenesis but also accumulation of cancer associated fibroblasts (CAFs) and epithelial to mesenchymal transition (EMT) of cancer cells. We recently noticed that pancreas cancer patients with higher VASH2 expression exhibited poorer prognosis. We therefore examined the role of VASH2 in pancreas cancer. We employed LSL-K-rasG12D; LSL-Trp53R172H; Pdx1-Cre (KPC) mice and characterized the function of Vash2 in the development of PDAC. Vash2 expression in pancreas was augmented during PDAC development in KPC mice. When KPC mice were crossed with Vash2LacZ/LacZ mice, the incidence of PDAC was same between KPC mice and KPC/Vash2 null mice. However, surprisingly, invasion and metastasis were completely absent in KPC/Vash2 null mice. Pathological analyses revealed that PDAC cells in KPC/Vash2 null mice remained immature phenotype. These results indicate that Vash2 is overexpressed and plays essential role in invasion and metastasis of highly metastatic pancreas cancer.

▲TOP

J-2041

Role of the actin-binding protein Girdin in pancreatic angiogenesis

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【Background】Girdin is an actin-binding protein identified as a substrate of Akt and is critical for migration and invasion of variety cancer cells. But the role of Girdin in pancreatic cancer (PaCa) was not clearly elucidated. On the other hand we have previously demonstrated the important role of angiogenesis in PaCa invasion and metastasis, In this study, we clarify the biological mechanisms of Girdin in PaCa from the standpoint of angiogenesis.【Methods】(1) The expression of Girdin in PaCa was examined by qPCR. (2) Girdin in PaCa was inhibited by siRNA technique. (3) We evaluated the alteration of VEGF expression by siGirdin using qPCR and ELISA.【Results】(1) Expression of Girdin was detected in all PaCa cells. (2) By transfection of siRNA, the expression of Girdin was inhibited in all cells. (3) Knocked-down of Girdin decreased the production of VEGF.【Conclusion】Girdin played the role in the production of angiogenic factor in PaCa. So Girdin may become a new molecular target in the treatment of pancreatic cancer.

J-2042

Combinatorial Histone Acetyltransferases (HATs) inhibition as a potential therapeutic approach for pancreatic cancer

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Type A HATs are involved in the regulation of gene expression through acetylation of nucleosomal histones in the context of chromatin. Gcn5, p300/CBP, and TAFII250 are some examples of type A HATs that cooperate with activators to enhance transcription associated with various cancers. Although the interactions of this cluster and required HATs combination for carcinogenesis are still not identified in pancreatic ductal adenocarcinoma (PDAC). To this end, we screened small molecule library which controls various combinatorial HATs. In cell viability assay, the HAT compounds had killing effect on PANC-1. We also confirmed this effect though shRNA-mediated knockdown experiment to understand the mechanism and confirm the efficacy of the combinatorial HATs inhibition, resulting induced apoptotic cell death in PANC-1 cell line. From analysis of public database in PDAC patients, we isolated one upregulated oncogenic pathway associated with chromatin modification. In this pathway, one transcription factor was decreased through the combinatorial HATs inhibition. Therefore, we proposed that this combinatorial HATs and this factor axis are promising targets toward PDAC treatment.

▲TOP

[PD] PD [Japanese]
Human papilloma virus (HPV) vaccines in Japan; current problems and future directions

2018 / 9 / 28 (Fri) 15:30-18:00 Room 11/12F Conference Hall, Osaka International Convention Center Room 11

Tetsuo Noda / Cancer Inst. of JFCR、Tomotaka Sobue / Div. Environ Med., Osaka Univ. Sch. Med.,

In Japan, more than one million of cancer patients arise every year, and it has been shown that more than 20% of these patients are involved in the preceding infectious diseases including viral infection. It is thus obvious that “prevention of infection” is an effective and practical approach for cancer prevention. Cervical cancer that deprives more than 3,000 Japanese women each year is caused by HPV infection, and protection against HPV infection by vaccine administration has been shown to suppress precancerous lesions, leading to reduce the incidence of cervical cancer. In Japan, routine use of HPV vaccines started in April 2013; however, “proactive recommendation” for routine use was stopped later, and has not yet restarted. In December 2015, statements concerning the future hazards brought by Japan’s current decision was announced by WHO.
Currently, five years have elapsed since the interruption of the proactive recommendation. In this panel discussion, recent incidence of cervical cancer and its precancerous lesions in Japan will be reviewed, and obstacles to restart the proactive recommendation will be discussed with stakeholders. Furthermore, what researchers should do in order to protect Japanese women from cervical cancer will be discussed.

PD

No Abstract

▲TOP

[ML10] ML10 [Japanese]
Morning Lectures 10

2018 / 9 / 28 (Fri) 8:00-8:50 Room 12/12F 1202, Osaka International Convention Center Room 12

Hiroki Kuniyasu / Dept. Mol. Pathol., Nara Med. Univ.

ML10

Identification of cancer stem cells by the multicolor lineage tracing method

Hiroo Ueno
Dept. Stem Cell Path., Kansai Med. Univ.

Discussant：Kouji Banno
Dept. OB& GY, Keio Univ., Sch. Med.

Adult tissue specific stem cells have important roles in tissue maintenance and in regeneration upon injury. On the other hand, accumulation of injuries in DNA can be a cause of aging or cancer. Therefore, understanding of the exact nature of adult stem cells is considerably important. However, many of the adult tissue-specific stem cells, especially in low-turnover tissues, have remained unidentified. To identify adult tissue specific stem cells, we have developed multicolor lineage tracing method. By using the method, we have identified lingual epithelial stem cells. They exist at the bottom of the interpapillary pit in the lingual epithelium. They are Bmi1 positive, and are usually slow cycling. However, upon irradiation-induced injury, they rapidly proliferate and regenerate the injured tissue. We could also identify candidate cancer stem cells in chemical-induced tongue cancer by the same strategy. We have also developed the system to culture lingual epithelial stem cells in vitro as an organoid in matrigel. We will present recent progress of our analyses on the function of lingual stem cells and taste bud stem cells and discuss about the cancer stem cells in these tissues.

▲TOP

[E-2043] E14-8 [English]
Novel biotherapy and molecular mechanism

2018 / 9 / 28 (Fri) 9:00-10:15 Room 12/12F 1202, Osaka International Convention Center Room 12

Yasushi Toh / Dept. Gastroenterol. Surg., Nat'l Kyushu Cancer Ctr.

E-2043

Effect of BuzhongYiqi Decoction on the T Cell Immunization of Gastric Cancer based on PD-1/PD-L1 Molecules

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Previous clinical studies have shown that Buzhong Yiqi Decoction (BYD) as an adjuvant drug for gastric cancer can significantly prolong patient survival. In order to investigate the effect and mechanism of BYD on cellular immunity, we firstly detected the proliferation of T lymphocytes by MTT and CFSE, and found that BYD could significantly promote the proliferation of T lymphocytes. Next, we established the xenografts gastric cancer model with MFC cells in 615 mice, and showed BYD increased mice spleen index, thymus index, inhibited tumors growth and significantly prolonged survival of mice. Flow cytometry result showed that BYD decreased Treg and PD-1+Treg cells in peripheral blood of mice. Immunohistochemistry results suggested that 5-FU+BYD reduced the expression of PD-1 and PD-L1, induced the number of tumor-infiltrating T lymphocytes compared with 5-FU group. The similar results were also certified by qRT-PCR and Western blot. Moreover, Compared with 5-FU group, the expression of P-PI3K, and P-AKT in 5-FU+BYD group was significantly down-regulated. In conclusion, BYD could suppress the immune escape of tumor to play a role in tumor inhibition and prolong survival.

▲TOP

E-2044

Expression and prognosis analysis of the Collagen genes in patients with gastric cancer under different treatment

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Collagen (COL) genes participate in the ECM-receptor interaction and focal adhesion in tumor, with a crucial role in invasion and metastasis. The prognostic values of COL genes have been shown in several malignancies. In the present study, to identified COL genes involved in the pathogenesis of gastric cancer (GC), we performed a comprehensive bioinformatics analysis of multiple microarray datasets by using the Oncomine database. We further evaluated the prognostic value of these different-expressed COL genes in GC by a systematic Kaplan-Meier survival analysis of the whole mRNA transcriptomics based on the Cancer Genome Atlas project (TCGA). We found that seven COL genes (COL1A2, COL4A1, COL4A2, COL6A1, COL6A2, COL6A3 and COL11A1) were over-expressed in GC. Among them, over-expression of COL1A2, COL4A1, COL4A2, COL6A1, COL6A2, COL6A3 were correlated with poor prognosis of GC patients treated by surgery only, while over-expression of COL4A1 and COL11A1 correlated with poor survival of patients treated by 5-FU based adjuvant therapy. Our results indicate that over-expression of COL genes may be novel prognostic markers for GC and serve as promising markers for therapy selection.

E-2045

Novel virotherapy for scirrhous gastric cancer with peritoneal metastasis

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Background: Scirrhous gastric cancer (SGC) often causes peritoneal metastasis, and no effective treatment has been established yet. Previously, we developed the oncolytic adenovirus (OBP-401), which could replicate only within the tumor cells selectively and express green fluorescent protein (GFP). In this study, we assessed the theranostic potentials of OBP-401 in combination with paclitaxel (PTX) against human SGC.Methods: The anti-tumor effects of OBP-401 and PTX against SGC cells (GCIY and KATOIII) were evaluated in vitro and in vivo. And the mechanism underlying the synergistic effect was investigated.Results: OBP-401 synergistically suppressed the viability of SGC cells in combination with PTX. And PTX enhanced the efficiency of OBP-401 replication in SGC cells. Moreover, the combination therapy with i.p. administration of OBP-401 and PTX significantly inhibited the tumor growth of peritoneal metastasis. Conclusions: OBP-401 has a potential to detect and eradicate peritoneal metastasis, and the i.p. OBP-401 administration in combination with PTX would be a novel promising treatment for SGC with peritoneal metastasis.

E-2046

Identification of genetic/epigenetic alterations based on wild-type TP53 in high methylation gastric cancer

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Gastric cancer (GC) is involved in multiple genetic/epigenetic alterations, but their synergistic contribution to gastric tumorigenesis was not fully clarified. We conducted targeted exome sequencing with DNA methylome analysis. Loss of MLH1 expression was enriched in high methylation GC cases (HME_MLH1(-)), and they commonly involved both KRAS mutation and frameshift mutation of TGF β -related genes. Extremely high-ME (E-HME) matched with Epstein-Barr virus-positive cases, and a part of E-HME cases also had KRAS mutation. Since HME_MLH1(-)/E-HME cases had no TP53 mutation, we screened critical genes for activated RAS-induced premature senescence using shRNA library. Bypass of senescence occurred through knockdown of five genetically/epigenetically inactivated genes other than TP53. Finally, we applied a chemical compound that we developed and found to induce apoptosis via TP53 pathway, and among a series of GC cell lines, TP53 wild-type cells including HME_MLH1(-)/E-HME GC cell lines specifically underwent apoptosis at low dose. HME_MLH1(-)/E-HME GC cases should have unique carcinogenesis pathway independent of TP53 mutation, and intact-TP53 might be utilized therapeutically.

▲TOP

E-2047

Uc.63+ contributes to gastric cancer progression through regulating NF-kB signaling

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The transcribed ultraconserved regions (T-UCRs) are a novel class of long non-coding RNAs and are involved in carcinogenesis of several types of cancer. Although several lines of papers have described the oncogenic role of Uc.63+, there are no reports mentioning its importance in gastric cancer (GC) biology. In this study, we evaluated Uc.63+ expression using clinical samples of GC by qRT-PCR and also assessed the correlation between Uc.63+ expression and clinico-pathological factors. The upregulation of Uc.63+ was significantly correlated with advanced clinico-pathological features. Knockdown of Uc.63+ significantly repressed GC cell growth and migration, whereas overexpression of Uc.63+ conversely promoted those of GC cells. In situ hybridization of Uc.63+ revealed its preferential expression in poorly differentiated adenocarcinoma. We further conducted a microarray analysis using Uc.63-overexpressed MKN-1 cells and found that NF-κB signaling was significantly upregulated in accordance with Uc.63+ expression. Our results suggest that Uc.63+ could be involved in GC progression through regulating GC cell growth and migration via NF-κB signaling.

E-2048

Genetic heterogeneity of high risk gastrointestinal stromal tumor (GIST)

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Background: GIST is the most frequent sarcoma in GI and its proliferation is driven by various genetic alterations which are mutually exclusive. We've conducted multiplex panel analysis for high-risk GISTs.Methods: Total 515 patients with high-risk GISTs diagnosed by central were prospectively registered from 127 hospitals between 2012 and 2015. Genotyping was performed using surgical specimens with conventional PCR/sequencing of KIT and PDGFRA genes, and targeted sequencing with NCC Oncopanel ver. 5.Results: KIT mutations were found in 456 GISTs (38 in exon 9, 406 in exon 11, 8 in exon 13, 4 in exon 17), PDGFRA mutations in 18 GISTs (1 in exon 12, 1 in exon 14, 16 in exon 18) and 19 GISTs were diagnosed as wild-type GIST. Multiplex analysis of 15 wild-type GISTs found NF1 mutations in 7, BRAF in 3, and SDHB in 1, and no causative mutation was found for 4. Wild-type GISTs located either the stomach and/or small intestine, and with a median follow-up of 4.5 years, 7 patients of 19 had relapses although most patients received imatinib-adjuvant therapy.Conclusions: GIST is a heterogeneous tumor and wild-type GIST with high-risk features may have poor prognosis in spite of adjuvant.

▲TOP

[J-2043] J14-6 [Japanese]
Molecular mechanism and diagnosis of peritoneal dissemination in gastric cancer

2018 / 9 / 28 (Fri) 10:15-11:30 Room 12/12F 1202, Osaka International Convention Center Room 12

Shuji Takiguchi / Nagoya City Univ. Hosp. Dept. Gastroenterological Surg.

J-2043
IL6 derived from intraperitoneal macrophages is involved in peritoneal dissemination of gastric cancer

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Gastric cancer (GC) often develops peritoneal dissemination (PD). However, involvement of intraperitoneal macrophages (ipMs) to PD remains unclear. We investigated the interaction between ipMs and GC cells and examined their roles on PD. Flow cytometric analysis of peritoneal washes from patients with GC revealed ipMs constituted the majority in the microenvironment of peritoneal cavity. In addition, the proportion CD163-positivity in ipMs were significantly higher in stage IV than stage I. Indirect co-culture of GC cells with macrophages potentiated migration/invasion ability of GC cells. Cytokine/chemokine array demonstrated that coculture of macrophages with GC cells increased IL-6 in the medium. IL6 was also elevated in peritoneal washes from patients and mice with PD of GC. In the absence of macrophages, IL-6 enhanced migration and invasion in GC cells in vitro. Administration of IL6 enhanced growth of peritoneal GC tumor in mice. On the other hand, anti-IL6 antibody tended to suppress their growth. The results suggested that intraperitoneal IL-6 secreted from ipMs are involved in aggravation of PD. ipMs-derived IL6 might be a therapeutic target to overcome the PD of GC.

▲TOP

J-2044

Extracellular vesicles from gastric cancer promote peritoneal dissemination via M2 differentiation of macrophages

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Gastric cancer (GC) often develops peritoneal dissemination, but its mechanism is still unclear. Extracellular vesicles (EVs) including exosomes are recently known to be involved in cancer metastasis. To explore their roles in peritoneal dissemination, we investigated their effects on macrophages as the intraperitoneal cancer microenvironment.Immunofluorescent assay of the ascites from patients with GC revealed that M2-like macrophages predominantly existed in peritoneal cavity. Whether EVs purified from the ascites or peritoneal lavage change the property of macrophages were explored. EVs from the ascites with positive cytology made macrophages M2-type, which was confirmed by their morphology and expression of CD163/206 (M2 marker). EVs purified from the culture medium of GC cell lines also induced the M2 differentiation in macrophages. Moreover, the co-culture of GC cells and differentiated macrophages enhanced migration of GC cells.These results suggested that EV derived from GC cells in the ascites affect the macrophages property, and thereby play critical roles in the intraperitoneal cancer microenvironment as the underlying mechanism of dissemination in GC.

J-2045

Molecular mechanism of peritoneal dissemination of gastric cancer involving adipocytes

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Introduction: We have demonstrated the possibility that extracellular vesicles (EV) released from cancer became the trigger for peritoneal metastasis. In the present study, we aim to elucidate the molecular mechanism of intercellular communication between adipocytes abundantly present in the peritoneal cavity and gastric cancer cells and peritoneal mesothelial cells.Methods: We examined the invasive and migration ability of gastric cancer cells by administering the condition medium and EV of adipocytes to gastric cancer cells and co-culturing with gastric cancer cells.Results: Although the changes in invasive and migration ability of the gastric cancer cells were not observed in condition medium or EV of adipose cell line, co-culture with adipocytes dramatically increased the invasive and migration of gastric cancer cells. Expression of SNAIL was higher in gastric cancer cells co-cultured with adipocytes. Expression of molecules involved in inflammation was elevated in co-cultured adipocytes.Conclusions: We demonstrated the possibility that adipocytes might be involved in acquiring the metastatic potential of cancer cells by intercellular communication with gastric cancer cells.

J-2046

In vivo imaging of peritoneal metastasis in gastric cancer by GGT-activatable fluorescence probe

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[Introduction] Diagnosis of peritoneal metastasis in gastric cancer (GC) is important for determining therapeutic strategies. The sensitivity of staging laparoscopy is reported 64-94%, so some cases are missed metastatic nodules. We have developed gGlu-HMRG, a γ-glutamyl transpeptidase (GGT) activatable fluorescence probe, and this can be useful for detection of ovarian and breast cancer. The aim of this study is to assess whether or not gGlu-HMRG can be applicable for peritoneal metastasis of GC. [Methods] We investigated GGT activity of seven GC cell lines (HGC27, MKN7, MKN28, MKN45, MKN74, Kato3, NUGC4). We prepared peritoneal metastasis model mouse with GC cell lines, and observed with gGlu-HMRG. Next, we assessed GGT activity of 96 human GC samples.[Results] GGT activity of HGC27, MKN45 and NUGC4 was comparatively higher than that of the other cell lines. We could detect metastatic nodules in model mouse of these three cell lines. GGT activity of 79 human samples (82%) was higher than that of HGC27, MKN45 and NUGC4.[Conclusion] gGlu-HMRG may be applicable for in vivo imaging of peritoneal metastasis in GC.

▲TOP

J-2047

Evaluation of living floating gastric cancer cells in peritoneal lavage by TelomeScan F35

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<Background>TelomeScan was reported to be possible to detect living circulating tumor cells in blood from cancer patients with high sensitivity and specificity. We applied TelomeScan F35 (TS-F35) to search the presence of floating cancer cells in peritoneal lavage and compared the results of cytology in gastric cancer. <Method>25 patients with advanced gastric cancer who underwent diagnostic laparoscopy were enrolled. Peritoneal lavage samples for cytology were stained with Papanicolaou and MOC-31, and that for TS-F35 were immunostained with CD45, CEA and EpCAM.<Results>Cytological results were positive (CY1) in 9 patients, suspiciously positive (Class III) in 5 patients and negative in 11 patients, respectively. The sensitivity of TS-F35 with selection by CD45 was 88.39%. However the specificity of that was very low (9.1%) , because of false-positive caused by peritoneal mesothelium cells. Combined diagnosis with CEA and EpCAM increased the specificity of TS-F35 to 81.8%, but decreased the sensitivity to 66.7%.<Conclusion>TS-F35 combined immunostaining was less sensitive but it is necessary to be evaluated as a predictive tool for peritoneal recurrence in gastric cancer.

J-2048

Troponin I2 as a specific biomarker for prediction of peritoneal metastasis in gastric cancer

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BackgroundPeritoneal metastasis is the most frequent recurrent pattern of gastric cancer (GC) and is difficult to be diagnosed by imaging and refractory to treatment.MethodsMetastatic pathway-specific transcriptome analysis was conducted by comparison of patient groups with no recurrence by comparison of patient groups with no recurrence and with peritoneal, hepatic, and nodal recurrence. Fifteen cell lines and 262 pairs of surgically resected gastric tissues were subjected to mRNA expression analysis. PCR array analysis was performed to explore coordinately expressed cancer-related genes. To evaluate the in situ protein localization and expression patterns, immunohistochemical staining was performed.ResultTroponin I2 (TNNI2) was found to be overexpressed specifically in the peritoneal recurrence group. High TNNI2 expression was significantly and specifically associated with peritoneal metastasis and served as an independent risk marker for peritoneal recurrence after curative gastrectomy. Prevalence of peritoneal recurrence increased in parallel with staining intensity of TNNI2.ConclusionsTNNI2 may serve as a biomarker for detection and prediction of peritoneal metastasis of GC.

▲TOP

[LS22] LS22 [Japanese]
Significance of 'inner' immunity at anti-cancer therapies

2018 / 9 / 28 (Fri) 11:50-12:40 Room 12/12F 1202, Osaka International Convention Center Room 12
：KOBAYASHI Pharmaceutical Co.,Ltd.

Shogo Kobayashi / Osaka University, Graduate School of Medicine, Department of Gastroenterological Surgery

LS22
Significance of 'inner' immunity at anti-cancer therapies

Mamoru Harada
Shimane University Faculty of Medicine, Department of Immunology

No Abstract

[J-2103] J14-12 [Japanese]
Genetic analysis of gastric cancer

2018 / 9 / 28 (Fri) 13:00-14:15 Room 12/12F 1202, Osaka International Convention Center Room 12

Hiroyuki Sugihara / Div. Mol. Diagn. Pathol., Dept. Pathol., Shiga Univ. Med. Sci.

J-2103

Search for predictive biomarkers of response to neoadjuvant chemotherapy in locally advanced gastric cancer

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Background: COMPASS trial is a randomized phase II study, which compares two and four courses of neoadjuvant chemotherapy (NAC) with S-1/cisplatin and paclitaxel/cisplatin for locally advanced gastric cancer. The present study explored biomarkers predicting the response of locally advanced gastric cancer to NAC in the COMPASS trial biomarker study. Methods: After extracting mRNA from biopsy specimens of primary tumor before NAC, the expression levels of 127 genes were quantified by real-time PCR. We identified genes with expression profiles that showed significant interactions with the pathological response or 3-year survival. Results: TIMP1, DSG2, RRM1, MUC2, EGFR, ZDHHC14, and CLDN18 were identified as predictive markers for pathological response. Among them, regimen selection by ZDHHC14 had the highest accuracy. THBS1, MSI1, and IGF2BP3 were identified as predictive markers of 3-year survival. Among them, regimen selection by THBS1 most significantly stratified 3-year survival. Conclusions: The effect of NAC in locally advanced gastric cancer was predictable by endoscopic biopsy before treatment. The possibility of personalized NAC based on biomarker analysis was suggested.

▲TOP

J-2104

miR-122-5p is a novel biomarker for liver metastasis in Alpha-fetoprotein producing gastric cancer

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Background: alpha-fetoprotein (AFP) producing gastric cancer (AFPGC) is recognized as one of the most aggressive tumors. We examined the microRNAs (miRNAs) expression in AFPGC tissue samples, and we investigated clinical usefulness of the AFPGC-specific miRNAs.
Methods: we performed comprehensive miRNA array-based approach, and the expression levels of each miRNA were compared between the AFP positive cells and AFP negative cells. Next, we validated the selected miRNA in tissue and plasma samples.
Results: in AFPGC tissue samples, the expression level of miR-122-5p was significantly higher compared to normal mucosa and AFP non-producing gastric cancer (non-AFPGC) tissues. The plasma expression level of miR-122-5p was also significantly higher in AFPGC patients compared to health volunteers and non-AFPGC patients, and strongly correlated with plasma AFP levels. Moreover, the expression of miR-122-5p in tissue samples more strongly correlated with malignant potential than plasma AFP level in AFPGC patients.
Conclusions: We revealed that miR-122-5p could be a useful biomarker for early detection, disease monitoring and predicting prognosis in patients with AFPGC.

J-2105

Gastric cancer derived RUNX3 mutation, R122C, induces intestinal metaplasia-like lesion in the antrum of knock-in mouse

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There has been long debate as to whether intestinal metaplasia (IM) is a precursor to gastric cancer. A part of the reasons why there is no definitive conclusion is that there is no suitable mouse model for IM. The RUNX3 mutation, RUNX3(R122C), was identified in gastric cancer and this mutation converted tumor suppressor to oncogene (Li et al., Cell 2002). We generated R122C knock-in mouse. Interestingly, we detected expression of Cdx2, intestine specific gene, in the antral glands at 6 weeks old mice and both Cdx2 and Muc2 at 6 months old mice. We generated organoids from the antrum of 6 weeks old R122C mice. These organoids also showed CDX2 expression. Although there is no massive appearance of goblet cells, we tentatively call the phenotype in R122C mice as IM. These IM regions did not show more malignant phenotype within 1 year. RNA was extracted from IM region of R122C mice and equivalent position of WT mice. We will discuss the changes of gene expression observed in R122C mice. We hope to use R122C knock-in mice for the study of step-wise gastric carcinogenesis.

J-2106

Functional analysis of CLDN18-ARHGAP26 fusion gene in gastric cancers

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【Introduction】The CLDN18 -ARHGAP26 fusion gene was first reported by TCGA but the prospective functions of this gene product remain unknown.【Methods】We have previously established 129 GC-Patient derived xenografts (PDXs) from surgically resected specimens in our institute. We first prepared tissue micro-array of formalin-fixed and paraffin-embedded (FFPE) specimen of PDXs and screened for the patients harboring a CLDN18-ARHGAP26 fusion genes by FISH. Then, we conducted several functional analyses, using the cell lines derived from PDX tumor with CLAN16-ARHGAP26 fusion.【Results】Among 124 GC-PDXs, we detected two CLDN18-ARHGAP26 fusion positive cases. We successfully established cell lines of these 2 cases. Furthermore, we would verify that PDX derived cells preserved the original patient properties. To address its function, we introduced siRNA into cultured cells to reduce their RNA and protein levels. Our data so far indicate that the CLDN18-ARHGAP26 fusion might promote anchorage independent cell growth. 【Conclusion】The CLDN18-ARHGA26 fusion gene potentially contributes to malignant phenotypes of GC.

▲TOP

J-2107

Integrated multigene expression panel to prognosticate patients with gastric cancer

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Aim; We aimed to build a new molecular-based model to predict prognosis in patients with gastric cancer. Methods;200 patients with gastric cancer were divided into learning and validation. In the learning cohort, mRNA expression levels of 15 molecular markers in gastric tissues were analyzed and concordance index (C-index) values for overall survival were calculated. The multigene expression panel was designed according to C-index values and the subpopulation index. The reproducibility of the panel was evaluated in the validation cohort. Results; Among 32,767 combinations, the optimal and balanced expression panel comprised four constituents (MAGED2, SYT8, BTG1, and FAM46) and the C-index value was 0.793. Using this panel, patients were provisionally categorized with scores of 1-3, and clearly stratified into favorable, intermediate, and poor overall survival groups. In the validation cohort, multivariate analysis revealed that the expression score was an independent prognostic factor for overall survival after curative gastrectomy. Conclusions; We developed an integrated multigene expression panel that simply and accurately stratified risk of patients with gastric cancer.

J-2108

Significant Role of Spondin2 expression in Gastric Cancer patients with Peritoneal Dissemination

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Background: peritoneal dissemination (PD) is a major cause of death in gastric cancer (GC). However, its molecular mechanism has not been elucidated. Here, we aim to identify driver genes for PD in GC.Method: (1) We identified a PD-associated gene using GC datasets (Singapore and GSE 62254) and performed survival analyses of the gene using the GC dataset and our patient cohort. (2) We assessed the clinicopathological significance of the gene expression using RT-qPCR and performed immunohistochemical analysis of the gene in our cohort. (3) Gene set enrichment analysis (GSEA) was performed using the GC datasets.Results: Spondin2 (SPON2) was identified as a PD related gene. High SPON2 expression was a poor prognostic factor ($p < 0.05$) and was associated with the depth of invasion ($p < 0.05$) in our cohort and the GC datasets. GSEA showed a positive correlation of SPON2 expression with EMT related genes. SPON2 was highly expressed in cancer-associated fibroblast (CAF) in immunohistochemical staining.Discussion: We identified SPON2 as PD-related gene in GC. SPON2-expressed CAF might contribute PD development possibly through promoting EMT of GC cells.

▲TOP

[J-2109] J19 [Japanese]
Radiation / photodynamic / thermal therapy

2018 / 9 / 28 (Fri) 14:15-15:30 Room 12/12F 1202, Osaka International Convention Center Room 12

Kazuhiko Ogawa / Dept. of Radiat Oncol, Osaka Univ. Scool of Med.

J-2109

Radiation increases invasive activity of breast cancer cells by lysosome exocytosis

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Some studies have shown that radiation may increase the invasive activity of cancer cells and potentially distant metastasis. Recently, lysosome exocytosis has been linked to cancer cell invasiveness and progression. In this study, we evaluate the role of lysosome exocytosis on invasive activity of breast cancer cells upon radiation. The invasive activity and lysosome exocytosis of tested breast cancer cell lines were increased after radiation treatment. Treatment with lysosome inhibitor bafilomycin A1 or chloroquine decreased the invasive activity of cancer cells, with or without radiation treatment. The protein level of ARL8B increased in the lysosome fraction upon radiation. Down-regulation of ARL8B with shRNA led to a decrease in lysosome exocytosis with a concomitant inhibition radiation induced invasive activity of the breast cancer cells without affecting the basal invasiveness. In addition, overexpression of ARL8B increased the invasive activity of breast cancer cells, which was similar to the result obtained after radiation. In summary, radiation enhances lysosome exocytosis in breast cancer cells that can lead to their increased invasive activity.

▲TOP

J-2110

Somatic Copy Number Alterations Associate with Chemoradiotherapy Response in Esophageal Squamous Cell Carcinoma

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Esophageal squamous cell carcinoma (ESCC) has extensive somatic copy number alterations (SCNAs) throughout the genome. To determine whether SCNAs associate with response to chemoradiotherapy (CRT), we performed whole-exome sequencing (WES) of pretreatment ESCC and paired-peripheral blood from 28 patients treated with definitive CRT. SCNAs were detected from WES data. Fourteen of 28 patients who had residual tumor or recurrence within 1 year after CRT were classified as non-responders. Furthermore, to reveal SCNA-dynamics following CRT, we performed multi-region WES of 12 pretreatment and 8 recurrent samples from 4 patients with local recurrence. In the analysis of pretreatment ESCC, 3q26-27 gain (putative driver gene is SOX2) and Yq11.2 loss were significantly associated with poor response to CRT and shorter prognosis. Consistent with these results, multi-region WES showed that these SCNAs were identified in pretreatment and recurrent ESCC. Furthermore, we identified 80% of SCNAs were consistently detectable in both primary and recurrent ESCC, indicating that pre-existing CRT-resistant clones persisted during CRT. Our findings demonstrated SCNAs associated response to CRT in ESCC.

J-2111

Vascular shut down effects of photodynamic therapy with Talaporfin

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Background:
Photodynamic therapy (PDT) is an attractive modality for cancer therapy. PDT consists of intravenous administration of a photosensitizer and irradiation a light, which produce reactive oxygen species that directly kill tumor cells and shut down tumor vessels. Here, we evaluated that PDT with Talaporfin (mono-l-aspartyl chlorin6, Laserphyrin®) shut down existing tumor vessels.
Materials and Methods:
(1)Talaporfin was administered to endothelial cell lines (HUVEC) followed by irradiation with the specific wavelength of light (664nm). The cell death-inducing effects were analized by WST-8 assay and antivascular factors were analized by immunofluorescent staining.
(2)Xenograft tumor mouse models were established with colon cancer cell lines (HCT116). Vascular shut down effects were assessed by using a laser speckle blood flow (LSBF) analyzing system (OMEGA ZONE).
Result:
(1)PDT treatment induced the cell death and the depolymerization of microtubules in endothelial cells.
(2)PDT significantly decreased blood flow in tumors but not in the neighboring healthy tissue.
Conclusions:
In summary, we could show the vascular shut down effects of PDT with Talaporfin in vitro and in vivo.

J-2112

Tumor suppression by magnetic hyperthermia treatment using a formulation loaded with iron oxide nanoparticles

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[Purpose] The purpose of this study was to examine the anti-tumor effect of local injection of the super carbonate apatite loaded with magnetic nanoparticles (sCA-MNPs) when combined with magnetic hyperthermia treatment (MHT) and the effect on magnetic particle imaging (MPI) images.[Materials and Methods] The suspension of sCA-MNPs or MNPs (pH 6.4-7.4) was scanned using by the MPI scanner to examine the pH sensitivity. The uptake of iron into Colon-26 cells was quantified using by the absorption photometer. In animal experiments, the following five groups were studied: Control, MNPs, MNPs + MHT, sCA-MNPs, and sCA-MNPs + MHT groups. The MPI study was conducted temporally after injection of MNPs or sCA-MNPs. MHT was started 4 h after the injection.[Results] The MPI signals were enhanced as pH decreased in the sCA-MNPs group. The cellular uptake study demonstrated that sCA-MNPs were taken up into tumor cells. The relative tumor volume growth in the sCA-MNPs + MHT group was significantly lower than that in the MNPs + MHT group on 1 to 5 days after MHT.[Conclusion] sCA-MNPs combined with MHT suppressed tumor growth and sCA-MNPs was successfully imaged by MPI.

▲TOP

J-2113

Effects of hyperthermia on DNA double-strand break repair machineries underlying hyperthermic radiosensitization

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Hyperthermia is known to exhibit inhibitory effects on DNA repair, resulting in radiosensitization, but its mechanisms have been largely unknown for many years. To address this long-standing question, we focused on DSB repair machineries: non-homologous end joining (NHEJ) and homologous recombination (HR). We treated HeLa and HCT116 cells with hyperthermia (43oC, 60 min) and systematically analyzed the level of NHEJ and HR factors by western blotting. We found that BRCA2, involved in HR, was mostly diminished, probably due to degradation, and that DNA ligase IV, involved in NHEJ, became insoluble. We previously found that Ku, involved in NHEJ, was inactivated in rodent cells, but not in human cells. In agreement with this, we did not observe any discernable change in Ku, suggesting the existence of the mechanisms protecting and recovering Ku from damage by hyperthermia. Elucidation and modification of such mechanisms might provide us with a new approach to improve cancer therapy through combination of hyperthermia and radiation. This work is partially supported by KAKENHI (17K20042). We thank Motoki Yamaguchi for conducting experiments and Isao Yoda for cooperation in irradiation.

J-2114

Oncothermia for progressive and recurred breast cancer patients

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IntroductionFor progressive recurrent breast cancer cases, our hospital conducts clinical studies using new hyperthermia (Oncothermia) as one of the options for multidisciplinary treatment.MethodsTen cases of advanced recurrent breast cancer who participated in clinical research since November 2015. Treatment time was 1 hour and the output was increased to 120 W step by step.ResultsIn 6 of 10 cases, other treatments such as anticancer drugs and radiation therapy were used in combination with oncothermia, and in 6 cases , 2 cases (30%) confirmed the shrinkage of the tumor lesion and it was judged as partial response (PR). In one case, there was no change in lesion and it was judged as stable disease (SD), and 3 cases (50%) increased metastasis during treatment and was judged as progressive disease (PD). Four out of ten patients were treated by oncothermia alone, and judged 1 case as PR, 2 cases SD, and 1 case PD. No apparent side effects appeared in all cases.ConclusionIt was suggested that oncothermia in progressive and recurred breast cancer patients may be effective for skin metastasis.

▲TOP

[J-2115] J24 [Japanese]
Cancer epidemiology

2018 / 9 / 28 (Fri) 15:30-16:45 Room 12/12F 1202, Osaka International Convention Center Room 12

Isao Miyashiro / Cancer Control Ctr., Osaka InterNatl. Cancer Inst.

J-2115

Cancer statistics -past trends and future perspectives

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Objectives: Cancer statistics is essential to cancer control. Along with a long history of cancer mortality data, cancer incidence and survival data have also recently been established especially after the legislative action. This study aims to analyze the past trends in cancer statistics and to show future perspectives.
Methods: Cancer mortality, incidence, and survival data were obtained from the vital statistics and Monitoring of Cancer Incidence in Japan (MCIJ). Age-adjusted trends in cancer mortality and incidence were examined using Joinpoint regression analysis developed by the U.S. National Cancer Institute.
Results & Discussion: Stomach, liver, lung and prostate cancers have been significantly decreasing in mortality. However cervical cancer has been significantly increasing, and colorectal and female breast cancers have been stable, though those cancers seemed to be improving in survival. All those cancers have been significantly increasing in incidence except for stomach, colorectal (stable), and liver (decreasing) cancers. Several activities to deepen the cancer statistics and its interpretation are ongoing including simulation approaches and geographical analysis.

▲TOP

J-2116

Smoking is a significant risk factor for acute myeloid leukemia : A pooled analysis of 9 cohort studies in Japan

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Smoking is an important modifiable risk factor for cancers, but epidemiological evidence on the risk of acute myeloid leukemia (AML) is scarce in Japan. Here, we evaluated the association of smoking habits with the risk of AML by a pooled analysis of nine population-based prospective cohorts in Japan. Hazard ratios (HRs) and 95% confidence intervals (95% CIs) in the individual cohorts were estimated using a Cox regression model and combined using a random-effects model. During an average 13.9 years of follow-up for a total of 344,676 subjects, we identified 245 newly diagnosed AML cases. Current smokers had a marginally significant increased risk of AML compared to never smokers (HR=1.44, 95%CI: 0.97-2.14). In addition, ever smokers with more than 30 pack-years had a statistically significant increased risk of AML compared to never smokers (HR=1.66, 95%CI: 1.06-2.63). Stratified by sex, this significant association was observed only among men (HR=1.69, 95%CI: 1.00-2.87). In conclusion, this study provides important evidence that smoking increases the risk of AML in Japan.
Collaborators: Nakayama T, Liu R, Sugawara Y, Tsuji I, Sadakane A, Mizoue T, Inoue M, Tsugane S.

J-2117

The association of PSCA gene and H. pylori-related gastric atrophy risk detected by GWAS and SKAT

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Helicobacter pylori (HP) is a well-known virulence factor causing gastric cancer in Japanese. We conducted the genome-wide association study and the gene-based SNP-set Kernel Association Tests (SKAT) on the risk of HP-related gastric atrophy (GA) using the data of the 3,385 participants from the four areas of J-MICC Study (Daiko, Aichi, Okazaki & Kyoto). 1,239 subjects positive for serum HP antibodies were analyzed in the GWAS and gene-based SKAT to detect the genes & SNPs associated with GA. GA was diagnosed by the serum pepsinogen levels, and the genotypes were determined by the Illumina SNP array. PSCA gene was detected as the top significant gene with the linear Kernel with the P-value of 5×10^{-7} and the FDR of 0.01, which finding was supported by the PSCA SNPs found to be genome-wide suggestive levels ($P < 1\times10^{-6}$). The replication study to verify these results with an independent data set is now ongoing. To the best of our knowledge, the present study is the first GWAS that clarified the roles of the PSCA gene with the risk of HP-related GA in Japanese as well as in East Asians.

J-2118

Association of cruciferous vegetable intake and all cause and cancer mortality among Japanese: the JPHC study

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Background: Cruciferous vegetables contain isothiocyanates, which effectively inhibit the bioactivation of procarcinogens, and enhance the excretion of carcinogens. However, a large cohort studies investigated the association of cruciferous vegetable intake with all cause and cancer mortality is sparse.Methods: The analysis included 88,184 subjects aged 45 to 74 years. Cox proportional hazard regression analysis was used to compute the hazard ratio (HR) and 95% confidence intervals (95% CI).Results: After 16.9 years of follow up, a total of 15,349 deaths of which 5,995 cancer deaths occurred. An inverse association was found between cruciferous vegetable intake and total mortality in both gender. Cruciferous vegetable intake was associated with lower cancer mortality in men with HR of 0.84 (95% CI: 0.75, 0.96); however no association was found in women. Conclusion: This prospective study suggests that a higher cruciferous vegetables intake is associated with reduced risk of mortality from all cause in both gender and cancer in men.Reference: Mori et al. “Cruciferous vegetable intake and mortality in middle aged adults: A prospective cohort study”. Clinical Nutrition (in press).

▲TOP

J-2119

Associations of cell-phone use and screen time with overweight: the Hekinan Children's Study

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Purpose: Obesity in childhood has been linked to adult obesity, which is a risk factor for some cancer. We examined whether cell-phone use and screen viewing were associated with excess body weight in schoolchildren.Methods: Subjects were 3,141 first-year students who participated in the baseline survey of Hekinan Children's Study, conducted annually in autumn between 2011 and 2015. The participant rate was 87.4%. They were asked to submit a questionnaire administered by parents. Heights and weights were measured at the schools. Overweight was defined according to the cut-off point for children specified by the Extended International Obesity Task Force. The associations of cell-phone use, time spent watching television, and time spent on a game and a computer with overweight were assessed using logistic regression models.Results: Among 2,594 analytic subjects, cell-phone users had a high relative risk for overweight compared with non-users after adjustments for potential confounders. Time spent watching television was positively associated with the risk of overweight.The authors thank Dr Nagai, Dr Itakura, Dr Harada, Dr Takahara, and Dr Yamanaka, the Hekinan Medical Association.

J-2120

Establishment of BioBank Japan searching system for biospecimen, based on clinical information database

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BioBank Japan (BBJ) project was launched in 2003 for the implementation of personalized medicine. Through the collaboration with more than 60 hospitals, BBJ collected DNA, serum, and clinical information derived from about 270,000 patients with 51 diseases. More than 16,000 DNA and 10,000 serum samples were distributed to 59 researchers in academic institutions or companies. In addition, genomic information of about 1 million SNP loci from 182,000 patients were deposited in public database. To accelerate utilization of the biospecimen and their genomic data, we constructed a large clinical information database and conducted epidemiological analyses. Positive family history was associate with 3-8 times higher risk of various cancers, indicating the important role of host genetic factor. We also established searching system for biospecimen and genomic information, including basic clinical measurements: age at baseline, sex, registration status of the target diseases, smoking and alcohol intake history, as well as disease-specific ones: histology of tumors and family history etc. This searching system may provide easier accessibility and promote utilization of the biospecimen of BBJ.

▲TOP

[ML11] ML11 [Japanese]
Morning Lectures 11

2018 / 9 / 28 (Fri) 8:00-8:50 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13

Toshinari Minamoto / Div. Transl. Clin. Oncol., Cancer Res. Inst., Kanazawa Univ

ML11

Transcription factors regulated by selective autophagy

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The discovery of Autophagy-related genes (ATGs) by Dr. Yoshinori Ohsumi opened the door to solve the molecular mechanisms of autophagy. But, the research on autophagy does not still mature, rather it contains a large number of issues that should be addressed. To date, a series of ATG genes has been identified, but working mechanism of each ATG gene product remains unclear. Moreover, while growing lines of evidence shed light on the importance of unconventional mode of autophagy such as selective autophagy, the role in our life course is still a mystery. Further, while autophagy is considered to be involved in various vital events such as cellular differentiation, stem cell homeostasis and anti-aging, those regulatory mechanisms through autophagy are still not clear. In this lecture, I show the cutting-edge of the autophagy-study and discuss about perspective of the research of autophagy-field.

▲TOP

[S9-1] S9 [English]
An update on hereditary tumors

2018 / 9 / 28 (Fri) 9:00-11:30 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13

Yoshio Miki / Dept. Mol. Genet., Inst. Med. Sci., Tokyo Med. & Dent. Univ.、 Naohiro Tomita / Div. Lower GI Surg. Dept. Surg. Hyogo Col. Med.

The era of genomic medicine is beginning in many disorders. Genomic medicine is making a major impact in guiding diagnoses and treatment, especially in the fields of oncology. In cancer genome medicine, hereditary tumor has been a typical example of development from basic research to clinical practice, and significant advances of technology in next-generation sequencing have led to major changes in the field of hereditary tumors. The genetic testing for hereditary tumors has become possible in a short time and at a low cost; however, there are many unresolved problems such as application for insurance, systems to provide genetic counseling, ethics issues, and so on. In Japan, clinical applications of cancer genome sequence are going to be implemented in diagnosis and treatment of sporadic cancers, but it is important to consider the management of hereditary tumors identified as secondary findings.
In this session, researchers and experts who are involved in hereditary tumors at the forefront of basic research or clinic will provide their latest information and exchange opinions on the current problems related to hereditary tumors.

S9-1

Up-to-date information on the clinical management of hereditary colorectal cancer, including immunotherapy

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Colorectal cancer (CRC) is the most common cancer and the second leading cause of cancer-related death in Japan. Approximately 30% of patients with CRC have a family history, and 5% of CRC cases can be attributed to high-risk variants of known CRC susceptibility genes. Correctly diagnosing hereditary CRC is of paramount importance to patient care. Inherited forms of CRC are often divided into two categories: polyposis syndromes and nonpolyposis syndromes. However, differentiation between polyposis, especially attenuated polyposis, and nonpolyposis is often challenging. Next-generation sequencing methods have revealed several conditions with similar phenotypes, such as Lynch syndrome, Lynch-like syndrome, constitutional mismatch repair deficiency syndrome, and polymerase proofreading-associated polyposis. Recently, immune checkpoint inhibitors have been shown to be a promising treatment for hyper-mutated cancers, including hereditary CRC, such as constitutional mismatch repair deficiency, polymerase proofreading associated polyposis, and Lynch syndrome.

▲TOP

S9-2

Hereditary breast and ovarian cancer syndrome

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Patients of hereditary breast and ovarian cancer syndrome (HBOC) caused by pathological mutation of BRCA1 and BRCA2 accounts for about 3 to 5% of all breast cancer (BC) patients, estimated about 3000 to 4500 new patients per year in Japan. Hospitals providing genetic counseling and testing service of HBOC are increasing to more than 140. However, only a few hospitals provide risk-reducing surgery for BRCA1/2 mutation carriers. Olaparib, a poly ADP ribose polymerase (PARP) inhibitor expected as a specific remedy for HBOC, has been used for ovarian cancer (OC) since January 2018, and will be available for BC from July in Japan. For BC patients, it can be used only for those with pathological BRCA1/2 mutation carriers diagnosed by means of a companion genetic test. Therefore, most of patients with advanced and unresectable BC will have genetic test in every clinic and hospital treating BC, and a lot of BRCA1/2 mutation carriers who need genetic counseling will be found among those patients and their family members. It is an urgent issue to establish ubiquitous genetic services for counseling, surveillance, risk-reducing surgery, and some economical support for those.

S9-3

Hereditary thyroid cancer

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Hereditary cancer, also known as a familial tumor, develops as a result of carrying a susceptibility gene. Among thyroid cancer, hereditary cancer also recognized. The different types of hereditary thyroid cancers (HTC) include medullary thyroid cancer (MTC)related to the MEN 2 or familial medullary thyroid cancer (FMTC), familial non-medullary thyroid cancer (FNMTC), cribriform morula variant papillary thyroid cancer (CMVPTC) related to the familial adenomatous polyposis (FAP) and follicular thyroid carcinoma (FTC) related to the Cowden disease. MTC with MEN2 or FMTC, such as RET gene mutation is recommended total thyroidectomy and lymph node dissection. Nowadays there is a risk classification by RET mutations among hereditary MTC. Patients carrying such mutations without occurrence of MEN2 are recommended prophylactic thyroidectomy due to the risk classification by RET mutation. RET mutation is also an important preoperative marker for MTC with or without hereditary, as it is dependent on the decision of total or hemithyroidectomy. In this session, I would like to introduce the characteristic and management of HTC compared to common thyroid cancers.

S9-4

Action and application of PARP inhibitors

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Poly(ADP-ribose) polymerase (PARP) inhibitors have been clinically shown to be effective in hereditary and sporadic cancers harboring BRCA1/2 mutations; the combination of PARP inhibitor and BRCA1/2 mutation induces "synthetic lethality" and it is explained by the severely disturbed homologous recombination (HR) repair (Bryant et al. , Farmer et al. , Nature, 2005) and PARP trapping on DNA (Murai et al. , Cancer Res, 2012). Mutations or epigenetic aberrations that lead to similar defects in BRCA1/2 mutated cancers are called "BRCAness". Gene mutations including ATM, ATR, CtIP, and PTEN have been reported to cause BRCAness. Pharmacologically induced synthetic lethality has been recently reported in prostate cancer models using androgen receptor signaling inhibitor, enzalutamide, that suppressed HR gene expressions (Li et al., Sci. Sig, 2017). Comprehensive screenings and other studies have shown the novel genes that affect sensitivity and resistance to PARP inhibitors. Recent progress on the biology of PARP inhibitors will be discussed.

▲TOP

S9-5

Construction of Integrated Database of Clinical and Genetic Information for Lynch syndrome in Japan

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Generally, lower prevalence rate of hereditary cancers results in scattered information about the diseases. Therefore, it is difficult to get or share the disease information for clinical practice. Lack of disease information makes appropriate medical management, such as diagnosis, prevention, surveillance and therapy difficult. To resolve these problems, information about the hereditary cancer should be gathered in one and accessed freely if necessary. Nevertheless, it may not be enough, international cooperation is also required. This time, we try to construct integrated database of clinical and genetic information for Lynch syndrome, as a model case of hereditary cancer database. Genomic information obtained from colorectal cancer susceptibility gene panel analysis and clinicopathological information are entered in the format for statistical analysis. Therefore, information you want to know are displayed as a graph or table through the image viewer developed for this database. By collecting the information about the other hereditary cancers with same format and gathering in one, the database constructed will be good usability for clinical practice.(Grant Number JP18kk0205004)

S9-6

A report from a genetic counseling outpatient clinic for hereditary cancer syndrome in the age of NGS

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Clinical NGS has brought about 2 major changes in the clinical practice of hereditary cancer syndromes (HCS). The 1st is the so-called "genome-first" approach, in which screening type of genetic tests have been offered increasingly to the clients, especially those with a borderline possibility of HCS. This is probably a welcoming trend in response to the gradual but steady increase in the awareness of genetic cancer risk in Japan, in spite of the relative decline of the available family history in the age of small family and personal information protection. However, such "genome-first" approach has been generating a number of VUS (variants with uncertain clinical significance) for both primary (PF, variants on the genes that may be responsible for the observed phenotype) and secondary findings (SF) and demands substantial effort in the clinical annotation/ curation of the variants. The 2nd NGS-originated new addition to the traditional HCS genetic counseling is SF from the somatic clinical sequencing. Here the major challenge would be how to communicate with the patients and families, who may be neither really seeking nor ready for their genetic risk of HCS.

S09-Special_Remarks

Clinical limitations, implications and ethical considerations of genetic analysis in hereditary tumors

Eiso Hiyama
Natural Sci. Ctr. for Basic Res. & Development, Hiroshima Univ.

Technological advances including next-generation sequencing (NGS) and genome-wide association studies (GWASs) have expanded in the last decades with significant improvements in reliability, sequencing chemistry, pipeline analyses, data interpretation and costs. GWAS has identified novel risk loci and likely causal genes in hereditary tumors, and NGS has also identified mutations in inherited cancer syndromes based on DNA-sequencing. Such advances make these technologies useful clinical strategies in diagnosis and prediction of hereditary tumors. However, some of these results may be the secondary or incidental findings and some may not be involved in pathogenesis. Moreover, genetic testing encounters many ethical problems among the wishes or interests of related people including future children, requiring genetic counselling before and after testing, and informed consent of patients.As conclusive remarks, I would like to summarize clinical limitations, implications and ethical considerations of genetic analysis in hereditary tumors.

▲TOP

[LS23] LS23 [Japanese]
New applications for Clinical Sequencing

2018 / 9 / 28 (Fri) 11:50-12:40 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13
：TAKARA BIO INC.

Junichi Mineno / TAKARA BIO INC. Bioindustry Business Unit

LS23

New applications for Clinical Sequencing

Yoshimasa Tsujimoto
Department of Clinical Genomics Graduate School of Medicine, Osaka University

No Abstract

▲TOP

[S12-1] S12 [English]
Animal models in cancer research

2018 / 9 / 28 (Fri) 13:00-15:30 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13

Takuro Nakamura / Div. Carcinogenesis, Cancer Inst., JFCR、Akira Suzuki / Div. Mol. & Cell. Biol., Kobe Univ. Grad. Sch. Med.

Generation of animal models for human cancers is very important for understanding the nature of human tumorigenesis and malignant progression. In addition, these models are very useful for exploring and evaluating the new anti-cancer drugs and cancer treatments. A lot of efforts have been paid to generate unique and useful models that recapitulate human malignancies.
In this symposium, we will invite five speakers who generate innovative mouse models showing quick cancer onset, utilizing a novel in vivo gene activation method via CRISPR/Cas9 system, clarifying genetic pathways by sleeping beauty transposon-mediated mutagenesis, investigating chromatin remodeling mechanisms in leukemia, and revealing genetic and epigenetic interactions in bone and soft tissue sarcomas.
Our objective with this symposium is to show these new animal cancer models or innovative methods that can attract the interests of researchers in many fields from basic to clinical, and that can assist the progress and the goals for cancer research, diagnotics and therapy.

S12-1

Critical role of Hippo signaling pathway at the onset of squamous cell carcinomas

Akira Suzuki
Div. Mol. Cell Biol, Grad. Sch. Med., Kobe Univ., Med. Inst of Bioregulation, Kyushu Univ.

Co-author：Miki Nishio[1]、Hirofumi Omori[1]、Yoko To[2]、Tomohiko Maehama[3]、Yukari Aono[3]、Tohru Kiyono[4]、Kenichi Taguchi[5]、Muneyuki Masuda[5]、Shinya Toyokuni[6]、Hironori Tashiro[7]、Hidetaka Katabuchi[7]
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Hippo pathway is regulated by the surrounding microenvironment such as cell-cell contact, extracellular matrices, and external forces. Dysregulation of this pathway leads to organomegaly, and tumorigenesis.
Core components of the Hippo pathway include the MST kinase/SAV1 and LATS-NDR kinases/MOB1 complexes. The Hippo core components function as negative regulators for cell proliferation by repressing the transcriptional co-activators YAP/TAZ that essentially promote cell proliferation. In fact, the loss of MOB1, a common adaptor protein of LATS and NDR kinases, strongly activates YAP/TAZ.
Squamous epithelial cells in Cervix and Head & Neck exhibit tight cell adhesion and are readily affected by rich extracellular matrices and external forces. We therefore generated uterus and tongue-specific MOB1-deficient mice, respectively. These mutant mice all show early onset of cancers and YAP is activated from precancerous stage of these tumors in human, indicating that Hippo pathway is the master regulator for the onset of these squamous carcinomas.
We believe these mice are quite useful as the spontaneous and quick onset tumor burden models to explore the new anti-cancer drugs.

▲TOP

S12-2

Genome editing technology-based epigenetic gene activation in vivo

Fumiyuki Hatanaka
Salk Inst.

Co-author：Hsin-Kai Liao、Juan Carlos Izpisua Belmonte
Salk Inst.

Current genome editing systems generally rely on inducing DNA double-strand breaks (DSBs). In the meantime, concerns about unwanted mutations caused by DSBs have never been alleviated, particularly for applying genome editing in clinical therapeutics. CRISPR/Cas9 system has been repurposed to enable target gene activation, which allows controlling endogenous gene regulation without creating DSBs. However, in vivo implementation of these gain-of-function systems has proven challenging. Here we report a robust system for in vivo activation of endogenous target genes with trans-epigenetic remodeling. The system relies on recruitment of Cas9 and transcriptional activation complexes to target loci by modified single guide RNAs. As proof-of-concept, we used this technology to treat several disease mouse models. Our results demonstrate that CRISPR/Cas9-mediated target gene activation can be achieved in vivo, leading to observable phenotypic changes, and ameliorate disease symptoms, thus opening new avenues for developing targeted epigenetic therapies against human diseases.

S12-3

Abnormal hematopoiesis and hematological malignancies induced by dysregulated polycomb gene functions in mice

Atsushi Iwama
Grad. Sch. Med., Chiba Univ., Chiba, Japan

Polycomb-group (PcG) proteins function as transcriptional repressors through histone modifications. Of great interest, inactivating mutations of polycomb repressive complex (PRC) 2 genes, such as EZH2, have been identified in patients with myelodysplastic disorders including myelodysplastic syndrome (MDS) and MDS/myeloproliferative neoplasms (MPN) overlap disorders. In line with these findings, deletion of Ezh2 alone was enough to induce MDS and MDS/MPN in mice, and Ezh2 loss cooperated with other mutations such as TET2, RUNX1 and JAK2V617F in the pathogenesis of MDS, MPN and MDS/MPN. PRC1.1, consisting of RING1B, PCGF1, KDM2B, and BCL6 corepressor (BCOR), represents one of the variant PRC1 complexes. Recently, inactivating mutations of BCOR have also been implicated in hematological malignancies. We found that deletion of Bcor induces acute T lymphoblastic leukemia (T-ALL), and concurrent depletion of Bcor and Tet2 induced lethal MDS and MDS/MPN. suggesting a tumor suppressive function of PRC1.1 in hematopoietic system. I would like to show several mouse models of hematological malignancies with PcG insufficiency and discuss the role of PcG genes in hematological malignancies.

S12-4

Forward genetics using CRISPR-Cas9 in intestinal organoids identified novel colorectal tumor suppressor genes

Haruna Takeda
Kanazawa Univ., CRI

Large scale transposon based genetic screening in mice identified numerous candidate cancer driver genes in the intestine. Likewise, next generation sequencing on CRC genomes identified numerous genes mutated in cancer. These comprehensive genomic datasets help to enrich cancer driver genes from passenger genes, however, it is necessary to experimentally validate the function of mutated genes in cancer development. To validate candidate tumor suppressor genes, we introduced Cas9 and the pool of gRNAs targeting multiple genes in mouse intestinal tumor organoids and transplanted the organoids subcutaneously to induce tumor development. We analyzed the tumor genome to identify the gRNAs whose fraction were dominated in the tumor tissue compared to the matched organoids, and enriched 5 candidate genes. We further performed single gene knockout in AK organoids and showed that knockout of 3 genes could promote tumorigenesis. Here, we have established the system to achieve efficient validation for cancer driver genes using a gene editing approach with CRISPR-Cas9 and identified 3 genes that function as tumor suppressor. These novel genes can be a new drug target to cure cancer.

▲TOP

S12-5

Modeling bone and soft tissue sarcoma to clarify fusion gene and epigenome interaction

Takuro Nakamura
Div. Carcinogenesis, Cancer Inst, JFCR

Childhood cancers are characterized by low mutation rates, disease-specific mutations, and a single cancer-driving mutation in contrast to adult cancers. These driving mutations frequently involve transcription factors and co-factors, suggesting the important role of epigenetic background in tumorigenesis. Fusion gene-associated bone and soft tissue sarcoma is one of such malignancies, of which the majority affect children, adolescents and young adults. We have generated a series of fusion gene-associated mouse sarcoma models. In these models, sarcoma cells are highly dependent on fusion gene expression, and tumor phenotypes and gene expression profiles are quite similar with those of human sarcomas. These data indicate that interactions between fusion genes and specific epigenetic status are important among the species. The presentation will focus on chromatin remodeling functions of EWS-FLI1 and ASPSCR1-TFE3 for Ewing sarcoma and alveolar soft part sarcoma, respectively. The roles of cis-acting elements and important co-factors will be discussed. Importance of mouse models in these studies will be emphasized.

S12-Special_Remarks

Special Remarks

Masayuki Miyasaka
Inst. of Academic Initiatives, Osaka Univ.

No Abstract

[S13-1] S13 [English]
Frontier of basic research and clinical practice targeting individualized medicine

2018 / 9 / 28 (Fri) 15:30-18:00 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13

Yuko Kitagawa / Dept. Surg. Keio Univ. Hosp.、Kazuto Nishio / Dept. Genome Biol, Kindai Uni., Sch. Med.

Progress in the field of molecular biology has facilitated the identification of multiple gene mutations and other abnormalities those are associated to the cellular transformation of cancer.
These changes in genes and its products are expected to serve as tumor biomarkers that could be utilized to predict therapeutic drug efficacy, facilitate classification and diagnosis, and prognosis. On this topic, Professors Takeuchi and Nishibeppu will introduce new biomarkers for early detection and resistance in this symposium and Dr. Suda will propose a new concept of “compound mutations” for lung cancer.
Increased CDx has made individual testing problematic because of limited sample availability and long turnaround times. NGS-based gene panel testing allows for detection of multiple genomic mutations to facilitate the selection of the best treatment option. Gene panel testing using liquid biopsy has been feasible and Dr. Sakamoto will present his advanced research using liquid-biopsy-based panel testing for urological cancers while Dr. Mukai will report progress on the Precision Medicine Initiative in Japan. Professor Tetsuichiro Muto will also deliver special remarks on the issue.

S13-1
Precision surgery for early-stage gastric cancer based on sentinel node concept

Hiroya Takeuchi
Dept. Surg. Hamamatsu Univ. Sch. Med.

Co-author：Yuko Kitagawa
Dept. Surg. Keio Univ. Sch. Med.

Background: Clinical application of sentinel node (SN) mapping for early-stage gastric cancer had been controversial for years. However, SN mapping may play a key role to obtain individual metastatic information and allows modification of the surgical procedures for early gastric cancer. Results: A Japanese group conducted a prospective multicenter trial of SN mapping for early gastric cancer. SN mapping had been performed for 397 patients with early gastric cancer. As results, detection rate of SN was 98%. The sensitivity to detect metastasis based on SN status was 93%, and accuracy of metastatic status based on SN was 99%. Based on the results, minimized gastrectomy such as partial gastrectomy, and segmental gastrectomy for early gastric cancer with negative SN has been developed. Conclusions: SN concept for cT1N0 early gastric cancer could be validated, and modified gastrectomy with personalized minimally invasive surgery which might retain the patients' quality of life (QOL) should be established. A multicenter prospective trial is now ongoing in Japan to evaluate function-preserving gastrectomy with SN mapping in terms of long-term survival and patients' QOL after surgery.

▲TOP

S13-2

Plasma microRNA profiles: identification of novel microRNA as a biomarker for chemoresistance in gastric cancer

Keiji Nishibeppu
Divi Dig Surg, Dept. Surg, Kyoto Pref Univ. Med.

Co-author：Shuhei Komatsu、Taisuke Imamura、Jun Kiuchi、Toshiyuki Kosuga、Kazuma Okamoto、Hirotaka Konishi、Takeshi Kubota、Atsushi Shiozaki、Hitoshi Fujiwara、Eigo Otsuji
Divi Dig Surg, Dept. Surg, Kyoto Pref Univ. Med.

BACKGROUND: This study aims to explore novel microRNAs in plasma for predicting chemoresistance in adjuvant chemotherapy of patients with gastric cancer (GC) using a microRNA array-based approach.MATERIALS AND METHODS: We used the Toray 3D-Gene microRNA array-based approach to compare preoperative plasma microRNA levels between patients with or without recurrences. All patients underwent an adjuvant chemotherapy regimen with S-1.RESULTS: (1) Of 2566 candidates, four candidate microRNAs, which were highly expressed in the preoperative plasma of patients with recurrences, were selected. (2) In a large-scale validation analysis by quantitative RT-PCR, we focused on plasma levels of miR-1229, which was an independent poor prognostic factor for relapse free survival (P=0.006, HR=5.99) (3) After overexpressing miR-1229 in GC cells, miR-1229 induced significant chemoresistance to 5-fluorouracil (5-FU), but not other anticancer drugs, up-regulating thymidylate synthase (TS), dihydroprimidine dehydrogenase (DPD) in vitro and vivo analyses.CONCLUSIONS: Plasma miR-1229 could be a useful next-generation biomarker for predicting chemoresistance to S-1 in GC patients.

S13-3

Compound mutations - focusing on EGFR-mutated lung cancers

Kenichi Suda
Div. Thoracic Surg., Dept. Surg., Kindai Univ. Faculty Med.

Before the discovery of the EGFR mutations in 2004, NSCLCs were treated as a single disease. We now treat NSCLCs based on the type of driver mutations: e.g. EGFR-TKIs for EGFR-mutated NSCLCs. However, recent studies have revealed that EGFR-mutated NSCLCs are not a single disease due to compound mutations. These studies observed that 14 - 25 % of EGFR-mutated NSCLCs have double EGFR mutations, usually a common TKI-sensitizing mutation (e.g. G719X, L858R, or exon 19 del.) together with an uncommon one (e.g. E709X, T790M, or S768I). The prevalence of compound mutations are highest in G719X, followed by L858R / L861Q mutations. Compound EGFR mutations may directly affect the efficacy of EGFR-TKIs and the prognosis of patients. In a broad sense, compound mutations include somatic aberrations in genes other than the EGFR. One of the examples is rare tumors with co-driver mutations such as co-existence of an EGFR mutation and an ALK fusion. Another example is concurrent mutations (e.g. TP53 or PTEN) / copy number aberrations (e.g. RB1 loss) which co-exist with EGFR mutations homogeneously or heterogeneously. These concurrent genetic aberrations may also have impact on patients' outcomes.

S13-4

Precision medicine in prostate cancer

Shinichi Sakamoto
Dept. Urol., Chiba Univ. Grad. Sch. Med.

Co-author：Keisuke Ando[1]、Maimaiti Maihulan[1]、Kazuto Nishio[2]、Tomohiko Ichikawa[3]
[1]Dept. Urology, Chiba Univ. Grad. Sch. Med.、[2]Dept. Genome Biol. Kindai Univ. Faculty of Med.、[3]Dept. Urol., Chiba Univ. Grad. Sch. Med.

Prostate cancer is the most prevalent male cancer in Japan. Since prognosis of prostate cancer is relatively long, the establishment of the treatment sequence during the course of treatment is of primary concern. The current treatment strategy is build based on Pathology, Clinical T stage, and PSA, which has not been changed for 30 years. However, recent evidence indicated the significance of monitoring somatic and genomic mutations. In castration sensitive prostate cancer, somatic mutations are less than 8 %. In castration resistant prostate cancer, the rate of androgen receptor (AR) amplification, TP53 miss sense mutations, and PTEN loss were 63%, 53%, and 41%, respectively. A clinical trial of PARP inhibitor revealed 88% of response rate among patients with a defect in DNA-repair genes. The emergence of AR V-7 is related to the resistance of the novel AR-targeted drugs. These pieces of evidence indicated the treatment sequence of prostate cancer will be decided based on mutation burden at the various steps as monitored by liquid biopsy. In the current presentation, we will discuss the current status of the precision medicine based on the clinical sequence in prostate cancer.

▲TOP

S13-5

Establishing a sustainable system for cancer genomic medicine in Japan

Yosuke Mukai
Health Service Bureau, Ministry of Health, Labour & Welfare

Co-author：Hideki Ueno、Masaharu Tanto、Masahiro Sasaki
Health Service Bureau, Ministry of Health, Labour & Welfare

Approximately one in two people in Japan still develop cancer within their lifetime. In order to promote cancer genomic medicine (CGM), Japanese authorities are gradually establishing a national system for providing CGM and building a institution that aim to collect, store and process genomic and clinical information based on " The Third Term Basic Plan to Promote Cancer Control Projects ".
In February 2018, Japanese authorities have designated 11 hospitals as " Designated core hospitals for CGM " which fulfill eight requirements such as implementing expert meetings and providing valid patient information to the Center for Cancer Genomics and Advanced Therapeutics (C-CAT). In order to provide CGM nationwide, Japanese authorities also declared 100 hospitals as " Cooperative hospitals for CGM " in March 2018. In addition, authorities have established the C-CAT, which maintains a master database of patient information and cancer knowledge database.
The authorities also plan to develop a Council that steers the direction of cancer genomic medicine in Japan, and further promotes the CGM project in collaboration with academia, industry and government.

S13-Special_Remarks

Special Remarks

Tetsuichiro Muto
Cancer Inst. Hosp.

No Abstract

[ML12] ML12 [Japanese]
Morning Lectures 12

2018 / 9 / 28 (Fri) 8:00-8:50 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Shinichiro Motohashi / Dept. Med. Immunol. Grad. Sch. Med. Chiba Univ

ML12

CAR T cell therapy

Naoki Hosen
Dept. Cancer Stem Cell Biol., Osaka Univ. Sch. Med.

Discussant：Kazuhito Yamamoto
Dept. Hematol. Cell Therap., Aichi Cancer Ctr.

Chimeric antigen receptor (CAR) T cell therapy is a novel immunotherapy. A CAR combines antigen-binding domains derived from the antibodies with the signaling domains of the TCRξ chain and additional costimulatory domains from receptors such as CD28 or 41BB. CARs are transduced into T cells ex vivo, creating expandable antigen-specific CAR T cells. On the basis of dramatic efficacy in clinical trials, CAR T cells targeting CD19 have recently been approved by the U.S. Food and Drug Administration (FDA) for treatment of B cell leukemia and lymphoma. Although serious cytokine release syndrome (CRS) associated with CAR T cell therapy was a major concern, tocilizumab has been proved to suppress CRS without impairing anti-tumor effect. Now many researchers aims to expand CAR therapy to other types of cancers. However, appropriate target antigens are lacking in many cancers. Recently, we discovered that the active conformer of an integrin could serve as a specific therapeutic target for multiple myeloma (MM), suggesting that more cancer immunotherapeutic targets can be identified in cell surface proteins that undergo conformational changes.

▲TOP

[IC1] IC1 [Japanese]
Introduction Course for Current Cancer Research 1

2018 / 9 / 28 (Fri) 9:00-9:35 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Hirofumi Yamamoto / Det. Mol Path, Osaka Univ.

IC1

Hiroshi Maeda
BioDynamics Res. Foundation., Dept. Microbiol., Grad. Sch. Med. Sci., Kumamoto Univ., Osaka Univ. Med. Sch., Tohoku Univ.

Please see the Japanese Abstract which I sent to JCA congre on May 11, 2018 by E-mail.

▲TOP

[IC2] IC2 [Japanese]
Introduction Course for Current Cancer Research 2

2018 / 9 / 28 (Fri) 9:35-10:10 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Kiyoko Kato / Ob&Gy Dept. Kyushu Univ., Sch. Med.

IC2

Recent progresses in ovarian cancer study focusing on genomic analyses

Tadashi Kimura
Dept. Obstet. Gynecol. Osaka Univ. Grad. Sch. Med.

Genomic or proteomic analyses drastically changed the world of ovarian cancer studies. In 2011, The Cancer Genome Atlas (TCGA) project analyzed mRNA expression, miRNA expression, promoter methylation, and DNA copy number in 489 high-grade serous ovarian adenocarcinomas (HGSOC) with their corresponding prognostic data. It allows a systematic genomic comparison of cell lines and HGSOC tumors and researchers do not have to collect their own cancer tissues any longer. In 2013, Domcke et al. revealed a panel of 47 ovarian cancer cell lines and identified those that have the highest genetic similarity to HGSOC. For instance, SKOV3, which had been firmly believed to be HGSOC and extensively used in tons of works, lacks TP53 mutation, although TCGA revealed TP53 mutations are seen in almost all HGSOCs. SKOV3 is considered as unlikely HGSOCs. A manuscript of which this cell line is used as the model of HGSOC is nowadays hard to be accepted by qualified journals. Genetically engineered mouse (GEM) models of HGSOC and patient-derived xenograft (PDX) models are recently developed by several groups. In this session, I would like to introduce these research progresses in this field.

▲TOP

[IC3] IC3 [Japanese]
Introduction Course for Current Cancer Research 3

2018 / 9 / 28 (Fri) 10:10-10:45 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Meinoshin Okumura / Dept. General Thoracic Surg., Osaka Univ. Grad. Sch. of Med.

IC3
Surgical treatment for lung cancer

Hiroshi Date
Dept. Thoracic Surg., Kyoto Univ. Grad. Sch. Med.

Surgical treatment is the first choice for early stage lung cancer. Minimally invasive surgery such as VATS (video-assisted thoracic surgery) and RATS (robot-assisted thoracic surgery) has been wildly practiced resulting in early recovery, less complications and possibly better survival. Lobectomy is the standard treatment but sublobar resection may be an alternative treatment for less invasive lung cancer with ground-glass opacity. Prospective randomized trial to compare these two options were completed as JCOG study.Surgical treatment is an important option for advance lung cancer as well. Several studies have suggested that induction therapy followed by surgical resection may improve the outcome in patients with stage IIIa (N2) non-small-cell lung cancer. The question remains whether aggressive surgery has a role in the treatment of T4 lung cancer. Patients with T4N0-1M0 cancers invading the distal trachea, carina, left atrium, aorta, superior vena cava, or vertebral bodies may be surgical candidates. Induction chemo-radiotherapy may shrink the tumor, which may result in obtaining better free surgical margin thus may improve surgical radicality.

▲TOP

[IC4] IC4 [Japanese]
Introduction Course for Current Cancer Research 4

2018 / 9 / 28 (Fri) 10:45-11:30 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Shinzaburo Noguchi / Dept. Breast Endocrine Surg., Osaka Univ. Sch. Med.

IC4
Current status and the future perspectives of breast surgical oncology

Seigo Nakamura
Div. Breast Surg. Oncol., Showa Univ. Sch. Med.

Surgery for breast cancer has changed markedly during the past 30 years. Radical mastectomy, referred to as Halstead's operation, has been completely disappeared at present, while breast-conserving surgery (BCS) has become the standard therapy for early breast cancer. The role for axillary management including sentinel lymph node biopsy has been shifting from local control to determination of the strategy for systemic therapy. Therefore, navigation surgery according to the image of MRI or CT has been taking the important role to assess the extension of cancer more accurately compared with the conventional modalities such as mammography and ultrasonography.

▲TOP

[LS24] LS24 [Japanese]
Tissue Biopsy and Liquid Biopsy: Detection and analysis of drug resistance in lung cancer

2018 / 9 / 28 (Fri) 11:50-12:40 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14
：Bio-Rad Laboratories K.K.

Masatoshi Soejima / Bio-Rad Laboratories K.K

LS24

Tissue Biopsy and Liquid Biopsy: Detection and analysis of drug resistance in lung cancer

Ryohei Katayama
Division of Experimental Chemotherapy, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research (JFCR)

No Abstract

[IC5] IC5 [Japanese]
Introduction Course for Current Cancer Research 5

2018 / 9 / 28 (Fri) 13:00-13:35 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Masahiko Watanabe / Dept. Surg. Kitasato Univ., Sch. Med.

IC5
Introduction Course for Current Cancer Research 4

Tsunekazu Mizushima
Dept. Surg. Osaka Univ. Grad. Sch. Med.

Co-author：Shiki Fujino、Norikatsu Miyoshi、Hidekazu Takahashi、Naotsugu Haraguchi、Taishi Hata、Chu Matsuda、Hirofumi Yamamoto、Yuichiro Doki、Masaki Mori
Dept. Surg. Osaka Univ. Grad. Sch. Med.

The data from cancer statistics in Japan expects colorectal cancer to be the most prevalent and the mortality rate to be the second after lung cancer in 2017 (https://ganjoho.jp/reg_stat/statistics/stat/short_pred.html). Various research results so far have revealed the characteristics of colorectal cancer. Standard treatment of colorectal cancer has been established as a guideline by the Japanese Society for Cancer of the Colon and Rectum. Prognosis of colorectal cancer has improved due to advances in chemotherapy with the application of new drugs since the 1990s, the introduction of minimally invasive surgical treatment including laparoscopic surgery and the increased usage of our guidelines. However, there are still many problems to be solved in order to enable patients to receive minimally invasive, functionality preservation and maintaining a quality of life. In this introduction course, we will outline the current state of colorectal cancer treatment according to our guidelines. In addition, we will introduce some clinical problems to be solved for the future advancement of colorectal cancer treatment and improvement of the QOL for patients.

▲TOP

[IC6] IC6 [Japanese]
Introduction Course for Current Cancer Research 6

2018 / 9 / 28 (Fri) 13:35-14:10 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Hisahito Matsubara / Dept. Frontier Surg., Chiba Univ., Grad. Sch. Med.

IC6

Literature review skills for clinical cancer research

Yosuke Adachi
CSME, Med. Kurume Univ.

When I was a resident, my supervisors taught me "Doubt, think, and try" "Both bed-side and bench-side" "See patients, read papers". When I was a senior surgeon at community hospital, I conducted textbook-reading with colleagues or residents, and when I became a chief surgeon at university hospital, I published evidence-based guidebooks for oncologic surgeons. To search for literature, I must have often gone to the library and taken real journals in hand. Nowadays, however, we can access a vast number of papers through online journals anywhere only by entering keywords. The world has become quite a convenient one, and it is easy for us to get abundant (often too much) information for clinical practice and research work. In this session, I try to introduce my experiences and practices concerning (1) how to select useful journals and papers, (2) how to read each paper efficiently and critically, (3) how to get significant findings of each study, (4) how to summarize findings and data of many studies or analyses, and (5) how to integrate literature review. Keep your eyes wide open and always ask yourself "Is it true?" "Why?" "Why not?" Skepticism is the source of research.

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[S14-1] S14 [English]
Inflammation and tumorigenesis

2018 / 9 / 28 (Fri) 14:10-16:40 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Tetsuo Takehara / Gastroenterology & Hepatology, Osaka Univ. Grad. Sch. Med.、Masanori Hatakeyama / Dept. Microbiol, Grad. Sch. Med, Univ. Tokyo

Cancer is a disease caused by abnormalities in genes. Many cancers, such as liver cancer and gastric cancer, develop on the basis of chronic inflammation of organs. Chronic organ inflammation causes genetic and epigenetic abnormalities in epithelial cells via oxidative stress and so on. At this time, cell death and modulation of autophagy influence such transformation and accumulation of transformed cells, resulting in failure of maintaining organ homeostasis. Chronic organ inflammation affects the establishment of the microenvironment of the tumor through changes in the collection and structure of various inflammatory cells or non-epithelial cells and influences the growth and engraftment or infiltration of the transformed cells. At this symposium, six experts will present their outstanding research results. At the end, Professor Yanaga will make special remarks. Subjects include many kinds of carcinomas - including liver, biliary tract, pancreas and mammary gland - and also include topics on cancer microenvironment. Participants will be able to learn about the latest academic progress in this area, as well as acquire knowledge and ideas useful for their own research.

S14-1

Signal peptide peptidase regulates propagation and pathogenicity of HCV

Toru Okamoto
Dept. Mol. Virol, RIMD, Osaka Univ.

Co-author：Junki Hirano[1]、Tatsuya Suzuki[1]、Shinji Kusakabe[1]、Makoto Tokunaga[1]、Takasuke Fukuhara[1]、Kohji Moriishi[2]、Kazuhiko Koike[3]、Yoshiharu Matsuura[1]
[1]Dept. Mol. Virol, RIMD, Osaka Univ.、[2]Dept. Microbiol, Grad. Sch. Med., Yamanashi Uni.、[3]Dept. Gastroenterol., Univ. Tokyo

Hepatitis C virus (HCV) is a major causative agent of chronic liver diseases including steatosis, cirrhosis and hepatocellular carcinoma (HCC). Core protein is the first viral protein to be translated and cleaved from the precursor polyprotein by a host signal peptidase. The immature core protein is further processed by signal peptide peptidase (SPP) to form mature core protein. Transgenic mice expressing HCV core protein exhibit insulin-resistance, steatosis and finally develop HCC, suggesting that core protein plays crucial roles in pathogenesis of HCV. We showed that SPP inhibition produced immature core proteins, which was specifically recognized by TRC8 and was induced proteasome-dependent degradation. We classified the inhibitors of γ-secretase, another intramembrane cleaving protease and found that the dibenzoazepine-type structure in the inhibitors is critical for the suppression of the SPP activity. Docking simulation of the SPP inhibitors based on 3D structure of SPP deduced in silico revealed that Val223 in SPP interacts with the dibenzoazepine-type structure in the inhibitors. These results provide a clue to develop specific inhibitors for SPP.

▲TOP

S14-2

Peribiliary glands: at the crossroads of inflammation, regeneration and cholangiocarcinogenesis

Hayato Nakagawa
Dept. Gastroenterology, The Univ. of Tokyo

Co-author：Yuki Hayata、Tomoharu Yamada、Kazuhiko Koike
Dept. Gastroenterology, The Univ. of Tokyo

The cellular origin of cholangiocarcinoma is a topic of interest. With regard to extrahepatic cholangiocarcnoma (ECC), peribiliary glands (PBGs), a potential stem cell niche of biliary epithelial cells (BECs), have attracted attention as the cellular origin of ECC. We recently reported a new mouse model of ECC through duct-cell-specific activation of Kras and deletion of TGFbR2 and E-cadherin, whose malignant progression depends on biliary epithelial injury and inflammation, and this mouse model suggested PBGs as the cellular origin of ECC. During inflammation, dying BECs released IL-33, as determined by microarray of biliary organoids, and stimulated a regeneration by PBGs via type 2 innate lymphoid cells, eventually leading to ECC. Additionally, we newly established another mouse model of ECC by duct-cell-specific deletion of TGFbR2 and PTEN, which also suggested PBGs as the cellular origin of ECC and the key role of TGFb signaling in PBGs-derived ECC. Furthermore, we identified a molecule expressed predominantly in PBGs and analyzed the behaviors of cells in PBGs by genetic lineage-tracing methods using mice expressing CreERT under the control of this molecule-specific promoter.

S14-3

Inflammation and liver/pancreatic cancer

Atsushi Umemura
Kyoto Pref. Univ. of Med.

Liver inflammation greatly enhances tumorigenesis and, correspondingly, chronic pancreatitis increases cancer risk.Interestingly, pancreatic cancer depends on mutations of driver genes such as KRAS, p16, p53, SMAD4, whereas no common driver mutations responsible for liver carcinogenesis were appreciated.This fact suggests that chronic inflammation gives rise to cancer beyond driver mutation, probably by inducing cell death, stimulating tissue regeneration, secreting cytokines/chemokines/growth factors, and modulating antitumor immunity.In addition, inflammation affects autophagy and metabolism which are tightly related to obesity.Obesity, a chronic systemic inflammatory state, promotes tumorigenesis and progression.Because most of liver cancer arises from chronic inflammatory livers, extensive studies of liver cancer to elucidate the relationship between inflammation and cancer has been performed. In regard to pancreatic cancer, there has been recent advances in understanding of disease progression.These two serious cancers are most affected by obesity, so that they may share common mechanisms which provide insights into " inflammation and cancer " .

S14-4

HMGB1 and other DAMPs in cancer and other diseases; therapeutic implication

Tadatsugu Taniguchi
Ins. Indust. Sci., Univ. of Tokyo

We have been investigating a class of cytokine-like molecules, called DAMPs (Damage-Associated Molecular Patterns), which are typically released by necrotic cell death. I will present our recent data on how HMGB1 functions in inflammation and tumor microenvironment. I also present our data on the efficacy of ISM ODN for cancer immunotherapy. If time permits, I will also present our recent results on other DAMPs, which may also participate to the regulation of cancer progression.

S14-5

CAFs induce formation of metastatic human breast tumor cell clusters with partial epithelial-mesenchymal transition

Akira Orimo
Dept. Mol. Path., Juntendo Univ.

Co-author：Yasuhiko Ito[1]、Yoshihiro Mezawa[1]、Kaidiliavi Sulidan[2]、Yataro Daigo[3]、Nadila Wali[1]、Okio Hino[1]、Kazuyoshi Takeda[4]、Michiaki Hamada[5]、Yuko Matsumura[2]
[1]Dept. Mol. Path., Juntendo Univ.、[2]Dept. Mol. Path., Juntendo Univ., Dept. Obstetrics & Gynecol., Juntendo Univ.、[3]Res. Hosp., Inst. of Med. Sci., The Univ. of Tokyo、[4]Div. Cell Biol., Biomed. Res. Ctr., Juntendo Univ.、[5]Dept. Electrical Engineering & Biosci.

Recent emerging evidence supports that tumor invasion and metastasis depend on non-cell autonomous effect of nearby stromal cells. A large number of carcinoma-associated fibroblasts (CAFs) are often present in the tumor-associated stroma of various human carcinomas. Tumor- and metastasis-promoting properties of these cells are demonstrated by the large body of previous reports. The invasion-metastatic cascade is composed of a series of multi-step processes and a single or clusters of tumor cells are proposed to invade and seed metastasis. However, roles of CAFs on formation of tumor cell clusters and the relevance to epithelial-mesenchymal plasticity have not fully understood yet. We show here that CAFs induce formation of breast tumor cell clusters with partial epithelial-mesenchymal transition. These CAF-primed tumor cell clusters increase an ability to collectively invade and metastasize into distant organs due to greater cell-cell adhesion, anoikis-resistant and lung-colonizing traits. Taken together, these findings strongly suggest crucial roles of CAFs regulating epithelial-mesenchymal plasticity on promotion of tumor malignancy.

S14-6

The Hippo signaling pathway in inflammatory tumor microenvironment

Masanori Hatakeyama
Dept. Microbiol., Grad. Sch. Med., Univ. Tokyo

Deregulation of YAP and TAZ, the Hippo signal-regulated transcriptional co-activators, are crucial for tumorigenesis. We found that TAZ/YAP interact with Parafibromin. Intriguingly, TAZ is stimulated by binding with tyrosine-dephosphorylated Parafibromin whereas YAP is activated through binding with tyrosine-phosphorylated Parafibromin. Thus, Parafibromin inversely regulates YAP and TAZ activities depending on its tyrosine phosphorylation status. High-molecular-weight hyaluronan (HMW-HA), a major component of the extracellular matrix, exhibits anti-oncogenic action whereas low-molecular-weight hyaluronan (LMW-HA), the degradation product of HMW-HA by HYAL2 hyaluronidase, acts oncogenically. However, the mechanisms underlying the size-dependent opposite actions of hyaluronan remain unclear. We show that treatment with HMW-HA stimulates Hippo signaling in mammary epithelial cells via CD44. Conversely, LMW-HA antagonizes the HMW-HA-mediated Hippo signal activation. Elevated HYAL2 inhibits the Hippo signal and thereby excessively activates YAP/TAZ in aggressive breast cancers. Thus, the HYAL2-Hippo axis is an attractive molecular target for the treatment of aggressive breast cancer.

S14-Special_Remarks

Special Remarks

Katsuhiko Yanaga
Dept. Surg., The Jikei Univ., Sch. Med.

No Abstract

▲TOP

[ML13] ML13 [Japanese]
Morning Lectures 13

2018 / 9 / 28 (Fri) 8:00-8:50 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15

Masakazu Toi / Breast Surgery. Kyoto Univ. Med.

ML13

Academic drug discovery for breast cancer

Noriko Gotoh
Div. Cancer Cell Biol., Cancer Res. Inst., Kanazawa Univ.

Discussant：Hiroji Iwata
Aichi Cancer Ctr. Hosp., Breast Oncol.

Recently, many molecular targeting drugs have been developed and many cancer patients have survived longer than before. On the other hand, number of breast cancer patients has been increasing and currently 1 out of 11 Japanese women suffer from the disease throughout life, raising the big problem. In particular, patients with triple negative subtype are still treated with conventional chemotherapy, leading to recurrence. Many of the recurrent cancer patients do not respond well for treatment and eventually die. Emerging evidence suggests that recurrence takes place among residual cancer cells after the treatment. These cells have stemness traits and thus called "cancer stem-like cells". Therefore, it is important to target the cancer stem-like cells to prevent recurrence and improve prognosis. Basic scientists have been working hard to find appropriate targets for cancer stem-like cells. I have an opportunity to be involved in the academic drug discovery program supported by AMED, though I am a basic scientist but not an expert for drug discovery. In this lecture, I would like to discuss how basic scientists are able to contribute to drug discovery.

▲TOP

[S10-1] S10 [English]
Overview of cancer genome research and new challenges

2018 / 9 / 28 (Fri) 9:00-11:30 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15

Johji Inazawa / Dept. Molec. Cytogenet, Med. Res. Inst, Bioresouce Res. Ctr., TMDU、Hidewaki Nakagawa / RIKEN IMS

S10-1

Pan-cancer Whole Genome Sequencing Project (PCAWG) in ICGC/TCGA

Hidewaki Nakagawa
Lab for Cancer Genomics, RIKEN IMS

Co-author：Satoru Miyano
Hum. Genom. Ctr., IMS, Univ. Tokyo

The PCAWG (Pan-cancer Whole Genome Sequencing) study was launched at 2014 and is an international collaboration to identify common patterns of mutation in more than 2,800 cancer whole genomes from the International Cancer Genome Consortium (ICGC). This genome project in 39 distinct tumor types produced large amount data with many types including SNVs, INDELs, structural variations (SVs), copy number alterations (CNAs), pathogens, germline variations, HLA, RNA expression profiles, gene fusions, and phenotypic annotations etc. First, ~0.8 Peta-byte raw sequence data were aligned and processed in the synchronized 10 cloud data centers including AWS and Super computer in Human Genome Center, IMSUT, and called variants in the uniform way by combining three established pipelines (Sanger, Broad, and DKFZ). Second, these variants data in the PCAWG samples (totally, 47M somatic events and 88M germline variants) were analyzed and interpreted by 10~ working groups (driver, SV, signature, evolution, germline, RNA effects, immunogenicity, etc). The methods and results of PCAWG study are overviewed in this session.

▲TOP

S10-2

Cancer Immunogenomics Analysis in ICGC/PCAWG

Seiya Imoto
HIC, Inst. Med. Sci., Univ. Tokyo

Genomic instability and inflammation or immune responses in the tumor microenvironment are major underlying hallmarks of cancer, and the interaction between the cancer genome and immune reactions could have important implications for the early and late phases of cancer development. The immune system is a large source of genetic diversity in humans and tumors. We here analyzed the whole genome sequencing of 2,834 donors and RNA-seq data from Pan-Cancer Analysis of Whole Genomes (PCAWG) project with respect to key immunogenomic aspects using computational approaches. Our results demonstrate that diverse genomic alterations in specific tumor types, variation in immune microenvironments, and variation in oncogenic pathways are related to immune escape, and we further observed immune editing during cancer development. To illustrate the history of immuno-editing history for each cancer genome and to explore underlying molecular pathways involved, we defined immuno-editing index by comparing exonic neoantigen s to virtual neoantigens in pseudogenes.

S10-3

Drug Discovery Concept for Diffuse-type Gastric Cancer revealed by Genomic and Immunegenomic Approach

Shumpei Ishikawa
Genomic Path., MRI, TMDU, Mol. Preventive Med., Univ. of Tokyo

Diffuse gastric cancer (DGC) has characteristic clinical & biological features such as single cell stromal infiltration, strong desmoplastic response. Except for CDH1, DGC-specific drivers explaining these features have not been discovered. But recent cancer genomic analysis revealed its genomic stability, and unique RHOA gene driver mutation. While the biochemistry of mutant RHOAs has been suggested to be complex, one of the possible mechanisms is the dominant negative character of the mutant. Deconvolution of gastric cancer tissue transcriptome data also shows that DGC is characterized by decreased activity of T cells and increased B cell infiltration, suggesting that simple use of checkpoint inhibitors is ineffective, as is evidenced by recent report of clinical trial. Comprehensive profiling immunogenomics reveals different behavior of T & B cell repertoire to the cancer environment and clonal expansion of tumor specific B cells. Those clones frequently recognize sulfated glycosaminoglycan, which has been found to be major humoral cancer antigens for DGC. These results attracted attention to another cancer immunotherapy, such ae stimulating the humoral cancer pre-immunity.

S10-4

Genomic landscape of hepatoblastoma

Hiroyuki Aburatani
Gen. Sci. Div., RCAS, Univ. of Tokyo

Co-author：Hidewaki Nakagawa[1]、Eiso Hiyama[2]
[1]IMS, RIKEN、[2]Natural Sci. Ctr. for Basic Res. & Development, Hiroshima Univ.

Hepatoblastoma (HBL) is the most common primary liver tumor in children and is usually diagnosed during the first 3 years of life. HBL is derived from hepatic precursor cells and morphologically similar to immature hepatocytes. Tumor specimens from 360 HBL cases were collected at the institutions of the Japanese Study Group for Pediatric Liver Tumors (JPLT) between 2000 and 2012. Comprehensive genomic data, including exome, RNA-seq, SNP typing and DNA methylome, were obtained for 168 cases. CTNNB1 mutation and loss of heterozygosity at 11p15.5, spanning IGF2/H19 locus, were observed as frequent driver events. Patient stratification based on their genomic/epigenomic information will lead to selection of an appropriate treatment option for each case. Distinct subgroups of HBL patients were identified based on DNA methylation profiles and will be discussed at the symposium.

▲TOP

S10-5

Super-enhancer and genome phase separation in cancer research

Hiroshi I. Suzuki
Koch Inst., MIT

Co-author：Phillip A. Sharp
Koch Inst., MIT

Changes in gene expression programs can be attacked therapeutically at the levels of signaling, transcription, post-transcription, and protein-mediated control. By multimodally integrating genomics, epigenetics, and RNA biology, recent advances have opened up new possibilities for constructing quantitative models of transcriptomes, discovering biomarkers, and targeting transcription in cancer. We here summarize the roles of super-enhancers and broad H3K4me3 domains, recently proposed new epigenetic signatures, in cancer biology (Suzuki et al. Cell 2017). Intriguingly, studies of super-enhancers suggest a new research field which connects the phase separation of multi-molecular assemblies to the function of non-coding genome. The phase separation model provides a conceptual framework to explore principles of gene control and predict and target genome function. In addition, we discuss about new aspects of transcription cycle involving divergent transcription, RNA splicing, and transcription pause (Chiu, Suzuki et al. Mol Cell 2018) and new phases of functional genomics for cancer gene screening (Suzuki et al. Nat Genet 2018) together with new other system and synthetic approaches.

S10-Special_Remarks

Special Remarks

Takashi Tokino
Genome Med. Sci., Sapporo Med. Univ.

No Abstract

[LS25] LS25 [Japanese]
Precision cancer biology by target capture sequencing

2018 / 9 / 28 (Fri) 11:50-12:40 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15
：Agilent Technologies Japan, Ltd.

Yutaka Suzuki / Laboratory of Systems Genomics, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo

LS25

1) Adult T-cell leukemia in genome-sequencing era

Kaoru Uchimaru
Laboratory of Tumor Cell Biology, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo

No Abstract

▲TOP

LS25

2) Development of target capture sequencing for host and virus

Makoto Yamagishi
Laboratory of Tumor Cell Biology, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo

No Abstract

▲TOP

[S15-1] S15 [English]
Cancer and cellular senescence signaling

2018 / 9 / 28 (Fri) 13:00-15:30 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15

Shigeomi Shimizu / Pathol. Cell Biol., Med. Res. Inst., TMDU、Makoto Nakanishi / Dept. Cancer Cell Biol. IMS. The Univ. of Tokyo

Accumulating evidence have suggested that cellular senescence plays an important role in organismal aging and pathogenesis of age-related disorders such as cancer. One important hallmark of senescence is an inability to proliferate upon any physiological mitotic stimuli. This durable cessation of cell proliferation is governed by various genotoxic stresses, including telomere shortening, which ultimately activate DNA damage responses. Thus, senescence has been proposed to act as an anti-tumorigenic barrier in a cell autonomous fashion. Another hallmark of senescence is the appearance of senescence-associated secretory phenotypes (SASP) which is defined as a robust secretion of growth factors, cytokines, and proteases. Therefore, SASP can cause deleterious effects on the tissue microenvironment, leading to the micro-inflammation that likely promotes carcinogenesis in a cell non-autonomous manner. In this session, the speakers will highlight recent advances in molecular understanding between senescence and cancer as well as other age-related disorders.

S15-1
Role of cellular senescence in carcinogenesis

Makoto Nakanishi
Div. Cancer Cell Biol. Inst. Med. Sci. Univ. Tokyo

Co-author：Yoshikazu Johmura
Div. Cancer Cell Biol. Inst. Med. Sci. Univ. Tokyo

P53-dependent mitosis skip is essential for senescence induction in normal human cells exposed to various senescence-inducing stimuli. However, the factors determining whether or not cells undergo senescence are largely unknown. We found that duration of G2 checkpoint activation plays a key role in the determination of cell cycle phase when p53 is activated after senescence-inducting stimuli. Delayed G2 checkpoint recovery increased a number of G2 cells with activated p53, thereby enhancing mitosis skip, and sensitized cells to senescence. In contrast, hastened G2 checkpoint recovery desensitized cells to senescence. On the basis of this concept, we generated a senescence-prone mouse strain carrying a Claspin 5M transgene. MEFs from these senescence-prone mice were highly sensitive to senescence even under very low level of DNA damage. Intriguingly, the senescence-prone mice manifested higher resistance to AOM/DSS-induced colonic carcinogenesis. Therefore, our results provide experimental and decisive evidence for senescence as a tumor suppressive mechanism in vivo.

▲TOP

S15-2

Senescence-associated secretory phenotype (SASP) in tumor microenvironment promotes liver cancer

Naoko Ohtani
Pathophysiol., Grad. Sch. Med., Osaka City Univ.

Co-author：Fumitaka Kamachi
Pathophysiol., Grad. Sch. Med., Osaka City Univ.

Cellular senescence is a permanent cell cycle arrest induced by irreparable DNA damage, acting as a barrier to tumorigenesis. Recent studies, however, reveal that senescent cells secrete a variety of inflammatory cytokines, chemokines and proteases, a signature termed the senescence-associated secretory phenotype (SASP). Certain SASP factors reveal deleterious effects such as tumorigenesis. We have recently shown that obesity promotes liver carcinogenesis through gut microbial metabolite, deoxycholic acid (DCA), which is known to cause DNA damage in mice. The enterohepatic circulation of DCA provokes DNA damage and consequent cellular senescence in hepatic stellate cells, which in turn, secretes SASP factors in the liver, thus facilitating hepatocellular carcinoma development in obese mice. We also identified not only cytokines but also a lipid mediator, PGE2, was produced as a SASP factor in the senescent hepatic stellate cells. In this symposium, we will show you a tumor promoting cross-talk of SASP factors in the tumor microenvironment.

S15-3

Yorkie/YAP drives tumor progression by antagonizing Pointed/ETS-mediated cellular senescence

Tatsushi Igaki
Grad. Sch. of Biostudies, Kyoto Univ.

Oncogene-induced senescence is one of the major tumor-suppressive mechanisms. However, the mechanism by which oncogene-activated mutant cells overcome cellular senescence to progress toward malignancy is unknown. Through a genetic screen in Drosophila, we found that the Hippo pathway effector Yorkie/YAP abrogates oncogenic Ras-induced cellular senescence by antagonizing the ETS transcription activator Pointed, thereby driving tumor progression. We found that Pointed acts as a novel tumor suppressor that is sufficient to induce cellular senescence downstream of Ras signaling. Interestingly, malignant tumors with Ras activation and cell polarity defects significantly downregulate Pointed expression, and forced expression of Pointed in malignant tumors dramatically suppressed their growth and metastatic behavior. Mechanistically, elevated Yorkie activity in malignant tumors represses Pointed expression by regulating FoxO signaling, thereby inhibiting cellular senescence. Our data provide a mechanistic understanding of a novel oncogenic cooperation between Ras and Yorkie/YAP via Pointed/ETS-mediated regulation of cellular senescence.

S15-4

Relationship between autophagy and cellular senescence

Shigeomi Shimizu
Pathol. Cell Biol., Med. Res. Inst., TMDU

Co-author：Masashi Narita[1]、Satoko Arakawa[2]
[1]Univ. of Cambridge, Cancer Res. UK Cambridge Inst.、[2]Pathol. Cell Biol., Med. Res. Inst., TMDU

Autophagy is a fundamental cellular process that degrades sub-cellular constituents and is well conserved among species ranging from yeast to humans. Autophagy contributes various biological events, including cellular senescence. We previously reported that autophagy participated in the senescence-associated secretory phenotype (SASP) by the generation of distinct cellular compartment at the trans-side of the Golgi apparatus, namely "TOR-autophagy spatial coupling compartment (TASCC), where autolysosomes and mTOR accumulate during Ras-induced senescence. The disruption of mTOR localization to the TASCC delays the induction of SASP during Ras-senescence. In this meeting, by describing some of these data together with more recent data, I will discuss autophagy and senescence.

▲TOP

S15-5

Cellular senescence in pulmonary aging and disease

Masataka Sugimoto
Sec. Immol., Dept. Mech. Aging, Natl. Ctr. Geriat. Gerontol., Dept. Aging Res., Nagoya Univ. Grad. Sch. Med.

Cellular senescence plays central roles in tumor suppression by halting the proliferation of cells at risk of malignant transformation. Besides tumor suppression, it has become evident that senescence contributes to tissue aging through their cell non-autonomous functions, namely SASP (senescence-associated secretory phenotype). We have recently established a transgenic mouse (ARF-DTR mouse) in which senescent cells can be eliminated using toxin receptor-mediated cell knockout system. Using ARF-DTR mice, we have shown that ablation of senescent cells ameliorated aging-associated phenotypes of lung tissue. Aging of lung tissue increases a risk of pulmonary diseases such as emphysema. However, it has remained to be clarified whether cellular senescence promotes the pathology of pulmonary emphysema. We herein discuss possible involvement of cellular senescence in the development of emphysema. The elimination of senescent cells prevented lung tissue from elastase- and cigarette smoke-induced lung dysfunction through suppression of pulmonary inflammation. Thus, our data imply the potential of senescent cells as a therapeutic/preventive target for pulmonary diseases.

S15-6

Escape from stem cell aging initiates cutaneous melanoma

Emi Nishimura
Med. Res. Inst., Tokyo Med. & Dent. Univ.

Cancer is a disease of aging due to the accumulation of DNA damage although the cellular mechanisms for the trade-off between aging and cancer are still largely unknown. Hair graying, a typical aging phenotype, is mediated by the progressive depletion of melanocyte stem cells (McSCs), the reservoir for pigment-producing melanocytes. We previously reported the existence of a self-renewal checkpoint that limits the self-renewal of stem cells under genomic stress through the induction of their differentiation. To determine whether the dysfunction of that checkpoint in McSCs causes cutaneous melanoma, a devastating skin cancer, we developed a stem cell targeting system in mice. We found that carcinogens modify the expression of niche factors that promote McSC self-renewal even under severe genomic damage/stress. Those stress-resistant clones prevented the expression of the stem cell aging phenotype but initiated melanomagenesis instead. Furthermore, attenuation of niche signaling in McSCs profoundly repressed carcinogen-induced stem cell renewal and melanoma formation. These results demonstrate that self-renewal of stem cells through the niche is the initial step for melanomagenesis.

S15-Special_Remarks

Special Remarks

Hidetaka Katabuchi
Dept. Ob

No Abstract

[J-2121] J2 [Japanese]
Animal models for cancer (2)

2018 / 9 / 28 (Fri) 15:30-16:45 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15

Takayuki Nakagawa / Lab. Vet. Surg., Dept. Agri. Life Sci., The Univ. Tokyo

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J-2121

Development of mouse brain tumor model and germline genome engineering method using in vivo electroporation

Nobuyuki Onishi
Div. Gene Reg. IAMR, Keio Univ. Sch. Med.

Co-author：Hideyuki Saya
Div. Gene Reg. IAMR, Keio Univ. Sch. Med.

Glioblastoma multiforme (GBM), is one of the most malignant brain tumors, has highly-proliferative and invasive characters. There is no established effective therapy for GBM has radio- and chemo-resistance. To understand these malignant characters of GBM, an appropriate model of brain tumor is required. For reproducing a clinical tumor initiating process, we are developing a model of mouse brain tumors by having the genetic engineering in mouse brain directly. Combination of *in vivo* electroporation and piggyBac system make the introduction of activated-RAS and sh*Ink4a/Arf* into mouse brain, result in efficiently formed brain tumors have the malignant behavior. On the other hand, we are developing a genome engineering method called improved-Genome editing via Oviductal Nucleic Acids Delivery (*i*-GONAD) that delivers CRISPR ribonucleoproteins to E0.7 embryos via *in situ* electroporation. *i*-GONAD generates mouse models containing single-base changes, kilobase-sized deletions and knock-ins. Based on these findings, we propose the *in vivo* electroporation technique is strategic manner to generate target mouse models.

▲TOP

J-2122

MicroRNA-29 may suppress colon carcinogenesis by inhibiting DSS-induced colitis

Naoto Tsujimura
Dept. Gastroenterological Surg., Grad. Sch. Med., Osaka Univ.

Co-author：Yuhki Yokoyama[1]、Akira Inoue[2]、Haruka Hirose[1]、Norikatsu Miyoshi[2]、Hidekazu Takahashi[2]、Naotsugu Haraguchi[2]、Taishi Hata[2]、Chu Matsuda[2]、Tsunekazu Mizushima[2]、Yuichiro Doki[3]、Masaki Mori[3]、Hirofumi Yamamoto[4]
[1]Dept. Mol. Pathol., Health & Sci., Grad. Sch. Med., Osaka Univ.、[2]Dept. Gastroenterological Surg., Grad. Sch. Med., Osaka Univ.、[3]Dept. Gastroenterol. Surg. Osaka Univ. Grad. Sch. Med.、[4]Dept. Gastroenterological Surg., Grad. Sch. Med., Osaka Univ., Dept. Mol. Pathol., Health & Sci., Grad. Sch. Med., Osaka Univ.

Chronic inflammation is an established risk factor for carcinogenesis, and inflammatory bowel disease (IBD)-related cancer is known as an example. Sodium dextran sulfate (DSS) is often used to induced colitis and carcinogenesis in mouse model. In this study we tried to break off the inflammatory cascade caused by DSS by systemic administration of microRNA 29 (miR-29) using super carbonate apatite (sCA) nanoparticles as a drug delivery system. Injection of sCA-miR-29a-3p or -29b-3p into the tail vein of mice markedly prevented colitis. RNA sequencing and Ingenuity Pathway Analysis revealed that miR-29a and -29b individually or in combination could inhibit interferon-associated inflammatory cascade. sCA-miR-29b efficiently targeted $CD11c^+$ dendritic cells (DCs) among various types of immune cells in the inflamed mucosa. RT-PCR analysis indicated that the miR-29 RNAs in $CD11c^+$ DCs suppressed the production of Interleukin (IL)-6, transforming growth factor (TGF)- β and Interleukin (IL)-23 subunits in DSS-treated mice. In conclusion, miR-29a or -29b may suppress DSS-induced carcinogenesis through efficient inhibition of colitis.

J-2123

The ATM inhibitor enhances the replication of oncolytic reovirus in canine and human cancer cell lines

Masaya Igase
Dept. Mol. Diag. Ther., Vet., Yamaguchi Univ.

Co-author：Shunsuke Noguchi[1]、Yuki Nemoto[2]、Takuya Mizuno[2]
[1]Dept. Vet. Radiol., Vet., Osaka Pref. Univ.、[2]Dept. Mol. Diag. Ther., Vet., Yamaguchi Univ.

Oncolytic virotherapy using mammalian reovirus is one of the novel therapies against human cancer. We have previously reported the oncolytic effects of reovirus in various kinds of canine cancer cell lines and tumor-bearing dogs. Moreover, to discover a new drug to enhance the oncolytic effects of reovirus, we screened the signaling pathway inhibitor library with 285 compounds. We found that the combination of an ataxia telangiectasia mutated kinase (ATM) inhibitor and reovirus resulted in the significant cell growth inhibition with the increased virus replication in canine melanoma cell lines as well as various kinds of human cancer cell lines. Moreover, we revealed that reovirus induced the phosphorylation of the ATM without DNA damage in the early stage of infection, which was abrogated by the ATM inhibitor. Furthermore, we found that the ATM inhibitor caused the acidification of endosomes accompanied by the enhancement of the viral disassembly. These findings suggest that combination of the ATM inhibitor and reovirus promises the clinical potential of cancer therapy.

J-2124

ERK/MAPK pathway upregulates COX2/PGE2 axis in BRAFV595E canine urothelial carcinoma

Ryohei Yoshitake
Lab. Vet. Surg., Univ. Tokyo., Grad. Sch. Agri. & Life Sci.

Co-author：Shotaro Eto[1]、Masahiro Shinada[1]、Kohei Saeki[2]、Rei Nakano[3]、Hiroshi Sugiya[3]、Ryohei Nishimura[2]、Takayuki Nakagawa[2]
[1]Lab. Vet. Surg., Univ. Tokyo., Grad. Sch. Agri. & Life Sci.、[2]Lab. Vet. Surg., Dept. Agri. Life Sci., The Univ. Tokyo.、[3]Lab. Vet. Biochem., Nihon Univ., Bioresource Sci.

Canine urothelial carcinoma (cUC) is a naturally-occurring cancer in the lower urinary tract of aged dogs with 70% of cases harboring $BRAF^{V595E}$ mutation homologous to human $BRAF^{V600E}$. Notable characteristic of cUC is high response rate to non-steroidal anti-inflammatory drugs. We previously revealed that $BRAF^{V595E}$ cUC cells produce enormous amount of PGE2. In this study, we performed molecular targeting agent screening to reveal mechanism of PGE2 production in the $BRAF^{V595E}$ cUC cells.
The results revealed that inhibitors targeting arachidonic acid cascade, RAF/MEK/ERK pathway and p38/JNK pathway significantly suppressed PGE2 production. Additional experiments suggested that COX2 expression/PGE2 production in the cUC cells were decreased by a selective BRAF inhibitor. It was also found that $BRAF^{V595E}$ cUC tended to show higher COX2 expression compared to wild-type cUC tissues. In conclusion, RAF/MEK/ERK signaling possibly play a significant role in COX2/PGE2 axis in cUC, which may be facilitated by activating mutation of BRAF gene. Further study is required to reveal relationship between BRAF mutation and cancer-associated inflammation.

▲TOP

J-2125

Sentinel lymph node detection using newly developed handheld magnetometer in animal models

Akihiro Kuwahata
Grad. Sch. of Engineering, The Univ. of Tokyo

Co-author：Kohei Saeki[1]、Takayuki Nakagawa[1]、Sachiko Matsuda[2]、Hiroyuki Takei[3]、Seigo Nakamura[4]、Itsuro Saito[5]、Masaki Sekino[6]、Moriaki Kusakabe[7]
[1]Agricultural & Life Sci., The Univ. of Tokyo、[2]Dept. Surg., Keio Univ. Sch. Med.、[3]Dept. Breast Surg., Nippon Med. Sch.、[4]Dept. Surg., Showa Univ. Sch. Med.、[5]iMed. Japan Inc.、[6]Grad. Sch. of Engineering, The Univ. of Tokyo、[7]Agricultural & Life Sci., The Univ. of Tokyo, Matrix Cell Res. Inst. Inc.

Background:The magnetic detection of sentinel lymph node (SLN) is a promising alternative to conventional radioisotope/indigo carmine techniques. This is the collaborated study to develop novel handheld magnetic probe and evaluate the feasibility of SLN detection with the developed magnetic technique in animal models.Methods:Magnetometer was developed by numerical calculations and experimental evaluations. Methodology for lymphography using a magnetic tracer (Resovist) was optimized using healthy rats and dogs. Thereafter, feasibility of the method was demonstrated in a clinical trial recruiting 40 client-owned dogs with naturally-occurring tumors.Results:Detection length of the magnetometer is about 10 mm with respect to 5 μL of Resovist, which indicates the magnetometer can detect the SLN. Accumulation of magnetic tracer in SLNs was significantly increased with volume and time passage. SLN detection with the magnetometer was successful in 28 cases (70%), which was comparable to the conventional method with indigo carmine.Conclusion:Magnetic detection of SLN with the developed handheld magnetometer may be a choice for intra-operative SLN biopsy without need for using radioisotope.

J-2126

Tumor endothelial cell-derived prostaglandin D2 inhibits vascular hyper-permeability and angiogenesis

Takahisa Murata
Dept. Animal Radiology, Tokyo Univ.

Co-author：Keisuke Omori[1]、Teppei Morikawa[2]、Akiko Kunita[2]、Masashi Fukayama[2]
[1]Dept. Animal Radiology, Tokyo Univ.、[2]Dept. Path., Tokyo Univ.

Tumor endothelial cells (TECs) are one key component of tumor microenvironment and known to possess irregular characteristics in motility and inflammatory reactions. We found marked increase of lipocalin-type prostaglandin D synthase (L-PGDS) mRNA expression in TECs isolated from mouse B16 melanoma compare to that of normal endothelial cells. In situ hybridization showed L-PGDS mRNA expression in the human melanoma and oral squamous cell carcinoma TECs. In vitro experiments showed that tumor cell-derived interleukin-1 and tumor necrosis factor-α increased mRNA expression of L-PGDS in isolated ECs. We next investigated the contribution of L-PGDS-derived PGD_2 in tumor growth. B16 melanoma implanted into the L-PGDS or endothelial L-PGDS deficient mice grew faster than those of WT mice. Immunohistochemical staining revealed that vascular endothelial cells mainly express L-PGDS in growing tumor. Host L-PGDS deficiency accelerated vascular hyper-permeability and angiogenesis. These observations identify TEC-derived L-PGDS/PGD_2 as a negative regulator of tumor microenvironment by restricting angiogenesis and vascular permeability.

▲TOP

[MV1] MV1 [Japanese]
The JCA-Mauvernay Award Lecture

2018 / 9 / 28 (Fri) 8:00-8:50 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16

Ryuzo Ueda / Dept. Tumor Immunol., Aichi Med. Univ. Sch. Med.

MV1

Identifying novel targets for leukemia therapy using the CRISPR/Cas9 gene-editing tool

Takahiro Maeda
Ctr. for Cell. & Mol. Med., Kyushu Univ. Hosp.

Acute myeloid leukemia (AML) is a devastating disease with a long-term survival rate of less than 30%. Sequencing studies now provide a near-complete picture of the AML genome; however, functional studies are necessary to devise novel therapeutic strategies and to assess the significance of AML-associated mutations. Successful application of the CRISPR-Cas9 system for genome editing is transforming the landscape of functional genomics. To identify novel drug targets, we employ genome-wide CRISPR-Cas9 screens utilizing AML lines with defined genetic backgrounds, which enables straightforward interpretation of screening results. Our ongoing studies include: 1) genome-wide CRISPR-Cas9 screens in the presence or absence of chemotherapeutic drugs to identify novel drug-resistant mechanisms and/or synthetic-lethal relationships between drug and gene knockouts and 2) CRISPR-Cas9-mediated comprehensive dense mutagenesis to systematically define functional protein coding sequences of potential drug targets. I will present our recent findings in our lab and discuss potential utility of the CRISPR-Cas9 system for the translational research in the field of hematology/oncology.

▲TOP

[SST3-1] SST3 [Japanese]
New insights and treatments for hematopoietic malignancy

2018 / 9 / 28 (Fri) 9:00-11:30 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16

Yuzuru Kanakura / Dept. Hematol. Oncol., Osaka Univ. Grad. Sch. Med.、Atsushi Hirao / Div. Mol. Gen., Cancer Res. Inst. Kanazawa Univ.

Advanced cancer genomics has provided us valuable information of molecular mechanisms of tumorigenesis. In addition, comprehensive approach by using state-of-art technologies, including transcriptome, proteome and metabolome, have contributed to the development of new molecularly targeted drugs and discovery of biomarkers for therapeutics. This symposium will focus on recent progress in the field of hematopoietic malignancy. Six distinguished speakers will present new insights and treatments for pediatric acute lymphoblastic leukemia, adult T-cell leukemia-lymphoma, chronic myelogenous leukemia and acute myelogenous leukemia. We sincerely hope this symposium will be an excellent opportunity to discuss how basic/clinical research contributes to benefit of patients with hematopoietic malignancy in future.

SST3-1

Identification of novel fusion genes for pediatric T cell acute lymphoblastic leukemia

Junko Takita
Dept. Ped., The Univ. of Tokyo.

To discover oncogenic drivers in pediatric T-ALL, we performed whole-transcriptome sequencing (WTS) in 121 cases. Intriguingly, novel recurrent in-frame SPI1 fusions were detected, and RT-PCR analysis in additional 60 cases revealed other 2 SPI1 fusions, indicating that SPI1 fusions accounted for 4% of pediatric T-ALL. SPI1 fusion cases showed a double-negative or CD8 single-positive phenotype and had a uniformly poor overall survival. These cases represent a subset of pediatric T-ALL distinguishable from the known T-ALL subsets, in terms of expression of genes involved in T-cell precommitment, and post b-selection and of mutational profile. SPI1 fusions retained transcriptional activities, and when constitutively expressed in mouse stem/progenitor cells, they induced an enhanced cell proliferation and a maturation block. Notably, SPI1 fusion cases have a distinct clinical and genetic features with extremely poor prognosis and frequent mutations of KRAS/NRAS. Our findings implicating that SPI1 fusions can be the first example of an oncogenic driver associated with aggressive phenotype in pediatric T-ALL, and cases with SPI1 fusions may be a novel clinical/genetic entity.

▲TOP

SST3-2

Genetic basis and its clinical implication in adult T-cell leukemia/lymphoma

Keisuke Kataoka
Div. Molecul Oncol, Natl Cancer Ctr. Res Inst.

Adult T-cell leukemia/lymphoma (ATL) is an aggressive peripheral T-cell lymphoma associated with HTLV-1 infection. We have carried out an integrated genetic analysis, in which whole genome, exome, and transcriptome sequencing as well as array-based copy number and methylation analyses were performed in more than 400 ATL cases. We found recurrent genetic alterations in T-cell receptor/NF- κ B signaling and other T-cell-related pathways. A conspicuous feature of ATL genome is the predominance of gain-of-function alterations, including activating mutations in PLCG1, PRKCB, CARD11, VAV1, IRF4, CCR4, and CCR7 and CTLA4/ICOS-CD28 fusion genes. We also discovered a unique genetic mechanism of immune evasion caused by structural variations (SV) disrupting 3'-untranslated region (UTR) of the PD-L1 gene. These SVs caused a marked elevation of aberrant PD-L1 transcripts, leading to immune escape of tumor cells in vivo. In addition, ATL subtypes are further classified into molecularly distinct subsets with different prognosis by genetic profiling. Our findings not only provide novel insights into the molecular pathogenesis in ATL, but also contribute to improve therapeutic strategy in ATL.

SST3-3

Molecular targeting therapy for CML and stop studies

Shinya Kimura
Div. Hematology, Respiratory Med. & Oncol., Saga Univ.

ABL tyrosine kinase inhibitors (TKIs) dramatically improves chronic myeloid leukemia (CML) prognosis. Stop imatinib (STIM) study was firstly conducted in France. Then, several stop studies of first-generation (imatinib) and second-generation ABL TKIs (dasatinib, nilotinib) have been started. We reported the dasatinib discontinuation (DADI) trial (Lancet Haematol 2015). These studies revealed that almost half of CML patients who achieved a certain period of sustained deep molecular response (DMR) with ABL TKI can stop it safely. Adverse effects of ABL TKIs withdrawal and predicting factors for successful discontinuation including immunity are becoming clear gradually. We recently reported that the combinations of some NK KIR and HLA alleles correlated with potent NK cell immunity against CML (Cancer Immunol Res 2018). We are currently carrying out another imatinib stop study (DOMEST) and first line dasatinib discontinuation (1st-DADI) trial. It is important to conduct a comprehensive examination of the results of stop studies with a wide variety of protocols in order to determine which discontinuation method results in the highest probability of treatment free remission.

SST3-4

Genome-wide CRISPR-Cas9 screen identifies leukemia-specific dependence on a pre-mRNA metabolic pathway

Takahiro Maeda
Ctr. for Cell. & Mol. Med., Kyushu Univ. Hosp.

Genome-wide knockout screening employing CRISPR-Cas9 genome-editing is a powerful tool for functional genomics. To identify novel targets for acute myeloid leukemia (AML) therapy, we established two mouse AML lines that exhibited a normal karyotype and harbored functionally-normal Trp53 and performed genome-wide CRISPR-Cas9 screening, followed by a second screen in vivo. We show for the first time that mRNA decapping enzyme scavenger (DCPS) is essential for AML cell survival. RG3039, a DCPS inhibitor that was originally developed to treat spinal muscular atrophy, exhibited anti-leukemic activity in human AML cell lines and in AML PDX models. DCPS protein interacted with components of pre-mRNA metabolic pathways, including spliceosomes as revealed by mass spectrometry. RG3039 treatment of AML cells caused aberrant pre-mRNA splicing leading to cell cycle arrest and apoptosis. Finally, we show that humans harboring germline bi-allelic DCPS loss-of-function mutations do not exhibit hematologic phenotypes indicating that DCPS is dispensable for steady-state hematopoiesis in humans. Our findings shed a new light on a pre-mRNA metabolic pathway regulated by DCPS.

▲TOP

SST3-5

Identification of BCAAs metabolism pathway as a common machinery for maintaining the stemness of human acute leukemia

Yoshikane Kikushige
Dept. Med. & Biosystemic Sci., Kyushu Univ., Dept. Med. & Biosystemic Sci., Kyushu Univ.

Co-author：Koichi Akashi
Dept. Med. & Biosystemic Sci., Kyushu Univ.

Recent advances in measuring cellular metabolites have revealed that several specific metabolism pathways actively contribute to the maintenance of stemness in several types of stem cells including ES cells, iPS cells and tissue stem cells. In this study, we comprehensively analyzed cellular metabolites of human undifferentiated CD34+ normal HSPCs and CD34+ acute leukemia cells to test whether specific metabolic pathways or metabolites could govern stem cell properties of malignant stem cells. We found CD34+ acute leukemia cells exhibited the significantly higher cellular contents of BCAAs as compared to normal CD34+ HSPCs. The immature CD34+ acute leukemia cells commonly expressed BCAAs-transporters and BCAT1, one of the critical molecules for BCAA metabolism pathways. Inhibition of BCAA metabolism pathway significantly attenuated leukemia progression via targeting malignant stem cells through the alteration of specific epigenetic enzyme activity. This study provides the novel evidence that human acute leukemia cells commonly utilize BCAA metabolism pathways to maintain the malignant stem cell properties.

SST3-6

Novel approach for cancer stem cell-targeted therapy for hemotological malignancy

Issay Kitabayashi
Div. Hematological Malignancy, Natl. Cancer Ctr. Res. Inst.

Selective targeting of cancer stem cells is a promising strategy for preventing relapse. EZH1/EZH2 are catalytic components of Polycomb repressive complexes 2 (PRC2), which trimethylates histone H3 at lysine 27 (H3K27) to repress transcription of target genes. We found that EZH1/2 are essential for maintenance of cancer stem cells (CSCs) in acute myeloid leukemia and multiple myeloma. The EZH1/2 dual inhibitor selectively reduced the number of CSCs and prevented tumor progression in xenograft models for acute myeloid leukemia, multiple myeloma, and lymphomas. Phase1 clinical trials of the dual inhibitor have been initiated in malignant lymphoma and acute leukemia. Mutations in isocitrate dehydrogenase (IDH) 1 and 2 are frequently observed in various cancers. We have found that mutant IDH is essential for progression and maintenance of cancers. And we have shown that mutant IDH1 inhibitor prevented growth of the tumors with IDH1 mutation in acute myeloid leukemia, glioma and condrosarcoma. Based on these results, Phase I clinical trial of the inhibitor has been initiated for patients of glioma with IDH1 mutation.

▲TOP

[LS26] LS26 [Japanese]
Treatment Strategy for 2nd-line mCRC

2018 / 9 / 28 (Fri) 11:50-12:40 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16
：Sanofi K.K./ Yakult Honsha Co., Ltd.

Naohiro Tomita / Division of Lower GI Surgery, Department of Surgery, Hyogo College of Medicine

LS26

1) Impact of VEGF on tumor immune micro environment

Hisato Kawakami
Department of Medical Oncology, Kindai University, Faculty of Medicine

No Abstract

LS26

2) Benefits of Angiogenesis inhibitor for mCRC

Yasutoshi Kuboki
Department of Experimental Therapeutics and GI Oncology, National Cancer Center Hospital East

No Abstract

▲TOP

[SST4-1] SST4 [Japanese]
New treatments for skin cancer

2018 / 9 / 28 (Fri) 13:00-15:30 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16

Ichiro Katayama / Dept. Dermatol. Osaka. Univ. Sch. Med.、Heiichiro Udono / Dept. Immunol., Okayama Univ. Grad. Sch. Med.

Dermatology covers a wide variety of both mesenchymal and epithelial tumors, such as melanoma, Paget disease of the external genitalia, basal cell carcinoma, Merkel cell carcinoma, angiosarcoma and cutaneous lymphoma. Although surgery and radiation therapy has been a main stream of therapeutic strategy for these malignancies, a paradigm shift has occurred over recent years, in which immune checkpoint inhibitors show extremely high efficiency, and gene therapy and regenerative medicine make it possible to cure or prevent refractory diseases. Furthermore, personalized medicine becomes reality along with a development of whole genome sequencing technology. In this symposium, experts in basic and clinical research field talk about recent findings and future perspectives of dermato-oncology.

SST4-1

New multidisciplinary treatment with Boron Neutron Capture Therapy (BNCT) against melanoma

Hiroyuki Michiue
Neutron Therapy Res. Ctr., Okayama Univ.

In cancer treatment, the melanoma therapy is the key test of cancer treatment at the point of immunotherapy. The NEJM paper showed the high correlation between tumor mutational burden and objective immune response rate with immunotherapy in 27 kinds of cancer. But the enhancement effect of immune reaction with X-ray radiotherapy might be very small and another adjuvant immunotherapy will be essential.In 2017, Okayama University established Neutron Therapy Research Center (NTRC) for creating new trial for BNCT (Boron Neutron Capture Therapy). BNCT is a high LET radiotherapy. The key in BNCT is to transduce high content of boron isotope 10B in the tumor tissues, while avoiding its accumulation in the healthy tissues. BPA was synthesized as melanoma BNCT drug with one B and L-phenylalanine. Recently, some reported that the l-amino acid transporter-1 (LAT-1) system imports BPA into melanoma and many kinds of cancer cells. That plays a major cancer-selective role in BPA-based BNCT.In this time, we will propose the new multimodality therapy with the combination of immunotherapy and BNCT and discuss new treatment vision against and progressive inoperable recurrent melanoma patients.

▲TOP

SST4-2

Novel type of cancer vaccine, artificial adjuvant vector cells with multiple immunopotentiating effects against melanoma

Shin-ichiro Fujii
Lab. for Immunotherapy, RIKEN Ctr. for Integrative Med. Sci.

Co-author：Kanako Shimizu
Lab. for Immunotherapy, RIKEN Ctr. for Integrative Med. Sci.

Immunotherapy is proving to be an effective therapeutic approach in a variety of cancers. Despite the clinical success of antibodies against the immune regulators CTLA4 and PD-L1/PD-1, only a subset of people exhibit durable responses, suggesting that a broader view of cancer immunity is required. For this purpose, simultaneous induction of innate and adaptive immunity should be an ideal mechanism. We therefore have focused on DCs, which play a pivotal role in determining the quality and magnitude of innate and adaptive immunity. To effectively utilize the DC in situ, we have developed an artificial adjuvant vector cells (aAVCs), comprised of a CD1d-NKT ligand complex on the cell surface and containing tumor antigen inside of the cells. This approach induces adjuvant effects by combining NKT cell activation with delivery of antigen to DCs in vivo. In addition to linking innate and adaptive immunity, aAVC therapy can lead to efficient trafficking of tumor specific cytotoxic T cells to the tumor and also the formation of long-term memory T cells. We will discuss the development of melanoma antigen-expressing aAVC and demonstrate the efficacy of this cellular drug.

SST4-3

Establishing non-inflammatory RNA adjuvant for vaccine immunotherapy for cancer

Tsukasa Seya
Dept. Pathol I., Hokkaido Univ., Grad. Sch. Med.

Co-author：Aya Miyazaki、Sumito Yoshida、Misako Matsumoto
Dept. Pathol I., Hokkaido Univ., Grad. Sch. Med.

PD-1 therapy brought us proof of consent on therapeutic efficacy of cancer immunotherapy. However, many problems remain unsettled in low remission rate, many side effects, limitation of adaptive cancer type and so on. Tumors with PD-L1 high and infiltration with cytotoxic T lymphocytes (CTL) tend to be targets for PD-1 therapy. Tumor-specific CTLs are derived from naive CD8 + T cells, and this process requires dendritic cell (DC)-priming by adjuvants. PD-1 antibodies make preexisting CTLs activate or reinvigorate but not DCs. Yet, DC-priming adjuvant has not been approved yet. This is in part because adjuvant is accompanied with cytokine toxicity, leading to flu-like side effects. The authors chemically synthesized nucleic acid adjuvant ARNAX, which, unlike polyI: C (a virus dsRNA mimic), exclusively targets TLR3 in DCs without activation of the systemic MAVS pathway, and sufficiently regresses tumors (Matsumoto et al. Nat Commun 2015) . ARNAX/antigen + PD-1/L1 therapy culminates in therapeutic efficacy in mouse model studies using PD-1-resistant tumor (Takeda et al. Cell Rep 2017). In the future you can provide high QOL vaccine immunotherapy to humans by using ARNAX.

SST4-4

Development of novel therapeutics targeting NUAK2 against acral melanomas

Takeshi Namiki
Dept. Dermatol., Grad. Sch., Tokyo Med. & Dent. Univ.

Acral melanomas account for approximately 50% of melanomas in Japan. But the development of therapeutics against acral melanomas has been hampered due to a substantially lower frequency of acral melanomas in Caucasian population. We previously reported that NUAK2 amplification is involved in the proliferation and migration of human melanoma cells (PNAS2011). We also reported that Aaray-CGH analysis revealed PTEN deficiency was associated with genomic amplification encompassing the NUAK2 locus, and increase of phospho-Akt was correlated with NUAK2 expression when immunohistochemically evaluated in acral melanoma samples (Cancer Res2015). Furthermore, we have investigated downstream targets of NUAK2 using microarray analysis and found out that the regulation of mTOR has a profound importance in cell proliferation as a downstream target of NUAK2. We also found that decreased phosphorylation of pS6K and pS6 in SM2-1 (acral melanoma cell) with NUAK2 silencing by western blots. These results indicate that mTOR pathway has a critical importance to promote melanoma development. A strategy targeting NUAK2 and mTOR pathway is a potentially promising therapeutics against acral melanomas.

▲TOP

SST4-5

Recent advances in therapeutic strategies for unresectable or metastatic melanoma and Merkel cell tumor

Hisashi Uhara
Dept. Dermatol., Sapporo Med. Univ., Sch. Med.

Immune checkpoint inhibitors (ICI) and low weight molecular targeting agents have produced a new era in the treatment of patients with advanced skin tumors. The following treatment options will be focused; ICI, adjuvant and neo-adjuvant setting and novel therapies including oncolytic viruses in melanoma and ICI for Merkel cell tumor (MCC). Melanoma: ipilimumab + nivolumab (ipi+nivo): 3 yr OS rate: 58% (ipi+nivo), 52% (nivo), and 34% (ipi). G3, 4 AEs : 59% (ipi+nivo), 21% (nivo) and 28% (ipi). Melanoma: adjuvant (not approved): 1 yr RFS: 70.5% (nivo), 60.8% (ipi)). Dabrafenib+trametinib showed a significantly lower risk of recurrence in Pts with stage III (combi vs placebo: 3 yr. RFS: 58%: 39%, 3yr. OS rate: 86%: 77%). Oncolytic virus: Talimogene laherparepvec (T-VEC) and canerpaturev (C-REV, HF10) are being investigated in clinical trials in combination with ICI. MCC: Anti-PD-L1 antibody (avelumab): ORR: chemoTx-refractory: 31.8%), 1st line: 62.1%. Avelumab became the first treatment for patients with metastatic MCC to receive approval in the USA, the EU, and Japan.

SST4-6

Skin disorders caused by novel cancer drugs and their countermeasures

Atsushi Tanemura
Dermatology Dept., Osaka Univ., Sch. Med.

There have been significant advances in the development of therapeutic drugs targeting molecules, enabling a remarkable anticancer treatment effect which could not be achieved with conventional anticancer drugs. Since these novel drugs involve skin side effects that have never been experienced, appropriate management of them is a major key in the continuation of treatment. Among the many molecules targeted for cancer treatment, EGFRi is one that inhibits epidermal growth factor (EGF), which is also highly expressed in skin component cells of the skin, causes a very high frequency of skin disorders. In severe cases, it is necessary to reduce or discontinue EGFRi. Hand-foot syndrome caused by tyrosine kinase inhibitors may also lead to serious problems in one's daily life. In addition, immunity checkpoint inhibitors have been used more and more frequently, resulting in various skin disorders such as systemic pruritus and vitiligo. We hereinafter outline the molecular mechanisms of the development of these skin disorders and discuss optimal countermeasures against them by presenting some of the cases that we have experienced.

▲TOP

[J-2127] J14-13 [Japanese]
Other cancers

2018 / 9 / 28 (Fri) 15:30-16:45 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16

Yae Kanai / Dept. Path., Keio Univ. Sch. Med.

J-2127

The prognostic impact of Programmed cell death 1 Ligand 1 in Thymic Carcinoma

Soichiro Funaki
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Co-author：Yasushi Shintani[1]、Kenji Kimura[1]、Yoko Yamamoto[1]、Meinoshin Okumura[2]
[1]Dept. Gen. Thoracic Surg., Osaka Univ., Sch. Med.、[2]General Thoracic Surg. Toneyama Natl. Hosp.

[Purpose] The clinical significance of Programmed cell death 1 Ligand 1(PD-L1) in thymic carcinoma (TC) remains unclear. In this present study, we investigated the relationships between and clinicopathological findings and PD-L1in TC. [Patients and Methods]A total of 35 patients with TC surgically resected with and without induction chemotherapy were retrospectively analyzed. The relationship between PD-L1 TPS (tumor proportion score) and clinical backgrounds were evaluated by immunohistochemical staining (IHC). [Results] 16 cases were positive in IHC of PD-L1 (45.7%). In disease free survival rate (DFS), the PD-L1-positive cases were significantly worse as compared to negative cases (P=0.01). Comparing of PD-L1 TPS according to induction chemotherapy showed the TPS with chemo-treatment was significantly higher than without chemo-treatment (P=0.02). [Conclusion] Our results suggest that high PD-L1 expression is a prognostic factor, and is enhanced by chemo-treatment. Moreover, anti PD-1/PD-L1 immunotherapy may be a reliable option to treatment in TC and provide more effective anticancer activities in combination with chemotherapy.

▲TOP

J-2128

Genetic analysis of pheochromocytoma

Tatsuki Ogasawara
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Co-author：Yoichi Fujii[1]、Masanori Fujimoto[2]、Yusuke Shiozawa[1]、Tetsuichi Yoshizato[1]、Hiromichi Suzuki[1]、Kenichi Yoshida[1]、Hideki Makishima[1]、Satoru Miyano[3]、Tomoaki Tanaka[4]、Seishi Ogawa[1]
[1]Dept. Path. & Tumor Biol., Kyoto Univ.、[2]Dept. Clin. Cell Biol., Chiba Univ. Sch. Med.、[3]Hum. Genom. Ctr., IMS, Univ. Tokyo、[4]Dept. Mol. Diag., Chiba Univ. Sch. Med.

Pheochromocytomas (PCs) are rare, catecholamine-secreting tumors of the adrenal medulla, molecular pathogenesis of which is still unclear in up to 30% of cases, particularly in those showing malignant clinical pictures. To elucidate the molecular basis of PC and to characterize genetic features of malignant cases, we performed whole exome sequencing of paired tumor/normal DNA from 22 PC patients, including 6 malignant cases. Predominantly showing age-related signatures, somatic mutations were detected in all cases with a median of 13.5 mutations per sample. As many as 63% of the cases had pathogenic mutations in known cancer drivers, including VHL, RET, NF1 and HRAS, or a fusion gene involving MAML3. Copy number abnormalities were detected in most cases (20/22), including del(1p), del(3q), LOH(11p), and LOH(17p). In addition, we identified a somatic PTEN frameshift mutation and a germline TP53 mutation in 2 cases, which have not previously been reported in PC. Of interest, both patients showed an aggressive clinical course. We will discuss the molecular classification of PC with an implication to PCs showing malignant clinical features.

J-2129

Functional analysis of diacylglycerol kinase γ in melanoma

Masahiro Kai
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We have previously shown that diacylglycerol kinase (DGK) α prevents tumor necrosis factor α -induced apoptosis in melanoma cells, suggesting its oncogenic function (BBA, 2007). In contrast, we reported that DGK γ may play a tumor suppressor role in colorectal cancer (Mol Carcinog, 2017). We thus aimed to clarify the function of DGK γ in melanoma. qRT-PCR analysis revealed that DGK γ was downregulated in melanoma cell lines and primary melanoma tissues, and that low DGK γ expression was associated with metastasis. Ectopic expression of DGK γ didn't affect melanoma cell proliferation, while it suppressed migration and invasion. Notably, both constitutively active and kinase-dead forms of DGK γ inhibited migration and invasion of melanoma cells, indicating its novel function independent of the DGK activity. Microarray analysis suggested that DGK γ may suppress NF-κB signaling in melanoma cells. Currently, we are investigating the function of DGK γ in melanoma, and we will discuss its biological and clinical implications.

J-2130

Genetic and epigenetic alteration in malignant melanoma

Yosuke Yamamoto
Dept. Dermatol., Chiba Univ., Grad. Sch. Med., Dept. Mol. Oncol., Chiba Univ., Grad. Sch. Med.

Co-author：Keisuke Matsusaka[1]、Kiyoko Takane[2]、Masaki Fukuyo[1]、Satoshi Ota[3]、Hiroyuki Matsue[4]、Atsushi Kaneda[1]
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Malignant melanoma (MM) is the most life-threatening disease among all the skin malignancies. Recent genome-wide analyses of MM revealed that BRAF, RAS, and NF1 are the most frequently mutated driver genes (TCGA, Cell 2015). However, involvement of epigenetic alteration has not been fully investigated. To elucidate molecular alteration in MM, we here performed genome-wide DNA methylation analysis of 51 clinical MM samples using Infinium 450k beadarray, and target exon sequencing analysis against 275 driver genes. Hierarchical clustering analysis stratified MM into high-methylation and low-methylation subtypes. High-methylation subtype tumors were significantly progressed in tumor thickness and showed significantly worse prognosis, and a surrogate marker gene correlating with tumor thickness and poor prognosis was identified. Target exon sequencing analysis revealed genes specifically mutated in each subgroup of MM. Our data indicate that MM consists of at least two different molecular subtypes showing distinct methylation epigenotypes and mutation patterns as well as clinicopathological features.

▲TOP

J-2131

Enhanced IL-32 γ expression in malignant pleural mesothelioma affects the growth rate, and VEGF and IL-8 production

Muneo Numasaki
Dept. Geriato., Inst. of Aging & Cancer, Tohoku Univ.

Co-author：Yoshihisa Tomioka
Tohoku Grad. Sch. of Pharm. Sci.

IL-32 has been demonstrated to be involved in the cell growth, invasion and metastasis of lung adenocarcinoma and colon cancer. However, until now, the biological effects of IL-32 on malignant pleural mesothelioma have not been elucidated yet. Thus, in this study, we constructed a retroviral vector carrying IL-32 γ cDNA and transfected into six malignant pleural mesothelioma lines, and investigated the effects of overexpressed IL-32 γ on growth and angiogenic factor expression such as VEGF and IL-8. Overexpression of IL-32 γ stimulated or suppressed the growth of malignant pleural mesothelioma cells. In addition, IL-32 γ transfectants secreted larger amounts of VEGF and IL-8 compared with neo resistant gene transfectants. In addition, PI3K inhibitor inhibited IL-32-induced enhanced cell growth and VEGF production. Moreover, p38 or NF- κ B inhibitor inhibited IL-32-induced enhanced IL-8 production. These findings indicated that IL-32 is critically involved in cell growth, and VEGF and IL-8 secretion through MAPK and PI3K signaling pathways. These findings also demonstrated that IL-32-targeting therapy of malignant pleural mesothelioma may be a promising therapeutic strategy.

J-2132

Evaluation of the tissue distribution of oncometabolite 2-hydroxyglutarate in gliomas using mass spectrometry imaging

Mitsuhiro Hayashi
Mol. Pharm, Natinal Cancer Ctr. Res. Inst.

Co-author：Makoto Ohno[1]、Koichi Ichimura[2]、Akihiko Yoshida[3]、Yoshitaka Narita[4]、Akinobu Hamada[5]
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Introduction: Mutations of the isocitrate dehydrogenase (IDH)-1/2 genes frequently occur in gliomas. The mutant IDH gain a new ability to produce oncometabolite 2-hydroxiglutarate (2HG); however, the concentration and distribution of 2HG is unclear.
Method: Matrix assisted laser desorption and ionization mass spectrometry imaging (MSI) and liquid chromatography-tandem mass spectrometry (LC-MSMS) are used for analyzing the 2HG evaluations.
Results: In the human gliomas with IDH mutation, a variety of 2HG distributions were shown by high mass resolution analysis. Multivariate analysis including certain endogenous molecules confirmed that most of 2HG localization were incompatible with the blood flow molecule as shown in the image and a few 2HG were co-localized. The 2HG concentrations measured by MSI and LC-MSMS correlated well and were significantly higher in the IDH mutation than in the wild type. High spatial resolution analysis revealed the concentration gradients of 2HG at a cellular level.
Conclusion: MSI showed the heterogeneous distribution of 2HG in gliomas. Distribution analysis of cancer-associated metabolites can be helpful in molecular biology and translational research.

▲TOP

[P-2001] P1-1 [English/Japanese]
Cell culture (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Masumi Tsuda / Dept. Cancer Path., Faculty of Med., Hokkaido Univ.

P-2001

Remarkable difference between 3D and 2D cultures of cancer cells in response to drugs

Takahiro Yoshida
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Co-author：Norio Nonomura
Dept. Urol. Osaka Univ. Grad. Med.

Tumor cells growing as multicellular spheroids in 3D culture, alternatively called organoids, are believed to more closely mimic solid tumors in situ. In this study, we compared 2D and 3D cultures in terms of chemosensitivity of bladder cancer cells. Bladder cancer cell lines RT4 and 5637 spontaneously formed round spheroids with time in poly-HEMA coated dishes. The cell lines proliferated more rapidly in 2D culture than in 3D culture. High-throughput chemosensitivity assay demonstrated that the cell lines were more resistant to cisplatin and gemcitabine in 3D culture than in 2D culture. Strikingly, CHIR99021, a GSK3 inhibitor and Wnt pathway activator, promoted proliferation of the cell lines only in 3D culture but inhibited proliferation in 2D culture. The enhancing effect of CHIR99021 on proliferation in 3D culture was also confirmed by examining patient-derived organoids. In conclusion, high-throughput drug sensitivity assay revealed remarkable difference between 3D and 2D cultures of cancer cells in response to drugs. Our data is a concrete example of why 3D culture is important for cancer research, e.g. drug screening in vitro.

▲TOP

P-2002

Combined gemcitabine and pitavastatin anticancer studies in pancreatic cancer

Ya-Hui Chen
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[1]Ctr. of Diabetes Res., Dept. Res., Changhua Christian Hosp.、[2]Ctr. of Diabetes Res., Dept. Res., Changhua Christian Hosp., Div. Endocrinology & Metabolism, Dept. Internal Med.

Pancreatic cancer (PC) is an aggressive cancer, with high rates mortality, a poor prognosis and limited therapeutic options. The objective of the present study was to demonstrate the synergistic anticancer effect of combined gemcitabine (GEM)-pitavastatin (PITA) in MIA PaCa-2 cells. Cell viability was evaluated using a CCK-8 assay. The effect of combined GEM-PITA on cell cycle phase distribution, cell death progression and mitochondrial membrane potential (MMP) were evaluated using flow cytometry. Western blotting was employed to detect target protein change in MIA PaCa-2 cells following combined GEM-PITA treatment. The results revealed that combined GEM-PITA induced a dose dependent inhibition in the growth of MIA PaCa-2 cells. Cell cycle analysis revealed that combined GEM-PITA induced sub-G1 and S-phase arrest in MIA PaCa-2 cells. Staining with Annexin V/PI revealed that apoptosis and necrosis occurred in these cells by PARP-1-caspase3/9 and RIP3-RIP1-MLKL dependent pathways. The results also demonstrated that combined GEM-PITA treatment resulted in a loss of mitochondrial MMP. Therefore, combined GEM-PITA may be used as a therapeutic agent in the treatment of human PC.

P-2003

Induction of apoptosis accompanied by G2/M phase arrest in mouse lymphoma cells by 9-(E,Z)-hydroxyoctadecadienoic acid

Makoto Tsuiji
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[Aim] It has been reported that linoleic acid oxide hydroxyoctadecadienoic acid (9-HODE and 13-HODE) have inhibitory activity on drug efflux pumps and cell proliferation and DNA synthesis as an agonist of PPAR γ. In this study, the effect of HODE on proliferation of mouse lymphoma cells was analyzed.[Methods] The isomers of 9-HODE and 13-HODE were added to the culture media for mouse lymphoma cells (EL4 and E.G7-OVA). After 24 hours, the numbers of cells were measured by the WST-8 assay. Cells stained with Annexin-V-FITC and PI were measured by FACS. The cell cycle analysis was also performed.[Results & Discussions] 9-(E,Z)-HODE has remarkable proliferation inhibitory activity. At 24 hours, the population of late apoptotic cell increased. The populations at G2/M phase and sub G1 increased. DNA ladder and fragmented caspase 3 appeared. In the previous studies, a mixture of isomers has been used for 9-HODE, but the results of this study indicate that only 9-(E,Z)-HODE among the isomers induced apoptosis accompanied by G2/M phase arrest and showed growth inhibitory activity.

P-2004

An ex-vivo culture system of ovarian cancer retains the pathological features of primary tumors faithfully

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We have succeeded to generate a series of xenografts faithfully recapitulating the histological phenotypes of the primary ovarian cancers. By modifying a culture condition which supports primary normal human oviductal epithelial cells, we established eighteen ovarian cancer cell lines out of eighteen patient samples obtained by surgical resection, which include all main four types of epithelial ovarian cancer histology. Cancer cells were enriched by the anti-EpCAM antibody-conjugated magnetic beads and propagated in the optimized condition. Histotype specific marker expression was confirmed in vitro and the xenograft tumor tissues from all subtypes except mucinous type. None of three typical mucinous type cell lines were tumorigenic in immune-deficient mice, but conditional expression of a set of oncogenes forced the non-tumorigenic cells to form tumors in nude mice with more poorly differentiated phenotype. However, termination of the oncogene expression resulted in well-differentiated mucinous phenotype recapitulating the original tissue. These novel cell lines and xenografts should be useful for better understanding of ovarian cancer.

▲TOP

P-2005

Translational three-dimensional culture method to study clinical status of colorectal cancer with liver metastasis

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For translational research, new research tools with structural materials and tissue engineering are necessary to reflect the precise condition of patients. Given that liver metastasis is the most common in recurrence of colorectal cancer (CRC), we developed a three-dimensional (3D) cultured organism method, which is not inferior to animal vivo experiments and can give an efficient platform to study mechanism and discover drug. To this end, we conducted 3D culture method composed of extracellular type I collagen and stromal fibroblasts, as well as endothelial cells, HUVEC, showing similarity to vivo organs. The results showed that co-culturing with HUVEC gave rise to the network formation in tumor. In contrast to 2D, the expression of hepatocyte function-related genes increased in 3D model, and the expression profiling mimicked mice in vivo. Utilizing this model, we then studied the involvement of extracellular exosomes, which contain miRNAs and metabolites and are proposed to form the pre-metastatic niches in liver at early stages of massive metastasis. The present study showed the feasibility of 3D model for translational study of live metastasis in CRC.

P-2006

Conditional reprogramming cells are novel tools for drug response assay in Luminal B breast cancer

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The aim of study was to develop an experimental system that allows us to search effective drug quickly and test the efficacy in vitro.METHODS: Cells of core needle biopsy samples of Luminal-B BC were transferred to conditional reprogramming conditions to generate immortalized cultures with ROCK inhibitor and Swiss 3T3 cells. Breast cancer panel target sequence was performed to compare the original tumor and conditional reprogramming cells (CR cells). Drug response was assessed by MTS assay with 4OH-tamoxifen, adriamycin and docetaxel. Oncotype Dx (Genomic Health) was performed to estimate the benefit of chemotherapy.RESULTS: Using this protocol, three of five CR cell lines were generated during 6 weeks. DNA target sequences were consistent within primary tumor and CR cells after several passages.The CR cells from Luminal B breast cancer with low recurrence score of Oncotype Dx was more sensitive to endocrine therapy than chemotherapy. CONCLUSIONS: CR cells of Luminal-B BC were promising experimental tool for drug screening and gene expression pathway analysis from small amount of clinical samples.

P-2007

Establishment of the substrata made of tissue/organ sections for histopathology based systems for nanotoxicity

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A culture model utilizing substrata made of tissue/organ sections for histopathology (TOSHI) appears to conserve both tissue composition and microarchitecture in an in vivo environment. We aim to establish a (TOSHI) based systems including gpt assay for toxicity of nanomaterials. In this study, A549 cells, human lung epithelial cells, were cultured as a monolayer form on sliced tissues prepared from liver and lung of F344 rats, exposed to Fe_3O_4 nanoparticles. Cell viability, ROS production, EGFR and integrin expressions was analyzed in this system. Different sliced tissues induced different cell response of A549 cells such cell adhesion, integrin and EGFR expressions. These results suggest that this system may provide a microenvironment affecting cell response after exposure of nanomaterials. We will also present results of gpt assay in this system after treatment of Fe_3O_4 nanoparticles.

▲TOP

[P-2015] P1-3 [English/Japanese]
DNA damage

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Kumiko Ogawa / Path., Natl. Inst. Health Sci.

P-2015
Withdrawn

No Abstract

▲TOP

P-2016

HDACi down-regulate proteomic UNG2 by ubiquitin-proteasome degradation

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Yantao Bao, Ge Liu, Xiaopeng Lu, Qian Zhu, Wei-Guo Zhu. Histone deacetylase inhibitors (HDACi) and 5-FU were found to have synergistic toxicity towards cancer cells. Co-treatment with these two drugs enhanced DNA damage. Previous studies found 5-FU to misincorporate into genomic during DNA replication. Misincorporated 5-FU can be recognized by Uracil DNA glycosylase and repaired by base excision repair (BER). Our work showed HDACi to down regulate UNG2 protein levels in cancer cells. Further studies showed HDACi degraded UNG2 by an ubiquitin-proteasome dependent manner. This study demonstrated the capability of HDACi to degrade UNG2, a glycosylase that was vital in BER pathway initiation, suggesting a mechanism that was involved in synergistic therapies by combining HDACi and 5-FU/other UNG2-targetting agents.

P-2017

Destabilization of linker histone H1.2 is essential for ATM activation and DNA damage repair

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Linker histone H1 is a master regulator of higher order chromatin structure, but its involvement in DNA damage response and repair is unclear. Here, we report that linker histone H1.2 is an essential regulator of ataxia telangiectasia mutated (ATM) activation. We show that H1.2 protects chromatin from aberrant ATM activation through direct interaction with the ATM HEAT repeats domain and inhibition of the MRE11-RAD50-NBS1 (MRN) complex-dependent ATM recruitment. Upon DNA damage, H1.2 undergoes rapid PARP1-dependent chromatin dissociation through poly-ADP-ribosylation (PARylation) of its C terminus and further proteasomal degradation. Inhibition of H1.2 displacement by PARP1 depletion or H1.2 PARylation-dead mutation compromises ATM activation and DNA damage repair, thus leading to impaired cell survival. Taken together, our findings suggest that linker histone H1.2 functions as a physiological barrier for ATM to target the chromatin, and PARylation-mediated active H1.2 turnover is required for robust ATM activation and DNA damage repair.

P-2018

Withdrawn

No Abstract

P-2019

PMS1 is involved in O6-methylguanine-induced apoptotic pathway

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The mismatch repair (MMR) protein complex, MSH2/MSH6 and MLH1/PMS2, plays important roles in tumor suppression by inducing apoptosis to cells carrying O^6-methylguanin in DNA, in addition to repairing mismatched bases. However, the molecular mechanism of the pathway is still obscure. To identify genes involved in the apoptotic pathway, we performed affinity purification of proteins interacting with and present proximally to MLH1, followed by mass spectrometric analysis. In this attempt, we identified PMS1, forming heterodimer with MLH1, but the function of which is still largely unknown. To unveil whether PMS1 is involved in either MMR and/or MMR-dependent apoptosis, we constructed a PMS1-knockout cell line using CRISPR/Cas9. The KO cells shows significant resistance to an alkylating agent in contrast to the parent cell line, HeLa MR. The appearance of sub-G1 population after administration of an alkylating agent is suppressed in the KO cells. However, the phosphorylation of ATR and CHK1 kinases during checkpoint activation is comparable to that of wild type cells. These results suggest that PMS1 functions as an executor of apoptotic induction during MMR-dependent apoptosis pathway.

P-2020

ATM regulates INO80 chromatin remodeling complex phosphorylation to prevent chromosomal translocations

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The mechanism of chromosome translocations is largely unknown. Chromosome aberrations involving 11q23 abnormalities are one of the most frequent chromosome abnormalities in secondary leukemia associated with etoposide treatment. Abrogation or loss of the ataxia-telangiectasia mutated (ATM) protein, increases the 11q23 chromosome translocations. We previously reported that ATM deficiency results in the excessive binding of DNA repair factors, RAD51 and INO80, at the breakpoint clustering region (BCR) of MLL gene in 11q23 chromosome translocations. Here, we show that ATM regulates the phosphorylation of ARP8, a subunit of the INO80 chromatin remodeling complex, to repress the excessive binding of INO80 and RAD51 to the BCR of MLL and reduce the incidence of 11q23 chromosome translocations. These findings suggest that ATM plays an important role in maintaining the fidelity of DNA repair to prevent the etoposide-induced 11q23 abnormalities through regulating ARP8 phosphorylation. The mechanisms of etoposide-induced 11q23 chromosomal translocations will be discussed.

▲TOP

[P-2028] P1-5 [English/Japanese]
Process of carcinogenesis (2)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Katsumi Imaida / Onco-Path., Kagawa Univ., Fac. Med.

P-2028

Expression of PD-L1 in carcinogen-induced lung tumor cells of rodents

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Recently, it is important to check the expression of programmed cell death ligand 1 (PD-L1) in tumor cells, for immuno-checkpoint therapy. It is known that PD-L1 negatively regulates T-cell signaling and frequently upregulated in some malignant tumors, including melanoma, ovarian and lung cancers.
In the present study, the expression of PD-L1 (CD274 polyclonal antibody, abnova, 1:400) was examined by immunohistochemical staining for the lung proliferative lesions by N-bis(2-hydroxypropyl)nitrosamine (DHPN) and the chronic inflammation by quartz (intratracheal instillation), in F344 rats, and by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in A/J mice.
PD-L1 expressions were observed in cytoplasm, nucleus and nucleolus of tumor cells in both of rats and mice. In rats, the expression of PD-L1 is not different among the histological types and with or without chronic inflammation. In mice, PD-L1 tends to be strongly expressed in adenocarcinoma (AC) than adenoma (AD) and hyperplasia. Especially, the expression in nucleolus is only observed in AD and AC. These results suggested the expressions in nucleus and nucleolus might be one of the factors for development to tumor cells.

▲TOP

P-2029

Development of neuroblastoma model from iPS-based neural crest cells for analysis of neuroblastoma tumorigenesis

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Neuroblastoma (NB) is thought to originate from undifferentiated neural crest cells (NCCs). In NB, MYCN seems to be the most powerful oncogenic molecule and govern NB tumorigenesis. Although MYCN activates apoptotic p53 pathways, we reported functional inactivation of p53 in NB cells (Kurata K et al., ONCOGENE, 2010; Yamaguchi Y et al., EJC 2014). The mechanism of MYCN and p53 inactivation to induce NB has not been elucidated. To investigate the mechanism, we prepared wild type iPSC, iPSC derived from Li-Fraumeni syndrome patient, and neural crest cells (NCCs) differentiated from iPSCs in vitro (Fukuta M et al., PlosOne, 2014). MYCN was successfully introduced into the NCCs by lentiviral vectors. By soft agar assay, transformed clones were obtained from wild type and Li-Fraumeni NCCs, indicating that MYCN overexpression and p53 inactivation promotes NB tumorigenesis. MYCN expression was significantly higher in the MYCN-transduced cells and downstream target genes, BMI1 and CDT1 were up-regulated. Together, MYCN-related transformation in iPS-derived NCC has achieved and it appears to be an ideal system to study the NB tumorigenesis.

P-2030

Content-dependent transformation with activated Ras isoforms in epithelial cells

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The classical mammalian ras genes exist three isoforms K-, H- and N-Ras. Ras mutations are genetic events that have been detected in 30 % of all human cancers. One of the issues of cancer therapy is that mutated Ras tumors are resistant to EGFR chimeric monoclonal antibody treatment. Therefore, there is a need for development of new therapeutic strategies to mutated Ras. One reason is that the occurrence of Ras isoform mutation is largely different in each tissue. In this study, we analyzed the carcinogenesis efficiency and function of each Ras isoform using human normal bronchus and small airway epithelial cells. Firstly, we induced carcinogenesis by introducing each Ras (G12V) retrovirus vector and evaluated carcinogenesis efficiency by soft agar assay. As a result, carcinogenesis efficiency showed difference between them. Also, we identify down-stream signaling of Ras involved in carcinogenesis by performing western blot analysis. These results indicated that the protein expression level which associated with growth-related PI3K/AKT and MAPK signaling affected differently in each Ras isoform.

P-2031

LAT1 inhibitor JPH203 inhibit cell proliferation, invasion, and migration through IGFBP-5

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Objectives: L-type amino acid transporter 1 (LAT1) that transports essential amino acids is highly expressed in many tumors and cancer cell lines. We examined the antitumor effect in human bladder cancer cells using LAT1 inhibitor JPH 203.Methods: Gene expression was compared by qPCR and expression of related proteins was examined by Western blotting. SiRNA was used for knocking down the target gene. A functional experiment of bladder cancer cell line was performed using LAT1 inhibitor JPH203. Genes related to LAT1 were analyzed using RNA-Seq.Results: LAT1 was highly expressed in 5637 and T24 cells. LAT1 knockdown and JPH203 10 μM suppressed cell proliferative, invasive and migratory ability. JPH203 inhibited phosphorylation of MAPK / Erk and p70S6K. IGFBP-5 was identified as a gene that most changed by SiLAT1 by RNA-Seq analysis. SiIGFBP-5 suppressed the cell proliferation in two bladder cancer cell lines like JPH 203.Conclusions: The study demonstrated that JPH 203 had an antitumor effect in bladder cancer patients. Since JPH 203 is currently undertaking the first domestic clinical trial in solid cancer patients, the clinical benefit of JPH203 is awaited to be investigated.

▲TOP

P-2032

Multifaceted roles of Ptger2 (Prostaglandin E receptor 2) in asbestos-induced inflammation and malignant mesothelioma

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Our current research focuses mainly on asbestos-induced malignant mesothelioma. We had established an animal model of malignant mesothelioma. As our microarray results reveal that the expression of PTGER2 is significantly upregulated in mesothelioma, we believe that this gene might play a potentially important role in mesothelioma. To investigate the potential role of PTGER2 in maintaining the malignant phenotypes of MM cells, we knocked down PTGER2 expression in both SM and EM cell lines, using two different shRNA constructs. Reduced PTGER2 expression resulted in the suppression of proliferation in both SM and EM, as assessed by two cell proliferation assays with Ki-67 expression and dead cell fraction. Moreover, PTGER2-depleted tumour cells displayed a reduced colony-forming ability. PTGER2 depletion also inhibited mesothelioma cell invasion. We also found PTGER2 is associated with COX2/PGE2 inflammatory pathway, stimulating the Akt signaling pathway in asbestos-induced malignant mesothelioma. Next step we will discuss the effect of the non-steroidal anti-inflammatory drugs and ptger2 inhibitors in tumorigenicity of asbesotos-induced malignant mesothelioma.

P-2033

Inflammatory microenvironment derived from asbestos increases mutagenesis to repairing mesothelial cell

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【Purpose】 Asbestos induced carcinogenesis was reported in 1950`s. However, the carcinogenesis mechanisms have not been elucidated yet. The aim of this study is to determined what plays a critical role in tumor initiation in asbestos exposure.【Methods】 Fischer 344 rats were used as a model of inflammation and injected asbestos to peritoneal cavity. From these rats, we collected peritoneal organs for pathological analysis and mesothelial cells of gene expression analysis. To demonstrate increasing mutagenesis, we used mesothelial and macrophage cell lines for co-culture assay.【Results】 Asbestos induced to tissue remodeling, and we found the macrophage not to mesothelial cell engulfing. The asbestos associated inflammation induced to intra- and extra cellular Fe(II) overload which contribute to mutagenesis. From co-culture analysis, we demonstrated inflammatory microenvironment as the same of in vivo analysis, and DNA double strand break and oxidative damage were induced in repairing mesothelial cells indirectly. Hence, we speculate the inflammatory microenvironment induced by asbestos play a critical role in mutagenesis during carcinogenesis.

P-2034

Estimated acquired gene variants contributing to the carcinogenesis of occupational cholangiocarcinoma

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Several approaches using the samples at the sequential stages of carcinogenesis have been tried to understand the molecular events during progression of cancer. However, it is difficult to spatiotemporally collect samples. Recently identified occupational cholangiocarcinoma among printing workers was a high incidence of early-onset with hypermutation and characterized by precancerous lesions at multiple sites of the bile ducts. We performed whole-exome analysis of multiple lesions, including the invasive carcinomas and precancerous lesions of four occupational cholangiocarcinoma cases, to understand the molecular events during carcinogenesis. The mutation rates of the cell cycle/apoptosis pathway and the chromatin modification pathway tended to be higher in the invasive carcinomas than in the precancerous lesions (100.0% vs. 57.1%; P = 0.06 and 100.0% vs. 71.4%; P = 0.18, respectively). Although there is a problem of small sample size, these results suggest that disruption of the genes including these pathways might be necessary for acquisition of the invasive phenotype and this approach using hypermutated tumors is useful for exploring the important genes for carcinogenesis.

▲TOP

[P-2042] P1-7 [English/Japanese]
Radiation carcinogenesis and oxidative stress (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yoichiro Kusunoki / Mol. Biosci., Radiat. Effects Res. Found.

P-2042

Glut3-siHIF1a-Ag@Mn Nanomedicine for Targeted MRI Guided Radiotherapy Sensitization in pancreatic cancer

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To develop a nanoplatform with imaging and radiotherapy sensitization is urgently needed for cancer. In this work, a multifunctional nanoplatform (Glut3-siHIF1a-Ag@Mn) was developed for the pancreatic carcinoma with T1-weighted magnetic resonance imaging (MRI) and radiotherapy sensitizing effect. TEM and UV-Vis results suggest that the Glut3-siHIF1a-Ag@Mn nanoplatform has uniform diameter of 95 nm with good dispersity. Glut3-siHIF1a-Ag@Mn can specially target pancreatic cancer cells with high expression of Glut1. The viability of mouse embryo fibroblasts and human pancreatic cancer cells is still greater than 90% by incubating with various concentration Glut3-siHIF1a-Ag@Mn(0, 0.1, 0.5, 1, 2umol/L). The Glut3-siHIF1a-Ag@Mn has good MRI signal and is shown to accumulate in the tumor region. Importantly, Glut3-siHIF1a-Ag@Mn shows great radiotherapy sensitizing effect on inhibiting tumor growth in vitro and in a Panc-1 xenograft model. Histopathological analysis further reveals that the combination Glut3-siHIF1a-Ag@Mn with radiotherapy results in most extensive cell apoptotic and necrotic in the tumor without inducing obvious side effect to major organs.

▲TOP

P-2043

Chemoprevention of radiation-induced intestinal tumors by trans-Resveratrol in ApcMin/+ mice

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Medical technologies such as radiotherapy and CT scan have progressed rapidly for cancer treatments or diagnoses. Therefore, second cancer after those treatments has been clinical problem in survivors, especially childhood cancer. In addition, children seem to be more sensitive the effects of radiation. Hence it is important to establish the protection against second cancer induced by radiation exposure in childhood. The aim of this study is to examine the possible chemopreventive effects of trans-Resveratrol (Res), one of phytochemicals, against radiation-induced intestinal tumors in $Apc^{Min/+}$ mice. Male C3B6F1 $Apc^{Min/+}$ mice were irradiated 0 or 2 Gy X-rays at 2 or 7 weeks old. After interval of 2 weeks, mice were administrated with a drink containing 0, 0.002 or 0.01% Res until dissected at 30 weeks old. The tumors were counted and measured their size. Both doses of Res significantly decreased the total number of small intestinal tumors in irradiated mice. Especially, expansion of tumor size was significantly inhibited. These findings suggested that Res has a potency to prevent the intestinal tumors induced by radiation in both young and adult mice.

P-2044

Effect of radiation exposure on mouse B-cell lymphoma development

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Epidemiological studies of the Japanese atomic-bomb survivors have demonstrated an increased risk of hematopoietic malignancies after exposure to ionizing radiation at young ages. Other studies on patients exposed to medical radiation provide evidence that there is a risk of secondary B-cell acute lymphoblastic leukemia. However, the effect of radiation on B-cell lymphoma development are yet to be fully elucidated, because cancer in humans occurs as a result of the combined effects of radiation with other factors such as age, diet, and family history. In this study, we aimed to investigate the effect of radiation on B-cell lymphoma development and related molecular mechanisms using a mouse model. We first performed immunohistochemical classification of B-cell lymphomas developed after whole-body irradiation of male and female B6C3F1 mice with 4 Gy of gamma-rays at 1 or 7 weeks of age. A fraction of B-cell lymphomas in irradiated group developed significantly earlier than those of the non-irradiated group. Whole-exome sequencing of B-cell lymphomas developed spontaneously or after irradiation is currently in progress and mutational patterns of these lymphomas will be discussed.

P-2045

Influence of diet-induced obesity (DIO) on tumorigenesis and tumor development after early life exposure to radiation

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It is known that children are highly susceptible to the carcinogenic effects of ionizing radiation. Since tumor development after radiation exposure is long process, influence of lifestyle has been concerned. Especially, diet-induced obesity (DIO) is tightly linked to cancer risk factor. We aimed to assess the influence of short term DIO at young adult age on tumorigenesis and tumor development after infant radiation exposure. B6C3F1 male mice were irradiated at one-week-old with 0, 0.1, 1 and 4 Gy of gamma-ray. After weaning, mice were fed with normal or high-fat (HF) diet for 4 weeks, then with standard lab diet. Measurement of body weight (BW), plasma insulin, adiponectin, leptin concentrations and histopathological analysis of liver tissue were carried out. Mice fed with HF diet were observed a gain of BW, higher level of plasma insulin, leptin, adiponectin and lipid accumulation in hepatocytes. These changes were rapidly recovered after the termination of HF diet administration. Thereafter, lipid re-accumulation in hepatocytes was accelerated by both DIO and/or radiation exposure. The influence of DIO on liver tumor development and lifespan shortening would also be discussed.

▲TOP

[P-2050] P1-9 [English/Japanese]
DNA damage and carcinogenic process

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-2050

DNA-PK inhibition releases PARP inhibitor-induced DNA replication stress

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DNA-dependent protein kinase (DNA-PK) is one of the component associated with DNA double-strand break (DSB) repair and has the critical role in non-homologous end-joining (NHEJ) pathway. On the other hand, recent studies indicated DNA-PK has some functions during DNA replication process, however the details have not been well known.In this study, we found DNA-PK inhibition caused the DNA replication restart from PARP inhibitor-induced replication stalling. This also showed resistant response to PARP inhibitor in cytotoxicity. Some data have indicated that the repair pathway could be independent of other major DSB repair pathways. We are considering the observation has a common mechanism with maintenance of genomic stability and now investigating the detailed mechanism.

▲TOP

P-2051

Role of human DNA polymerase θ in double-strand break repair and foreign DNA integration

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Human cells possess at least two genetically distinct mechanisms for homology-independent repair of DNA double-strand breaks (DSBs): non-homologous end joining (NHEJ), in which DNA ligase Ⅳ (Lig4) is indispensable, and alternative end-joining (A-EJ) whose molecular mechanism remains elusive. Here we show that DNA polymerase θ (Pol θ) is essential for A-EJ, which is the sole homology-independent DSB repair route in the absence of NHEJ and is typically characterized by either 2-6-bp microhomology or templated insertions at the junctions. Remarkably, despite its minor role in chromosomal DSB repair, Pol θ substantially contributes to random integration of transfected DNA, and cells doubly deficient in Pol θ and Lig4 exhibit 100% gene-targeting efficiency by virtue of undetectable levels of random integration. Further, we provide the first direct evidence that the dual loss of Pol θ and Lig4 reveals rare Alu-Alu recombination events, which are reliant on Rad52 and suppressed by Msh2. Our findings provide new insights into the mechanics of foreign DNA integration and the role of Pol θ in preserving genome integrity.

P-2052

Inflammation-related DNA damage and cancer stem cells in bladder cancer

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Chronic inflammation is an important risk factor for carcinogenesis. Under inflammatory conditions, reactive oxygen species and reactive nitrogen species are generated from inflammatory and epithelial cells and result in oxidative and nitrative DNA damage. We previously reported that COX-2 was co-localized in nuclei with stem cell biomarkers, Oct3/4 and CD44v6, in bladder cancer tissues of patients with chronic inflammation by parasite infections. In this study, we investigated the relationship of cancer stem cell with nitrative DNA damage, 8-nitroguanine, in inflammation-related carcinogenesis, by immunohistochemical analysis for bladder tissues. The cancer stem cell biomarker, CD44v9, and 8-nitroguanine, were positively stained in bladder cancer tissues. High mobility group box 1 (HMGB1), which plays a role in inflammatory signals, tended to be highly stained in the COX-2 -positive cancer tissues.
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P-2053

Localization of CD44v6 expression in NNK-induced mouse lung adenocarcinoma at the advanced stage

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Expression of CD44 variant isoform encoded by variable exon 6 (CD44v6) have been observed in various human cancers and is correlated with poor prognosis through a variety of signaling pathways. In this study, in order to determine whether CD44v6 is involved in advanced stage of lung cancer, expression of CD44v6 in the 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) -induced mouse lung adenocarcinomas was examined immunohistochemically. Female 7-week old A/J mice were administered NNK (2 mg/0.1 ml saline/mouse, i.p.) at days 0 and 7, then maintained without any additional treatment until sacrifice at weeks 78. Advanced adenocarcinomas were observed, and the small clusters of adenocarcinoma cells in air spaces surrounding the edge of the tumor were observed in 8/12 mice (67%). This small tumor clusters were composed of papillary structure or solid nest, and expressed CD44v6 strongly. In the adenocarcinoma nodule with the small tumor clusters, level of CD44v6 expression was elevated in papillary structures of peripheral area. These results suggest that CD44v6 is associated with growth pattern of lung adenocarcinomas at the advanced stage of NNK-induced mouse lung carcinogenesis.

▲TOP

P-2054

Effects of nicotine on rat urinary bladder carcinogenesis

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Tobacco smoking is a major risk factor for human cancers including urinary bladder carcinoma. Nicotine is a major component of tobacco smoke, but it is not clear whether nicotine is carcinogenic on the urinary bladder. In the present study, we examined the promoting activities of nicotine in two-stage urinary bladder carcinogenesis in rats using N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN). In results, nicotine significantly increased the incidence and number of urothelial carcinomas in a dose-dependent manner. Ki67 and pSTAT3 labeling indices and expression of nicotinic acetylcholine receptor (nAChR) alpha 7 were significantly increased by nicotine, but the TUNEL assay for apoptosis showed no increase. In a 4 week study, inhibitors of nAChRs decreased nicotine-induced urothelial simple hyperplasia and Ki67 labeling index in the bladder and kidney pelvis. These data suggest that nicotine enhances urinary bladder carcinogenesis via nAChR and STAT3 signaling pathways.

P-2055

Gastric carcinogenesis mechanism due to abnormal expression of chromatin reconstitution factor, ARID1A

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It has been suggested that AT-rich interactive domain-containing protein 1A (ARID1A) plays a role in the suppression of various cancers. This study was designed to unravel the pathobiological role of impaired ARID1A expression in gastric carcinogenesis. We examined ARID1A expression immunohistochemically in 100 gastric cancer tissue specimens with regard to the clinicopathological features. Based on the proportion and intensity of ARID1A immunoreactivity at the cancer invasion front, we subdivided the specimens into low- and high-expression ARID1A groups. Notably, low ARID1A expression was significantly correlated with poor disease-free and overall survival of the patients. A comprehensive gene profiling analysis followed by immunoblotting revealed that a pro-apoptotic member of the Bcl-2 family of proteins, Harakiri (also known as death protein 5), was less expressed in ARID1A low/poor prognosis than ARID1A high/good prognosis gastric cancers. The present findings indicate that impaired ARID1A expression might lead to gastric carcinogenesis, putatively through gaining resistance to the Harakiri-mediated apoptosis pathway.

▲TOP

[P-2062] P4-5 [English/Japanese]
p53-related genes (1)

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P-2062

Immunohistochemical analysis of P53 and MAF expression in colorectal cancer

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MAF is reported as oncogene in breast cancer and multiple myeloma. However, our in vitro experiments indicated that knock down of MAF expression led to increased proliferation and enhanced colony formation in intestinal cells. To investigate the role of MAF expression in colorectal cancer, we performed immunohistochemistry for MAF expression (n=101) and examined the impact on patients' prognosis. p53 staining was also performed in a subset of resected specimens.Immunohistochemistry for the MAF protein revealed that MAF expression significantly decreased in tumor tissues as compared to the normal counterparts (P=0.024). This occurred at early stage cancers. Kaplan Meier curve of RFS (relapse free survival), or OS (overall survival) showed that low expression group had a worse tendency than high expression group (P = 0.095, P = 0.061). We also found that MAF expression was inversely correlated to intense p53 staining compatible to mutant p53.Our data suggest that MAF protein appears to function as tumor suppressor rather than oncogene in intestinal cells and colorectal cancer. MAF may suppress cell proliferation in cooperation with p53.

▲TOP

P-2063

Mitotic surveillance by nucleolar stress response and its role in cancer therapy

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Nucleolar stress response (NSR) helps to maintain nucleolar integrity and inhibits cell growth by activating p53 pathway during nucleolar dysfunction. To determine the role of NSR in physiological setting and tumor therapy, we developed a novel fluorescence reporter system for NSR in living cells. Chemical screening using the reporter system showed mitotic inhibitors as NSR inducers. Time-lapse imaging analysis showed that NSR was transiently induced at nucleolar disassembly at the beginning of mitosis and decreased at nucleolar reassembly after mitosis. Nucleolar reassembly disruption by chemicals resulted in high NSR even after mitosis. Prolonged mitotic inhibition increased multinuclei with no nucleoli, which show abnormal NSR induction, demonstrating the relationship between nucleolar reconstitution in mitosis and NSR. Mitotic inhibition induced NSR-dependent p53 activation and tumor growth inhibition. The breast cancer patients received mitosis-targeted cancer therapy with high expression of NSR regulatory gene displayed prolonged recurrence-free survival periods. Thus, NSR has a role in mitotic surveillance via nucleolar reassembly, with sensitivity to cancer therapy.

P-2064

Influence of nucleoside analogue-induced DNA replication stress on mutant p53-mediated cell fate decision

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TP53 is the most frequently mutated gene in cancers. Most of the mutations exist within the DNA-binding domain as a single amino acid substitution. The most common missense mutations in the p53 gene not only abrogate its tumor suppressive function, but also lead to a gain-of-function effect. In our current research, Trifluridine (FTD; nucleoside analogue)-induced DNA replication stress (DRS) activates p53 and subsequently induces cellular senescence in p53-proficient cells, whereas FTD triggers apoptosis via aberrant mitosis progression in p53-knockout cells. However, how the expression of missense mutant p53 affects cancer cell fate outcomes modulated by DRS remains unclear. Here, we generated p53 missense mutation knock-in HCT-116 cells using CRISPR/Cas9 gene-editing system. Our study provides the first comprehensive characterization of DRS against wild-type p53, p53-null, and (conformational and contact) mutant p53 in isogenic cancer cell line model. These analyses may unveil key therapeutic vulnerabilities of mutant p53 cancer.

P-2065

Switching of p63 from the beta-catenin suppressor mode to the co-activator mode

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The most prominent isoform of p63 (TP63) in carcinomas and keratinocyte stem cells, ΔNp63α (here referred to as p63), was originally characterized as a dominant negative-type suppressor of the p53 family trans-activators. As we reported, p63 also binds to TCFs/LEFs to suppress β-catenin. However, different lines of evidence indicated unusual cases of β-catenin activation by p63. This study aimed to investigate the puzzling results. We and others found that p63 interacts with p300 and NF-Y known as transcriptional co-activators. In the stage of moderate β-catenin activation, p63 played as the suppressor by binding to TCF4. However, when the maximum activation was achieved with nuclear-targeted S33Y β-catenin, p63 was converted to the co-activator. This mode did not require a p53/p63 binding site, and was distinguished from trans-activation. The C- and N-terminal domains were found to bear the suppressor and co-activator functions, respectively. Thus, p63 may switch from the β-catenin suppressor mode to the co-activator mode in cooperation with the binding partners depending on the magnitude of β-catenin activation.

▲TOP

P-2066

Acute phase proteins as p53-targets in carcinogenesis

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Acute phase proteins (APPs) have been reported to be involved in innate anti-cancer response, however their regulatory mechanism remains largely unexplored. To evaluate the role of p53, a key transcription factor in DNA damage response and tumor suppression, in APP mediated anti-tumor activity, we carried out screening of APPs that are regulated by p53 using whole body transcriptome dataset. p53 wild-type mice ($p53^{+/+}$) and p53 knock-out mice ($p53^{-/-}$) were exposed to X-ray irradiation, and 56 of 88 APP genes exhibited more than 2-fold upregulation in wild-type $p53^{+/+}$ irradiated mice. This was then correlated with transcriptomic analyses on human cancer from The Cancer Genome Atlas (TCGA) data in which 5 genes (APCS, HP, ITIH4, MBL2 and SERPINA3) exhibited significantly reduced expression in hepatocellular carcinoma with TP53 mutation. Further functional analysis on these 5 APP genes shed light on p53-target genes of the innate immune system and its mechanism in carcinogenesis.

P-2067

Combination of gain-of-function mutation and lost of wild-type allele in p53 promotes colon cancer tumorigenicity

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More than 50% of TP53 mutations in colorectal cancer (CRC) are missense-type, and in most cases, wild-type TP53 is lost of heterozygosity (LOH). Previously, we generated $Apc^{\delta 716}$ $Trp53^{R270H}$ mice expressing mutant $p53^{R270H}$ in the intestinal tumor cells, and found that $Trp53^{R270H}$ promoted submucosal invasion, indicating gain-of-function (GOF) mechanism of mutant p53. Moreover, nuclear accumulated mutant p53 induced global transcriptome change with increased promoter accessibility. Notably, p53 was nuclear accumulated in $Apc^{\delta 716}$ $Trp53^{R270H/R270H}$ mouse tumor cells, while it was distributed to the cytoplasm in $Apc^{\delta 716}$ $Trp53^{+/R270H}$, suggesting that wild-type p53 interferes nuclear accumulation of mutant p53. To further examine the mechanism of p53 mutation/LOH, we generated $Apc^{\delta 716}$, $Kras^{G12D}$, $Tgfbr2^{-/-}$, $Trp53^{+/R270H}$ compound mice and established "AKT/P (+/mut)" organoid cells. We also constructed AKT/P (mut/LOH) cells, in which wild-type p53 was lost by LOH, and found that cloning efficiency was significantly increased by p53 LOH. These results indicate that p53 LOH in addition to GOF mutation is a crucial factor of tumor malignancy, even when other driver genes are mutated.

▲TOP

[P-2074] P4-7 [English/Japanese]
p53-related genes (2)

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P-2074

Identification of cell surface proteins on lung cancer cells interact with p53-depleted fibroblasts

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Communication between cancer cells and their microenvironment controls cancer progression. Cancer-associated fibroblasts (CAFs) are one of the major components of cancer microenvironment. We have previously revealed that p53-depleted fibroblasts enhance proliferation and invasion of lung cancer cells through increasing expression of TSPAN12, belonging to tetraspanin family, in fibroblasts. In this study, we explored cell surface proteins expressed in lung cancer cells, which interact with cell surface of p53-depleted fibroblasts. Mass spectrometry analysis revealed AXL receptor tyrosine kinase expressed in lung cancer cells interacted with p53-depleted fibroblasts. Moreover, we showed that recombinant soluble extracellular region of TSPAN12 was bound to surface of lung cancer cells. These bindings were suppressed by knockdown of AXL in lung cancer cells. Furthermore, we indicated that knockdown of AXL repressed proliferation of lung cancer cells enhanced by p53-depleted fibroblasts. These results suggested that AXL on surface of lung cancer cells interacts with TSPAN12 in p53-depleted fibroblasts, and these interactions are required for lung cancer progression.

▲TOP

P-2075

The roles of p120 catenin family protein as a novel p53 target in cancer

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p53 is the one of the most important tumor suppressor gene and frequently mutated in human cancers. It is activated by various cell stresses and inhibits cell proliferation through cell cycle arrest and apoptosis by modulating transcriptional regulation. Therefore, it is important that identify novel p53 transcriptional targets to clarify unknown functions of p53. To identify them, we performed ChIP-seq and RNA-seq using p53 overexpressing cells and cells treated with Nutlin-3a, a small-molecule that activates endogenous p53. As a result, we found candidate genes to which p53 directly binds and upregulated by activated p53. We analyzed one of these genes which is a member of p120 catenin family. We revealed that both their transcriptional activities and the expression of mRNA and protein are increased by p53 activation. The knockdown of the gene significantly suppressed the Caspase activity induced by the Nutlin-3a. Furthermore, low expression of the gene in cancer patients was correlated with decreased survival and poor prognosis. Collectively, our results show that the gene may be a novel prognostic predictor and therapeutic target for cancers.

P-2076

Identification of novel receptors for secreting protein p53PAD7 that triggers inhibition of cell proliferation

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p53 is activated by cellular stresses and transactivates its target genes that contribute to tumor suppression. We have identified p53PAD7 as a p53 target gene and overexpression of p53PAD7 results in inhibition of cell proliferation. However, molecular function of p53PAD7 is totally unknown. We found p53PAD7 is a secreting protein, and the addition of purified p53PAD7 to the culture cells stopped cell proliferation. We hypothesized that p53PAD7 acts as a ligand that should be received by specific receptors. We, therefore, performed immunoprecipitation of extracellular p53PAD7 in the presence of heterobifunctional crosslinker to identify p53PAD7 receptors on the cell surface. Subsequent mass spectrometric analysis successfully identified protocadherin factors, FAT1 and FAT4, as p53PAD7 interacting proteins. Human FAT1 and FAT4 are homologs of FAT found in Drosophila melanogaster, which is a receptor of Hippo signaling pathway that regulates cell proliferation. Consistently, we observed activation of Hippo signaling pathway when purified p53PAD7 is added to cell culture. These data suggest that p53PAD7 is a novel regulator of Hippo signaling pathway and cell proliferation.

P-2077

Role of p53-GATA3-RuvB2 Pathway in Regulating Malignant Transformation of Breast Cancer

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The two major tumor suppressor pathway, p53 and Rb have been shown to regulate the reprogramming of somatic and/or progenitor cells, whereas GATA3 functions in the upstream of Rb. Moreover, mutant p53, a gain of function, can actively have functional consequences with tumorigenesis. However, a little is known about the crosstalk and role of p53-GATA3 in cancer malignant regulation. Here, we generated p53 mutant KI cells and single and/or combination of KO cells for p53 and Rb or GATA3, and then performed organoid culture. We found that the introduction of p53 and Rb/GATA3 double KO or mutant p53 was solely able to mediate the transformation to malignant formation, whereas the knockout of p53 or Rb/GATA3 increased intermediate. To elucidate its mechanism, we have identified GATA3 binding proteins using LC-MS/MS analysis and then performed functional analysis. We found that RuvBL2 was associated with GATA3 and exerted repressive function for GATA3. Indeed, KM-plotter showed that RuvBL2 is significantly upregulated in poor prognosis group of breast cancer patients. Thus, our results suggest novel mechanistic insight into the cancer malignant regulation through p53-GATA3-RuvB2 pathway.

▲TOP

P-2078

Crucial roles of DDX31 as related to the status of TP53 in bladder cancer progression

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The elucidation of the molecular mechanisms of bladder cancer (BCa) are priority issues because patients of advanced BCa is poor prognosis. DEAD box polypeptide 31 (DDX31) are high frequently upregulation and TP53 are about 50% altered (mostly missense mutation) in BCa, but their contributions to the progression of BCa are unclear. Here, we report the crucial roles of DDX31 depending on the status of TP53 in BCa progression. We demonstrated that DDX31 bound to wildtype p53 (wtp53) in SW780 cells, and deletion of DDX31 led to the upregulation of the apoptosis-specific p53-target genes PUMA and Ei24, resulting in suppression of cell growth of bladder cancer suggesting that DDX31 potentially suppresses apoptosis induced by PUMA and Ei24 genes via its binding to wtp53. On the other hand, DDX31 bound to mutant p53 (mutp53) /SP1, thereby enhancing mutp53 (gain of function) transcriptional activation, resulting in enhancement of the aggressive behavior (cell invasion, migration and anchorage independent growth) of UM-UC-3 cells bearing TP53 mutation.These findings suggest that DDX31 may play the distinct progressive roles depending on the status of TP53 in BCa.

▲TOP

[P-2086] P6-2 [English/Japanese]
Cell cycle / genomic instability

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-2086

Functional relationships between tau deficiency and early breast carcinogenesis

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The microtubule associated-protein tau has been identified as a good positive prognostic indicator in breast cancer. To explore the role of tau in early carcinogenesis, we knocked it down in primary human mammary epithelial cells. This resulted in micronucleation in a katanin-like1 dependent manner. Chromosome bridges and loss of the kinetochore fibers on which tau is physiologically localized were also observed. Physical fragility was also confirmed following mitotic spindle isolation from tau-knockdown cells, supporting the relevance of microtubule damage. The karyotypes of tau-knockdown cells further suggested the involvement of tau in breast tumorigenesis. Consistently, we observed that the katanin-like1 induction of cellular transformation is prevented by tau in rat fibroblasts. The mutant katanin-like1 (L123V) identified in breast cancer showed an increased transformation capacity as well as microtubule severing activity. We propose that tau may contribute to tumor progression when there is an excessive imbalance in katanin-like1 pathways. We also present the microtubule-interacting octapeptide NAP as a candidate modifier against the tau deficiency in tumor cells.

▲TOP

P-2087

Mouse model predicts that driver mutations for tumorigenesis may arise in very early embryonic developmental stage

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It has been well known that the tumorigenesis is dependent on the multistep mutagenesis of the genomic sequences. Thus, the estimation of mutation rate is one of the critical factors to assess tumorigenesis. The “next generation sequencing” technologies now allow us to identify germline mutations by comparing the genomic sequences between the parents and their children. We further enhanced the detection of developmental stage-specific spontaneous mutations by combining the whole genome- and amplicon-sequencing technologies and applied them to the originally developed “complete outbreeding” pedigrees of the mouse. As a result, we found that 26.2% (56/214) of the so far identified germline mutations had originally arisen as mosaic somatic mutations between 2 cell- and 32 cell-embryonic stages. This unexpected extremely high mutation rate at the very early developmental time predicts that the de novo mutations that is responsible to trigger the very first step of tumorigenesis, namely, driver mutations, may mostly arise in the very early developmental stage.

P-2088

Non-coding RNA upregulated in cellular senescence provokes chromosomal instability

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Cellular senescence has been known as an important tumor suppression mechanism by inducing permanent cell cycle arrest. Recently, we have reported that epigenetic modification of chromatin is altered during cellular senescence. However, the effect of the alteration on transcriptional regulation remains elusive. Here, we found out that cellular senescence decreased trimethylations of histone 3 lysine 9 in some regions on genome which is tandemly repeating sequence, and enhanced the expression of non-coding RNA in human diploid fibroblasts. Especially, the expression of human non-coding RNA was dramatically upregulated. Similarly, the expression of murine non-coding RNA was increased during cellular senescence in MEF cells. Next, to analyze the function of senescent specific non-coding RNA, we performed overexpression of non-coding RNA in MEF cells. Interestingly, chromosomal instability (CIN) was observed in these MEF cells, and the cells were capable of anchorage-independent growth. These results suggest that cellular senescence may promote the expression of non-coding RNA having the risk for developing carcinogenesis.

P-2089

Novel anti-cancer compound CGK733 inhibits cell cycle progression and induces apoptosis

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CGK733 has been reported as an ATM/ATR inhibitor. CGK733 also induces or inhibits apoptosis depending on the experimental conditions. However, the molecular target of CGK733 and its mode of action have been unknown. Here, we found that low dose of CGK733 causes cell cycle progression delay. On the other hand, high dose induces cleavage of PARP, which is a target of activated caspase, suggesting that high dose of CGK733 induces apoptosis under our experimental condition. To investigate the molecular mechanism of the cell cycle progression delay, we checked the expression levels of cyclins and found that CGK733 treatment causes downregulation of cyclins. Among the cyclins, Cyclin A2 and Cyclin D3 were dramatically decreased upon CGk733 treatment. We will discuss the detailed molecular mechanisms of cell cycle progression delay by CGK733 treatment.

▲TOP

P-2090

The ataxia telangiectasia and Rad3-related kinase inhibitor AZD6738 sensitizes bladder cancer cells to gemcitabine

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Introduction: Ataxia telangiectasia and Rad3-related (ATR) kinase is activated at damaged replication forks and resected DNA double-strand breaks. Gemcitabine-induced DNA double-strand breaks are often repaired by ATR kinase, which attenuates gemcitabine's antineoplastic activity. In the present study we investigated the combined effect of gemcitabine and ATR kinase inhibitor AZD6738 in bladder cancer cells.
Methods: Using bladder cancer cells (5637, J82, T24, UM-UC-3), the combined effect of gemcitabine and AZD6738 was evaluated.
Results: The combination of gemcitabine and AZD6738 induced drastic apoptosis and killed bladder cancer cells synergistically (combination indexes < 1). It also suppressed colony formation significantly. Mechanistically, the combination synergistically caused DNA double-strand breaks evidenced by increased expression of pH2A.X. Furthermore, decreased expression of Rad51 confirmed that AZD6738 inhibited the repair of gemcitabine-caused DNA double-strand breaks by suppressing homologous recombination of DNA.
Conclusions: AZD6738 enhanced the sensitivity of bladder cancer cells to gemcitabine by inhibiting DNA repair.

P-2091

Identification of new proteins that specifically interact with a Kinetochore protein D40/Knl1/CASC5

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D40/Knl1/CASC5 (D40) gene encodes a kinetochore protein, which plays essential role in mitosis. Recently, it turned out that D40 is also one of causing genes for autosomal primary microcephaly (MCPH).
Purpose. Although several mitotic proteins were reported to interact with D40, we expect that there are unidentified D40-binding proteins. In this study, we tried to identify new proteins that specifically bind to D40.
Methods. Yeast Two-Hybrid system was used to screen and identify the cDNAs which encode D40-binding proteins. A c-terminal D40 cDNA fragment was cloned into pGBT9 to make a fusion with Gal4 DNA-binding protein. This plasmid was introduced into a yeast strain Y153, and the resultant clone was named as Y#134. The library plasmid, in which cDNAs derived from an immortalized B cell line was fused with Ga4 Activation domain, was transformed into Y#134 to screen both Histidine (+) and LacZ(+) clones.
Results. More than a dozen of positive yeast clones were identified, and the retransformation experiment of cDNAs recovered from the yeast clones identified that at least one cDNA was positive for both phenotypes, encoding a potential new D40-binding protein.

▲TOP

[P-2097] P7-1 [English]
Development of novel device / tool in genomic analysis [English]

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-2097

Accurate prediction of chromatin conformation status using deep learning

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Many reports have demonstrated that destructions of 3D structures of chromatin conformations lead to the activation of oncogenes. Hi-C method have made it enabled to display 3D proximities among each genomic locus. However its reproducibility is still uncertain in the current procedures. To obtain reproducible results without heavy computational task, we built a model to predict chromatin structures using deep learning based on genomic sequences, map positions on reference sequence, and epigenetic data. The model was constructed and evaluated based on the information of lymphoblastoid cell line NA12878. We showed ROC curves and measured the areas under the ROC curve (AUC) to quantify the prediction performance. The model we selected as the best-performed one displayed its high accuracy (96.25%), sensitivity (99.35%), and specificity (95.22%). The AUC of the model was 0.997 against a test data. These results suggest that our model is an effective method for predicting chromatin 3D structures substituted for Hi-C analysis. We expect our algorithm is useful to recognize the alterations of chromatin conformation that lead to the activation of oncogenes.

▲TOP

P-2098

Development of a single-cell sequencing platform enabling simultaneous detection of both gene mutation and expression

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Leukemic cells comprises a heterogeneous population of clones having different combinations of driver mutations, which might differentially impact the hematopoietic machinery. Recent development of single-cell analysis platforms has provided a novel opportunity to understand this heterogeneity of leukemic cells, which however, is largely hampered by the difficulty to detect both mutations and gene expression profiles at the same time. To overcome this, we newly developed a robust method for single cell sequencing. Exploiting both genomic DNA and RNA as sequencing templates combined with improved chemistry, we minimize allele-dropouts to achieve accurate determination of the allelic status of mutation together with gene expression status for hundreds of cells at a single run. By applying this methods to the analysis of longitudinal samples from a patient with normal karyotype AML, we successfully separated a set of leukemic cell types having different combinations of mutations, which had unique expression profiles corresponding to distinct steps during sequential acquisitions of mutations. Our method helps understand the clonal evolution of AML.

P-2099

Quantification of ultra-rare somatic mutations using molecular barcode and a small number of template DNA

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Accumulation of genetic changes in normal tissues is important for cancer development. However, measurement of mutations in normal tissues is difficult due to its very low frequency. Recently, we developed a simple method for quantification of rare somatic mutations using sequencing libraries prepared from 100 copies of genomic DNA as a template [Cancer Lett. 403: 152, 2017]. The method enables us to scan variants within a wide (>15kbp) region using limited number of sequencing reads. In this study, we combined the method with the molecular barcode technology to perform more accurate analysis. We prepared sequencing libraries with molecular barcodes from less than 2 ng (660 copies) of human genomic DNA using a targeted DNA panel (22 genes, 651primers, 15,160 bases). We optimized parameters to identify rare and true mutations, whose theoretical allele frequencies are 1/(copy number of template). Analysis of increasing mutation frequencies in cells treated with increasing doses of mutagens, and their signature mutations is in progress. When our new method is established, ultra-rare somatic mutations can be accurately and cost-effectively measured.

P-2100

Withdrawn

No Abstract

▲TOP

[P-2105] P7-3 [English]
Comprehensive genomic analysis / whole genome / exome sequencing in solid tumor [English]

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-2105

Genomic Landscape of Upper Urinary Tract Urothelial Carcinoma

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BackgroundsUpper urinary tract urothelial carcinoma (UTUC) is clinically similar to bladder urothelial carcinoma but its molecular pathogenesis is poorly understood. Materials & methodsGenomic DNA/RNA was extracted from 200 surgical specimens and subjected to whole exome/RNA sequencing and SNP array karyotyping. Results95% of cases harbored either TP53/MDM2, FGFR3, or RAS pathway mutations in an almost mutually exclusive manner, based on which UTUCs are classified into 3 distinct subgroups with unique molecular and clinical features.Gene expression analysis identified 3 clusters; cluster 1 was characterized by high expression of basal markers, enriched in TP53 mutations. In contrast, cases in cluster 2 and 3 showed high expression of luminal markers, enriched in RAS and FGFR3 mutations, respectively. Notably, cases in cluster 1 showed significantly high expression of immune blockade targets, CD274 and PDCD1LG2.ConclusionsUTUC showed distinct genetic landscape, associated with various clinical features. Our findings of molecular characteristics in UTUC will contribute to the development of novel diagnostics and therapeutics.

▲TOP

P-2106

Comprehensive Analysis of Indels in Whole-genome Microsatellite Regions across 21 Cancer Types

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To reveal the mutational landscape of the microsatellite repeat regions in whole genome level, we here analyzed approximately 9.2 million microsatellites in 2,913 ICGC PanCancer samples across 21 tissues. First, we developed an insertion and deletion caller considering error patterns of different types of microsatellites. Among the 2,717 pancancer samples, our analysis identified 31 samples with higher microsatellite mutation rate, which we defined as microsatellite instable (MSI) cancers. As expected, colorectal, uterus, and stomach cancers had a larger number of MSI samples, but MSI was also observed in a minority of samples in liver, pancreas, ovary, esophageal, and skin cancers. Next, we selected 20 microsatellites which mutated frequently in MSI-positive cancer in PCAWG, and defined a novel microsatellite marker set to detect MSI cancers with high sensitivity. Third, we found that replication timing, DNA accessibility, and DNA shape, were significantly associated with mutation rate of the microsatellites. Our analysis provides a comprehensive picture of mutations in the microsatellite regions, and revealed possible source of mutations, and a useful marker set.

P-2107

Mutational profiles of anal squamous cell carcinomas using whole-exome sequencing

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Anal squamous cell carcinoma (ASCC), either with human papillomavirus (HPV) (+) or (-), is a neoplastic disease with frequent recurrence and metastasis. To characterize ASCC genomes, we attempted to disclose novel alterations of ASCC genomes as well as genetic features including mutation signatures. We found known ASCC mutations (TP53, CDKN2A and PIK3CA) and CNAs (gains on 3q and 19q and losses on 11q and 13q). In addition, we discovered novel mutations in HRAS and ARID1A and CNAs (gain on 8q and losses 5q, 9p, 10q and 19p) that had not been reported in ASCCs. We identified 4 signature patterns of the mutations (signatures 1 and 2 with deamination of 5-methyl-cytosin, signature 3 with APOBEC and signature 4 with mismatch repair) in the ASCCs. In addition, we first found that ASCCs harbored chromothripsis, copy-neutral losses of heterozygosity and focal amplification of KLF5 super-enhancer. Analyses of primary and recurrent/metastatic pair genomes revealed that driver events in development and progression of ASCC might not be uniform. Our data indicate that ASCCs may have similar mutation and CNA profiles to other SCCs, but that there are unique genomic features of ASCCs as well.

P-2108

A novel analytical method of full-length cancer cDNA sequencing and its application to breast cancer

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The analysis of cancer genomics has progressed dramatically since the advent of next generation sequencing (NGS) and numerous oncogenic mutations have been identified by NGS. However, not only mutation is essential for carcinogenesis, but also epigenetic regulation is. Recently, alternative splicing has been found contributing to cancer progression, e.g. switching of PKM isoforms by PTBP1 promotes gemcitabine resistance in pancreatic cancer cells (S. Calabretta et al., Oncogene 2016). Unfortunately, it is difficult to identify novel isoforms by NGS, for its unavoidable process of short-read reconstruction. Therefore, we adopted Iso-seq, the full-length cDNA sequencing application on Sequel (Pacific Biosciences), and developed a novel workflow for accurate determination of tumor-specific splice variants. This method successfully classified alternative splicing events into 5 categories: exon skipping/inclusion, alternative 5'/3', and intron retention. In addition, it enabled the extraction of splicing event combinations. We applied it to breast cancer for identification of subtype-specific variants. This technology can provide new insight about carcinogenic process.

▲TOP

P-2109

Clonal structures of regionally synchronous gastric adenomas and carcinomas

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We performed whole-exome sequencing for 15 synchronous gastric adenoma (GA) and gastric carcinoma (GC) pairs and found no significant difference in driver mutation or copy number alteration numbers between GAs and GCs. In addition to TP53, APC, RNF43, and RPL22, we discovered novel KDM6A, PREX2, FAT1, KMT2C, GLI3 and RPL22 mutations and hypermutation in GAs, but did not identify recurrent drivers for GA-to-GC progression. Most GA/GC pairs exhibit parallel evolution with early divergence rather than stepwise evolution during progression. Of note, three cases were identified as clonally non-related GA/GC pairs despite the lack of histologic differences. We found differences in dominant mutational signatures 1, 6, 15, and 17 in GA/GC trunks, GA-branches, and GC-branches. Compared to our previous work on synchronous colon adenoma/carcinoma genome structures, mutational events during GA-to-GC progression might be more context-dependent than colon adenoma progression. Our results show that non-clonal synchronous GA/GC is common, and that GA genomes have already acquired distinct genomic alterations, suggesting caution in the diagnosis of synchronous GA and GC.

P-2110

Characterization of genome-wide p53 binding sites comprising cancer associated SNPs

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The p53 gene, one of the most frequently altered tumor suppressor gene in human cancers, encodes a transcription factor that regulates numerous target genes through the p53 target motif in the genome. Here, we conducted comprehensive analysis of SNPs associated with cancer risks, along with a location in p53 binding sequences. To screen functional p53 binding sites in the genome, we used 3 datasets: p53 ChIP sequence data originating from ReMap database, p53 binding motif predicted by JASPAR transcription factor binding site database or our in-house prediction algorithm, and GWAS dataset of various cancers. Our results indicated the significant association of at least 3 SNPs located in p53 binding sites on chromosome 4, 5, and 8 associated with stomach, lung, and prostate cancer, respectively. These SNPs have the potential to modify p53's binding efficiency depending on the genotype. Our study would elucidate the molecular mechanisms underlying significant heterogeneity in the response to DNA damaging agents and cancer risk.

▲TOP

[P-2116] P7-5 [English/Japanese]
Genomic analysis in solid tumor

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yuko Murakami-Tonami / Dept. Clin. Lab. Med., Juntendo Univ. Grad. Sch. Med.

P-2116

Mutational landscape of 4000 cancer tissues with whole exome sequencing and panel-based deep sequencing - Project HOPE

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The Project HOPE (High-tech Omics-based Patient Evaluation) has been under progress for five years since its onset in 2014 using whole exome sequencing (WES), cancer related gene panel, fusion gene panel and gene expression profiling (GEP). In the current study we performed comprehensive analysis of multi-omics dataset to identify causal genomic aberrations in cancer tissues. Integrative analysis by focusing on subset of data (2652 samples) identified driver genomic alterations in 1698 (64%) samples in 144 genes. Among them 1456, 48 and 445 are detected by WES, fusion panel and GEP, respectively. Although 86% of them (1456 out of 1698) can be identified by WES, detection of remaining 14% requires measurements by another platform. Analysis of pan-cancer somatic mutation data identified 110 genes as driver gene candidates. Of these, 78 and 32 genes are known and novel driver genes, respectively. Two genes in the latter case are supported by multiple methods indicating that they are good candidates for further evaluation. Thus combinatorial analysis is powerful approach to delineate driver genomic alterations.

▲TOP

P-2117

Development of the gene expression database of renal cell carcinoma cases to identify the tumor markers

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High coverage expression profiling (HiCEP) is a comprehensive gene expression analysis invented in Japan. However, it requires complicated processes to obtain the annotation and sequence information of the detected peaks. We combined the HiCEP and analyses by the next-generation sequencer, and try to establish the gene expression database of renal cell carcinoma (RCC) cases to identify effective tumor markers.
We collected the cancerous and macroscopically non-cancerous regions from 83 RCC cases. Among them, six cases with clear cell type RCC were analyzed by HiCEP. HiCEP fragments were also sequenced by the next-generation sequencer (ion PGM, Thermo Fisher Scientific). Furthermore, we investigated the expressed peaks in cancer and normal tisuues.
We could develope the HiCEP fragment database for the first time in mammals including humans, and detected several candidate markers for RCC.
We will perform the replication analyses with the other RCC cases, and further analyses of the blood samples from RCC cases. Then, we aim to identify diagnostic and prognostic markers of RCC.

P-2118

Next generation sequencing approach for detecting 1,084 known fusion genes and novel fusion gene partners - Project HOPE

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The detection of chromosomal translocations and fusion genes is expected to play a critical role in exploring the mechanisms of tumorigenesis. Recent advances in next-generation DNA sequencing (NGS) has enabled new discoveries in cancer genome research. We recently reported the successful design and development of a panel of 491 fusion genes in the analysis of 600 clinical tumor specimens using NGS. Here, we revolutionized the fusion genes panel using the unique single primer extension approach. We demonstrate the design and development of a panel that enables the extended detection of 1,084 fusion genes as well as novel fusion gene partners in the analysis of cultured cancer cell lines and clinical tumor specimens for Project High-tech Omics-based Patient Evaluation (HOPE).

P-2119

Functional analysis of a rare HER2 variant (G776S) identified by clinical NGS in a colorectal cancer patient

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Introduction/Purpose: Clinical next-generation sequencing (NGS) identifies various actionable mutations as well as variants of uncertain significance (VUS). Recently, we found a rare HER2 VUS (G776S) in a 50-year-old man diagnosed with colon cancer. The aim of this study was to clarify the potential functional role of this HER2 VUS (G776S).
Method: We constructed plasmids including cDNA encoding wild type or mutant HER2 G776S. These plasmids were transfected into colon cancer cells (COLO 320) in which neither EGFR nor HER2 are highly expressed or mutated, and thus stable colon cancer cells expressing either wild type HER2 or HER2 G776S mutation were established. We evaluated their oncogenic function and drug sensitivity.
Results: Mutant HER2-G776S-expressing colon cancer cells showed increased phosphorylation of HER2, as well as anchorage-independent growth activity. Moreover, these cells were more sensitive to the HER2 tyrosine kinase inhibitors (TKIs).
Conclusion: This study revealed a potential role of the HER2 VUS identified by clinical NGS in the cancer patient. These analytical methods may be exploited to develop a precision cancer medicines for cancer patients.

▲TOP

P-2120

Comprehensive analysis of Hepatoblastoma

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Hepatoblastoma is a rare malignant tumor that occurs in the liver of children which outcome is not determined only by clinical staging. To elucidate an element that reflects prognostic factors and/or malignancy of hepatoblastoma, we carried out integration analysis including genome mutation (exome and whole genome sequence), copy number alteratio, DNA methylation array and gene expression profiles for clinically annotated 168 hepatoblastoma which collected by JPLT-2 study. CTNNB1 mutation was extremely frequent (>75% cases), however only very few number of genetic mutations (0.52 SNV/Mb in median) was observed. Copy number alterations indicated recurrent 11p15.5 UPD/LOH including IGF2/H19 region (30%), 4q35 focal deletion (19%) and 2q24.3 focal amplification (8%). DNA methylation profile was classified into four, in one class enriched elder onset cases (>3 yo) which prognosis was good and TERT promoter mutation was enriched, whereas the other elder onset hypermethylation class tended to poor prognosis. Parts of the molecular mechanism which clarifying a factor reflecting the prognostic prediction factor and malignancy, leading to selection of the hepatoblastoma treatment.

P-2121

Molecular cytogenetic analysis of three phenotypically different tumor cell lines derived from the same individual mouse

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We have established 3 cell clones, named B12, D2 and D11, derived from a spontaneous sarcoma in an old female C57BL/6-EGFP mouse. All 3 clones represented similar morphology and growth rate but tumorigenicities were quite different. To understand how these phenotypically differences have occurred during the process of establishment of cell clones, we have perfomed multi-color FISH techniques to investigate molecular cytogenetic analysis using the mouse chromosome specific painting probes. We found several unique characteristics with chromosomal numerical changes such as chromosomes 13, 17, and X, and structural rearrangements involving chromosomes 8, 14, 15, and 19. B12 cell clone has two X chromosomes and three 17 chromosomes but the other two both clones have three X chromosomes and four 17 chromosomes. Structural rearrangements such as Robertosonian translocations involving chromosomes 1 and 11 were identified on D11 and B12, respectively. Genomic differences and rearrangements among 3 cell clones would be affecting the different immunogenicities and vasculogenic activities, thus these cytogenetic data will be providing important information for further tumorigenesis studies.

P-2122

Genomic amplification of DEAD-Box Helicase56 on Ch.7p induces oncogenic splicing abnormalities in colorectal cancer

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Backgrounds: We reported that genomic amplification of chromosome 7p (Ch.7p) occurs in early stage of colorectal cancers (CRC). DEAD-Box Helicase (DDX) family on Ch.7p is related to splicing and induces splicing abnormalities.Material and Methods: mRNA expression of DDX56 was compared with DNA copy number and clinicopathologic features. Gene set enrichment analysis (GSEA), cell proliferation and cell cycle assays using siDDX56 were also performed. RNA sequence was performed to clarify the effect of DDX56 on splicing event.Result: DDX56 expression was significantly higher in CRC tissues and was positively correlated with DNA copy number of Ch.7p, lymphatic invasion, distant metastasis and poorer prognosis. GSEA showed DDX56 expression was positively correlated with gene set of cell cycle progression. siDDX56 inhibited the proliferation and the progression of cell cycle. The rate of intron retention and the protein expression of normal WEE1; cell cycle checkpoint regulator, were lessened by siDDX56.Conclusion: We identified that the genomic amplification of DDX56 on Ch.7p promoted tumor progression through splicing abnormalities of cell cycle checkpoint regulator gene in CRC.

▲TOP

[P-2129] P10-2 [English/Japanese]
Angiogenesis / Extracellular matrix

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-2129

Investigation of the functions of endothelial Apelin on tumor formation

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Apelin is the endogenous ligand for APJ receptor that is broadly expressed at the surface of cells in some organs, including heart, endothelium etc. In previous study, overexpression of Apelin in tumor cells significantly suppressed tumor growth by inducing tumor vascular maturation. In my case, I found absence of Apelin in Apelin KO mice supported tumor growth compared with WT type mice, which indicated Apelin as a tumor suppressive factor. Basically, endothelial Apelin is supposed to regulate cell biological functions through binding to its receptor APJ. In order to find out what other kinds of cell types did Apelin influenced in MC38 tumor, I separated cells in tumor microenvironment. As results, Endothelial cells expressed high level of APJ, while immune cells also exhibited APJ expression. Based on these results, I have made two hypotheses: Apelin supresses tumor through regulation of tumor vessel formation, or immune cell infiltration. Now I am trying to find out how Apelin regulates tumor vessel formation and through what kind of signal pathways, and also which type of immune cells play the core role in this case and how they supress tumor growth.

▲TOP

P-2130

Search for characteristic genes in the onset pattern of salivary duct carcinoma

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Salivary duct carcinoma (SDC) is a highly aggressive type of salivary gland cancer, arising de novo or ex pleomorphic adenoma (Ca-ex-PA).Due to its morphological similarity and HER2 overexpression, SDC has been considered to include multiple entities like breast cancer. However due to its rarity, its molecular biological profiles have not been elucidated.In this study, focusing on the difference between its onset pattern, we aim to clarify its entities and explore the characteristic genes of each entity by comparative test and randomforest method.We retrospectively analyzed 19 patients with SDC (Ca-ex-PA: 13, de novo: 6) in Hokkaido University between 1991 and 2015.We assessed 78 gene expressions by PCR array. VEGFA, AKT1, and IGF1R were shown to be enhanced in whole SDCs. VEGFA, ERBB2, and IGF1R were more expressed, while SLIT2 and PTEN less expressed in Ca-ex-PA than de novo. Moreover, randomforest method showed that VEGFA could be a characteristic gene of Ca-ex-PA. In conclusion, AKT/PI3K signaling pathway to angiogenesis were hyperactivated in whole SDCs, especially in Ca-ex-PA. VEGFA could be a crucial factor of malignant conversion from PA to SDCs.

P-2131

Development of simple assay for isolating invasive living cells

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We developed a modified invasion assay using a three-dimensional collagen gel that enables isolation of invasive living cells, which we named the invading cell trapping (iCT) assay. We used a small cell strainer consisting of a nylon mesh with 40- μ m^2 pores and collagen gel layers formed across the membrane. The pores of the nylon mesh allowed transmembrane migration of cells. To carry out multiple assays simultaneously, strainers were used in a multi-well chamber plate. Test cells were seeded in the lower gel layer and invasive cells were attracted upward by serum stimulation and trapped in the upper gel. At the end of the assay, the collagen gel layers in each cell strainer were easily separated and living cells in the gel were collected and analyzed. An advantage of the iCT assay is that it can capture living invasive cells in the upper gel while leaving non-invasive ones in the lower layer. Further enrichment of the two cell populations can be achieved by repeating the assay. Thus, the iCT assay allows comparative analysis of invasive vs. non-invasive cells.

P-2132

Induction of EMT of colon cancer cells on E-cadherin-Fc matrix

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[Background & Aim] Epithelial mesenchymal transition (EMT) is considered as the initiation of metastasis in cancer progression. Decades of research revealed that epithelial-like cells acquired a spindle-shaped morphology and enhanced mobility via EMT. Recent studies reported that E-cadherin Fc domain fusion protein matrix (E-cad-Fc) could maintain the embryonic stem cells as the undifferentiated status with a spindle-like solitary formation. Thus E-cad-Fc may have a potential to induce EMT. However, the effect of E-cad-Fc on EMT in tumor cells has not been elucidated. [Methods & Results] We cultured colon cancer cell lines SW480 and HCT116 with E-cad-Fc, or/and EMT inducers such as TGF-beta and EGF. Results showed that both cultures with E-cad-Fc or EMT inducers alone could promote EMT properties including down-regulation of E-cadherin, up-regulation of Snail1 and vimentin, and spindle-shaped morphology. These characteristics were further enhanced in combination cultures of E-cad-Fc and EMT inducers. [Conclusion] E-cad-Fc effectively works on promoting EMT properties, and combination of E-cad-Fc and EMT inducers might be a useful tool for investigating EMT.

▲TOP

P-2133

The intranuclear PEX domain of MMP involves proliferation, migration, and metastasis of aggressive adenocarcinoma cells

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Members of matrix metalloproteinase (MMP) family promote cancer cell migration, invasion, and metastasis through alteration of the tumor milieu, intracellular signaling pathways, transcription. We examined gene expression signatures of colon adenocarcinoma cell lines with different metastatic potentials and found that rapidly metastatic cells powerfully expressed genes encoding MMP3 and MMP9. The non-proteolytic PEX isoform and proteolytic isoforms of MMPs were significantly expressed in the metastatic cells in vitro. Knockdown of MMP3 attenuated cancer cell migration and invasion in vitro and lung metastasis in vivo. Profound nuclear localization of MMP3 / PEX was found in tumor-stroma marginal area. In contrast, MMP9 was localized in central area of subcutaneous tumors. Overexpression of the PEX isoform of MMP3 promoted proliferation and migration of the rapidly metastatic cells in vitro. Taken together, the non-proteolytic PEX isoform of MMPs locating in cell nuclei involves proliferation, migration, and subsequent metastasis of aggressive adenocarcinoma cells.

▲TOP

[P-2140] P10-4 [English/Japanese]
Cell adhesion / invasion (2)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Kazuyuki Itoh / Res. Inst. Nozaki Tokushukai

P-2140

IFT20 promotes invasiveness of colorectal cancer cells by regulating microtubule dynamics

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We have previously shown that signaling elicited by the Ror2 receptor tyrosine kinase promotes invadopodia formation for tumor invasion in human osteosarcoma cells and that intraflagellar transport 20 (IFT20) mediates its signaling by regulating Golgi structure and transport. Herein, we investigated the role of IFT20 in regulating invasiveness of colorectal cancer cells, in which Ror2 gene is often silenced. Transwell and 2D-invasion assays have revealed that IFT20 promotes invasiveness of colorectal cancer cells, irrespective of Ror2 expression. Interestingly, immunofluorescence analysis has shown that microtubules are accumulated highly in cells leading collective invasion, in a manner dependent on IFT20. Moreover, time-lapse live cell imaging of the microtubule plus-end binding protein EB1 has revealed that IFT20 is required for polarized elongation of microtubules toward the front side of invading cells. These findings have pointed out that IFT20 promotes invasiveness of colorectal cancer cells by regulating microtubule dynamics.

▲TOP

P-2141

Ror1 induces filopodia formation and invasion of lung adenocarcinoma cells via SmgGDS-Rif axis

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The Ror-family of receptor tyrosine kinase Ror1 is expressed highly in various types of cancer cells and regulates their proliferation, survival, invasion, and metastasis, depending on their cell types. Despite of recent advances in our understanding how Ror1 promotes tumor cell proliferation and survival, its role in regulating tumor cell invasion remains rather elusive. Here, we show that ectopic expression of Ror1 induces filopodia formation in a manner dependent on the Rho-family small GTPase, Rif (rho in filopodia, also known as RhoF). In lung adenocarcinoma cell line PC-9 in which Ror1 and Rif are expressed highly, suppressed expression of either Ror1 or Rif inhibited filopodia formation and invasion of PC-9 cells. We also found that SmgGDS (small GTPase guanosine diphosphate dissociation stimulator) is a Rif binding protein and acts as a guanine nucleotide exchange factor (GEF) for Rif in vitro. Furthermore, SmgGDS could bind both Rif and Ror1 in PC-9 cells, and knockdown of SmgGDS inhibited filopodia formation and invasion of PC-9 cells. These results suggest that Ror1 promotes filopodia formation and invasion of the lung adenocarcinoma cells through SmgGDS-Rif axis.

P-2142

Fascin-1 promotes breast cancer cell invasion by regulating expression of LGR5

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Fascin-1 (FSCN1) is an actin-binding protein and promotes cancer cell invasion in various cancer cells. However, the detail molecular mechanism of invasion regulated by FSCN1 is not well known. Therefore, we studied the downstream genes of FSCN1 in the signal pathway of invasive activity. We used human breast cancer cells (MDA-MB-231) and assessed the invasiveness by using Matrigel invasion assay. This assay confirmed that MDA-MB-231 shows high invasiveness, whereas FSCN1 knock-downed cells exhibit low invasiveness. We next investigated the downstream genes of FSCN1 by using real-time PCR. We compared expression of 100 genes of FSCN1 knock-downed cells with that of control cells. We revealed that FSCN1 knock down in MDA-MB-231 significantly decreases mRNA expression of leuicine-rich repeat containing G protein-coupled receptor 5 (LGR5), which promotes breast cancer motility, to approximately 25%. We also found that FSCN1 knock down decreases LGR5 protein expression and diminishes invasiveness in MDA-MB-231. Thus, our findings suggest that FSCN1 promotes cancer cell invasion by regulating expression of LGR5.

P-2143

Involvement of CD36 in cell proliferation and invasion in oral squamous cell carcinoma

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CD36, the receptor of free fatty acids, has been demonstrated to play an important role to initiate metastasis in oral squamous cell carcinoma(OSCC). In the present study, we aimed to evaluate the involvement of the expression of CD36 in proliferation and invasion of OSCC. 4 human OSCC cell lines, HSC-2, HSC-3, HSC-4 and Ca9-22, were used in this study. Proliferation was evaluated by Ki-67 intracellular staining. Epithelial cell adhesion was analyzed by flowcytometric analysis of E-cadherin and β-catenin. Furthermore, migration activity was assessed by flowcytometric analysis of PDGFRs, and migration assay. Expression of E-cadherin was decreased in CD36+CD44-cells. Upregulation of Ki-67 and PDGFRβ was observed in both CD36+CD44+cells and CD36+CD44-cells. Moreover, migration activity was significantly increased in CD36+CD44+cells. Our results suggested that CD36 involved in proliferation and migration activity in OSCC.

▲TOP

P-2144

Mechanism of pattern formation of cancer cell invasion

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Many different patterns of collective invasion of cancer cells are observed in tumors. How these varying patterns can be determined remain poorly understood. We use complementary experimental and computational approaches to investigate how interactions between cancer cells, CAFs and the ECM affect the invasion patterns of SCC. Cellular Potts modelling was used to determine the key factors determining patterns of invasion. This analysis revealed interesting and unexpected roles of cell-cell adhesion and matrix proteolysis in determining the pattern of collective SCC cell invasion. Matrix proteolysis determines the width of invasive strands and, interestingly, the favorability of cell-cell adhesion for collective invasion depends on the concordance of the directional cues. To test the veracity of these predictions we used two different 3D invasion assays with A431 SCC cell delivertive lines in which cell-cell adhesion or matrix remodling abilities were modified. These analyses confirm the importance of those abilities for determining the pattern of collectively invading strands. Further, in vivo tumour model suggested unexpected role of cell-cell adhesion in metastasis.

P-2145

Effect of antagonized peptide derived from CXCR4 in A549 cells tumorigenesis

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Molecular targeted therapies contribute to raising cancer patient survival. CXCL12(SDF-1)/CXCR4 axis has been shown to involve in tumor growth and metastasis. It also has been well documented that CXCL12/CXCR4 regulates lung cancer progression, including migration and metastasis. A 20 amino acid peptide based on CXCL12/CXCR4 specific sequence to antagonize CXCR4 was designed. After CXCL12 treatment, migration and invasion abilities of non-small cell lung cancer cell A549 were increased. Blocking the CXCL12/CXCR4 interaction by peptide suppressed CXCL12-induced A549 cells migration and invasion. The EMT markers thrombomodulin, fibronectin, and vimentin, were also up-regulated or reduced by peptide treatment. Altogether, our designed peptide would provide another therapeutic method for lung cancer.

▲TOP

[P-2152] P10-6 [English/Japanese]
Cell adhesion / invasion (4)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Masami Suganuma / Grad. Sch. Sci. Engi. Saitama. Univ.

P-2152

Girdin/GIV Regulates Collective Cancer Cell Migration by Controlling Cell Adhesion and Cytoskeletal Organization

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Pathological observations show that cancer cells frequently invade the surrounding stroma in collective groups rather than through single cell migration. The identification of genes and proteins that are specifically involved in the collective behavior of cancer cells has thus far been limited. Here, we studied the role of the actin-binding protein Girdin in collective cancer cell migration. We found that Girdin was essential for the collective migration of the skin cancer cell line A431 on collagen gels as well as their fibroblast-led collective invasion in an organotypic culture model. We provide evidence that Girdin binds to &beta-catenin in E-cadherin-mediated cell-cell adhesion. Girdin-depleted cells displayed scattering and impaired E-cadherin-specific cell-cell adhesion. Importantly, Girdin depletion led to impaired cytoskeletal association of the &beta-catenin complex, which was accompanied by changes in the supracellular actin cytoskeletal organization of cancer cell cohorts on collagen gels. Finally, we showed the significance of Girdin expression in the progression of human skin cancer.

▲TOP

P-2153

Role of CXCL12/CXCR4 signaling axis in radiation resistant pancreatic cancer cells

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【Background】Chemoradiation therapy is provided as treatment for locally advanced pancreatic cancers(PaCas), but the effect is insufficient. One of the reason is acquisition of radiation resistance. We established radiation resistant (RR) PaCa cells to elucidate the mechanism of RR. In RR PaCa cells, the gene expression of CXCR4 was enhanced. Previously, we reported CXCL12/CXCR4 signaling axis played an important role in metastasis of PaCas. From these results, we focused CXCL12/CXCR4 signaling axis in the mechanism of radiation resistance. 【Methods and Results】(1) We performed fractionated irradiation to PaCa cells and established RR PaCa cells. (2) We performed cDNA microarray analysis to RR and normal PaCa cells. (3) In the genes which were up-regulated in RR cells, we focused CXCR4 and confirmed the enhancement of CXCR4 in RR cells by qRT-PCR and ELISA. (4) In PaCa cells, we performed invasion assay and confirmed that CXCL12 enhanced invasion abilities and these effects were down-regulated by CXCR4 antagonist. 【Conclusions】From these results, possibility of the new treatment using CXCR4 antagonist for RR PaCa was suggested.

P-2154

Modeling of tumor progression signaling pathway via EphA2 and EGFR

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EphA2 is a family of receptor tyrosine kinases, known to be associated with cancer progression and metastasis. Previous reports demonstrated that EphA2 transmits two opposite signals, anti- and pro-oncogenic signaling. In the presence of EphA2 ligands, tyrosine phosphorylated EphA2 suppresses EGFR-downstream signaling involved in tumor growth and survival. On the other hand, in the absent of the ligands, EphA2 is phosphorylated at its serine residues by EGFR-downstream signaling molecules, AKT and/or RAK, and promotes tumor progression. Recently we found that EphA2 cleavage by membrane proteinase, MT1-MMP, converts it from tumor suppressor to oncoprotein accompanying with EGFR downstream signaling. Since the EphA2 signaling pathway is complicatedly cross-talked with EGFR and its downstream signaling, it is not easy to identify the essential pathway and molecule by biological study.
In this study we constructed a mathematical modeling to understand key signaling pathways and molecules involved in EphA2-induced tumor progression and discuss numerical results of simulation of signal network transmitted by pro-oncogenic EphA2 signaling.
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P-2155

Metformin inhibits epithelial-mesenchymal transition in human pancreatic cancer cell lines

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Epithelial-mesenchymal transition (EMT) is considered to be a crucial event in the development of cancer metastasis. Metformin is an antidiabetic drug used in treatment of type 2 diabetes. Recently, increasing evidence has indicated that metformin can decrease the developing cancer, however, effect of metformin on EMT is unknown in pancreatic cancer. We investigated whether metformin inhibits EMT in the human pancreatic cancer cell lines(PANC-1, BXPC-3, and MIAPaCa-2). Pancreatic cancer cells were exposed to metformin (48h) and evaluated for EMT-related factors using western blot analyses and RT-PCR. Wound healing assay was performed to determine cell invasion and proliferation. Metformin inhibited TGF-β induced changes in morphology. Metformin inhibited the expression of TGF-β induced Vimentin, and facilitated the expression of E-cadhelin. Metformin reduced TGF-β induced cell migration. Furthermore, cell viability was no significantly change between exposing metformin or not. These results indicate that metformin inhibits EMT in human pancreatic cancer cells.

▲TOP

P-2156

The binding of FGF2 and integrin enhances the ability of invasion of breast cancer cells

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Background：Integrins and growth factor receptors are critical for cancer progression. We previously showed that the binding of fibroblast growth factor-2 (FGF2) and integrin is a required for tumor angiogenesis in endothelial cells. In this study we focus the effect of the binding on cell invasiveness of breast cancer cells.
Method：Integrin binding defective FGF2 mutants (Lysine-119 to Glu and Arg-120 to Glu , K119E/R120E and Lysine-125 to Glu , K125E) were expressed in Escherichia coli and purified. We performed migration assay and invasion assay using breast cancer cell lines. We investigated the activity of matrix metalloproteinases (MMPs), which induces cancer invasion. We also analyzed a signal transduction caused by the binding of FGF2 and integrin.
Result：Wild-type FGF2 induced cell migration while FGF2 mutants suppressed cell migration and MMP activation. FGF2 mutants suppressed cell invasion. Moreover, FGF2 mutants suppressed FGF signaling induced by wild-type FGF2 in vitro.
Conclusion：Our study suggested that FGF2 mutants suppress cancer cell invasion and metastasis. We propose that the binding of FGF2 and integrin promote cancer cell invasion and metastasis.

P-2157

Activation of intercellular integrin-β1 promotes collective invasion in human squamous carcinoma cells

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Collective invasion, which shows both invasive ability and characteristics of collective migration, has been reported as a critical phenomenon of invasive cancers. In this study, we aimed to elucidate the mechanism of collective invasion by focusing on the intercellular interaction in the population of human squamous carcinoma cells. We cultured A431 cancer cell line in collagen gel matrix. The population of A431 cells expressed integrin-β1 in the intercellular site, and lost the ability to invade collectively in the presence of integrin-β1 inhibitory antibody. Integrin-β1 is a representative extracellular matrix（ECM）receptor, therefore it was suggested that ECM proteins exist in the intercellular site. By examining the localization of several ECM proteins with immunofluorescence staining, we found that Laminin-5 and type-17 collagen localized to the intercellular site. Furthermore, phosphorylated focal adhesion kinase（FAK）, downstream of integrin-β1 was also localized to the intercellular site. Therefore these findings suggest that interaction between integrin-β1 and ECM proteins in the intercellular site promotes collective invasion in collagen gel matrix by activating FAK.

P-2158

HSP22 reduces the migration of hepatocellular carcinoma cells through the suppression of the PI3K/AKT pathway

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Among small heat shock proteins (HSPs), HSP22 ubiquitously exsists in a variety of cells. In the present study, we investigated the implication of HSP22 in hepatocellular carcinoma (HCC) cell migration. The migration of human HCC-derived HuH-7 cells stimulated by transforming growth factor (TGF)-α or hepatocyte growth factor (HGF) was significantly enhanced by the knockdown of HSP22 expression. Down-regulation of HSP22 protein in the cells markedly strengthened the AKT phosphorylation induced by TGF-α or HGF. Inhibitors of the phosphatidylinositol 3-kinase (PI3K)/AKT pathway, which suppressed the TGF-α-induced migration, significantly reduced the amplification of HuH-7 cell migration by HSP22 knockdown. PI3K but not AKT was coimmunoprecipitated with HSP22 in HuH-7 cells. Taken together, our results strongly suggest that HSP22 represses HCC cell migration by TGF-α or HGF thorugh the down-regulation of the PI3K/AKT signaling pathway.

▲TOP

[P-2166] P10-8 [English/Japanese]
Extracellular matrix

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yasuhiko Kitadai / Pref. Univ. Hiroshima

P-2166

The role of histone deacetylase 1 in distant metastasis of pancreatic cancer

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Backgrounds: Current therapies for pancreatic cancer (PC) do not sufficiently control distant metastasis after curative resection for resectable PC. Numerous studies have suggested that the epithelial-mesenchymal transition (EMT) is pivotal for metastasis of carcinomas. The fact that the EMT is reversible suggests that it is induced by an epigenetic mechanism. In this study, we aimed to investigate the role of histone deacetylase 1 (HDAC1), which is an epigenetic mechanism on distant metastasis of PC.
Methods: We investigated the HDAC1 expression in 103 resected PC specimens using immunohistochemistry. In vitro, the HDAC1 activity, the EMT-associated genes, the invasion ability and HDAC1 inhibitor assay were examined.
Results: The high expression of HDAC1 in clinical samples was significantly associated with distant metastasis. In vitro, an HDAC1 inhibitor decreased the invasion ability and reversed the EMT change. The dominant factor in the EMT-related transcriptional genes was SNAIL.
Conclusion: The targeting HDAC1, which could suppress metastasis by inhibiting the EMT, would be a promising treatment option for resectable PC.

▲TOP

P-2167

Clinical significance of Cofilin-1 expression in pancreatic cancers

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The prognosis of patients with pancreatic cancer is still poor, with a 5-year survival rate of less than 10%. As a result of genetic research involving prognosis of pancreatic cancer using public database, we focused on Cofilin-1. In recent years, it has been revealed that cancer cells form invadopodia to destroy extracellular matrix and cause hematogenous metastasis, and it is suggested that Cofilin-1 has a key role in stabilizing the structure of the invadopodia. At first, we examined the relationship between Cofilin-1 expression level and clinicopathological factors and prognosis using GSE21501 and TCGA, which are public databases of patients with pancreatic cancer. We set the cut-off value, and divided into the two groups, the Cofilin-1 high and low expression groups. The overall survival was significantly worse in the high expression group. Cox proportional hazard analysis was performed, and high expression of Cofilin-1 and pathological stage were independent poor prognostic predictors. We'll report together results of immunohistochemical staining for Cofilin-1 in clinical samples of our own department.

P-2168

Utilizing transposon mutagenesis to elucidate the mechanisms of metastasis-associated HCC

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Metastasis is the spread of primary cancer to distant sites and the primary cause of cancer mortality. A drastic reduction was shown in the survival rate in patients with advanced metastasis-associated HCC compared to early stage localized HCC. However, the metastatic cascade in HCC-associated metastasis remains ambiguous. Therefore, a Sleeping Beauty (SB) insertional mutagenesis screen for driver genes associated with metastasis-associated HCC was performed.Truncated epidermal growth factor receptor (Egfr) gene was frequently detected in both mouse primary liver tumors and lung metastases, indicating the importance of Egfr mutations for HCC progression. More importantly, potential metastatic genes were discovered and shown at a high relevance to human metastatic HCC patients. Their roles in metastasis were validated in in vitro assays and determined with significant contributions to metastasis at different aspects.Taken together, our study shows that the SB screen can provide valuable information on the mechanism of metastasis-associated HCC. In addition, we have shown genes contribute to metastasis in a cooperative manner under the Egfr-associated HCC context.

P-2169

High-throughput screening to identify upstream regulators for HCC metastasis

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Metastasis is the major cause of cancer modality but its mechanism is largely unclear. In our previous studies, we indicated aberrant up-regulation of FNDC3B highly associated tumor metastasis in HCC and other cancers. To further understand its metastasis signaling, we tried to potential upstream regulators for FNDC3B. By reanalysis ChIP-seq and gene expression data resulted from public domain database, we defined 53 transcription factors cloud be binding to promoter of FNDC3B. Then we knock-down these factors by shRNA and observed the expression of FNDC3B by QPCR. At least 2 different shRNAs for each gene and triple repeat in 3 cell lines were applied for screening. Finally, we indicated E2F1 and EP300 could be activators but MAX and USF1 could be suppressors for FNDC3B. Then ChIP coupled with Droplet Digital PCR (ddPCR) was used to confirmed protein-DNA interaction. In vitro and in vivo assays were applied to indicated their role in tumor metastasis. More interesting, up-regulation of E2F1 was significant associated with poor patients' survival (p-value $=7.2 \times 10^{-5}$). Our finding provided new regulation mechanism for FNDC3B and could serve more therapeutic targets for metastasis.

▲TOP

P-2170

CXCL12 is involved in liver metastasis of intrahepatic cholangiocarcinoma

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[Background]Recurrences often happen in patients with intrahepatic cholangiocarcinoma (ICC) after radical surgeries. How to control liver metastasis would be important to improve patients' prognosis.[Methods] Samples of ICC (primary and metastatic tumors) were collected as a multicenter study of the Kyushu Study Group of Liver Surgery. cDNA microarray was performed using three pairs of primary and metastatic ICCs. Protein expression was evaluated by immunohistochemical staining (IHC) using 30 primary ICC and 38 metastatic lesions. In vitro experiments, SSP-25 and HuH-28, which are human ICC cell lines, were used.[Results] Using cDNA microarray, genes of high expression (KRT83, CXCL12) and low expression (REG3G, OLFM4) were extracted in the metastatic ICC as compared with the primary ICC. In IHC, only CXCL12 was significantly higher expressed in metastatic ICC compared to primary ICC ($p = 0.0329$); however, there was no significant difference in the other 3 genes. In vitro experiments, invasive and migration ability were suppressed in both cells by suppressing CXCL12. [Conclusions]CXCL12 would be a key molecule in liver metastasis of ICC.

P-2171

Notch signaling enhances the mutual association with epithelial ovarian cancer and mesothelial cells

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Mesothelial Cells (MCs) are the primary components of the tumor microenvironment for Epithelial Ovarian Cancer (EOC) cells. The Notch signaling-mediated alteration of the tumor microenvironment can play a crucial role in tumor progression; however, the exact role of Notch signaling between EOC and MCs remains uncertain. We have identified MCs that have been altered by humoral factors, mainly TGF- β 1 derived from ovarian cancer cells, and have been defined as Cancer-Associated Mesothelial cells (CAM). The aim of this study is to clarify the molecular mechanism of CAM and EOC interactions that constitute the EOC peritoneal dissemination microenvironment. We examined Notch pathway activity in human EOC tumors and EOC peritoneal dissemination mouse model by immunostaining for Hes1, a transcriptional target of the pathway. We also confirmed the effects of CAM on EOC by co-culture. Furthermore, NICD3 overexpressing cell line was prepared and the phenotype was observed. We aim to clarify the role of Notch signal activation EOC in microenvironment formation by preparing a reporter cell line of Notch signal which can be observed with time by genome editing technology.

P-2172

Activation of c-Src by neddylation blockade enhanced cancer cell migration through Akt signaling pathway

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Neddylation regulates the function of the proteins through the small ubiquitin-like molecule NEDD8, thereby promoting or suppressing cancers. We performed neddylomics and found that neddylation block increased prostate and GBM cell migration in concert with increased c-Src phosphorylation. Recently, it was demonstrated that a neddylation inhibitor MLN4924 stimulated tumor-sphere formation and wound healing. We found that C-CBL acts as an E3 ligase for neddylation of c-Src. After being neddylated, c-Src is poly-ubiquitinated and degraded through the proteasome. When the neddylation is blocked, c-Src becomes stabilized and phosphorylated, which stimulates cell migration. In human lung cancer tissues, the down-regulation of C-CBL was found to be associated with phosphorylation of c-Src, cancer metastasis, and poor survival of patients. Moreover, Akt signaling pathway mediated c-Src phosphorylation by neddylation blockade. Therefore, neddylation may be intrinsic inhibiting system of cancer metastasis by suppressing Akt-mTOR- c-Src pathway. This study provides a new insight about the role of neddylation in cell migration and cancer metastasis.

▲TOP

[P-2180] P10-10 [English]
Invasion and metastasis (1) [English]

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Atsushi Osoegawa / Dept. Surg. & Sci., Grad Sch. Med. Sci., Kyushu Univ.

P-2180

Inhibitors of MRLC phosphorylation suppressed cholangiocarcinoma cell invasion and MMP-2 secretion

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Cholangiocarcinoma (CCA) is a malignant of biliary tract. Metastasis of this cancer is a major cause of death among cancer patients. Tissue invasiveness by malignant cells is a multistep process. Myosin regulatory light chain regulates myosin activity which underlies most contractile activities of the cells including migration and invasion of cancer cells. The function of myosin regulatory light chain (MRLC) is activated by phosphorylation catalyzed by myosin light chain kinase (MLCK). In this study, the role of MRLC in the invasiveness of cholangiocarcinoma was investigated by inhibiting the MRLC activity using ML-7, a MLCK inhibitor. ML-7 reduced MRLC phosphorylation dose dependently, correlating with suppression of cell proliferation as determined by MTT assay. However, non-toxic dosage of ML-7 showed a trend toward reduction of invasiveness and MMP-2 gelatinase activity, but the reduction was not statistically significant. These data indicate that MRLC activity is vital to cell proliferation but not the metastatic properties when treated at sub-toxic concentrations.

▲TOP

P-2181

c-Myc promotes lymphatic metastasis of pancreatic neuroendocrine tumor through VEGFC upregulation

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Lymphangiogenesis is essential for tumor metastasis via lymphatic system. c-Myc overexpression has been found in many cancers and is involved in tumorigenesis. However, whether c-Myc participates in lymphangiogenesis of pancreatic neuroendocrine tumor (pNET) microenvironment is unclear. We have found that high expression of c-Myc was commonly observed in pNETs previously. In this study, we found that VEGFC expression was increased in human and mouse pNET cell lines with c-Myc overexpression. Mechanistically, c-Myc transcriptionally upregulated VEGFC expression through direct binding to E-box of VEGFC promoter and enhanced VEGFR3 phosphorylation and tube formation of lymphatic endothelial cells. In mouse model, mice bearing pNET cells with c-Myc overexpression had more lymph node metastasis. Combine treatment of mTOR inhibitor, RAD001, with c-Myc inhibitor, 10058-F4, or VEGFC neutralizing chimera protein, VEGFR3/Fc, reduced lymph node metastasis of the mice. Taken together, we demonstrated that c-Myc enhances lymph node metastases via upregulating VEGFC/VEGFR3 axis. Simultaneously targeting mTOR and c-Myc or VEGFC may attenuate c-Myc induced lymphangiogenesis of pNET.

P-2182

Peritoneal liquid biopsy to predict the recurrence after curative surgery for advanced gastric cancer

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Background: To elucidate recurrence pathophysiology, the prognostic effect of peritoneal cancer cell spillage during surgery was evaluated in patients (pts) who underwent curative(R0) gastric cancer (GC) surgery.
Methods: Pts with advanced GC (≥pT2 MP) who underwent R0 surgery were enrolled. Peritoneal washing (PW) fluid before (PW-Pre) and after surgery (PW-Post) was collected and cultured to detect viable cancer cells.
Results: In 100 consecutive pts with advanced GC negative for PW-Pre-cytology and PW-Pre cancer cell culture [CCC(-)], 53 pts showed PW-Post CCC(+) and 47 had PW-Post CCC(-). No clinicopathological difference was observed between the two cohorts. Recurrences (peritoneum:7, liver:3, distant lymph node:9, local:1) occurred in 20 (37.7%) pts with PW-Post CCC(+). No recurrence was observed in pts with PW-Post CCC(-). Multivariate analysis showed that PW-Post CCC(+) was an independent risk factor for recurrence (Odds 60.3, P=0.005). The 5-year relapse-free survival rate was 45.4% in pts with PW-Post CCC(+), and 100% in pts with PW-Post CCC(-) (P<0.0001).
Conclusion: PW-CCC as peritoneal liquid biopsy is a promising predictive biomarker for recurrence after R0 GC surgery.

P-2183

Inhibition of metastasis with a peptide having the conserved amino acid residue of integrin α6 in breast cancer cells

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Inhibition of metastasis may improve survival in breast cancer patients. Our previous study have reported that integrin α6 is a migration-promoting factor for metastasis in breast cancer cells. This study reveals the amino acid residue required for integrin α6 function, and demonstrates reduction in metastatic properties via peptide-mediated inhibition of integrin α6.To identify functional amino acid residues of integrin α6, we performed comparative genomics analyses among vertebrates, and found Asp-358. Integrin α6 overexpression enhanced cell migration in low-metastatic breast cancer cell lines, whereas overexpression with D358A mutation did not, indicating that Asp-358 is required for integrin α6 function on cell migration. We designed a 8 amino acid peptide having the sequence around Asp-358. We observed reduced cell migration and inhibition of integrin α6 complex formation in the cells treated with the peptide. Moreover, in zebrafish metastasis assay, the peptide inhibited metastasis. These indicate that the peptide inhibits integrin α6 function for metastasis. This study contributes to establishment of therapies targeting breast cancer metastasis.

▲TOP

P-2184

Metastatic phenotype and acidic microenvironment

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Objective: Extracellular pH (pH_e) is often acidic in tumor tissue due to aerobic glycolysis (also known as Warburg effect). We have shown that acidic pH_e. We recently reported that TRPM5 mediates acidic-pH_e signaling to increase matrix metalloproteinase-9 (MMP-9) expression and that inhibition of TRPM5 reduced lung metastasis. In this study, we will report that adaptation to acidic pH_e induces metastatic phenotype in Lewis lung carcinoma (LLC) model. Methods: Acidic pH_e-adapted LLC (LLCm1A) cells were prepared from low metastatic variant (LLCm1) of LLC cells by culturing with DMEM/F12 + 10% FBS at pH 6.2, for more than 70 passages to compare metastatic phenotype with LLCm1 cells. Results: LLCm1A cells metastasized into lung two times higher than LLCm1 cells. The metastatic ability was correlated with MMP-2 production, whose level was not affected with acidic pH_e, and also correlated with acidic pHe-induction rate of MMP-9. Conclusion: Our data suggests that metastatic phenotype is induced under respective control of chronic and acute effect of acidic pH_e.

P-2185

In silico designed peptide can suppress the interactions between CXCL12 and CXCR4 for against lung cancer

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Lung cancer is not only the most common cancer in male but also the leading cause of cancer deaths worldwide due to the high mortality and low five-year survival rate. In accordance with the seed and soil theory, the eligible organ microenvironment with high expression of chemokine CXCL12 would attract tumors. Chemokine receptor CXCR4 and its monogamous ligand, CXCL12, have been implicated in the development of primary tumor and metastases. Upon binding, CXCL12 activates CXCR4 which is associated with disparate types of cancers. A peptide-based antagonist derived from CXCL12 based on molecular docking of CXCL12 to CXCR4 and MM/PBSA binding free energy calculations was determined to block the interactions between CXCR4 and CXCL12. The simulation results revealed that electrostatic interactions dominate the binding. The cell experiments also confirmed that the designed peptide can inhibit CXCL12-induced monocytes adhesion and transmigration. The peptide also suppressed the lung cancer cell A549 proliferation and downstream CXCL12/CXCR4 signaling. This study demonstrates that in silico prediction based functional peptide design can be effective for developing anti-cancer drugs.

P-2186

Genistein Enhances Doxorubicin Cytotoxic Activity and Inhibit Cells Migration on 4T1 Breast Cancer Cells

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Genistein (gen) is an active flavonoid robustly found in soybean. In this study, we evaluated the cytotoxic and anti-migration effects of gen in combination with doxorubicin (dox) on 4T1 cells.Cell viability was investigated using MTT assay. Cell cycle modulation and apoptosis event were determined using flowcytometry with propidium iodide (PI) and Annexin V staining, respectively. Migration and invasion inhibitory was examined using scratch wound healing assay, gelatin zymography, and Westernblot.We found that gen, at 5-200 μM, inhibited cells proliferation in a dose and time dependent manner. In combination with dox, gen at 1 μM and 50 μM enhanced the cytotoxicity of dox. The combination increased cells accumulation at G2/M phase and induced apoptosis. Furthermore, the combination inhibited cells migration and formation of lamellipodia. The combination also inhibited the expression level of the protein regulators of migration and invasion, i.e. MMP-9, MMP-2, and Rac-1 proteins. Taken together, gen is potential to be developed as co-chemotherapeutic agent by inducing apoptosis and inhibiting cells migration on triple negative breast cancer cells.

▲TOP

[P-2193] P10-12 [Japanese]
Invasion and metastasis (3)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-2193

Oncolytic virotherapy for inhibition of epithelial-mesenchymal transition in esophageal cancer

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Epithelial-mesenchymal transition (EMT) is a biological process, by which epithelial cancer cells acquire mesenchymal properties. EMT is highly associated with invasion, metastasis, and drug resistance in various types of cancer including esophageal cancer. However, there was no promising antitumor strategy to inhibit the EMT program in esophageal cancer. We developed a telomerase-specific oncolytic adenovirus OBP-301, and a phase I/II clinical study of OBP-301 in combination with radiotherapy for esophageal cancer is underway in Japan. In this study, we explored the biological effect of OBP-301 in the EMT program of human esophageal cancer TE-4 cells. Transforming growth factor-TGF-β) induced the EMT program with mesenchymal marker activation and increased migration ability in TE-4 cells. OBP-301 attenuated the TGF-β-induced EMT in TE-4 cells. Interestingly, EMT-induced TE-4 cells exhibited more resistance to chemotherapeutic agents but similar sensitivity to OBP-301 compared with parental cells. Our results suggest that oncolytic virotherapy is a promising antitumor strategy to inhibit the EMT program and the viability of EMT-undergoing tumor cells in esophageal cancer.

▲TOP

P-2194

Deciphering the molecular network for the onset of metastasis by CMTM6 in sarcomas

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Sarcoma is a highly aggressive and metastatic malignant tumor of bone and soft tissues. Control of metastasis based on the molecular targeting contributes to improve patient prognosis. To this end, we screened microRNAs (miRNAs) to identify target molecules, which regulate malignant properties of Ewing's sarcoma (EWS) cells. miRNA microarray analysis indicated that the expression profiles are hardly changed among the clinical samples analyzed. However, miR-451a is significantly repressed in clinical samples from patients with poor prognosis. miR-451 was found to induce remarkable repression of CMTM6, a chemokine-like membrane protein. Depletion of CMTM6 strongly inhibited cell migration of EWS cells. Interestingly, miR-451a was upregulated in the culture condition with high glucose. On the other hand, CMTM6 was down-regulated in the same condition, suggesting the possibility that glucose levels affects cell migration. Our data suggest that the onset of metastasis is induced by the change of intracellular nutrient availability in the sarcoma microenvironment, and CMTM6 is involved in the migration of sarcoma cells. Downstream network of CMTM6 will be also discussed.

P-2195

Endoglin mediates myofibroblastic and tumor-promoting carcinoma-associated fibroblasts in human breast carcinomas

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Carcinoma-associated fibroblasts (CAFs) rich in the tumor microenvironment actively influence tumor growth and progression. However, biological markers that identify CAF populations actively involved in promoting tumor progression have yet to be fully elucidated. We show here that endoglin, a TGF-β coreceptor playing a crucial role in angiogenesis, is substantially displayed on tumor-promoting CAFs. The expression level of endoglin was increasingly upregulated in human breast CAFs during the series of tumor progression. Endoglin expression was also detected in tumor-associated myofibroblasts in breast carcinoma patients. Moreover, inhibition of endoglin expression by shRNA in CAFs attenuated both TGF-β-Smad2/3 and SDF-1-CXCR4 signaling that mediated the myofibroblastic and tumor-promoting properties. Furthermore, the inhibition of stromal endoglin expression notably attenuated an ability of the co-injected breast carcinoma cells to form the primary tumor and lung metastasis in recipient mice. Taken together, these findings indicate that endoglin expression on CAFs may serve as a potential marker to determine tumor-promoting mesenchymal cells in human carcinomas.

P-2196

The functional and clinicopathological analysis of hypoxia inducible factor-1 α in head and neck squamous cell carcinoma

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Introduction: The effect and mechanism of Hypoxia inducible factor-1 α (HIF-1 α) on tumor metastasis in head and neck squamous cell carcinoma (HNSCC) is still unclear. The aim of the present study was to investigate the molecular targets of HIF-1 α relevant to metastasis, and to reveal the impact of HIF1-α on HNSCC metastasis. Methods: The cell proliferation rate of HNSCC cell lines (FaDu, Detroit512) under HIF-1 inhibitor was evaluated by MTS assay. Gene expression levels related to cancer stem cell (CSC) and HIF-1 α were measured by quantitative real-time PCR. Immunohistochemical expression of HIF-1 α in 33 cases of hypopharyngeal cancer was evaluated, and clinicopathological factors regarding lymph node metastasis were analyzed. Results: HIF-1 inhibitor suppressed the cell growth of HNSCC cell lines, and HIF-1 inhibitor downregulated the expression of CSC markers. Multivariate analysis revealed that high expression of HIF-1 α was independently correlated to lymph node metastasis (P=0.029). Conclusion: These results suggest that HIF-1 α plays a key role in tumor progression of HNSCC through CSC properties and HIF-1 α inhibition may have the possibility to suppress tumor metastasis.

▲TOP

[P-2203] P11-1 [English/Japanese]
Metabolism / metabolome (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-2203

Mitochondrial pyruvate career expression controls epithelial mesenchymal transition and radiation resistance

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As a carrier protein in the inner membrane of mitochondria, mitochondrial pyruvate carriers (MPCs) play an important role in uptake of pyruvate, and is causative to Warburg effect in tumors. Previous reports showed that MPC protein family expressed differentially between cancer and non-cancerous cells, and indicated that MPC is involved in the tumor suppressive function, suggesting the possibility as a novel target specific for cancer. To this end, we studied the MPC function in gastrointestinal cancers including pancreatic and colorectal cancer cells, and showed that the reduced expression of MPC resulted in the gain of epithelial-mesenchymal transition (EMT) phenomenon, which is associated with glycolytic and glutaminolytic metabolism in flux analysis. We found that the MPC deficient expression induced an apparent spindle shape-like phenotype, which was associated with EMT marker expressions such as N-Cadherin and Fibronectin, but also with cell survivals in colony formation assay after irradiation, indicating that MPC function links to radiation resistance. The present study demonstrated the feasibility that MPC can be useful for a radiation-sensitizing, druggable target.

▲TOP

P-2204

FOXO3a-driven alternation of metabolism dictates the gemcitabine sensitivity

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Gemcitabine has been a first line therapeutic agent for pancreatic ductal adenocarcinoma (PDAC); however, the acquisition of resistance to gemcitabine remains a major challenge. Here, we investigated the metabolite profiles by liquid chromatography mass spectrometry between gemcitabine resistant PDAC and parental PDAC cells, and found that lactic acid amount and lactate dehydrogenase activity were increased in gemcitabine resistant PDAC cells. We observed the elevated lactate dehydrogenase A (LDHA) expression significantly correlated with recurrent pancreatic cancer patients following gemcitabine treatment and with cancer stem cell (CSC) properties. We further identified that FOXO3a induced miR4259 directly targeted the 3'untranslated region of LDHA and reduced LDHA expression, leading to decreased gemcitabine resistance and a reduction in the CSC phenotypes of pancreatic cancer. Our findings provide evidence of an underlying epigenetic regulation of LDHA by FOXO3a/miR4259, which appears to be involved in cancer stemness and the chemoresistance of pancreatic cancer.

P-2205

A mitochondorial enzyme MTHFD1L confers growth and cancer stem-like poperties in breast cancer cells

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Breast cancer is the most common type of cancer and the increasing rate of mortality due to breast cancer raises serious problems. Cancer cells need large amounts of purine and pyrimidine nucleotides for DNA replication, RNA transcription, and cell proliferation. One-carbon (1C) metabolism has emerged as one of the key pathways for synthesis of purine and pyrimidine nucleotides. There are two parallel pathways of 1C metabolism in cytoplasm and mitochondria and it is reported that the latter is highly up-regulated in cancer cells. We searched database to find 1C metabolic enzymes that show increased expression levels in breast cancer tissues compared with normal tissues and associate with poor prognosis in breast cancer patients. As a result, we identified SHMT2, TYMS, MTHFD2, and MTHFD1L. It is reported that SHMT2, TYMS, and MTHFD2 are good molecular targets for cancer. We thus focus on MTHFD1L for which there are very few reports about cancer. We found that MTHFD1L is expressed in various breast cancer cell lines. Knockdown of MTHFD1L greatly reduced cancer cell growth and stem-like properties. Our findings suggest that MTHFD1L would be a novel molecular target for cancer therapy.

P-2206

Transcriptome analysis of pro-oncogenic alterations caused by Mieap inactivation

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Background: Mitochondria-eating protein (Mieap), a p53-inducible protein is frequently inactivated in human cancer. We aimed to investigate the influence of Mieap inactivation on gene expression patterns.Methods: We used four cell lines: HepG2, A549, and LS174T which are all TP53 wild-type and Mieap-unmethylated, and HMEC4 derived from normal mammary epithelial tissue. Mieap knockdown (KD) sublines under normoxic, hypoxic, and post-irradiated conditions were subjected to microarray analysis. Results: We identified 815, 547, 826, and 455 significantly up-regulated genes (fold change ≥ 2.0) and 911, 896, 658, and 1093 significantly down-regulated genes (fold change ≤ 0.5) after Mieap KD in HepG2, A549, LS174T, and HMEC4, respectively. Gene ontology of recurrently up-regulated genes after Mieap KD included Vitamin transport (GO: 0051180) and Lipid metabolic process (GO: 0006629), and those of recurrently down-regulated genes included Developmental process (GO: 0032502). Transferrin receptor was commonly up-regulated in Mieap KD cancer cell lines under normoxic and post-irradiated conditions.Conclusions: Mieap inactivation in cancer may alter metabolic status including iron metabolism.

▲TOP

P-2207

A metastasis protein CERS6 is transcriptionally regulated by miR-101 and YB-1

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Sphingolipids contribute to unique biological properties, though their functions in cancer pathogenesis are unknown. Our analysis of 149 NSCLC specimens showed that the ceramide synthase gene CERS6 was overexpressed in lung cancer. Alteration in CERS6 expression level was associated with a decreased cell migration in vitro, as well as a decreased RAC1-positive, C16 ceramide-dependent lamellipodia formation and attenuation of lung metastasis in mice, while forced expression of CERS6 showed an opposite phenotype. CERS6 expression may be regulated by a tumor-suppressor miR-101, because CERS6 and miR-101 expression levels were negatively correlated in clinical specimens, and because a luciferase analysis suggested binding of miR-101 to the 3' UTR of CERS6 mRNA. Beside miR-101, a transcription factor YB-1 may also regulate CERS6 expression. YB-1 knockdown suppressed both CERS6 mRNA and protein expressions, lamellipodia formation as well as migration activity. Furthermore, expression levels of YB-1 and CERS6 mRNAs were positively correlated in clinical specimens. These data suggest that a metastasis protein CERS6 is transcriptionally regulated by multiple mechanisms in lung cancer.

▲TOP

[P-2213] P11-3 [English/Japanese]
Metabolism / metabolome (3)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Masaaki Miyo / Dept of Surg, Kinan Hosp.

P-2213

A distinct function of the retinoblastoma protein in the control of lipid composition identified by lipidomic profiling

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By using lipidomics analysis, we demonstrate that Rb depletion in MEFs induces significant alterations in their lipid composition. Analysis of the acyl chain composition revealed an increase of saturated and mono-unsaturated acyl chains with specific carbon chain length. Consistently, we observed that Rb depletion increased the levels of fatty acids with the corresponding carbon chain length and number of carbon-carbon double bonds. Microarray analysis revealed that Rb depletion induced significant upregulation of enzymes involved in elongation and desaturation of fatty acids. Among these, we found that Elovl6 and Scd1 are the most robustly controlled by Rb possibly through E2F and SREBP transcription factors. Depletion of Elovl6 or Scd1 significantly suppressed colony formation, sphere formation and xenograft tumor growth of Rb-deficient tumor cells. Suppression of self-renewal by the SCD1 inhibitor was rescued upon supplementation of the mono-unsaturated fatty acids generated by this enzyme. This study suggests a novel role for Rb in suppressing the malignant progression of tumors by controlling the lipid composition.

▲TOP

P-2214

Role of RB-KDM5A in glucose metabolism

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The tumor suppressor gene RB controls cell cycle and cell differentiation in cooperation with various factors. In particular, histone demethylase KDM 5A is a major binding partner of RB, its function is suppressed by RB. It has been reported that terminal differentiation is inhibited by activation of KDM5A along with RB inactivation and abnormal expression of KDM5A is involved in carcinogenesis and metastasis in gastric cancer (GC). Therefore, we speculated that the abnormality of RB-KDM5A is important in the context of GC and tried to find out what gene is controlled by RB-KDM5A. Analysis of nascent RNA in RB inactivated GC cell line revealed that 91 genes including PGAM1 directly controlled by RB-KDM5A. Next, we investigated the impact of RB inactivation on metabolism, and found that rate of glucose utilization and lactate production was decreased in GC cells. When we evaluated the glycolytic activity when stimulated with 8.5 mM glucose using XF24, ECAR was decreased by RB knockdown. Furthermore, various specific rates of glycolysis and ECARs were rescued by overexpression of PGAM1, suggesting that RB-KDM5A regulates glucose utilization via PGAM1.

P-2215

Targeting carbonyl stress induced by tyrosine kinase inhibitors for cancer treatment

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[Purpose] Tyrosine kinase inhibitor (TKI) treatment is generally long-term and can induce resistance to TKIs. Therefore, it is important to conduct more strategic approach based on intracellular modulation mechanism in TKIs-therapy. We have previously reported that TKIs-mediated suppression of glycolysis disturbs energy balance in cancer cells. Here, we focused on TKI-induced carbonyl stress and investigated the mechanism to avoid the stress of cancer cells.[Methods and Results] Human chronic myelogenous leukemia K562 cells were used as model cells. Imatinib exposure increased intracellular glucose level, and also increased the mRNA levels of Aldo-Keto Reductase (AKR) 1B family proteins in K562 cells. AKR1B proteins reduce carbonyl compounds, which are derived from excessive glucose and generate intracellular stress. Imatinib exposure also induced autophagy, which can eliminate carbonyl proteins. Dual inhibition of AKR1B (using Epalrestat) and autophagy (using Chloroquine) significantly enhanced anti-cancer effects of TKIs.[Discussion and Conclusion] Inhibiting ingenious machineries to avoid TKI-induced carbonyl stress may provide more efficacy for cancer therapy.

P-2216

Mechanism of energy metabolism regulation by Ertredin, a 3D-spheroid formation inhibitor of EGFRvIII-transformed cells

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EGFRvIII is a mutant form of the epidermal growth factor receptor gene (EGFR) that expressed constitutively activated receptor tyrosine kinase without ligand-binding domain. The expression of EGFRvIII is likely confined to various types of cancer, particularly glioblastomas. We recently identified Ertredin in the course of the screening for 3D-spheroid formation inhibitors of cells overexpressing EGFRvIII. Ertredin induced apoptosis in the 3D -spheroids and the tumors. On the other hand, it did not inhibit 2D-growth of EGFRvIII -overexpressing cells or parental normal cells remarkably. In the last report, we described that Ertredin perturbed mitochondrial function. In addition, not only electron transport chain but also glycolysis was significantly suppressed under 3D-condition by Ertredin. Here, we investigated the effect of Ertredin on TCA cycle and mitochondrial electron transport chain complex I. We discuss the different effects of Ertredin on EGFRvIII expressing-cells and on normal parent cells. Also, we show the hypothetical mechanism of the metabolism suppression model of Ertredin in the cancer cells. Collaborators: C Nosaka, S Shimamoto

▲TOP

P-2217

Effects of Progesterone and Estrogen on Release of Lipoprotein Lipase from Mouse Mammary Tumor FM3A cells

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We have found that the release of lipoprotein lipase (LPL) are stimulated by medroxyprogesterone acetate (MPA) and β-estradiol (Est), and that these actions are partly due to the various mitogen-activated protein kinase (MAPK) pathway. In this study, we determined whether the synthesis of new LPL molecule involved in effects of these hormones. The tumor cells were incubated with these hormones under an addition of [3H] leucine in the presence of cycloheximide (CHX). The incorporation of [3H] leucine into the protein of the cells was increased up to 20nM of Est in a dose-dependent manner, but decreased at the concentration of MPA over 5nM. The incorporation of [3H] leucine by Est and MPA was suppressed with CHX. Although the incorporation was inhibited to 75% and 25% of the original level by 1 and 5 μM of CHX, respectively, the release of LPL by Est and MPA was well preserved and increased. These results suggest that the release of LPL by Est and MPA are associated with MAPK-pathway with different sensitivity rather than an elevation in enzyme molecule synthesis. [We thank Governor Keiji NISHIMOTO of Yasuura Hosp.(Kure) and Inst.Edu. Med.(Hiroshima) for his cooperation].

▲TOP

[P-2223] P12-3 [English]
Cancer immunity (1) [English]

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Sadamu Homma / Div. Oncol. Jikei Univ., Sch. Med.

P-2223
Withdrawn

No Abstract

▲TOP

P-2224

Withdrawn

No Abstract

P-2225

Influence of smoking on the immune microenvironment in breast cancer

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Background: The immune microenvironment (IME) in cancer is involved in therapeutic effects of chemotherapy. Tumor-infiltrating lymphocytes (TILs) is an indicator of IME, and the strength of expression varies depending on only subtype in breast cancer. Smoking is involved in carcinogenesis of breast cancer, and in lung cancer, correlation between smoking and IME has also been reported. In this study, we examined whether smoking also affects IME in breast cancer.Methods: 149 patients with HER2-enriched breast cancer or triple-negative breast cancer received neoadjuvant chemotherapy. TILs were assessed in biopsy specimens at diagnosis. Smoking measures was calculated using Brinkman index (BI) at diagnosis.Results: The expression of TILs and the pathological complete response (pCR) rate in the high BI group was higher than in the low BI group (p=0.043, p=0.042, respectively). However, there was no correlation between disease free survival or overall survival and BI (p=0.114, p=0.347, log-rank, respectively).Conclusions: This study shows smoking may cause activation of IME. A good IME resulted in a high pCR rate, but did not affect prognosis because of carcinogenicity of smoking.

P-2226

Roles of asialo-series ganglioside GD1alpha in human cancer cell lines

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Some gangliosides have been considered as tumor-associated antigens. GD1alpha or its synthase gene St6galnac5 was reported to be involved in the metastasis of murine lymphomas or human breast cancers, respectively. But roles of asialo-series gangliosides in human cancers have not yet been reported. Using monoclonal antibodies against GD1alpha and GM1b, we found that GD1alpha and its synthase genes were highly expressed in retinoblastoma, Y79. To investigate roles of GD1alpha, we established GD1alpha-positive (+) and -negative (-) clones of Y79 by cell sorting and then limiting dilution, and analyzed genes expression, phenotypes, and signals in cells. GD1alpha (+) Y79 clones showed higher expression of GD1alpha synthase, lower proliferation and invasion compared with GD1alpha (-) clones. Exogenous addition of GD1alpha to GD1alpha (-) clones also showed lower proliferation. Analysis of signaling molecules showed lowered phosphorylation of p-p130Cas (Y410), p-FAK (Y397), p-paxillin (Y118), p-AKT (Ser473) and p-ERK-1/2 in GD1alpha (+) clones than in GD1alpha (-) clones. Addition of exogenous GD1alpha to GD1alpha (-) clones also showed lowered phosphorylation of the signaling molecules.

▲TOP

P-2227

Oxidative phosphorylation-related complexes in human T cell (MT-2) and its sublines continuously exposed to asbestos

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For observation of the long-term exposure of asbestos to human T cells, HTLV-1 immortalized human T cell line, MT-2 (ORG), was used and sublines which exposed chrysotile (CH) or crocidolite (CR) on continuous and long-term exposure were established. All sublines acquired resistant to asbestos-induced apoptosis. Expression of oxidative phosphorylation related complexes (OXPHOS-Cs) after transient high-dose exposure (to CH or CR) was compared between ORG and sublines. Exposure to ORG did not change OXPHOS-Cs except Complex (C)-I attenuated compared with non-exposed control on CR exposure. In CB1, expression of C-III decreased markedly, NNT (nicotinamide nucleotide transhydrogenase) was markedly elevated. In CR1, expression of C-III and -V was elevated, and NTT was markedly elevated as in CB1.Thereafter, NNT was knocked down and the amount of reactive oxygen spices (ROS) was measured. The ROS level decreased in CB1 compared to ORG, on transient exposure. The ROS under asbestos exposure was significantly increased in NTT-knocked down-CB1 compared to CB1. Thud, resistance to asbestos-induced apoptosis in long-term exposed sublines may be due to increased expression of NNT

P-2228

Serum YKL-40 as a novel diagnostic marker for cervical squamous cell carcinoma patients

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Cervical cancer is the most common and the leading cause of death in gynecologic cancer worldwide. Although Pap smear is a gold standard for screening cervical cancer, a false–negative rate of 15% to 20% has been demonstrated in many series of re–reviewed Pap smears. Moreover, inadequate sampling of the transformation zone, poor collection and fixation of the specimen and inclusion of excessive blood, inflammatory material, or necrotic material can obscure or preclude the correct cytopathology. YKL–40 is a glycoprotein reported to be associated with metastasis and poor survival of cervical adenocarcinoma patients. Our study aimed to detect serum YKL–40 of cervical squamous cell carcinomas (SCC) compared to healthy controls using ELISA. We found that serum YKL–40 in SCC was significantly higher than control. Assessment of serum YKL–40 as a diagnostic marker for SCC using ROC curve showed AUC of 0.958 (0.916–0.999), the sensitivity of 94.6% and the specificity of 87.5%. Our study indicated the potential of serum YKL–40 as a novel diagnostic marker and when combined with Pap smear may increase sensitivity and specificity, and reduce the false–negative rate in SCC screening.

P-2229

Tumor-immune system analysis. The effects of T cell movement, its density, etc. for systematic quantitative approach

Mitsuo Takase
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This is the analysis of tumor-immune system behaviors with its computer software. This uses the data like T cell movements, local T cell density distribution, local IL2 density distribution calculated from other data, tumor cell distribution, etc. This mass action can be expressed as eigen value problems. Then, there can be eigen value &lambda=1 where any strong immune system response can be made according to the necessity. Here, the state is named local ignition.This mass action must exist, can be calculated quantitatively and makes the strong systematic firing at eigen value &lambda=1 have a so big effect and quite different from each T cell activation. On the other hand, the distribution levels of the activated T cells and CTL can be kept so low under eigen value &lambda <1.This time, &lambda, the effects of T cell movement and its density, etc. are shown by the simulation. The data used are not actual ones. But as the first step many case studies will be conducted knowing the behaviors, then their aspects will be matched to actual cases in a future.The target in a future is the systematic quantitative total evaluation of effective elements against a tumor using analysis.

▲TOP

[P-2008] P1-2 [English/Japanese]
Cell culture (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Toshio Imai / Ctr. Anim. Div., Natl. Cancer Ctr. Res. Inst.

P-2008

Capture of CTC using SS-Chip

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[Background and purpose] CTC has characteristics different from cells of the primary, and analyzing it leads to deepening of knowledge about metastasis.Previously The Cellsearch® was the main way to capture CTC, but many methods are currently being developed and we are capturing CTC by Proprietary size sort chip using the principle of fluid dynamics (SS-chip). SS-chip can capture living CTC unlike the method by magnetic beads. This chip does not require a large-scale apparatus and can be used without cost. Since oral cancer CTC reports are few, we tried isolation of oral cancer CTC using SS-chip. [Methods] First, we mixed the cultured oral cancer cell line with healthy human blood, let blood flow to the chip, and calculated the recovery rate of cells. Then, the high metastatic strain of adenoid cystic carcinoma cell lines in which GFP gene is transferred was injected into the tail vein of a mouse. 24hours later, capturing of CTC was attempted using SS-chips from blood collected from that mouse. [Result] We could use this chip to obtain a good recovery rate of oral cancer cells. [Conclusion] We were able to capture oral cancer CTC with high sensitivity and low cost method.

▲TOP

P-2009

E-cadherin gene status affected by morphology of mammary cancer cells

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We set up the hypothesis that the morphology of cultured cells from breast cancer patients may represent gene status and drug resistance potential. It would be useful to make clinical decisions only by biopsy samples from breast cancer patients, and a morphology of cultured cells could be useful for developing an innovative diagnosis method. To confirm our hypothesis, we used nine 4T1E subgroups obtained by limited dilution of 4T1 cells derived from spontaneously developed mouse mammary gland cancer. Using these sub-clones, we compared two clones and found that the clones were constructed of different organoid shapes in 3-dimension (D) culture, although the morphology of the cells was almost the same when compared in 2-D culture conditions. The other remaining clones that showed different organoid shapes in 3-D culture showed different cell morphology in 2-D culture. Some cells with spindle type morphology led us to investigate the gene expression levels of E-cadherin (CDH1), and we found there are differences in CDH1 expression levels. In the presentation, we would like to show and discuss the organoid shapes in 3-D culture and E-cadherin status, with some further speculation.

P-2010

Potential role of the mitochondria-eating protein Mieap in p53-dependent cell death

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Mieap was identified as a p53-inducible protein to have critical roles in mitochondrial quality control. Mieap is transcriptionally upregulated by p53 in response to various cellular stresses. Inactivation of Mieap causes accumulation of unhealthy mitochondria, leading to promotion of cancer development in vivo. Recently we found that non-canonical mitophagy regulated by Mieap induces cell death via iron-dependent ROS production and mitochondrial apoptotic pathway. However, the physiological significance of the Mieap-induced cell death is still largely unclear. Here we report that Mieap mediates the p53-dependent cell death in response to DNA damage. A2780 is an ovarian cancer cell line containing the wild-type p53 and Mieap. We established the p53-knockdown (KD) and/or Mieap-KD A2780 cell lines. The DNA damage by ADR treatment strikingly induced cell death in the A2780 control cells whereas the cell death was severely impaired in both the A2780 p53-KD and Mieap-KD cells. The similar results were obtained from the LS174T (colorectal cancer) and A549 (lung cancer) cell lines. These results suggest that Mieap plays a crucial role in the p53-dependent cell death.

P-2011

Cellular force assay: analysis on the effect of KRAS mutations

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Recent progress in elucidating the roles of mechanical forces in regulating various cell functions expands the field of biology to one where interdisciplinary approaches with engineering techniques become indispensable. Contractile forces - inherently present in proliferative cells including cancer cells due to the activity of ubiquitous nonmuscle myosin Ⅱ - are one of the cellular forces, but because the cellular myosin works downstream of diverse signaling pathways, it is often complicated to evaluate how the inherent cellular forces change upon perturbations to particular molecules including oncogenes. Here, we determine whether the contractile forces of individual cells are upregulated or downregulated using an assay we developed, particularly focusing on the effect of mutations in KRAS.References from our group:Fujiwara, S., et al., PLOS ONE. Published: April 19, 2018.Ichikawa, T., et al., Journal of Cell Science, 130, 3517-3531, 2017.Fukuda, S.P., et al., Development, Growth & Differentiation, 59(5), 423-433, 2017.Sakane, Y., et al., Molecular Biology of the Cell, 27(20), 3095-3108, 2016.

▲TOP

P-2012

Generation of iPS cells as a model for NBCCS by using CRISPR/Cas9 System

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NBCCS, also known as Gorlin syndrome, is an autosomal dominant disease characterized by developmental malformation and increased occurrence of basal-cell carcinomas (BCCs) and medulloblastomas (MBs). NBCCS is caused by mutations in the PTCH1 gene, encoding the receptor for the sonic hedgehog (SHH) pathway. Dysregulation of the SHH signaling pathway contributes to the development of NBCCS-related MBs or BCCs because of the mutation in the wild-type PTCH1 allele, following Knudson's hypothesis also known as the two-hit hypothesis. In this study, we performed gene editing experiments using CRISPR/Cas9 system in human iPSCs derived from NBCCS Patients. We tried to introduce a mutation in the remaining wild-type allele of PTCH1 in NBCCS-iPCSs ($PTCH1^{+/-}$ iPSCs), and obtained several $PTCH1^{-/-}$ iPSC clones. We then transplanted $PTCH1^{-/-}$ iPSCs into immunocompromised mice subcutaneously. After 10-14 weeks, teratomas of undifferentiated type have developed from $PTCH1^{-/-}$ iPSCs. Furtermore rossete formation, commonly found in MBs, was observed in the teratomas from $PTCH1^{-/-}$ iPSCs. These results demonstrated that this experimental system can be a model recapitulating NBCCS-associated tumors.

P-2013

Exprolatory study for regulatory molecules expressed in pancreatic cancer cell lines showing various behaviors

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[Introduction]We have subcloned highly invasive cells from pancreatic cancer cell line. Since clones with morphology and behavior were further obtained in these processes, the molecules expressed in these cells were sought. [Materials and Methods]Additional subclone cells were obtained by subculturing established highly invasive cell lines. Furthermore, by observing the morphological change, the factors showing cell proliferation ability and intracellular expression were identified. [RESULTS]Several types of cells with different cell morphology and behavioral distortion were obtained. It is a group that grow isolatedly due to poor mutual adhesion, a group that strongly adhere between each cells and slow proliferation, a group that tends to float from aggregates with high adhesion and a group that collect floating cells, respectively. Expression of not only cell proliferation ability but also several molecules observed between these groups. [Summary] Although it is an established cell line, it is possible to obtain a single subclone having different characteristics from the property of a molecule different from a cell group having various characteristics.

P-2014

Three-dimensional organoids reveal therapy resistance of esophageal squamous cell carcinoma cells

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Background and Aims: Esophageal squamous cell carcinoma (ESCC) is a lethal disease due to cell heterogeneity and therapy resistance. To facilitate ESCC therapy in personalized medicine, three-dimensional (3D) organoids may be useful for functional characterization of ESCC cells. We investigated the feasibility and the utility of patients-derived 3D ESCC organoids. Methods: We generated 3D organoids from paired biopsies representing tumors and adjacent normal mucosa from therapy-naive ESCC patients and ESCC cell lines. We evaluated morphology, proliferation and molecular markers in 3D ESCC organoids. Results: 3D ESCC organoids were grown successfully from 11 out of 16 tumors (68.8%) and passaged with recapitulation of the histopathology, proliferation properties, p53 and CD44 expression present in the original in situ tumors. Successful 3D organoid formation was associated significantly with poor response to chemotherapy or chemoradiation therapy (P=0.0357, progressive and stable diseases, n=10 vs. partial response, n=6). Conclusions: 3D ESCC organoid system may serve as a highly efficient platform to explore cancer therapeutics and therapy resistance mechanisms.

▲TOP

[P-2021] P1-4 [English/Japanese]
Process of carcinogenesis (1)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Reo Maruyama / Project for Cancer Epigenomics, Cancer Inst., JFCR

P-2021

The function and mechanism of HNF1A-AS1 in the development of hepatocellular carcinoma

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By detecting the expression of HNF1A-AS1 in a total of 138 paired clinical HCC tissues and paracancerous tissues and found that HNF1A-AS1 expression was significantly over-regulated in 91 paired clinical HCC tissues. HNF1A-AS1 expression levels in HCC were significantly associated with hepatitis B (P=0.036), cirrhosis (P=0.009), metastasis (P=0.01), clinical stage (P=0.002). The patients in the high HNF1A-AS1 expression level have worse overall survival compared to the low expression level. The expression of HNF1A-AS1 in the HCC cell lines HepG2, HCCLM3, SK-Hep1, HUH7, Hep3B, SMMC7721 was significantly upregulated compared with the normal liver cell line QSG-7701.Knock-down of HNF1A-AS1 in HUH7 and SMMC-7721 cell lines resulted in a significant decrease in cell viability, colony formation, the invasion and migration and induced cell-cycle arrest and apoptosis. HNF1A-AS1 knock-down suppressed HCC tumor growth in vivo. HNF1A-AS1 promotes HCC migration and invasion by regulate the expression of PIGR in EMT pathway.

▲TOP

P-2022

Deposition of platelets in sinusoids in dysplastic and neoplastic hepatic lesions in human and mice

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We previously reported that in the transgenic mice with liver specific human BRAFV600E mutation, which corresponds to the mouse BrafV637E that is highly prevalent in mouse DEN-induced hepatic tumors, overexpress thrombopoietin, exhibit megakaryocytosis/thrombocytosis, and large numbers of platelets were deposited in the liver sinusoids. Platelets can activate hepatocyte proliferation not only via releasing platelet factors, but also promoting for liver sinusoidal endothelial cells (LSEC). In the present study, we investigated whether platelets are deposited in liver sinusoids in hepatic tumors in human and mice. CD61 immunohistochemical staining revealed increased numbers of platelets were deposited in the sinusoids in foci, adenomas and hepatocellular carcinomas (HCC) induced by neonatal treatment with DEN compared to surrounding normal hepatic tissues. Moreover, increased numbers of platelets were deposited in sinusoids of dysplastic nodules and well-differentiated HCC compared to normal liver/cirrhotic nodules in human. These results indicate that platelets contribute to development of dysplastic/neoplastic lesions via interacting sinusoidal cells.

P-2023

Metabolome changes in NASH liver tissue and tumors developed in metabolic syndrome model TSOD mice

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The TSOD mouse exhibits both obesity and diabetes associated with marked pancreatic islets hypertrophy and hyperinsulinemia, thus resembling a common form of obese type 2 diabetes in humans. To uncover mechanisms of nonalcoholic steatohepatitis (NASH)-associated hepatocarcinogenesis, we performed the metabolome analysis of 20-week-old TSOD mice liver tissues and tumors using the CE-TOFMS, CE-QqQMS, MassHunter and Ingenuity Pathway analyses. In the livers of TSOD mice, development of liver tumors and increase in depositions of lipid, fibrosis and inflammatory cell infiltration were observed resembling the histopathological changes of human NASH liver tissue. In TSOD mice liver tumors, metabolome analysis demonstrated significant activation of glycolysis, Warburg effect and elevation of GSH/GSSG reflecting the resistance of tumors to oxidative stress. Furthermore, significant increase of arginine and decrease of glycine and methionine levels were found. The changes in arginine level were likely be related to the decrease of arginase expression in tumors. The metabolome alterations could be induced to the activation of mTOR pathway in the liver tumors of TSOD mice.

P-2024

Induced Tumorigensis by in vitro Reconstitution of Genetic Alterations in Biliary Tract Cells

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We previously demonstrated that intestinal niche is dispensable for intestinal organoids to achieve Apc-dependent tumor development in nude mice. To explore the potential of this approach in generating models for biliary tract cancers, we transduced primary liver or gallbladder cells from KrasLSL-G12D/+ mouse with lentiviral Cre and shRNAs targeting tumor suppressor genes (TSGs). Upon inoculation in nude mice, we noted that any single genetic alteration was insufficient, but combination of mutant Kras and repression of TSGs was basically sufficient, for tumor development. Induced lesions histologically varied from premalignant to full-blown adenocarcinoma, likely recapitulating the broad spectrum of multistep carcinogenesis. Tumors consisted of only Kras-mutant cells, indicative of its oncogenic potential. In contrast, two oncogenes for intrahepatic biliary tract, FGFR2-AHCYL1 and a mutant Pik3ca, occasionally induced solid tumors, and frequently developed multiple cysts, respectively, when combined with repression of TSG. Thus, the organoid-based approach might allow quick validation of tumorigenic potentials of genetic interactions, independently of biliary tract niche.

▲TOP

P-2025

Candida albicans infection creates a microenvironment favoring MDSC and Th17 and promotes mouse oral cancer incidence

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Microorganism infection has been reported to associate with many cancers. We adopt a carcinogen-induced mouse oral cancer model to investigate the effect of oral Candida albicans infection on the development of oral cancer. We observed that exposure of Candida increased the incidence of oral cancer. The expression of genes involved in the Th17 development pathway was elevated in the tumor microenvironment. A co-localization of Candida mycelium and presence of IL-1beta, IL-6, IL-17A, IL-17F, and IL-22 was demonstrated. Exposure of mouse oral epithelial cells to curdlan, a component of Candida cell wall, triggered the release of MCP-1 which can recruit monocyte and macrophage. Exposure of mouse monocyte to curdlan in turn modulated the differentiation of macrophages and dendritic cells and promoted the production of arginase, iNOS, IL-1beta, IL-6 ad IL-23. In addition, curdlan promoted the differentiation of myeloid-derived suppressor cells. These conditions may further promote the development of Th17 cells. We demonstrated that Candida albicans infection creates a microenvironment favoring MDSC and Th17 development and promotes mouse oral cancer incidence.

P-2026

Study of the mechanism of carcinogenesis by prenatal exposure to dimethylarsinic acid in mice

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In our previous study, prenatal exposure to dimethylarsenic acid (DMA), which is the main metabolites of inorganic arsenic, occurred lung and liver tumor in male adult mice by transplacental exposure of DMA. Though, the mechanism of carcinogenesis by prenatal exposure to DMA is unclear. The purpose of this study is to clarify the mechanism of carcinogenesis by prenatal exposure of DMA in male neonatal mice lung. DMA was administered to female CD-1 mice at dose of 0 and 200 ppm for 10 days from 8 to 18 days of gestation, and male newborn mice prepared. And various analyzes described below were performed.As a result of quantitative analysis of arsenic in the lung, no significant change in TMAO was observed, whereas it was revealed that DMMTA and DMDTA were significantly increased. Furthermore, it was revealed that S-adenosylmethionine (SAM) was significantly increased and histone H3K9me3 was significantly increased.From the above results, it was revealed that histone H3K9me3 was increased in neonatal male mice lung prenatal exposed to DMA. In addition, involved as its mechanism suggested that SAM increase caused by metabolic process of arsenic.

P-2027

Age-related remodeling of apparently normal esophageal epithelia by common cancer drivers

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Clonal expansion in aged, apparently normal tissues has been implicated in the development of blood and skin cancers. However, the dynamics of such expansion and its relation to cancer development is poorly understood. To understand clonal expansion in apparently normal esophageal epithelia, we performed whole exome sequencing of 410 micro-scale esophageal samples combined with high-density sampling (22 cancer, 12 dysplasia and 376 normal tissues), The age-related clonal expansion was driven by acquired mutations in common cancer drivers, which were overrepresented by NOTCH1 mutations. This showed a sharp contrast to cancer mutations, which almost invariably affected TP53 (100%), while less frequently involved NOTCH1 (24%). Driver-mutated clones multi-focally emerged from early adulthood and over years, increase their number and size, ultimately remodeling the entire esophageal epithelia in extreme elderly. Our results suggest that clonal expansion in esophageal epithelia is an inevitable consequence of normal aging, differentially impacting the development of cancer depending on mutation type and exposure to drinking and smoking.

▲TOP

[P-2035] P1-6 [English/Japanese]
Detection and assessment of carcinogens

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Satoru Takahashi / Dept. Exp. Pathol. Tumor Biol., Nagoya City Univ. Sch. Med.

P-2035

Genome-wide chemical mutation signature analysis using a novel highly- accurate genome sequencing method

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Research on cancer mutation signatures has furthered the understanding of relationships between cancer and environmental mutagens. However, there is limited data on the mutation signatures of environmental mutagens due to the difficulty in detecting rare somatic mutations caused by exposure to mutagens. In this study, we developed a novel, highly accurate genome-sequencing method using a next generation sequencer and analyzed genome-wide rare mutations caused by chemical exposures. We optimized our sequencing method and achieved high accuracy with an error frequency of less than 1 per 10^{7-8} bp. We analyzed DNA samples of mutagen- (e.g., ENU)-exposed *Salmonella typhimurium* TA100 strains or gpt delta mouse and obtained large-scale genome-wide somatic mutation data. In both experiments, the 6- or 96-type (trinucleotide) mutational signatures were similar to known signatures of each mutagen (e.g., GC to AT in ENU), and also indicated other minor patterns that were not elucidated in previous studies. These results indicate that our highly accurate sequencing method is effective in characterizing genome-wide chemical mutation signatures and linking them to mutations in the cancer genome.

▲TOP

P-2036

In vivo positive mutagenicity of 1,4-dioxane and quantitative analysis of its mutagenicity and carcinogenicity in rats

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1,4-Dioxane is a widely used synthetic industrial chemical and its contamination of drinking water and food is a potential health concern. It induces liver tumors when administered in the drinking water to rats. However, the mode of action (MOA) of the hepatocarcinogenicity of 1,4-dioxane remains unclear. To determine the in vivo mutagenicity of 1,4-dioxane, gpt delta transgenic F344 rats were administered 1,4-dioxane at various doses in the drinking water for 16 weeks. The overall mutation frequency and A:T to G:C transitions and A:T to T:A transversions in the gpt transgene were significantly increased at 5000 ppm. A:T to T:A transversions were also significantly increased at 1000 ppm. Furthermore, the DNA repair enzyme MGMT was significantly induced at 5000 ppm implying that extensive genetic damage exceeded the repair capacity of the cells in the liver. These findings demonstrate that 1,4-dioxane is a genotoxic hepatocarcinogen and induces hepatocarcinogenesis through a mutagenic MOA in rats. We also characterized the dose-response relationship of the mutagenicity and carcinogenicity using the no-observed effect level approach and the Benchmark dose approach.

P-2037

Induction of cell proliferation and DNA damage by Acetoaceto-o-toluidide in the urinary bladder of rats

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Acetoaceto-o-toluidide (AAOT) is made from o-Toluidine (OTD) and used for the synthesis of azo pigment. OTD is an aromatic amine and a well-known human urinary bladder carcinogen. However, little is known about the carcinogenicity of AAOT. The aim of the present study is to evaluate the effects of AAOT on the urinary bladder of rats. Six-week-old F344 rats were fed a diet supplemented AAOT at the dose of 0, 1.5% and 3% for 4 weeks. At the end of week 4, histopathological analysis of urinary bladder was conducted. To determine the cell proliferation and the DNA damage, Ki67 index and γ H2AX expression were assessed in the bladder epithelium by immunohistochemistry.Body weight depression and simple hyperplasia of the urinary bladder epithelium were observed in the rats administered AAOT in a dose dependent manner. Ki67 index and γ H2AX expression were also significantly increased in the bladder epithelium of the rats administered AAOT in a dose dependent manner. These results showed that AAOT increased cell proliferation and DNA damage in the urinary bladder epithelium of rats. Long-term study to evaluate the carcinogenicity of AAOT in urinary bladder in rats is ongoing.

P-2038

Effects of Heterocyclic Amine on the Proteome of earthworms

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Heterocyclic amines are carcinogenic compounds formed when proteinaceous foods are cooked at high temperature. They have also been detected in environmental media such as river water, domestic waste, cigarettes smoke and haze. They can induce various toxicities, such as hepatotoxicity, mutagenicity and genotoxicity effects in animals. In this work, we characterized the effects of 2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) in earthworm Eudrilus eugeniae using the liquid chromatography-mass spectrometry. 28 protein groups were identified from the control while 26 protein groups were identified from the PhIP-treated group. 18 proteins which were exclusive to both groups were further quantified using the bioinformatic tools, Perseus. 8 proteins including Calmodulin, Intermediate filament protein, Superoxide dismutase and Calcium-transporting ATPase were found to be downregulated while 2 proteins specifically Nitrous oxide reductase and Calretinin were upregulated. Proteomic responses revealed that PhIP induced cell apoptosis (or injury), oxidative stress, disturbance in energy metabolism in E. eugeniae in terms of differential proteomic biomarkers.

▲TOP

P-2039

Early detection of urinary bladder carcinogens by immunohistochemistry for stem cell markers

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Cytokeratin 14 (KRT14), aldehyde dehydrogenase 1A1 (ALDH1A1), and CD44 are reported to be the promising markers for identifying cancer stem cells in bladder cancer. The aim of the present study was to evaluate the applicability of these markers for early detection of bladder carcinogenicity. Six-week-old male F344 rats were orally treated with 6 bladder carcinogens (BCs) such as BBN, 2-NA, and NTA or 2 non-bladder carcinogens (NBCs) including TBPP and KBrO3 for 4 weeks. Animals were sacrificed at the end of administration, and immunohistochemistry for KRT14, ALDH1A1, and CD44 in the bladder epithelium was performed. Increased expression level of KRT14, ALDH1A1, and CD44 were observed in all BC-treated groups except KRT14 in NTA-treated rats. In NBC-treated groups, there was no increase in the expression level of these markers. In quantitative analysis of KRT14 expression by immunofluorescent staining, the ratio of KRT14-positive area to the whole bladder mucosa in rats treated with BBN or 2-NA was shown to be higher than that in the control group. These results suggest that the stem cell markers may be useful for the early detection of bladder carcinogens.

P-2040

Immunohistochemical detection of possible gastric carcinogens using DNA double-strand break marker, γ-H2AX

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DNA damage caused by chronic inflammation with Helicobacter pylori is considered as an important risk factor of gastric carcinogenesis. In this study, we have evaluated double-stranded DNA damage response utilizing focus formation of Ser 139 phosphorylated histone H2AX (γ-H2AX) against various chemical carcinogens. Six-week-old male rats were administered N-methyl-N-nitrosourea (MNU), 3, 2'-dimethyl-4-aminobiphenil (DMAB), dimethylnitrosamine (DMN), 1,2- dimehtylhydrazine (DMH), and water for control intragastrically for 5 days / week x 4 weeks and were sacrificed at day 28. Immunohistochemical analysis revealed that γ-H2AX focus formation was significantly increased in pyloric glands in MNU group and in fundic glands in the MNU and DMAB groups. γ-H2AX positive apoptotic cells were observed on the surface layer in MNU group. H2AX (h2afx) mRNA expression was significantly decreased and p21waf1 was in increasing trend in MNU and DMN groups compared to the control. In conclusion, this system, utilizing γ-H2AX, may be useful for screening undetermined gastric carcinogens.

P-2041

Gene expression profile in the early stage of aromatic amine-induced bladder carcinogenesis in rats

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[Background] Although aromatic amines are widely used as raw materials for dyes, there have been concerns about carcinogenicity, particularly in the urinary bladder. In this study, gene expression profile in the bladder mucosa of rats treated with aromatic amines was analyzed to clarify the effect in the early stage of carcinogenesis.
[Methods] Six-week-old male F344 rats were administered 0.8% o-toluidine or 1% o-anisidine in diet for 4 weeks. Gene expression level in 5 categories, which are known to be associated with bladder carcinogenesis, in the bladder mucosa at week 1 and 4 was analyzed by PCR array.
[Results and Discussion] Increased expression of genes associated with cell cycle, DNA damage, and Hedgehog pathway was induced by both o-toluidine and o-anisidine, suggesting the important role in the early stage of bladder carcinogenesis. Expression changes of chromatin modification/remodeling factors were relatively low, suggesting that they may be important in more later stage. Number of genes with altered expression level in o-toluidine group peaked at week 1 and decreased at week 4, showing a tendency similar to sequential changes of mucosal injury in the urinary bladder.

▲TOP

[P-2046] P1-8 [English/Japanese]
Radiation carcinogenesis and oxidative stress (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-2046
Withdrawn

No Abstract

P-2047

Protective role of JLP-JNK pathway against oxidative stress-induced cell death

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Tumor cells are known to maintain an increased level of reactive oxidative species (ROS), which is believed to be involved in tumor progression. However, high levels of ROS might induce oxidative stress that could lead to cell death. Several signaling pathways, including c-Jun NH_2-terminal kinase (JNK), play an important role to maintain the balance of ROS generation and elimination, and therefore to protect the cell from oxidative stress-induced cell death. Recently, JNK-associated leucine zipper protein (JLP, also known as SPAG9 or JIP4), a scaffold protein for JNK MAPK was identified as a biomarker of cancer. Although JNK is known to be activated in response to oxidative stress, the involvement of JLP remains elusive. Here we analyzed the role of JLP in response to oxidative stress-induced cell death. Our results showed that knockdown of JLP enhanced the cell death and attenuated the JNK activation in response to H_2O_2. These responses were almost completely reversed by re-expression of a wild type JLP, but not its mutant lacking JNK-binding domain. Collectively our data suggest JLP-JNK pathway protects tumor cells from oxidative stress-induced cell death.

P-2048

Erastin induced Ferroptosis in human pancreatic ductal adenocarcinoma cells depending on AMPK and autophagic pathway

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Ferroptosis is a noval mode of cell death involving the production of iron-dependent reactive oxygen species (ROS). AMP activated protein kinase (AMPK) is an established indicator of metabolic stress and can be induced by high intracellular ROS. However, the molecular mechanisms link between AMPK and Ferroptosis were remain obscure. In this study, we used Erastin as an inducer of ferroptosis and identified AMPK and its downstream effectors as positive regulator of ferroptosis. Erastin activates AMPK and induces a reduction in glutathione peroxidase 4 (GPX4) activity through an AMPK/Srebp1/BCAT2/Gln pathway. Additionally, activated AMPK promoted autophagy through direct phosphorylation of Ulk1, which further results in GPX4 degradation. Inhibition of autophagy or overexpression of Srebp-1 abrogated Erastin induced cell death. Conclusion: These findings demonstrated AMPK/Srebp1/BCAT2/Gln and autophage as novel molecular mechanisms involving in Eristin induced ferroptosis.

P-2049

Risk evaluation of radiation-induced cancer risk using ApcMin/+ mice

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It is well known that exposure to ionizing radiation (IR) may produce deleterious consequences in humans, including cancer induction. Fukushima nuclear accident and the increasing usage of radiation in medical and industrial field have brought us a great concern about health risks of low dose radiation. A mechanistic understanding of cancer induction by low dose and low-dose rate radiation is required for accurate estimation of cancer risk following exposure.$Apc^{Min/+}$ mouse is a murine model of human cancer syndrome familial adenomatous polyposis (FAP), which develops spontaneously many intestinal adenomas. $Apc^{Min/+}$ mice are highly susceptible to DNA damage agents such as IR. Here, we explored IR-induced cancer risk at using $Apc^{Min/+}$ mice with two different genetic backgrounds, B6/B6-F1-$Apc^{Min/+}$ mice and B6/B6-Ch18^{MSM}-F_1-$Apc^{Min/+}$ mice. Our data shows that IR increased the number of intestinal adenomas dose-dependent fashion even at low doses. The relationship between dose and tumor multiplicity seems to be linear in both $Apc^{Min/+}$ mouse models. Our data provides that $Apc^{Min/+}$ mouse might be a useful model for the evaluation of radiation induced cancer risk.

▲TOP

[P-2056] P4-4 [English/Japanese]
Oncogenes and tumor-suppressor genes

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Hiroki Nagase / Chiba Cancer Cent. Res. Inst.

P-2056

The RASSF6 tumor suppressor protein regulates apoptosis and the cell cycle via Retinoblastoma protein

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RASSF6 is a member of the tumor suppressor Ras-association domain family (RASSF) proteins. RASSF6 expression is epigenetically suppressed in human cancers and its low expression is associated with poor prognosis. RASSF6 plays a tumor suppressor role by regulating cell cycle arrest and apoptosis. Mechanistically, RASSF6 blocks MDM2-mediated p53 degradation and enhances p53 expression. However, RASSF6 also induces cell cycle arrest and apoptosis in the p53-negative background, which implies that the tumor suppressor function of RASSF6 does not solely depend on p53. In this study, we have revealed that RASSF6 enhances the interaction between pRb and protein phosphatase and induces CDKN2A. In this way, RASSF6 increases unphosphorylated pRb and augments the interaction between pRb and E2F1. Moreover, RASSF6 increases *TP73*-target genes *via* pRb and E2F1. Finally, we confirmed that RASSF6 depletion induces polypoid cells in the p53-negative background. In conclusion, RASSF6 plays as a tumor suppressor in cancers with the loss-of-function of p53 and pRb is implicated in this function of RASSF6.

▲TOP

P-2057

Interaction between tumor suppressor Rb and the circadian rhythm

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Recent progress of molecularly targeted therapy suggests that finding of new connection between cancer and other pathways such as immunology is beneficial to design new medicine. Epidemiological and clinical studies suggest that circadian clock disruption and the cancer progression are related. International Agency for Research on Cancer (IACR) classified shift work with circadian disruption as a group 2 human carcinogen. However, the molecular mechanism of how the circadian clock is disrupted in cancer and the consequence of circadian clock disruption in cancer are still unclear. The retinoblastoma protein (RB) is known to regulate cell cycle, differentiation and metabolism. Here we report several lines of evidence implicating RB in the circadian clock regulation. These findings uncover a new layer of circadian clock regulation and shed some fresh lights on the molecular mechanisms underlying circadian rhythm, cell cycle, and cancer.

P-2058

Identification of malignant mesothelioma-specific molecule whose expression is induced by deficiency of p16 / NF2 gene

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Malignant pleural mesothelioma (MPM) is a highly refractory tumor caused by exposure to asbestos. MPM is currently incurable due to difficulty of early diagnosis method and medication, both of which are urgently needed. CDKN2A and NF2 genes are frequently mutation/deleted in MPMs, but there is no established molecular marker for MPM patients with both NF2 and CDKN2A mutation. Our aim is to identify such biomarkers, which would be utilized for early diagnosis and could be used as therapeutic targets. In this study, we generated NF2/p16INK4A double knockout cells (NF2/INK4A-DKO) using a CRISPR/Cas9 system with the human mesothelial cell line Met-5A and explored its biological characteristics. Western blot analysis indicated that that TGF-beta, p-p70s6K CDC2 and p-c-Jun increased and those of CDK4, p27 and E2F1 decreased in DKO cells. Our findings suggest that disruption of both NF2 and CDKN2A enhances colony formation and migration significantly more than singly knock-out cells. DKO cells expressed abundant RNAs for secretory molecules (CD24, PTN, BMP7), the detection of which may help to identify DKO subtypes in clinical settings.

P-2059

Inactivation of p16INK4a retaining p14ARF function enhances the development of invasive oral cancers

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[Background]
The $p16^{INK4a}$ and $p14^{ARF}$ tumor suppressor genes are encoded within the CDKN2A locus on chromosome 9p21 and function as cell cycle regulatory proteins. Inactivation of these genes by genetic and epigenetic changes has been described in human oral cancers. However, it is unclear that importance of inactivation of $p16^{INK4a}$ gene retaining normal $p14^{ARF}$ function in the pathogenesis of oral squamous cell carcinoma(OSCC).
[Methods]
A total of 50 mice were used for the study (25 $p16^{INK4a}$ knockout, which has normal $p14^{ARF}$ function, and 25 wild-type C57BL/6 mice). All the mice were administrated with drinking water containing 4-nitroquinoline 1-oxide (4NQO) for 15 weeks to induce OSCC. We analyzed the tumorigenesis in oral cavity.
[Results]
Histological analysis revealed that the incidence of OSCC with invasion was increased in the tongue of $p16^{INK4a}$ knockout mice compared with the control group. There were not differences in $p16^{INK4a}$ knockout and the control group on the incidence of dysplastic and carcinoma in situ lesions.
[Conclusion]
Our results suggest that inactivation of $p16^{INK4a}$ retaining normal $p14^{ARF}$ function has a pivotal role in the pathogenesis of invasive OSCC.

▲TOP

P-2060

Molecular Docking to Identify a Novel Inhibitors for Tyrosine kinase in CML from Alkaloids

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Chromosomal abnormality so-called Philadelphia chromosome is the hallmark of chronic myelocytic leukemia (CML). More than 90% of CML caused by Philadelphia chromosome which mainly causes by a fusion gene called BCR-ABL1 coding for a hybrid protein: a tyrosine kinase signaling protein that is, causing the cell division uncontrollably. Targeting this signaling molecule is a novel approach for the resist of CML. In the recent year virtual high throughput screening has emerged as a widely accepted powerful technique in the identification of novel and diverse lead. The high-resolution X-ray structure of tyrosine kinase signaling protein has opened the way to introduce new small molecular inhibitors by structure-based virtual screening. In this study using different alkaloid molecules as potential novel inhibitors of tyrosine kinase signaling protein and proposed three candidate compounds with high scoring function. Thus from complex scoring and binding ability, it is clarified that these alkaloids might be developed as novel lead compounds to design new drugs against CML.

P-2061

STAT1 plays a crucial role in ETV6-NTRK3-mediated tumorigenesis

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The ETV6-NTRK3 (EN) fusion has been implicated in various cancers and has exhibited in vivo and in vitro transforming ability. In the present study, we analyzed transcriptome alterations using DNA microarray and RNA-Seq in EN-transduced NIH3T3 fibroblasts to identify the mechanisms that are involved in EN-mediated tumorigenesis. The functional profile assessment of EN-regulated transcriptome alterations correlated with the transforming potential and increased proliferation in EN-transduced cells. Notably, KEGG pathway, Ingenuity Pathway and Gene Regulatory Network analysis identified STAT1 as a significant EN-regulated molecule. We further demonstrated that EN enhanced STAT1 phosphorylation but attenuated STAT1 acetylation, eventually inhibiting the interaction between the NF-κB p65 subunit and acetylated STAT1. Consequently, nuclear translocation of NF-κB p65 and subsequent NF-κB activity were increased by EN. Notably, inhibition of STAT1 phosphorylation attenuated tumorigenic ability of EN in vitro and in vivo. Taken together, here we report, for the first time, STAT1 as a significant EN-regulated transcription factor and a crucial mediator of EN-induced tumorigenesis.

▲TOP

[P-2068] P4-6 [English/Japanese]
Cancer related genes

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Masuyuki Noguchi / Div. Cancer Biol, Inst for Genetic Medicine, Hokkaido Univ.

P-2068

miR-143/MSI2/KRAS expression system positively contribute to carcinogenesis in human bladder cancer

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It has been well established that microRNA (miR) - 143 was downregulated in human bladder cancer (BC). Recent precision medicine demonstrated that the mutations in BC were frequently observed in FGFR3, RAS and PIK3CA, which were strongly correlated with RAS signaling networks. We have previously demonstrated that miR-143 suppressed cell growth by inhibition of RAS signaling networks, mainly KRAS, in several cancers including BC. In the present study, we developed a novel synthetic miR-143 and demonstrated that the syn-miR-143 negatively regulated the RNA-binding protein Musahi-2 (MSI2) in human bladder cancer cell line T24. MSI2 is an RNA-binding protein and regulates the stability of mRNAs and their translation by binding to the target sequences of the mRNAs. Of note, the present study clarified that MSI2 positively regulated KRAS expression through directly binding to the target sequence of KRAS mRNA, which contributed to the maintenance of KRAS expression. Thus, miR-143 silenced KRAS directly and MSI2, which further downregulated KRAS expression trough perturbing MSI2/KRAS expression system.

▲TOP

P-2069

Identification of genes regulated by KRAS G12 mutations in colorectal cancers from the HOPE datasets

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KRAS is a commonly mutated driver gene in colorectal cancer (CRC). However, the full spectrum of downstream genes driven by KRAS-activating mutations has not been elucidated, and there are no effective targeted drugs clinically available for KRAS-mutated CRC. Here, we performed a comprehensive molecular evaluation of CRC using WES and gene expression profiles (GEP). KRAS mutations were detected in 41.1% of cases (372/906). Among KRAS-mutated CRC, KRAS G12 mutations were observed in 64.5% of cases (240/372). Of the known downstream genes in the KRAS pathway, increased expression was observed for CCND1, DUSP2, DUSP4, ETS2, JUN, RAC2, RAC3, SPRY4, ELK1, RALGDS and RASAL1 in KRAS-mutated CRC. To exploit novel targets of mutant KRAS G12, GEP was assessed in CRC with KRAS G12-mutated (n=240) and KRAS-wild type (n=390). KRAS-wild type CRC harboring mutation in HRAS, NRAS, PIK3CA, PIK3CD, PIK3CG, RALGDS, RGL1-3, BRAF, ARAF or RAF1 was excluded from analysis. We identified promising candidates that showed differential expression between these two groups. This approach allowed for identification of more specific and effective anti-cancer targets that will facilitate drug development.

P-2070

Abrogation of RhoA expression and activity is associated with its aberrant splicing in diffuse-type gastric cancer cells

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RhoA is a member of Rho family small GTPases that regulates diverse cellular functions. Recent studies identified recurrent somatic mutations of RHOA in diffuse-type gastric carcinoma (DGC). In this study, we investigated the possible abnormalities of RHOA in a panel of gastric carcinoma (GC) cell lines. Pulldown assay and immunoblot analysis showed that the activity and expression of RhoA were detectable in all cell lines tested, except for two DGC cell lines, HSC-59 and GSU. RHOA coding region sequencing revealed that aberrant alternative splicing of RHOA occurred in these cell lines. Quantitative real-time PCR analysis showed that the expression of wild-type RHOA was nearly undetectable, whereas splicing variants were almost exclusively expressed in HSC-59 and GSU cell lines. However, the expression levels of RHOA splicing variants were very low and the corresponding proteins were not detected by immunoblotting. Moreover, the splicing isoforms of RhoA protein were neither efficiently expressed nor activated even if ectopically expressed in cells. These results indicate that aberrant alternative splicing of RHOA results in the loss of its activity and expression in DGC cells.

P-2071

Mutant KRAS increases the surface expression of CD155 on human colorectal cancer spheroids

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Background/Aim: The interaction between CD155 (expressed by cancer cells) and TIGIT (expressed by lymphocytes) triggers a novel immunesuppressive mechanism in several types of cancer cells. However the precise role of mutant (mt) KRAS in the CD155-TIGIT pathway is unclear. Materials and Methods: HKe3 human colorectal cancer (CRC) cells which stably expressing wild-type (wt) KRAS (HKe3-wtKRAS) or mtKRAS (HKe3-mtKRAS) were used for two-dimensional (2D) and three-dimensional floating (3DF) culture. The expressions of CD155 were examined by flow cytometry. 3DF co-culture model was established using HKe3-mtKRAS and human Cytokine-induced killer (CIK) cells. Results: The expression of CD155 on HKe3-mtKRAS cells was 2.4-fold higher compared to that of HKe3-wtKRAS cells in 2D culture and a greater difference of 7.6-fold was observed in 3DF culture. In the 3DF co-culture model, a CD155 inhibiting antibody suppressed the growth of HKe3-mtKRAS spheroids and induced apoptosis in HKe3-mtKRAS spheroids. Conclusion: Blocking the CD155-TIGIT pathway, represents inhibition of a novel immune checkpoint which could act as a potential therapeutic strategy for the treatment of tumors with mtKRAS.

▲TOP

P-2072

Controllable NRAS expression system and analysis of different signals

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Klara Noble-Orcutt[2], Susan K. Rathe[2], Alexandra Hillesheim[2], Zain Qarni [2], Marie L Antony[2], Zohar Sachs[2], David Largaespada[2] 2. Masonic Cancer Center, Univ. of Minnesota NRAS has an important role in cell signaling in neoplastic cells and is well known as a major regulator of PI3 and MAP kinase signaling. Activation of NRAS signaling can induce proliferation; however, analyzing the signaling in the whole range of RAS expression would be difficult. In the present study, endogenous NRAS with a G12D mutation was knocked out by CRISPR in Thp-1 cells and transduced with a tetracycline (Tet)-inducible NRAS harboring an NRAS G12V mutant. Depletion of Tet inhibited cell proliferation and induced “G1-arrest”. Interestingly, Tet overdose also inhibited cell proliferation. Next-generation-sequencing confirmed exogenous NRAS mutant can be specifically inducible by Tet and mass cytometric analysis also confirmed multiple ERK and AKT activation. 162 genes are differently expressed with over expression of NRAS. This controllable NRAS expression can be a valuable source to reveal complicated NRAS signals.

P-2073

LGR6 overexpression induced by constitutive activation of the Wnt signaling pathway in NSCLC cells

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The Wnt signaling pathway implicates multiple cellular functions and molecular abnormalities of its pathway components, including mutations of CTNNB1 encoding β-catenin, cause constitutive activation of the pathway, leading to oncogenic transformation. Previous studies reported that the Wnt pathway activation occurs at high frequency in non-small cell lung cancer (NSCLC). To identify the genes associated with activation of the Wnt pathway, we investigated microarray expression profiling affected by small interfering RNAs (siRNAs)-mediated β-catenin knockdown in HCC15 NSCLC cells harboring CTNNB1 mutations. Consequently, we identified LGR6 as a gene downregulated by β-catenin knockdown. Quantitative RT-PCR analysis revealed that approximately one third of NSCLC cell lines including HCC15 exhibit LGR6 overexpression. Intriguingly, LGR6 expression levels were significantly higher in small cell lung cancer (SCLC) cell lines than NSCLC cell lines. siRNA-mediated LGR6 knockdown inhibited cell growth of LGR6-overexpressing NSCLC cells. These results suggest that constitutive activation of the Wnt pathway induces overexpression of LGR6, which may confer malignant phenotypes in NSCLC.

▲TOP

[P-2079] P6-1 [English/Japanese]
DNA repair / genomic instability

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Hiroshi Itoh / Dept. Mol. Pathol., Yamaguchi Univ., Grad. Sch. Med.

P-2079

Defective system-level regulation of Aurora B underlies chromosome instability in cancers

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The chromosomal passenger complex (CPC) including mitotic kinase Aurora B is enriched at centromeres and ensures accurate chromosome segregation. Previous studies have found that cancer cells consistently show a decrease in CPC/Aurora B function and its centromere localization. However, it is still unclear how the reduced CPC/Aurora B activity generates chromosome instability (CIN) and ultimately how it leads cells to cancer. Here, in order to address these questions, we studied trisomy cells and investigated changes in the CPC system. We found that the CPC system insufficiencies and aneuploidy are closely related to each other for promoting chromosome instability. We also adapted mouse cancer stem cells, which are undifferentiated cancer cells with a high tumorigenic potency, and examined that CPC function is decreased with malignant transformation. Our observations indicate that the defective CPC system causes chromosome missegregation resulting in aneuploidy and this aneuploidy in turn impairs CPC function. The understanding of this malignant cycle toward CIN would be a key clue to elucidate the intricate relationship between chromosome instability and cancer progression.

▲TOP

P-2080

Functional significance of cancer-testis antigens in genomic instability

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Cancer-testis antigens are normally expressed only in the germ cells but are also expressed at varying levels in cancer in a demethylation-dependent manner. Because of their cancer-specific pattern of expression, they have been considered to be promising targets for cancer therapy. However, their clinical applications have been limited to immunotherapy, because their functions in somatic cancer cells remain largely unknown. We have investigated the somatic roles of the synaptonemal complex proteins which are recently recognized as cancer-testis antigens, and found that these proteins can affect genome instability in various ways. For example, we found that SYCE2 directly binds to the heterochromatin-related protein HP1α through its N-terminal hydrophobic sequence and dissociates it from heterochromatin. We also found that this SYCE2-HP1α interaction is crucial for potentiation of the ATM-dependent DNA repair activity both in the steady states and upon induction of exogenous DNA damage. Thus SYCE2 plays a role in the link between the nuclear structure and the DNA damage response potentials in cancer, serving as a molecular basis for developing novel personalized cancer therapies.

P-2081

SUMO modification system regulates DNA damage-dependent exchange of histone variant H2A.Z-2

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Reorganization of damaged chromatin plays an important role in DNA repair to prevent carcinogenesis. Histone variant H2A.Z has two isoforms, H2A.Z-1 and H2A.Z-2, in vertebrates. In our previous study, we found that H2A.Z-2 is exchanged immediately after the induction of DNA double strand breaks. However, how the damage-dependent exchange of H2A.Z-2 is regulated remained to be clarified. In this study we investigated the role of SUMOylation, a post-translational modification, in the regulatory mechanism of the exchange of H2A.Z-2 at DNA damaged sites. We found that the depletion of PIAS4 significantly repressed the DNA-damaged dependent increase of the H2A.Z-2 mobility after induction of DNA damage. This suggests the involvement of SUMOylation in the regulation of H2A.Z-2 movement at damaged sites. We also found that PIAS4 regulated the SUMOylation of H2A.Z-2 after induction of DNA damage. These findings suggest that SUMOylation system regulates the exchange of H2A.Z-2 at DNA damaged sites to facilitate DNA repair. The biological significance of the reorganization of damaged chromatin by the exchange of H2A.Z-2 will be discussed.

P-2082

Aberrant (pro)renin receptor expression induces genomic instability by SMARCA5 disruption in pancreatic cancer

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<Background>We previously reported that aberrant (pro)renin receptor [(P)RR] expression induced genomic instability in human pancreatic ductal epithelial (HPDE) cells which are transforming into pancreatic ductal adenocarcinoma (PDAC). Here, we aimed to elucidate this molecular mechanism. <Methods> We performed nanoscale liquid chromatography with tandem mass spectrometry (LC-MS/MS). <Results> (P)RR overexpression induced genomic instability, i.e., dysfunctions of DNA replication, DNA repair and telomere maintenance in HPDE cells. Disorder of molecular network in imitation switch (ISWI) chromatin remodeling complex showed that (P)RR overexpression significantly increased SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5 (SMARCA5). SMARCA 5 overexpression downregulated the components regarding genomic stability pathways. Coimmunoprecipitation analyses showed that endogenous (P)RR has a direct molecular binding with SMARCA5. <Conclusions> Aberrant (P)RR expression induces genomic instability through the disorder of SMARCA5, suggesting that aberrant (P)RR expression is marked as a trunk of an evolutionary tree of PDAC.

▲TOP

P-2083

The role of chromosomal instability in cancer cell proliferation

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Chromosomal instability (CIN) in cancer cells is widely known to be related to the acquisition of drug resistance and malignant progression. However, there are many conflicting experimental results showing that CIN is detrimental for cell proliferation. Therefore, whether CIN plays a causative role in cancer development remains unclear. Mitotic dysfunction, which is characterized by chromosome missegregation, is a hallmark of chromosomally-unstable cancer cells. In this study, we isolated clones from HeLa cells showing either high or low CIN depending on the rate of chromosome missegregation, and examined proliferation of these clones. In normal culture condition, high-CIN clones showed growth disadvantage compared to low-CIN clones. However, in cancer stem cell enrichment condition, high-CIN clones formed bigger cell spheres than low-CIN clones. These results suggest that even though CIN is detrimental for cell proliferation in normal condition, it accelerates proliferation of cells with cancer stem cell-like features. In this symposium, we provide results of single-cell fate tracking and karyotypic diversity analysis, and discuss involvement of CIN in cancer cell proliferation.

P-2084

Transition from tetraploidy to aneuploidy is determined by Eg5-dependent spindle pole positioning

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Chromosomal instability is one of the most prominent features of tumor cells and causes aneuploidy. Although tetraploidy appears to be an intermediate state leading to aneuploidy, the underlying pathway to generate aneuploid cells through tetraploid state is poorly understood. Here, we report that spindle pole positioning in tetraploid cells depends on the spindle localization level of functional phosphorylated Eg5, a mitotic kinesin. Variation in the level of phosphorylated Eg5 localized at the spindle was associated with changes in spindle polarity and subsequent generation of aneuploid cells from tetraploid cells. Tetraploid cells with a high level of phosphorylated Eg5 underwent multipolar cell division, resulting in a highly heterogeneous aneuploid population, whereas those with a low level of phosphorylated Eg5 continued to undergo bipolar cell division and remained tetraploid. We propose that functional phosphorylated Eg5 behaves as a key regulator of spindle pole positioning and subsequent cell fate decisions in tetraploid cells.

P-2085

Paradoxical genomic destabilisation in human cells with Lynch syndrome MSH2 mutations introduced by CRISPR/Cas9 system

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Using the CRISPR/Cas9 technique, mutations reported in Lynch syndrome (LS) patients have been introduced into HeLa cells. HeLa clones carrying MSH2 variations, G674R, G674D and G674A, have been respectively established. An important hallmark of defective MMR is resistance to alkylating agents. These clones were indeed extremely resistant to N-methyl-N-nitrosourea (NMU), which confirms their MMR-deficient phenotypes. Consistently, the mutation frequencies determined in the HPRT locus were uniformly elevated in the clones. Another hallmark of MMR deficiency is microsatellite instability (MSI), and microsatellites are known to widely and drastically undergo length changes in MMR-defective tumors. However, microsatellite alterations in these clones were generally modest. Alterations in dinucleotide microsatellites were rare and, in all cases, within 6-bp, which corresponds to Type A instability that we have previously reported in Msh2-knockout animals. Mononucleotide repeats were stable in the clones. These observations strongly suggest that previously unrecognized molecular defects may underlie genomic instability observed in MSI^+ human tumors including ones occurring in LS patients.

▲TOP

[P-2092] P6-3 [English/Japanese]
DNA repair

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Akira Tomokuni / Osaka International Cancer Inst.

P-2092

Function of chromatin remodeler SMARCAD1 in the induction of apoptosis triggered by DNA mismatch

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The mismatch repair (MMR) complex, composed of MutSα (MSH2-MSH6) and MutLα (MLH1-PMS2), specifically recognizes mismatched bases during DNA replication. O^6-methylguanine, produced by *N*-methyl-*N*-nitrosourea (MNU) treatment, forms mismatched base-pair, O^6-methylguanine/thymine, and induces G/C to A/T transition mutation. To prevent such an outcome, the cell carrying this DNA mismatch is eliminated by the MMR-dependent apoptosis. Here, we provide the evidence showing that SMARCAD1, which has an ATP-dependent nucleosome remodeling activity, is a novel factor associated with the induction of MMR-dependent apoptosis. SMARCAD1 knockout cells (ΔSMARCAD1), as compared with control, were resistant to MNU, and the appearances of a sub-G1 population as well as caspase-9 activation were suppressed. Moreover, the MNU-induced mutant frequencies were increased in ΔSMARCAD1. In immunoprecipitation analysis, the formation of MMR complex in ΔSMARCAD1 was decreased as compared with control, and this effect was strongly dependent on the ATPase activity of SMARCAD1. Thus, we proposed the role of SMARCAD1 in the induction of apoptosis through the chromatin remodeling activity.

▲TOP

P-2093

Inhibiting the MCM8-9 selectively sensitizes cancer cells to DNA-crosslinking agent and PARP inhibitor

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MCM8-9 are paralogues of the MCM2-7 and play a crucial role in a homologous recombination-mediated repair process to resolve replication stress by fork stalling. Cancer cells undergo more replication stress than normal cells due to hyper-stimulation of growth. Therefore, inhibiting MCM8-9 could selectively hypersensitize cancer cells to platinum compounds and PARP inhibitors, both of which hamper replication fork progression. Here, we found that knockout of MCM9 or knockdown of MCM8 selectively hypersensitized transformed cells to cisplatin and olaparib. In agreement with reported findings, RAS- and HPV16 E7-mediated transformation of human fibroblasts increased replication stress, as indicated by induction of multiple DNA damage responses. Such replication stress was further increased by knockdown of MCM8, providing a rationale for cancer-specific hypersensitization. Finally, we showed that knocking out MCM9 increased the sensitivity of HCT116 xenograft tumors to cisplatin. Taken together, the data suggest that conceptual MCM8-9 inhibitors will be powerful cancer-specific chemosensitizers for platinum compounds and PARP inhibitors.

P-2094

Telomeric ssDNA-binding CST complex is involved in UV damage repair

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Ultraviolet (UV) light, one of the common environmental stresses, causes lesions in genomic DNA. The repair of DNA damage by UV irradiation requires excision of damaged nucleotides followed by local DNA replication to fill the gap. In recent years, accumulating evidence indicates that DNA replication of specific genome regions at S phase involves the ssDNA-binding CST (CTC1-STN1-TEN1) complex, which was originally identified as a telomere component (Miyake Y. et al., Mol. Cell, 2009). However, it remains unclear whether this protein complex also plays any role in other replication-dependent processes such as the nucleotide excision repair. To address the question, we constructed STN1-knockdown human cells and examined their response to UV irradiation. We found that the STN1-depleted cells were significantly sensitive to UV. The lack of STN1 led to the accumulation of unrepaired DNA. Interestingly, local UV-irradiation caused punctate UV damage signals colocalized with STN1 foci, both at telomeric and non-telomeric sites. We propose that the CST complex is involved in the DNA repair of UV damage in a genome-wide manner.

P-2095

BET inhibitors synergize with WEE1 inhibitor by impairing non-homologous end joining and enhancing DNA damages in NSCLC

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Background: The bromodomain and extraterminal domain (BET) proteins have been reported to interact with DNA repair pathways. AZD1775, a WEE1 G2 checkpoint kinase inhibitor, induces DNA damage by promoting premature mitotic entry. We hypothesized that BET inhibitors would increase AZD1775-induced cytotoxicity by impairing DNA damage repair. Here, we evaluate the efficacy and mechanisms of combining BET inhibitors and AZD1775 for non-small cell lung cancer (NSCLC).
Methods: Anti-tumor activities of AZD1775 and BET inhibitors (JQ1 and AZD5153) were analyzed in vitro and in vivo. γ-H2AX was evaluated as a marker for DNA double-strand break (DSB). Activity of non-homologous end joining (NHEJ) was evaluated using NHEJ reporter plasmid.
Results: The combination of BET inhibitors and AZD1775 showed synergistic effects for NSCLC cell lines. The BET inhibitors increased and prolonged AZD1775-induced DSB. BET inhibitors significantly repressed NHEJ-related genes and diminished NHEJ activity. In addition, BET inhibitors repressed MYT1 and promoted mitotic entry.
Conclusion: The combination therapy of BET inhibitors and AZD1775 can be a novel therapeutic strategy for NSCLC.

▲TOP

P-2096

Cornification-like differentiation induced in mammary epithelial cells is mediated by AP-1, NF- κ B, and PKA

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Detachment-induced cell death (DICD) is important for preventing cancer cell survival during metastasis. Previously, we reported that DICD in human mammary epithelial cells (HMECs) comprises three types of cell death: necrosis, entosis, and cornification-like differentiation (CLD) but not apoptosis. CLD is characterized by the upregulation of cornification-related genes, such as keratin 10, filaggrin, and caspase-14. However, little is understood about the mechanisms by which CLD is induced in HMECs under detached conditions. In this study, we analyzed CLD signaling involved in the induction of caspase-14. First, we found that the induction of caspase-14 was regulated by a transcriptional pathway that was regulated by activator protein-1 (AP-1) and nuclear factor (NF)- κ B. Further analysis revealed that protein kinase A (PKA) was activated under detached conditions and was involved in the induction of caspase-14. These results suggest that CLD is regulated by multiple pathways mediated by AP-1, NF- κ B, and PKA, which are potential targets for therapeutics inducing CLD in breast cancer cells.

▲TOP

[P-2101] P7-2 [English/Japanese]
Genomic analysis in hereditary / familial disease

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-2101

Germline mutations in breast cancers of 2004 Japanese women

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Germline predisposition has been implicated in 5% of all breast cancers (BCs), of which 20% are familial, and 75% are sporadic. In previous studies, ~30% of familial BCs in Japan were estimated to have a known predisposing allele, which most frequently affected *BRCA1*. However, the role of germline risk alleles has not been evaluated in the general population of Japanese BC patients. In this study, we enrolled a total of 2004 unselected Japanese women with BC including 386 with family history (FH), where pathogenic germline variants were interrogated among 11 genes implicated in familial/sporadic BCs using targeted sequencing of blood DNA. Pathogenic variants were found in 112 (5.6%) patients; 94 loss-of-function, 14 missense, and 4 splice site variants. Unexpectedly, *BRCA2* was most frequently mutated (N=62), followed by *BRCA1* (N=20) and *PALB2* (N=9). Variants were more prevalent among FH(+) BCs (11.4%), but also found in FH(-) cases (4.6%). Mutations were more frequent in early-onset BCs regardless of FH. Our findings highlight the importance of germline risk variants not only in hereditary/familial BCs but also in sporadic BCs with a higher prevalence of BRCA2 variants in Japan.

▲TOP

P-2102

Deep whole-genome sequencing identifies very recent selection signatures linked to evolution of Japanese

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Understanding natural selection signatures is crucial to unveiling demographic adaptation and disease genetic risk of modern humans. We report natural selection signatures in the Japanese population using 2,234 high depth whole-genome sequence (WGS). Using rare singletons, we evaluated genome-wide very recent natural selection signatures for the past 3,000 years by calculating the singleton density score (SDS). We identified multiple loci with significant selection signatures (ADH cluster, MHC region, BRAP-ADLH2, and SERHL2). Demographic analysis using large-scale GWAS data (n = 171,176) identified that variants with very recent selection signatures showed enrichment in heterogeneity of derived allele frequency spectra in the regions of Japan, which was highlighted by the two major regional clusters of the Japanese population (Hondo and Ryukyu). We observed significant overlaps between selection signature and the genetic risk of the modern human phenotypes, especially those of the alcohol or nutrition metabolism-related traits and esophageal cancer. Our study illustrates the value of high depth WGS to understand the evolution of modern humans and their relationship to disease risk.

P-2103

Whole-genome sequencing of multiple tissue samples isolated from two patients with Multiple endocrine neoplasia type 1

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Background: The Shizuoka Cancer Centre launched Project HOPE in January 2014. To date, 4,000 cases have been analyzed by whole-exome sequencing, and two cases of multiple endocrine neoplasia type 1 (MEN1) have been detected.Methods: We performed whole genome sequencing (WGS) on blood samples and tumour tissues (case 1: pancreas, duodenum; case 2: pancreas, mediastinum, parathyroid) isolated from the two MEN1 cases, and confirmed our findings by Sanger sequencing, real-time PCR, immunochemistry and aCGH analysis. Results: Novel germ-line MEN1 alterations were identified by WGS to be the only common mutations in either case. The first case was already reported. In the second case, we both detected a deletion that included MEN1, and inferred the presence of chromosomal structural changes, such as abnormal numbers of chromosome.Conclusions: Hereditary cancer syndromes are rare morbidities; consequently, there are few opportunities to analyze somatic mutations in multicentric tumours obtained from a single patient. Furthermore, few previous studies investigating MEN1 have used WGS. Thus, we believe that this study provides invaluable novel insights into MEN1 pathogenicity.

P-2104

Germline gene aberration profile of tumors of adolescent and young adult females

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There has been little improvement in the prognosis for adolescent and young adult (AYA) tumor patients, such as those of breast and ovarian cancers. Hence, there is an urgent need to understand the etiology of tumor development. Our mutational signature analysis of 67 those AYA tumors, based on whole exome sequencing, do not indicate environmental factors specifically underlying mutagenesis in their AYA ages. On the other hand, screening of germline mutations in 25 known cancer susceptibility genes revealed that 3 (4.5%) of these AYA cases carry pathogenic germline mutations in the BRCA2 gene. Two of them had also additional pathogenic germline mutation either in the TP53 or CHEK2 genes. Interestingly, germline structural aberrations have also been reported to be responsible for a subset (1-20%) of patients of hereditary breast and ovarian cancer (HBOC). Thus, a search for structural aberrations in several HBOC genes by Multiplex Ligation-dependent Probe Amplification (MLPA) method is underway. Magnitude of hereditable genetic factors for AYA breast and ovarian cancers will be discussed in comparison with that in European/US individuals.

▲TOP

[P-2111] P7-4 [English/Japanese]
Genomic analysis for tumor microenvironment in solid tumor

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yasuhiro Minami / Div. Cell Physiol., Kobe Univ., Grad. Sch. Med.

P-2111

Immune cytolytic activity linked to anti-tumor immunity and intra-tumor heterogeneity impact clinical outcome in BrCa

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We aimed to comprehensively assess whether intra-tumor immune cytolytic activity (CYT) associate to genetic alterations and tumor immune microenvironment, which impacts survival in breast cancer (BrCa) utilizing genomic data sets. High-CYT associated to somatic mutational events and genomic instability, while low-CYT increased somatic copy number alteration. CYT also has strongly positive correlation with immune checkpoint molecules and immune-response related gene sets. High-CYT also associated with higher composition of infiltrating immune-elimination cells, as opposed to inversely associated with infiltrating immunosuppressive cells. High-CYT associated with lower intra-tumor genetic heterogeneity and higher T-cell receptor diversity. High-CYT patients showed significantly improved prognosis independent of the other known prognostic factors. Moreover, high-CYT significantly associated with therapeutic response to chemotherapy in breast cancer patients. We concluded that high-CYT associates to improved clinical outcome, likely due to high anti-tumor immunity followed by alteration of intra-tumor genetic clonality in BrCa.

▲TOP

P-2112

Clonal evolution of non-malignant proliferative lesions into breast cancers

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Non-malignant proliferative lesions (NMPLs) in the breast have been implicated in breast cancer (BC) development, but their clonal dynamics during progression from NMPL to cancer is poorly understood. In this study, we performed whole exome sequencing of 39 micro-dissected samples from 6 patients with multi-focal proliferative lesions, including 5 normal ducts, 9 NMPLs, and 21 non-invasive and 4 invasive cancers. No somatic alterations were shared across samples from two bilateral cases with a germline TP53 or BRCA2 mutation. By contrast, in the remaining 4 unilateral cases, all proliferative lesions had one or more driver alterations shared within a long phylogenic tree, while harboring private mutations of their own. Our results suggest that early development of sporadic BC is shaped by a precancerous expansion of cells positively selected by a number of driver mutations and originated from a single cell that acquires a founder mutation long before the onset of cancer, most likely in early adolescent. This process is accelerated by a germline mutation shared by all somatic cells in hereditary cases, leading to a multi-focal expansion of independent precancerous clones.

P-2113

Characterization of hypermutated tumors based on tumor microenvironment immune types classification

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The Project HOPE (High-tech Omics-based Patient Evaluation) using whole-exome sequencing (WES) and gene expression profiling (GEP) has been launched since 2014. The hypermutated tumors with more than 500 single nucleotide variants (SNVs) were 118 cases, accounting for 3.7% of 3,174 tumors registered in HOPE project until Jan 2017. The PD-L1 expression and high tumor mutation burden might be possible biomarkers capable of predicting immune responses. Thus, we established a tumor microenvironment (TME) immune types classification based on the expression level of PD-L1 and CD8B genes using the category of four types (A: adaptive immune resistance, B: intrinsic induction, C: immunological ignorance, D: tolerance). Most of hypermutators belonged to immune types A, B and C except for type D ($PD\text{-}L1^{+}CD8B^{-}$). Meanwhile, the expression of 174 immune response-associated genes was compared between hypermutator cases in each immune type, which indicated that IL-6 gene is positively associated with PD-L1 level irrelevant to CD8B expression level. The classification of TME immune types might be a useful tool for evaluating the immunological status and predicting anti-tumor responses and prognosis.

P-2114

Examination for genetic and clinicopathological findings for earlu-stage serrated adenocarcinoma using CCS group

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Background: Serrated adenocarcinoma (SAC) prognosis has not been widely recognized, the serrated pathway-associated subtype consistently exhibits unfavorable prognosis in genetic studies. The aim of this study was to classify molecularly distinct subtypes for serrated carcinomas and clarify clinicopathological characteristics and genetic change for each subtype. Methods: We examined to classify 24SACs into 3 molecularly distinct groups by colon cancer subtyping (CCS) (De Sousa de Melo et al., Nat Med 19, 2013). We compared clinicopathologic characteristics, Ki 67 labeling index (LI), epithelial serration in SACs, and somatic mutation in 15 cancer-related genes among 3 groups which were classified by CCS. Results: CCS groups included CCS1: CDX (+) and MSI-L/MSS (14 cases), CCS2: MSI-H (5 cases), and CCS3: CDX (-) and MSI-L/MSS (5 cases). Prevalence of invasive carcinoma was significantly higher in CCS3 than CCS1. Ki67 LI and epithelial serration were higher in CCS3 than in CCS1. CCS2 showed the highest mutation number, whereas KRAS and/or BRAF mutation number were higher in CCS3 than CCS1. Conclusions: Early-stage SACs can be classified into 3 molecularly distinct subtypes.

▲TOP

P-2115

Genome sequencing of DNA isolated from long-term preserved FFPE thyroid cancer tissues

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This study evaluated the viability of formalin-fixed paraffin-embedded (FFPE) tissue samples in long-term storage for use in genomic analyses through the DNA isolation for genome sequencing process.First, a total of 50 thyroid cancer tissues from three different periods (<1976, 1976 to 2000, and >2001) from three Hiroshima hospitals. Then, the yields of extracted DNA and prepared DNA libraries and sequenced insert sizes were compared.The yield of extracted DNA and the efficiency of library preparation were low for DNA preserved before 1975. However, whole exome sequencing (WES) using the libraries prepared from the DNA of old samples was possible and, the results of BRAF mutation V600E that was detected by the WES were consistent with those obtained by Sanger sequencing.These results indicate that the DNA obtained from the FFPE thyroid cancer tissues preserved before 1975 are viable for genome sequencing, although such DNA has degraded and fragmented considerably compared to DNA preserved after 2001. The present study was conducted with the support of NCI, NIH, and HHS Contract HHSN261201400009C.

▲TOP

[P-2123] P10-1 [English/Japanese]
Angiogenesis

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yasufumi Sato / Dept. Vasc. Biol., IDAC, Tohoku Univ.

P-2123

X-ray CT imaging of the effect of VEGF-targeted antibody drug on tumor vessels

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Tumor blood vessels are necessary for tumor growth. Tumor induces angiogenesis by producing various angiogenic factors. VEGF is known as one of the main growth factors for tumor vascular growth. Recently, VEGF-targeted antibody drug, Bevacizumab, is applied in a clinical setting and it suppresses angiogenesis by inhibiting VEGF function. However, some cancer patients don't respond sufficiently to Bevacizumab. In this study, to know the mechanisms of Bevacizumab resistance, we employed two types of human cancer cells, ovary cancer cells and pancreas cancer cells, subcutaneously-implanted these cells into the mouse, and then administered Bevacizumab into the tumor-bearing mice. After administration of the antibody drug, tumor vessels were visualized using high resolution X-ray CT imaging with newly-created gold nanoparticles contrast agent (15 nm). First, we examined structural change of main artery which supplies nutrients and oxygen to tumor in the mice. Next, we investigated change of tumor vascular density depending on tumor size in the tumor-bearing mice. In this presentation, we will report interesting results obtained from these experiments.

▲TOP

P-2124

Induction of Periostin by Sulfatase 2-TGF β 1-SMAD Signaling Axis Mediates Tumor Angiogenesis in Hepatocellular Carcinoma

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Existing antiangiogenic approaches to treat metastatic hepatocellular carcinoma (HCC) are weakly effectual, prompting further study of tumor angiogenesis. Here we report a novel role for sulfatase 2 (SULF2) in driving HCC angiogenesis. Under diethylnitrosamine treatment, Sulf2-deficient mice (Sulf2 KO) developed smaller and less tumors with a markedly lower microvascular density compared to wild-type mice (Sulf2 WT) and had no metastases like Sulf2 WT. In human HCC cells, SULF2 overexpression increased endothelial proliferation, adhesion, chemotaxis and tube formation in a paracrine fashion. Mechanistic analyses identified the extracellular matrix protein periostin (POSTN) as an effector protein in SULF2-induced angiogenesis, where the TGF β 1/SMAD pathway functions as a critical signaling axis. POSTN silencing in HCC cells attenuated SULF2-induced angiogenesis and tumor growth in vivo. In clinical HCC specimens, elevated levels of SULF2 correlated with increased microvascular density, POSTN levels, and relatively poorer patient survival. Together, our findings define an important axis controlling angiogenesis in HCC and a mechanistic foundation for rational drug development.

P-2125

Identification and characterization of a tumor endothelium-related gene in colorectal cancer

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In this study, we aimed to clarify the mechanism of tumor angiogenesis in colorectal cancer (CRC). To this end, we isolated endothelial and epithelial cells from surgically resected CRC tissues (n = 14), and performed RNA-seq analysis. We identified a series of 18 genes which were upregulated in the tumor endothelial cells than in normal endothelial cells. We then validated the RNA-seq results by qRT-PCR, and identified AEBP1 as a novel tumor endothelium-related gene in CRC. Knockdown of AEBP1 in human umbilical vein endothelial cells (HUVECs) suppressed cell proliferation and in vitro tube formation. Injection of siRNA against AEBP1 in mice transplanted with human CRC cells suppressed microvessel formation in the xenografted tumors. Microarray analysis revealed that knockdown of AEBP1 in HUVECs significantly affected genes involved in tumor angiogenesis, including AQP1 and POSTN. Analysis using TCGA dataset suggested that higher expression of AEBP1 in primary CRC is associated with worse survival of CRC patients. Our results suggest that AEBP1 may play an important role in the angiogenesis in CRC, and that it could be a potential therapeutic target.

P-2126

ADAM9 promotes lung cancer progression through vascular remodeling by VEGFA, ANGPT2, and PLAT

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Lung cancer has a very high prevalence of brain metastasis, which results in a poor clinical outcome. Vascular remodeling and early angiogenesis are essential for successful brain metastasis formation. A disintegrin and metalloproteinase 9 (ADAM9), a membrane protein containing metalloprotease activity for ectodomain shedding is up-regulation in lung cancer cells and is correlated with metastasis to the brain. However, the underlying molecular mechanism of this correlation remains to be elucidated. In this study, the microarray experiments were used to explore ADAM9-regulated genes that function in vascular remodeling in lung cancer progression. The results showed the expression levels of vascular endothelial growth factor A (VEGFA), angiopoietin-2 (ANGPT2), and tissue plasminogen activator (PLAT) were suppressed in ADAM9-silenced cells, which in turn leads to decreases in angiogenesis, vascular remodeling, and tumor growth in vivo. These findings suggest that ADAM9 promotes tumorigenesis through vascular remodeling.

▲TOP

P-2127

Increased ABCB1 expression in tumor blood vessels of urothelial carcinoma after chemotherapy

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ABCB1, ATP binding cassette transporter, one of the stem markers, causes drug resistance. We have reported that tumor endothelial cells (TECs) are resistant to paclitaxel (PTX) with ABCB1 upregulation. PTX is often selected in 2nd line chemotherapy for metastatic urothelial carcinoma (mUC), however its outcomes are limited. We hypothesized that TEC ABCB1 is the cause of this situation. In this study, we investigated the ratio of ABCB1 positive (+) TECs before and after 1st line chemotherapy in UC tissues (n=66) by ABCB1 and CD31 immunostaining. In 42 cases (64%), the ratio of ABCB1+ TECs increased after 1st line chemotherapy. As the mechanism, chemotherapy elevated ABCB1 expression levels in ECs via increasing tumor IL-8 secretion. In vivo assay, when the ABCB1 inhibitor was combined with PTX, tumor growth and metastasis were more reduced with anti-angiogenic effect compared to PTX alone. It was suggested that chemotherapy causes inflammatory changes in tumors, which induce ABCB1 expression in TECs and cause drug resistance. Targeting ABCB1 in TECs can be a novel strategy to overcome cancer drug resistance.

P-2128

Silencing of MTA1 in endothelial cells induced anti-tumor effect by inhibiting angiogenesis via downregulation of S100A4

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Metastasis-associated proteins, such as S100A4 and MTA1, have been studied for over two decades, however correlation between them is still unknown. A recent report suggesting that silencing of S100A4 in endothelial cells markedly suppresses in vitro capillary formation and in vivo tumor angiogenesis, motivated us to examine MTA1 from the same perspective. In this study, we showed that the suppression of MTA1 in endothelial cells by murine MTA1-specific small interference RNA (mMTA1 siRNA) induced inhibition of capillary formation in vitro and new blood vessel formation in vivo using Directed In Vivo Angiogenesis Assay (DIVAA). Moreover, we found that mMTA1 siRNA inhibited tumor angiogenesis in a xenograft model. Further, we revealed that inhibition of angiogenesis by MTA1 siRNA mediates downregulation of S100A4 followed by promoting phosphorylation of non-muscle myosin heavy chain IIA (NMIIA). Interestingly, this signal pathway including MTA1 and S100A4 might be independent of VEGF-VEGFR pathway in endothelial cells. These results suggested that silencing of MTA1 in endothelial cells could be used as a new strategy to induce tumor regression via inhibiting tumor angiogenesis.

▲TOP

[P-2134] P10-3 [English/Japanese]
Cell adhesion / invasion (1)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yasunori Okada / Pathophysiol., Juntendo Univ. Sch. Med.

P-2134

Angiotensin II promotes hematogenous cancer metastasis through the activation of vascular endothelial adhesion molecules

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Angiotensin II (Ang II), a pressor peptide, has been reported to accelerate cancer metastasis. In this study, we elucidated the mechanisms by which Ang II exacerbates hematogenous metastasis, focusing the adhesion pathway in vascular endothelial cells (VECs). For this purpose, B16/F10 mouse melanoma cells (MMCs), which do not express the Ang II type 1 receptor (AT1R), were intravenously injected into C57BL/6 mice. Two weeks after cell injection, the number of lung metastatic colonies was significantly higher in the Ang II-treated group than in the vehicle-treated group. The AT1R blocker, but not the calcium channel blocker, significantly suppressed the effect of Ang II. In endothelium-specific AT1R knockout mice, Ang II-mediated acceleration of lung metastases of MMCs was significantly diminished. Ang II treatment significantly increased E-selectin (E-sel) mRNA expression in VECs collected from lung tissues, and thus promoted adherence of MMCs to the VECs. Ang II-accelerated lung metastases of MMCs were also suppressed by treatment with anti-E-sel antibody. Taken together, Ang II-treatment exacerbates hematogenous metastasis by promoting E-sel-mediated adhesion of MMCs to VECs.

▲TOP

P-2135

Withdrawn

No Abstract

P-2136

Overexpression of superoxide dismutase 2 (SOD2) promoted the invasive ability of human melanoma cells

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Oxidative stress contributes to the development of tumors, which can result from decreased activities of antioxidant enzymes. One of antioxidant enzymes, superoxide dismutase 2 (SOD2), which is located in mitochondria, has been shown to function as a tumor suppressor in different types of tumors, but the effect on invasive and metastatic properties was still undefined. We have previously reported that knockdown of SOD2 reduced the invasion through inhibiting motility of A2058 melanoma cells. In this study, we investigated the effect of SOD2 overexpression on invasive ability of human A2058 melanoma cell lines. A2058 cells transfected with expression vector containing SOD2 cDNA showed an increase of invasive ability compared to mock-transfected cells in Matrigel invasion assays. Overexpression of SOD2 also led to the enhanced motility of A2058 cells without affecting their growth. In addition, SOD2 overexpression reduced the homotypic aggregation in A2058 cells, while it had no effect on the adhesion to both fibronectin and laminin. These results suggest that overexpression of SOD2 may contribute the enhancement of invasion through increasing motility of A2058 melanoma cells.

P-2137

Type I myosin 1E regulates cell motility through interaction with the membrane-bending protein SNX9

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We previously revealed that intracellular localization of myosin 1E (Myo1E) is regulated by its cytoplasmic anchor protein SH3P2. Serum-induced phosphorylation of SH3P2 at Ser202 by RSK results in dissociation of Myo1E from SH3P2, and released Myo1E localizes to the leading edge where it promotes cell motility. Here, we show that Myo1E interacts with sorting nexin 9 (SNX9) at the leading edge. SNX9 is known to interact with several proteins involved in membrane traffic regulation through its SH3 domain and also with cell membrane through its PX-BAR domain, regulating membrane ruffle formation and endocytosis. The proline-rich region located within the tail homology 2 domain of Myo1E directly interacted with the SH3 domain of SNX9. Myo1E was colocalized with SNX9 and F-actin at the peripheral region of the serum-stimulated cells. siRNA-mediated knockdown of SNX9, as well as that of Myo1E, inhibited the cell motility. These results suggest that Myo1E utilizes SNX9 to regulate membrane traffic at the leading edge and promote cell motility.

▲TOP

P-2138

Anti-tumor progression effects of de novo designed peptide on breast cancer cells

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Breast cancer is one of the most common metastatic tumors. Metastasis of breast cancer involves a complex set of events, including epithelial-mesenchymal transition, proliferation, and migration. In this study, we designed and synthesized inhibitory peptide based on in silico investigated into the interleukin 8 (IL-8)-receptors binding which links to metastasis in breast cancer. It is showed that the peptide did not cause cytotoxicities on the MDA-MB-231 and MCF-7 cells. However, the mRNA and protein expression levels of EMT markers in the cells were regulated by the designed peptide. The peptide also inhibited the migration and colony formation of breast cancer cells induced by IL-8. Overall, our results demonstrated that the peptide has the ability to suppress IL-8-induced metastasis of breast cancer cells.

P-2139

Cleavage of extracellular domain of CDCP1 by MTSP1 regulates cancer cell migration

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CUB domain-containing protein 1 (CDCP1) is a type-I transmembrane protein which regulates cancer progression such as cell migration, invasion and anoikis resistance. CDCP1 has three extracellular CUB domains containing cleavable site by several proteases. Previously, we reported that CDCP1 forms homophilic complex via the extracellular CUB2 domain and is involved in the motility of cancer cells. However, little is known about the regulatory mechanism of CDCP1 homophilic complex formation in cancer cells. In this study, we found that cleavage of CDCP1 in cancer cells can be dependent on the expression of membrane-type serine protease MTSP1. Immunofluorescent analysis revealed that CDCP1 and MTSP1 were co-localized on cell surface in BxPC3 cells. In addition, BxPC3 cells treated with MTSP1 siRNA reduced CDCP1 cleavage and intracellular CDCP1 signaling and rescued by expression of siRNA resistant MTSP1. Interestingly, addition of recombinant CUB2 domain fused with maltose binding protein inhibited the cleavage of CDCP1. These results suggest that MTSP1 regulates homophilic complex formation of CDCP1 by cleavage of CDCP1 extracellular domain and may stimulate cancer cell migration.

▲TOP

[P-2146] P10-5 [English/Japanese]
Cell adhesion / invasion (3)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Shiro Suetsugu / NAIST

P-2146

Influence of CD40 to cancer proliferation and invasion in esophageal squamous cell carcinoma

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Background: Cluster of Differentiation 40 (CD40) is a costimulatory molecule expressed in antigen presenting cells and plays an important role in the immune system. Recently, it is reported that CD40 expression on esophageal squamous cell carcinoma (ESCC) correlates poor prognosis, however, the role of CD40 expression in ESCC is still unclear. Aim: We analyzed the molecular function of CD40 in ESCC cell lines and examined the mechanism contributing to cancer cell biology. Results: CD40 expression in 10 types of ESCC cell lines was evaluated by Western Blotting and quantitative PCR, and four cell lines were up regulated. CD40 ligand (CD154) stimulation to those CD40 up regulated ESCC cell lines showed that the MMP-9 secretion into the culture supernatant was amplified. The MMP-9 activity varied in 10 types of cells, but it was revealed that the activity was enhanced by CD40-CD40L interaction regardless of the level of CD40 expression. Conclusion: CD40 expression on ESCC cells may be involved in the acquisition of malignancy via MMP-9 activity.

▲TOP

P-2147

ARHGEF 10 is involved in cell invasion by modulating Rab8a-localization

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The function of ARHGEF10, a known guanine nucleotide exchange factor (GEF) for RhoA with proposed roles in various diseases, is poorly understood. To understand the precise function of this protein, we raised a monoclonal antibody against ARHGEF10 and determined its localization in HeLa cells. ARHGEF10 was found to localize to vesicles containing Rab6a, Rab8a, and/or the secretion marker neuropeptide Y (NPY)-Venus in a Rab6-dependent manner. These vesicles were known to originate from the Golgi and contain secreted or membrane proteins. Ectopic expression of an N-terminal-truncated ARHGEF10 mutant led to the generation of large vesicle-like structures containing both Rab6 and Rab8. Additionally, small interfering (si)RNA-mediated knockdown of ARHGEF10 impaired the localization of Rab8 to these exocytotic vesicles. Furthermore, the invasiveness of MDA-MB231 cells was markedly decreased by knockdown of ARHGEF10, as well as of Rab8. From these results, we propose that ARHGEF10 acts in exocytosis and tumor invasion in a Rab8-dependent manner.

P-2148

Cancer cells migrate on networks of elongated fibroblast protrusions - a new tumor invasion model

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Tumor microenvironment supports tumor progression in many ways. Especially, cancer-associated fibroblasts (CAFs) are known to play critical roles in tumor invasion. Despite great accumulation of our knowledge about tumor progression, it is poorly understood how cancer cells invade stromal tissues. Recently we found that various kinds of invasive cancer cells such as Panc-1 and A549 actively adhere to normal fibroblasts and this interaction promotes their invasion in 3D collagen gel. We established a new invasion model which allows rapid tumor cell invasion in the collagen matrix. In this model, chimeric spheroids of cancer cells and fibroblasts were prepared in EZSPHERE non-adherent micro-fabricated vessels for the spheroid formation. When the spheroids were embedded in the 3D collagen gel, both types of cells rapidly invaded the collagen matrix. Time-lapse movies clearly demonstrated that cancer cells adhered and migrated on long strings of fibroblasts in the 3D matrix. Some signal inhibitors suppressed the tumor invasion. The molecular mechanism for the tumor invasion will be discussed. (Coworker: Hiromichi Kumagai, Innovat. Technol. Res. Ctr., Asahi Glass)

P-2149

HSP47 augments a metastatic potential of triple negative breast cancer

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Although triple negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer and is a leading cause of cancer-associated death from malignancies in women worldwide, there is currently no effective antitumor therapy due to the difficulty for the regulation of a high metastatic potential of TNBC. Heat shock protein 47 (HSP47), a collagen-specific chaperone, has reported to be correlated with malignancy of TNBC, but the molecular mechanism by which HSP47 functions in TNBC remains largely unclear. Here we showed that a small population of TNBC derived from the tumors in TNBC-bearing mice expresses HSP47 and HSP47-positive TNBC has a high metastatic potential that is abolished by silencing of HSP47. We also found that HSP47 binds to non-muscle myosin IIA (MYH9), a key player of cell motility, and its interaction contributes to a high metastatic potential of HSP47-positive TNBC. Furthermore, forced expression of both HSP47 and MYH9 conferred a high metastatic potential on HSP47-negative TNBC and on non-TNBC that does not express MYH9. Overall, we identify HSP47 as a key regulator for metastasis of TNBC and suggest HSP47 as a candidate for a therapeutic target of TNBC.

▲TOP

P-2150

Fibroblasts-dependent invasion of cancer stem cells in squamous cell carcinoma

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Fibroblasts-dependent invasion is known as one form of cancer cell invasion. In this study, we examined whether podoplanin positive (PDPN+) cancer stem cells in squamous cell carcinoma have higher invasion potential undergoing fibroblasts-dependent invasion. Fucci labeled A431 cells were sorted into PDPN+ and PDPN- subpopulations which were used for collagen invasion assay. After 72h time-lapse imaging, we analyzed the total number and frequency of S/G2M phase of PDPN+ and PDPN- cells invading into collagen I. Without fibroblasts, there was no significant difference in the number of invaded PDPN+ and PDPN- A431 cells. On the other hands, when A431 cells were cocultured with fibroblasts, the number of invaded PDPN+ cancer cells was significantly higher than PDPN- cells. The frequency of S/G2M cells was not different between PDPN+ and PDPN- cells. During fibroblasts-dependent invasion process, the number of invaded cancer cells whose PDPN expression was suppressed by shRNA was significantly decreased.Our current study cleared that the invasion of cancer stem cell was enhanced when fibroblasts exists and PDPN was functional molecule of the invasion of cancer stem cell.

P-2151

Identifying epithelial-mesenchymal transition factor in relation with therapeutic resistance of malignant glioma

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BACKGROUND: Identifying primary factor of epithelial-mesenchymal transition (EMT) related with invasion and stemness of glioblastoma is critical in setting a therapeutic strategy. METHODS: Hypermotile glioma cell lines were established through a repetitive scratch method using U87, U251, and U118 cell lines. Changes in expression levels of EMT and stemness factors were investigated. RESULTS: Established hypermotile glioma cell lines showed enhancement of invasion and migration capacity. Up-regulation of SLUG expression resulted in increased invasion capacity. While overall survival rates were not significantly affected by SLUG expression levels, the group with low SLUG expression had longer progression-free survivals than the group with high SLUG expression (P=0.042). In addition, infiltrating single glioma cells frequently exhibited strong SLUG expression in the invasion front of human glioblastoma samples.CONCLUSIONS: Through establishment of hypermotile glioma cell lines using a repetitive scratch method, SLUG was named as a promising candidate that is expected to play a pivotal role in glioma progression in terms of invasive phenotype and stemness.

▲TOP

[P-2159] P10-7 [English/Japanese]
Transcription and gene expression (1)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Genichiro Ishii / Div. Path. EPOC Natl. Cancer Ctr.

P-2159

Gene expression profiling of organ tropism related upregulation in osteosarcoma derived sub-cell lines

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It is largely unknown how cancer cells find the accommodation in newly metastasized organs. To find the key molecules to control the organ tropism of metastasis, we repeatedly injected the cells taken from several different metastasized lesions into heart. The cell line, 143B is the metastatic osteosarcoma cell line, however, we accidentally made the sub-cell line frequently metastasized into adrenal gland (143Bad). We used this sub-cell line as the start, finally, we got the sub-cell line directly metastasized into kidney and liver (143Bkid) and lymph nodes (143Blym) before the adrenal gland metastasis. Then, among these sub-cell lines we performed gene profiling. The changes in ECM compositions, cell-cell adhesion molecules, and several signaling molecules were observed. We further validated the gene expressions, and we confirmed the upregulations of Slitrk6, Amelotin, DDIT4, and Pigment epithelium derived factor. PEDF is known to inhibit angiogenesis or cause apoptosis even in osteosarcoma. However, 143B+PEDF showed aggressive phenotypes in vivo similar to 143Bkid phenotypes. The results implied that PEDF could be a key molecule of organ tropism during metastasis.

▲TOP

P-2160

Microarray for screening of culprit genes related with pulmonary metastasis of colorectal cancer by using a model mouse

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<Background>Distant metastasis is crucial for prognosis of colorectal cancer (CRC). However, its specific mechanism remains undetermined. Therefore, clarifying a novel and critical molecule would be promising for improving prognosis of CRC. <Methods and Results> The pulmonary metastasis mouse model was established by injection of mouse CRC cell line CMT93 (106 /0.1ml) into the tail vein. A single cell was separated and cultured from the metastatic lesions in the lung and novel cell lines were established (CMT93PM). Invasion assay revealed approximately 1.2 times higher grade of invasion in CMT93PM compared to CMT93. DNA samples were extracted from them and microarray analysis was performed. The results showed that more than 90 kinds of molecules were over 10 times different in gene expression between CMT93 and CMT93PM. Ingenuity pathway analysis indicated that TGF-β, TNFR signaling, MAPK signaling, and RANK signaling pathways were significantly activated in CMT93PM. <Prospective>Refining of the results of microarray analysis is on the way, which could shed the light on the specific mechanism of pulmonary metastasis of CRC.

P-2161

Involvement of lymphoid enhancer binding factor 1/Cytoglobin axis in lung metastasis of osteosarcoma

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Lung metastasis remains a major cause of poor prognosis in osteosarcoma (OS) patients. Understanding molecular mechanisms of OS lung metastasis is greatly needed for developing new therapeutic strategies. High-lung metastatic (LM8-H) and low-lung metastatic (LM8-L) sublines from LM8 murine OS were isolated by using in vivo image-guided screening and analyzed their molecular signatures. The analysis uncovered that lymphoid enhancer binding factor 1 (LEF1), a transcription factor involved in the Wnt signaling pathway, increase extravasation of LM8-H. To identify target genes of LEF1 that are involved in the extravasation of LM8-H, genome wide meta-analyses were performed and cytoglobin (Cygb) was identified as a candidate gene. CYGB is a novel member of the globin family and known to act as a tumor suppressor. However, CYGB-overexpression increased the extravasation ability of LM8-L, whereas Cygb-knockout in LM8-H significantly suppressed lung metastasis in a syngeneic murine model of lung metastasis. Our study reveals a novel function of CYGB and new axis of LEF1-CYGB in cancer metastasis. This may open up new avenues of research to develop therapeutic strategy for lung metastasis.

P-2162

CD146 contributes the metastatic properties of human colon adenocarcinoma cells

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CD146 (MCAM) expressed on not only endothelial cells but also various malignant tumor cells. Several reports have shown that CD146 is involved in cell adhesion, angiogenesis and migration. However, contribution of CD146 in tumor metastasis is still controversial. To investigate the effect of CD146 on tumor metastatic properties, we establish two subclones from DLD-1 (human colon adenocarcinoma cells) expressing CD146 or not. Both cell lines express epithelial tumor marker EpCAM and TROP2 equally, while only CD146 (+) DLD-1 co-express CD44 variant. Consist with the morphological changes to mesenchymal features, the expression of E-cadherin and claudin-3 were decreased in CD146 (+) DLD-1. In addition, CD146 (+) DLD-1 significantly increased the production of VEGF and EMT related genes. Next, we aimed to induce MET in CD146 (+) DLD-1 using several phytochemical agents. We found that treatment of CD146 (+) DLD-1 by resveratrol could induce the morphological changes to epithelial phenotype and inhibited the EMT related genes. These data indicate that CD146 contributes in EMT, and resveratrol might be an effective candidate for treatment of aggressive feature of CD146 expressing tumors.

▲TOP

P-2163

Establishment of Spatial Transcriptomics for Analysis of Tumor Microenvironment and Heterogeneity

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Interaction between tumor cells and stromal cells contributes to malignancy of cancer. However, the cells present in tumor tissue are heterogeneous, furthermore, they are exposed to different microenvironmental stresses such as hypoxia and starvation for each locus. To elucidate tumor micro-heterogeneity, it is necessary to have a technique for segmenting the tissue and analyzing each of divided regions.We developed an automated punching micro-dissection system and established a Spatial Transcriptomics which analyzes gene expression at arbitrary micro-spots in tumor tissue.We analyzed a large amount of micro-tissues from xenograft tumor of human breast cancer cell line MDA-MB-231 which was orthotopically transplanted into immunodeficient mice. We then separated gene expression of tumor cells (human origin RNA) and stromal cells (mouse origin RNA), and estimated tumor-stromal interaction in each microscopic region. We detected specific markers of stromal cell from mouse RNA, and network analysis revealed co-expressed genes modules in each spot. Collaborators: K Arikawa, T Maruyama, H Matsunaga, T Yoda, M Hosokawa, H Kanbara, H Takeyama (Waseda Univ.)

P-2164

The hallmarks of long non-coding RNA associated with metastasis in human scirrhous gastric cancer

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Metastasis is considered as a major cause of death in cancer patients, however elucidation of the molecular basis of cancer metastasis does not proceed well. We focus on the function of long non-coding RNA (lncRNA) in metastasis of human scirrhous gastric cancer. To understand the molecular mechanisms of metastasis in scirrhous gastric cancer, we have previously established a patient-derived gastric cancer cell line HSC-44PE and its metastatic subcell line 44As3. By using two cell lines, we performed a microarray to clarify transcriptional dynamics associated with metastasis. We observed that 64 lncRNAs were upregulated more than 5-fold, and 52 lncRNAs were downregulated more than 5-fold in associated with metastatic properties. Most of lncRNAs are uncharacterized with regards to metastasis, whereas a part of lncRNAs such as H19 is well-established. We validated the result of the gene profiling by performing a real-time PCR for each lncRNA. Further, the function of lncRNAs was estimated by proliferation and migration assay. Our findings indicate novel signatures of cancer metastasis, and would be a key for understanding of metastasis in human scirrhous gastric cancer.

P-2165

CEACAM6 interacts with EGF receptor in the lipid-rafts and promotes oral squamous cell carcinoma metastasis

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Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6), a glycophosphoinositol (GPI)-anchor protein, is a heavily-glycosylated tumor antigen and we recently reported that CEACAM6 promotes EGFR signaling of oral squamous cell carcinoma (OSCC) metastasis via the complex N-glycosylation . However, it is unclear how glycosylated CEACAM6 interacts with EGFR. Galectin-3 (Gal-3), a gaclactoside-binding protein, can form pentamers to cross-link glycoproteins like a "lattice" microdomain in the lipid-rafts of cancer cell membrane. Here, we showed that Gal-3 was involved in the CEACAM6/EGFR complex in the lipid-rafts and blocking the N-glycosylation of CEACAM6 inhibited the CEACAM6/Gal-3 and CEACAM6/EGFR interactions. Furthermore, knockdown of Gal-3 suppressed EGFR signaling in CEACAM6-expressing cells. We thus conclude that the co-clustering of CEACAM6 and EGFR glycans by endogenous Gal-3 increases CEACAM6/EGFR interactions and enhances EGFR signaling of OSCC metastasis.

▲TOP

[P-2173] P10-9 [English/Japanese]
Transcription and gene expression (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Susumu Itoh / Lab. of Biochem., Showa Pharm. Univ.

P-2173

Activation of Src signaling mediates carcinoma-associated fibroblast-promoted metastasis in human breast cancers

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Tumor invasion and metastasis are substantially influenced by carcinoma-associated fibroblasts (CAFs) rich in tumor-associated stroma. However, the precise cellular and molecular mechanisms are poorly understood. We show here that CAFs promote metastasis via Src signaling in apposed human carcinoma cells. The Src activation also induced the highly epithelial traits with intermediate epithelial-mesenchymal transition (EMT). The highly epithelial traits notably mediated the tumor cell cluster formation and metastatic colonization in distant organs. Moreover, the breast cancer cells with intermediate EMT allowed the retention with highly epithelial cancer cell clusters leading to their collective cell invasion. CAF-released TGF-β1 and SDF1 importantly mediated the highly epithelial and intermediate EMT traits through activation of Src signaling in human breast cancer cells. These findings demonstrate that the CAF-induced highly epithelial and intermediate EMT traits mediate metastasis through Src signaling in human breast carcinomas.

▲TOP

P-2174

RhoGDI β functions as a critical regulator of spindle orientation in keratinocytes surviving after caspase-3 activation

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RhoGDI β is a metastasis-related protein. The C-terminal isoprenyl binding site is essential for preventing the aberrant Rac1 activation, which leads to increased metastasis. On the other hand, the N-terminal region, which can be cleaved by caspase-3, is essential for ensuring proper subcellular localization. The N-terminal cleaved fragment is persistently expressed in surviving cells following caspase-3 activation. RhoGDI β preferentially interacts with and functions as a Rac1 inhibitor, but the fragment represses Cdc42 activity, exhibiting aberrant intracellular localization. Cdc42 is known to be a key regulator of mitotic spindle orientation that is required for the maintenance of homeostasis in epithelial tissues. To determine the role of the fragment in keratinocytes surviving after caspase-3 activation, here we analyzed the effect of its expression on spindle orientation during mitosis. Using confocal fluorescence microscopic 3-D reconstruction techniques, we found that the fragment induced misorientation of the mitotic spindle axis. We proposed a novel mechanism of mitotic regulation that is critical for epithelial tissue homeostasis and function after caspase-3 activation.

P-2175

Cooperation of oncogenic K-Ras and PKC signaling downregulates E-cadherin expression by modulating ZEB1 function

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Tumor cells receive various signals from the tumor microenvironment. Protein kinase C (PKC) is activated by diverse extracelluar signals and is involved in various cellular functions.
In this study, we examined the effect of PKC signaling on E-cadherin expression in A549 lung adenocarcinoma cells. Treatment with phorbol myristate acetate (PMA), an activator of protein kinase C, induced the downregulation of E-cadherin while it did not upregulate N-cadherin. PMA-induced E-cadherin downregulation was reversed by PKC inhibitors, indicating that PKC signaling downregulates E-cadherin expression.
PKC-induced downregulation of E-cadherin was also observed in PANC-1 and SUIT-2 that are KRAS-mutated cancer cells like A549, but not in other cancer and normal cell lines. Moreover, knockdown of KRAS attenuated PKC-induced E-cadherin downregulation in A549, PANC-1, and SUIT-2 cells. Intriguingly, knockdown of ZEB1 also attenuated PKC-induced E-cadherin downregulation in these cell lines though ZEB1 expression was not upregulated by PKC.
These results suggest that cooperation of oncogenic K-Ras and PKC signaling downregulates E-cadherin expression by modulating ZEB1 function.

P-2176

Intrinsic cell property contributes to tumor cell dormancy in bone marrow

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Dormant or slow-cycling disseminated tumor cells (DTC) in bone marrow (BM) are resistant to conventional therapy in cancers including head and neck squamous cell carcinoma (HNSCC). Recently, we reported that the intrinsic TGF- β 2-triggered SDF-1-CXCR4 signaling axis was crucial for drug resistance dependent on a slow-cycling state in BM-DTC but not in lung metastatic cells. This study aimed to elucidate intrinsic molecular characteristics in BM-DTC. We used the human HNSCC cell line HEp3-originated sublines [i.e. parental line (P-HEp3), BM-DTC-derived (BM-HEp3), and lung metastases-derived sublines (Lu-HEp3)]. Our transcriptome analyses for these sublines revealed that slow-cycling and drug-resistant BM-HEp3 cells had unique gene expression signatures. In addition, our in vivo studies using HEp3 labeled with DNA barcode library showed that there were certain dominant clones in dormant BM-DTC distinct from those in tumor cells at other sites including inoculated site, lung, and peripheral blood. Further studies should contribute to better understanding of biology of minimal residual disease in BM and metastatic initiation.

▲TOP

P-2177

Aberrant glucose metabolism associates with invasiveness and metastasis of colon cancer cells

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Colon cancer is the most common cancer and the second most common cause of cancer-related mortality. Metastasis in colon cancer is the leading cause of poor prognostic outcomes. Aberrant cancer metabolism, especially aerobic glycolysis is one recognized metabolic hallmark of cancer. In order to find novel biomarker for metastatic colon cancer, we collected and analyzed primary and liver metastatic colon cancer tissues. Interestingly, we found changes in genes associate with cellular metabolism in liver metastatic colon cancer. We established metastatic colon cancer cells 116-LM and found that the glycolytic capacity as well as expression of glucose transporter were up-regulated in 116-LM cells. We further demonstrated that overexpression of glucose transporter regulates the expression of epithelial-mesenchymal transition and promotes tumor invasiveness and stemness properties. Moreover, mice treated with high fat sucrose diet increased blood sugar and xenograft tumor metastasis, whereas suppression of glucose transporter significantly impeded metastatic capacity. These evidence indicate that glucose transporter plays an important role in colon cancer metastasis.

P-2178

Screening of metastasis-related genes by microarray analysis of lung cancer cells using experimental lung metastasis

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Metastasis is the final feature of malignant tumors. To identify the novel molecular mechanisms underlying metastasis, we established highly metastatic sublines from a murine lung adenocarcinoma cell line, LMSMP1 by sequential experimental lung metastasis. Microarray analysis was performed using poly(A) RNA from highly metastatic sublines and parental LMSMP1. The expression of some groups of genes were upregulated in highly metastatic sublines in comparison with the parental cells. These include genes encoding cell-cycle regulators, stemness-related proteins, cell adhesion molecules, and transcription factors. Further analysis of the gene expression profiles, 302 genes and 341 genes were identified as the candidates of metastasis-promoting genes and metastasis-suppressor genes, respectively. The functional analysis of the biologically interesting genes within the candidates showed that Podnl1 enhanced the ability of experimental lung metastasis and Stc2 decreased the ability of experimental lung metastasis and soft agar colony formation. Analysis of this in vitro metastatic model using LMSMP1 would be useful for the studies of molecular and cellular mechanisms of cancer metastasis.

P-2179

Identification of EMT-related Target Genes Induced by the Mutation of Smad3 Linker Phosphorylation

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Smad3 linker phosphorylation plays essential roles in tumor progression and metastasis. We have previously shown that mutation of Smad3 linker phosphorylation sites (Smad3-EPSM) markedly reduced the tumor progression while increasing the lung metastasis in breast cancer. In this study, we identified genes which are differentially regulated in the presence of Smad3-EPSM. We confirmed that Smad3-EPSM strongly enhanced a capability of cell motility and invasiveness as well as expression of epithelial-mesenchymal transition marker genes, CDH2, SNAI1, and ZEB1 in response to TGF-beta 1 in pancreatic and prostate cancer cell lines. To identify the genes regulated by Smad3-EPSM, we performed high-throughput RNA-Sequencing of the prostate cancer cell lines infected with adenoviral Smad3-EPSM. We identified GADD45B, CTGF and JUNB genes in the expression profiles associated with cell motility and invasiveness induced by the Smad3-EPSM. These results suggested that inhibition of Smad3 linker phosphorylation may enhance cell motility and invasiveness by inducing expression of GADD45B, CTGF and JUNB genes in various cancers. Supported by KHIDI grant(HI14C2640) and NRF-2015R1D1A1A01057623.

▲TOP

[P-2187] P10-11 [Japanese]
Invasion and metastasis (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Motoko Shibanuma / Cancer Cell Biol., Showa Univ., Sch. Pharm.

P-2187

Identification of novel target molecules involved in spontaneous bone metastasis of mouse breast

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Triple negative breast cancer (TNBC) is a subtype of breast cancer, which lacks the expression of estrogen receptor (ER), progesterone receptor (PR) and HER2. TNBC has a tendency to metastasize to bone, thereby deteriorating the patients' quality of life. From a murine TNBC breast cancer cell line, 4T1, we established a subclone, 4T1.3, which can metastasize to bone upon its orthotopic injection into mammary fat pad. Using this model, we reported that 4T1.3 cells induced the intra-bone accumulation of fibroblasts, which can provide cancer cells with a growth factor, connective tissue growth factor. Additional comprehensive gene expression analysis detected the enhancement in the expression of EGF receptor (EGF-R) and IGF1-R and that of their cognate ligands, EGF and IGF1, in 4T1.3 cells and fibroblasts at bone metastasis sites, respectively. Moreover, abrogation of EGF-R or IGF1-R gene had few effects on in vitro properties of 4T1.3 cells, but reduced their growth when directly injected into the bone. Thus, the EGF or the IGF1 axis can contribute to bone metastasis by promoting the survival of 4T1.3 in bone cavity.

▲TOP

P-2188

Combined treatment of statins and dacarbazine inhibit tumor growth and metastasis in melanoma

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<Purpose>Malignant melanoma is a highly aggressive skin cancer, and the overall median survival in patients with metastatic melanoma is only 6—9 months. Although molecular targeted therapies have recently been developed and have improved the overall survival, melanoma patients may show no response and acquisition of resistance to these drugs. Thus, other molecular approaches are essential for the treatment of metastatic melanoma. <Methods> B16BL6 cells were subcutaneously injected in C57BL/6 mice. For spontaneous metastasis, primary tumors were removed and animals were monitored for another 3 weeks. Effects of statins on signal molecules were determined by western blots. <Results> Co-treatment with dacarbazine and statins significantly inhibited tumour growth and metastasis via suppression of the RhoA/RhoC/LIMK/SRF/c—Fos pathway. Moreover, the co—treatment significantly improved the survival rate in metastasis—bearing mice. <Discussion> Co—treatment with dacarbazine and statins may thus serve as a new therapeutic approach to control tumour growth and metastasis in melanoma patients.

P-2189

Establishment of murine colon cancer cell lines with high metastatic ability to mesenteric lymph nodes

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Purpose: The purpose of this study was to establish colon cancer cell lines and reveal the molecular mechanism involved in lymph node (LN) metastasis. Method: Murine colon cancer cell line (CT26) was orthotopically implanted to cecum. Metastatic LNs were harvested and cultured repeatedly. Metastatic LNs cell line harvested from Para-Aorta was named as CT26-ParaAoLN. Cellular features and gene expressions were evaluated. Results: The number of mesenteric LN metastasis after orthotopic transplantation in the cecum increased in CT26-ParaAoLN compared to CT26. There was no difference in cell migration ability. However, cell proliferation ability decreased in CT26-ParaAoLN. For gene expressions, differences were observed in cell adhesion factors and immunity-related genes between these cell lines. In addition, gene expression of TGF- β 1 involved in epithelial-mesenchymal transition and stromal fibrosis was significantly higher in CT26-ParaAoLN. Conclusion: CT26-ParaAoLN had highly metastatic ability to LNs. In addition, our study suggested that cell adhesion factors and immunity-related genes were involved in LN metastasis.

P-2190

Exosomes derived from murine colon cancer cell line inhibit peritoneal dissemination in vivo

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【Introduction】 Cancer-derived exosomes have an important role for the formation of distant metastasis, however the influence of cancer-derived exosomes in peritoneal dissemination still remains unclear.【Methods】 Exosomes were isolated from the supernatant of the murine colon cancer cell line utilizing ultracentrifugation method. Cancer-derived exosomes were intraperitoneally injected, subsequently, cancer cells were intraperitoneally administered as a peritoneal dissemination model. 【Results】 Peritoneal dissemination was significantly decreased in the exosome group compared with the PBS group in immune competent BALB/c mice (n=5, peritoneal nodule; PBS: 4±3.10 vs exosome: 0) and in nude mice (n=8, PBS: 8±5.26 vs exosome: 1.12±1.53, p=0.010). In addition, the numbers of peritoneal macrophage were significantly increased after administration of cancer-derived exosome compared with PBS.【Conclusion】 Pre-administration of CT26 cancer cell-derived exosomes decreased peritoneal dissemination in the mouse model. Innate immunity might be involved to inhibit the formation of peritoneal disseminations after cancer-derived exosomes administration.

▲TOP

P-2191

Development of a novel S100A8/A9 neutralizing monoclonal antibody for suppression of cancer metastasis

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Cancer metastasis is the most serious problems for cancer processes because of its life-threatening, so that an establishment of advanced therapeutic approaches for regulating metastasis is desired. As an interesting feature of metastasis, cancer cells frequently show organ specific metastasis. It has been compiled growing mass of evidence that S100A8/A9 plays a significant role in cancer organ specific metastasis through the binding with diverse receptors such as TLR4 and RAGE. In addition to them, we further succeeded to identify another important receptors, EMMPRIN, ALCAM and MCAM. Hence, these novel molecules might become promising therapeutic targets. In this study, we prepared 10 kinds of S100A8/A9 neutralizing antibody. In addition, we selected the higher effective S100A8/A9 antibody to suppress the cancer migration and evaluated the inhibition of the lung metastasis of melanoma in mouse model. Our selected antibody was significant effective for inhibition of the cancer metastasis. We therefore suggest that the developed biologics comes into a valuable appliance to regulate cancer metastasis.

P-2192

Increased susceptibility of highly metastatic human gastric scirrhous cell variants to chemotherapeutic reagents

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Highly metastatic variants, 44As3Luc, 58As9Luc、60As6Luc, were established from human gastric scirrhous cancer cell lines originally isolated from different patients by repeated transplantation of these cells (HSC-44PE, HSC-58 and HSC-60 cell lines, respectively) to the stomach of nude mice. To examine relationships between metastasis and susceptibility to chemotherapeutic reagents, these 3 sets of the metastatic variants and the parental cell lines were seeded onto 96 wells-plastic microplates and cultured in the presence or absence of varied concentrations of 5-FU and cisplatin (CDDP). The susceptibility to the reagents were tested by using WST-1 reduction and LDH release assays. The results showed that all three variants with highly metastatic characters showed more susceptible to 5-FU and CDDP than the parent cell lines, respectively. Furthermore, combination of 5-FU and low concentration of CDDP resulted in much increased susceptibility of 44As3Luc cells than HSC-44PE cells. Although from in vitro cell culture experiments, these results implies advantages of the combination of 5-FU and CDDP for the treatments of metastatic human scirrhous gastric cancer.

▲TOP

[P-2197] P10-13 [Japanese]
Invasion and metastasis (4)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Tomoya Yamaguchi / Dept. Cancer Biol., Grad. Sch. Med. Sci., Kumamoto Univ.

P-2197

Galectin-3, a novel tumor suppressor, contributes to metastasis regulation in cancers

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Galectin-3 (Gal-3) is expressed in some tissues across species and functions as a regulator of cell proliferation, adhesion, macrophage chemotaxis, angiogenesis, and anti-apoptosis. Although several reports have suggested that some tumors are progressively caused by the increase Gal-3 expression, we showed that Gal-3 affects and performs negatively on tumor metastasis. We found that B16 mouse melanoma cells express Gal-3 higher than highly metastatic B16-BL6 melanoma cells and the number of lung metastatic foci was decreased when Gal-3 was overexpressed in B16-BL6 cells. In contrast, knockout of Gal-3 expression in B16 cells (B16/Gal3KO) upregulated the expression of integrin beta3 and promoted cell aggregation ability with platelet and fibrinogen in vitro. Furthermore, B16/Gal3KO cells increased the number of metastatic foci in vivo. These results indicate that Gal3 negatively correlates with malignant transformation of cancer cells and works as suppressor in tumor metastasis.

▲TOP

P-2198

Analysis of tumor cell invasion using three-dimensional cultured tissue model with blood-capillary network

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Cancer cell invasion and metastasis occurs in human three-dimensional (3D) living tissues. However, a conventional cancer research analysis does not reflect all phenomenon of in vivo human body; In vitro experiment based on 2D cultured method is not same conditions of 3D human body, and In vivo experiment for example transplantation to animals have issues of species difference. In this study, we present a 3D cultured tissue model mimicking a human living tissue organization and evaluate the availability of analysis for tumor cell invasion ability using a 3D cultured tissue model compared to using conventional 2D-cell cultured method (taranswell migration assay/matrigel invasion). We can observe the behavior of human oral squamous cell carcinoma cells in 3D cultured tissue model as cancer microenvironment. In conclusion, the 3D cultured tissue model allow detail analysis of cell migration and invasion(for example, time- or area-specific in gene or protein expression) in an environment that imitate actual human tissue. It is expected that the tool in this study will be potent diagnostic system for predicting behaviors of cancer cells in preclinical assay.

P-2199

Development of a novel orthotopic peritoneal dissemination model using newly established pancreatic cancer cell line

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Peritoneal dissemination (PD) is an important cause of morbidity and mortality among patients with pancreatic ductal adenocarcinoma (PDAC). We developed and characterized a novel PD mouse model using newly established PDAC cell line. Tumor cells were cultured from the PD of a PDAC. The newly established TCC-Pan2 cell line was characterized for growth rate, tumor marker, histology and somatic mutations. The cells were implanted orthotopically to produce PD. TCC-Pan2 cells from these metastatic foci were expanded in vitro and subsequently implanted orthotopically of mice. The antitumor effect of PTX, NK105 and GEM was compared using this PD model. TCC-Pan2 cells caused tumor formation and PD with high frequency in the mice by orthotopic implantation. The potent metastatic subline Pan2M was obtained. NK105 exerted a stronger antitumor effect than PTX and GEM against Pan2M (Pan2MmLuc) cells. The survival rates on day 80 in the Pan2MmLuc mouse model were 100% for the NK105 and 0% for the PTX and GEM groups, respectively. TCC-Pan2 cell line and Pan2MmLuc PD-model were found to be useful tools for monitoring the responses to anticancer agents and for studying the biology of the PDAC.

P-2200

Establishment and characterization of a novel C57BL/6 mouse model of bone metastasis of breast cancer

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Bone is one of the most common sites of metastasis in breast cancer patients. Although C57BL/6 is the most commonly used strain for the generation of transgenic and knockout mice, there have only been a few reports in which C57BL/6 mice were used for the study of bone metastases of breast cancer. In this study, we established a highly bone-metastatic clone of the C57BL/6 mouse-derived breast cancer cell line E0771 by sequential in vivo selection (E0771/Bone). All mice intracardially inoculated with E0771/Bone developed bone metastases in C57BL/6 mice within 2 weeks. Of note, E0771/Bone exhibited enhanced cancer stem-like properties compared with parental E0771 cells. Furthermore, the expression of parathyroid hormone-related protein (PTHrP), the most common mediator of osteolytic bone metastases, was significantly upregulated in E0771/Bone. Thus, cancer stem-like properties and elevated PTHrP expression likely contribute to the enhanced bone-metastatic potential of E0771/Bone. We believe that this new mouse model is a useful tool for in vivo studies of bone metastases of breast cancer, especially for those using genetically engineered mice with a C57BL/6 background.

▲TOP

P-2201

Analysis of the metastasis process of scirrhous gastric carcinoma by multicolor fluorescent imaging

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Scirrhous gastric carcinoma (SGC) rapidly and diffusively infiltrates in the gastric submucosa and frequently causes peritoneal dissemination. However, the mechanisms underlying the progression of SGC remain poorly understood. To understand the process of SGC peritoneal dissemination, we investigated clonality of peritoneal tumors by multicolor fluorescent imaging. SGC cell lines were simultaneously infected with lentiviruses expressing three different fluorescent proteins, Tomato, mVenus, and mTFP1. The labeled cells (RGB cells) individually exhibited different colors on fluorescent microscope, enabling cell lineage tracking. RGB cells were i.p. or orthotopically injected into nude mice and peritoneal tumors were analyzed by fluorescent microscopy. Each of the peritoneal tumors, and even micrometastases at early stage, displayed multiple colors, demonstrating that they were comprised of multiclonal cell populations. Interestingly, multicellular clusters of RGB cells were detected in peritoneal lavage fluid as early as 4 h after i.p. injection. These results indicate that the multicellular clusters formed in the peritoneal cavity are the source of peritoneal metastases of SGC.

P-2202

Establishment and characterization of murine breast cancer cell line with highly lung metastatic potential

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Because metastatic breast cancer accounts for majority of deaths from breast cancer, it is important to elucidate the mechanism of breast cancer metastasis. Especially, triple negative breast cancer (TNBC), characterized by absence of ER, PR and lack of overexpression of HER2, is typically associated with poor prognosis due to high rate of recurrence and metastasis and lack of targeted therapies. In this study, to elucidate the mechanism of metastasis of TNBC cells to lung, 4T1 cells, highly metastatic breast cancer cells were used. We injected 4T1 cells into BALB/c mouse mammary fat pad and collected metastatic 4T1 cells from lung. Then, we re-injected the lung-metastatic 4T1 cells into mouse mammary fat pad again. By repeating the process, we acquired more highly lung metastatic 4T1-LuM cells compared to the parental 4T1 cells. Moreover, transplanted into mouse mammary fat pad, the 4T1-LuM cells showed to grow faster in the local and shorten mouse survival compared to the 4T1 cells. Therefore, we are going to unveil a key molecule for malignancy of 4T1-LuM cells by using a microarray.

▲TOP

[P-2208] P11-2 [English/Japanese]
Metabolism / metabolome (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-2208

Mitochondrial respiratory chain complex I activity has emerged as a potential target for cancer therapy

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Mitochondria play an essential role in metabolism and redox control and have been implicated in the malignant transformation of cells. Recently, we reported that the inhibition of mitochondrial replication/transcription suppressed the proliferation of human mammary tumor and hepatocellular carcinoma cells. The ATP levels in these cells were unaffected but the cells exhibited arrested growth at the G1/S and G2/M boundaries through the downregulation of cell cycle regulators such as E2F1. In this study, we demonstrated that the inhibition of the electron transport chain (ETC) activity with siRNA specific for NDUFV1, a NADH dehydrogenase that comprises a subunit of mitochondrial complex I, suppressed cancer cell proliferation. This suggests that cancer cell proliferation is dependent on the complex I activity of the ETC. Cancer cells with a lower mitochondrial activity were more sensitive to a chemical inhibitor of the complex. This inhibitor suppressed cell proliferation and/or induced cell death at doses that had no effect on normal cells. Thus, the complex I activity of the ETC has emerged as a potential therapeutic target for cancers with low mitochondrial activity.

▲TOP

P-2209

Single-cell analysis of mitochondrial function in human cancers: role of cancer-specific abnormal mitochondria

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Mieap, a p53-inducible protein, regulates mitochondrial quality control. Inactivation of p53/Mieap causes accumulation of abnormal mitochondria in cancer cells, leading to promotion of cancer development and aggressiveness. Therefore, cancer-specific abnormal mitochondria play a pivotal role in driving cancer initiation and progression. This study aims to characterize “cancer-specific abnormal mitochondria”. Nearly 95 years ago, Otto Warburg suggested that cancer mitochondria are abnormal. However, so far there are few studies that evaluated cancer mitochondria at single-cell level. Here we report the results of single-cell analysis on the mitochondrial function in human cancer. In this study, we evaluated the mitochondrial functions including the mitochondrial membrane potential activity, citric acid circuit activity, and ATP synthesis ability at the single-cell level in the Mieap-deficient, and wild type (WT) cancer cells, and normal cells. Moreover, we carried out the same analysis of the mitochondria in the p53-deficient, and WT cancer cells. In this paper, we discuss the significance of “cancer-specific abnormal mitochondria” on the basis of the results.

P-2210

Reprogramming of energy metabolism via autophagy in pancreatic cancer cells

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[Purpose] Cancer cells mainly obtain their energy for survival, ATP, from glycolysis. However, merely inhibiting the glycolysis is insufficient for the eradication of cancer cells. Here, we investigated reprogramming mechanism of energy metabolism focusing on autophagy in pancreatic cancer cells whose glycolytic pathway is suppressed.
[Methods] Human pancreatic cancer PANC-1 cells were used as model cells. Glycolysis was suppressed by replacement of sugar source, glucose, in the culture medium. Autophagic activity, mitochondrial function and metabolic profiles were evaluated. Furthermore, we focused on mitophagy, which is a selective degradation machinery of mitochondria by autophagy.
[Results and Discussion] We observed glycolytic suppression induced autophagy, mitophagy and increased mitochondrial function in PANC-1 cells. Glycolytic suppression also changed metabolic profiles, that were reversed by inhibiting autophagy. These data suggests that autophagy contributes to reprogramming of energy metabolism and maintenance of mitochondrial function. Here, we propose inhibiting autophagy (including mitophagy) plus glycolytic suppression is efficient for pancreatic cancer treatment.

P-2211

Lipidomic Analysis in Liver Cancer Cells Regulated FABP5 Expression

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Hepatocellular carcinoma (HCC) is a highly prevalent cancer. Our group has reported that the fatty acid binding protein 5 (FABP5)-down-regulated animal model showed the suppression of tumors. The lipid metabolism is also likely to involve cancer development. In this study, we aimed to reveal the lipid profile by FABP5 regulation in liver cancer cells. Li-7 cells down-regulated FABP5 and HepG2 cell over-expressed FABP5 were cultured in different mediums with/without 250 μM fatty acids, such as palmitic acid and oleic acid (1:2). After these cultures, lipids were analyzed by liquid chromatography mass spectrometer. In FABP5-down-regulated Li-7 and FABP5-over-expressed HepG2 cells, the volume of triglyceride (TG) increased higher in fatty acids-added culture medium than in normal one. Not FABP5 but other FABP subtype would transport fatty acids in the cells following the synthesis of TG. It has been reported that free fatty acid accumulation is a risk factor of hepatitis C virus-hepatocellular steatosis and hepatitis B virus-HCC. Our results suggest that regulating FABP5 as well as other subtypes could be useful for the prevention and therapy for highly malignant HCC.

▲TOP

P-2212

Evaluation of phospholipids expression in prostate cancer cell lines in LCMS

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Lipid metabolisms have been considered to play important roles in oncogenesis and progression of prostate cancer, but the precise roles remain unknown. Herein we established experimental methods to analyze the phospholipids in Liquid Chromatography-Mass spectrometry (LCMS), and we analyzed the profiles of phospholipids in prostate cancer cell lines. First, using the synthetic phospholipids, we confirmed that we could measure phosphatidylcholine, phosphatidylinositol, lysophosphatidylcholine, lysophosphatidylethanolamine simultaneously. The dilution series of each phospholipid showed linear relationship between concentration and intensity in LCMS. We evaluated phospholipid compositions of four prostate cancer cell lines, LNCaP, PC3, DU145 and AI-LNCaP which is androgen-independent cell line established from LNCaP. Analyzing the profile of phospholipid in the cell lines, we found that the expression of polyunsaturated fatty acids (PUFAs) were higher in PC3 and DU145 than in LNCaP. PC3 and DU145 have more malignant potential than LNCaP. These finding suggest that PUFAs might be related to aggressiveness of prostate cancer.

▲TOP

[P-2218] P11-4 [English/Japanese]
Metabolism / metabolome (4)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-2218

Osteosarcoma stem-like cells have the metabolic features of high aerobic glycolysis demands mediated by LIN28B

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Osteosarcoma (OS) is a highly malignant bone tumor and the prognosis for non-responders to chemotherapy is still poor. Previous studies revealed human sarcomas contained sarcoma-initiating cells (SICs). SICs have the characteristics of high tumorigenesis and resistance to chemotherapy and could be a cause of recurrence. In this study, we characterized SICs of an OS cell line, screened for SICs-related genes, and tried to regulate the proliferation of OS by interference of metabolism.
At first, we established a new human osteosarcoma cell line OS13, and we isolated clones showing higher tumorigenesis as SICs (OSHIGH) and the counterpart clone. OSHIGH cells showed chemo-resistance and their metabolism highly depended on aerobic glycolysis and suppressed OXPHOS. Using RNA-seq, we identified LIN28B as SIC-related gene highly expressed in OSHIGH cells. Knockdown of LIN28B reduced tumorigenesis, reduced chemo-resistance and reversed OXPHOS function. Inhibition of glycolysis could inhibit the proliferation of OS cells. Taken together, the results indicated that LIN28B-silencing and manipulation of glycolysis might be attractive treatments for refractory OS.

▲TOP

P-2219

PKM1 stimulates latent PKM2's activity via direct interaction

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The glycolytic enzyme PKM exists as two isoforms: PKM1, which is constitutively active and promotes glucose catabolism, and PKM2, which is activated only in response to increased levels of allosteric activator(s) such as FBP. Although most cancer cells predominantly express Pkm2 over Pkm1, some subsets of lung cancer including small-cell lung cancers (SCLCs) express both PKM1 and PKM2. Here we provide evidences that PKM1 stimulates latent PKM2's activity in SCLCs. Pkm1 and Pkm2 can form a complex in vitro and in intact cells. Importantly, in the absence of FBP, combined activities of Pkm1/Pkm2 varied exponentially rather than linearly relative to the amount of Pkm1. Km and Vmax values of a 1:1 Pkm1/Pkm2 mixture were similar to those of Pkm1 or Pkm2 in the presence of FBP. When cells were cultured in low glucose, Pkm2 seen in SCLC cells resided primarily in an active tetramer, which was not the case when we used other types of lung cancer cell lines, which show little PKM1 expression. These results strongly suggest that expression of PKM1 in SCLC cells boost PKM2 activity in a factor-independent manner via direct interaction.

P-2220

Tumor suppression by controlling intercellular levels of ROS through ROS metabolic enzymes by curcumin derivatives

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The intracellular level of reactive oxygen species (ROS) is generally increased in most tumor cells compared with that of normal cells, which contributes to enhanced proliferation as well as the higher rate of mutation in tumor cells. Because the extremely high levels of ROS trigger oxidative stress to induce apoptosis and senescence, tumor cells have developed their own mechanism to maintain ROS at the moderately elevated levels. We previously showed that the expression levels of various ROS scavenging enzymes (CBR, GST, NQO, ADH1, AKR1, and GLO1) were elevated in human leukemic cells, and that curcumin inhibited these enzymes by direct binding, leading to an increase in ROS levels and suppression of tumor cell growth. These data suggest that curcumin has a promising potential in cancer therapy. However, curcumin has its own problem, low solubility in water, low stability in vivo, etc. Here we developed various curcumin derivatives with improved activity. These compounds exhibited lower IC50 for suppression of tumor cell proliferation, enhanced induction of ROS levels and senescence, suggesting that curcumin derivatives are good candidates for a novel cancer therapy.

P-2221

Autophagy inhibition synergizes with calcium mobilization to achieve efficient therapy of malignant gliomas

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Autophagy plays a critical role in tumorigenesis, but how autophagy contributes to cancer cells' responses to chemotherapeutics remains controversial. To investigate the roles of autophagy in malignant gliomas, we knocked out the ATG5 gene, which is essential for autophagosome formation, in tumor cells derived from glioblastoma patients. ATG5 disruption did not change the phenotypes of glioma cells and did not alter their sensitivity to temozolomide, an agent used for glioblastoma patient therapy. Screening of an anti-cancer drug library identified compounds that show greater efficacy to ATG5-knockout glioma cells compared to control. One of candidates, nigericin, in combination with ATG5 deficiency synergistically suppressed spheroid formation by glioma cells in a manner mitigated by Ca^{2+} chelation or CaMKK inhibition. Using a patient-derived xenograft model, we demonstrated that chloroquine, a pharmacological autophagy inhibitor, dramatically enhanced the efficacy of compounds selected in this study. Our findings propose a novel therapeutic strategy in which calcium-mobilizing compounds are combined with autophagy inhibitors to treat glioblastoma patients.

▲TOP

P-2222

The value of global metabolomics in association with clinical factors for diagnosis of renal cell carcinoma

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Any biomarkers have not been clinically useful for diagnosis and therapy for renal cell carcinoma (RCC). We aimed to identify metabolites which have clinical importance in cancer tissues by using LC-MS/MS based on our global metabolomics protocol. Tumor and non-tumor tissues were sampled from 20 patients who underwent surgical treatment. We identified 58 metabolites significantly increased levels in tumor tissues, 34 of them could indicate a high diagnostic potential for RCC (area under curve (AUC)>0.8; Group A), but 24 were not discriminators between tumor and non-tumor (AUC<0.8; Group B). We identified 6 pathways from 9 metabolites in Group A and 8 pathways from 10 metabolites correlated with high malignant potential (clinical and pathological stage, Fuhrman grade, coagulation necrosis etc.) in Group B. Although only two pathways (glycolysis and tryptophan pathway) were common between the two groups, malignant parameters found to be significantly correlated with Group B compared with Group A. Our study suggested that metabolic pathways were different between cancer diagnosis and high malignant potential for RCC.

▲TOP

[P-2237] P12-5 [Japanese]
Cancer immunity (2)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Takuro Saito / Dept. Surg., Osaka Police Hosp.

P-2237

The relationship between Warburg effect and M2-like macrophage polarization in head and neck cancer

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Lactic acid, which is secreted from tumor cells by reprogramming of glucose metabolism, has important roles as a proinflammatory and immunosuppressive mediator. FDG-PET/CT measures glucose uptake in tumors. The concentration of lactic acid, FDG-PET/CT parameters and the expression of M2 macrophage markers in head and neck squamous cell carcinoma (HNSCC) were measured.We analyzed the expression of M2 macrophage markers, colony stimulating factor 1 receptor (*CSF1R*) or *CD163*, normalized using a pan-macrophage marker, *CD68* by real-time RT-PCR or immunohistochemical staining (IHC). M2 macrophage polarization assessed by RT-PCR was compared with intratumoral lactic acid concentration. M2 macrophage polarization assessed by IHC was compared with FDG-PET/CT parameters. Tumors with higher levels of *CSF1R* showed a significantly higher concentration of lactic acid. A similar tendency was observed for *CD163*. The CD163/CD68 ratio is positively correlated with SUVmax.These results suggest that tumor-secreted lactic acid promotes induction of M2-like macrophage polarization in human HNSCC, and that FDG-PET/CT could predict M2-like macrophage polarization within tumor microenvironment.

▲TOP

P-2238

Tumor-associated macrophages are correlated with better prognosis in stage IIIc or IVb endometrial carcinomas

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【Introduction】Endometrioid carcinoma of uterine corpus shows an excellent prognosis in the early stages. However, the patients at advanced stages show worse prognosis and less reactivity to chemotherapy. Thus, treatments focusing on the tumor microenvironment including immunotherapy have attracted attention.【Material and methods】Formalin-fixed paraffin-embedded tissue samples of 24 endometrioid carcinoma (FIGO stage IIIc; 16, IVb; 8) were included in the study. All cases were stained immunohistochemically with CD3, CD8, Foxp3, CD68, and CD163. For each section, we selected one high-power-field and counted the total number of CD3-, CD8- and Foxp3-positive cells in invasive fronts. We also selected three intermediate-power fields for each section, and evaluated the density of CD68 and CD163 cells in the invasive fronts.【Results】Most of the tumor associated macrophages (TAMs) were positive for CD163. The higher density of TAM in the invasive fronts was correlated with better prognosis.【Discussion】This results might indicate that TAM infiltration not only high activity of M2, that is advantageous to tumor, but also reflect immnoactivity or other microenvironments.

P-2239

Function of CD163, a macrophage scavenger receptor, in tumor microenvironment of sarcoma

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Recent findings showed that the significance of CD163-positive macrophages (MØ) in tumor progression. However, few studies related to the functions of CD163 in MØ have been published. Therefore, we tried to uncover the involvement of CD163 in MØ activation using CD163-deficient mice and human samples. At first we performed immunohistochemistry of CD163 using 45 samples of undifferentiated pleomorphic sarcoma (UPS). High density of CD163-positive MØ was closely related to shortened progression free survival time and higher histological grade. Tumor development of sarcoma (MCA205 and LM8) cells were significantly abrogated in CD163-deficient mice as compared with WT mice. Co-culture study using peritoneal MØ and MCA205 cells was performed, and we found that proliferation of MCA205 was significantly increased by co-culture with WT MØ whereas this MØ -induced proliferation of MCA205 was suppressed when CD163-deficient MØ were used. Therefore, CD163 is related to protumor activation of macrophages and considered to be closely involved in tumor development and progression in murine and human malignant tumors.

P-2240

Multifunctionality of Peripheral Blood CD8 T Cells in Esophageal Cancer Patients with Preoperative Chemotherapy

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INTRODUCTION: The recent studies have revealed the role of CD8 T cells in cancer microenvironment. However, tumor infiltrating CD8 T cells are difficult to be assessed their function in clinical settings. Then, we focus on multifunctionality (triple cytokine production: INF- γ ,TNF- α ,IL-2) of peripheral blood CD8 T cells, and apply it on patients with esophageal cancer preoperative chemotherapy and consider whether it would be a predictive marker of chemotherapeutic effect.METHODS:Blood sampling is performed before the introduction of preoperative chemotherapy, after each course finished and peripheral blood mononuclear cells are isolated. These cells are incubated with PMA/Ionomycin and analyzed with flowcytometry. Drug-induced immunogenic modulation is also assessed in vitro.RESULTS: The degree of multifunctionality of peripheral blood CD8 T cells has moderately correlated with tumor reduction brought by following chemotherapy. The CD8 T cell function may be affected by drug-induced immunogenic modulation. Conclusions:These data suggested that multifunctionality of CD8 T cells would be a possible biomarker for good responder of cancer chemotherapy.

▲TOP

P-2241

CD4+CD8+ double positive T cells infiltrating in tumor microenvironment among various cancer types

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Introduction:CD4+CD8+ double positive(DP) T cells differentiate into CD4+ or CD8+ single positive(SP) T cells in the thymus, resulting in the distribution of only SP T cells in periphery. However, it has been reported that DP T cells infiltrating in several cancer tissues are observed. Purpose: The expression of DP T cells in tumor infiltrating lymphocytes(TILs) among various cancer types was investigated. Materials and Methods: Fresh tumor and peripheral blood were obtained from 218 cancer patients (15 esophageal cancers, 15 gastric cancers, 15 colorectal cancers, 15 uterine body cancers, 15 ovarian cancers, 15 non-small-cell-lung-cancers, 15 thymic tumors, 90 renal cell carcinomas (RCC), 23 urothelial carcinomas). %DP T cells (DP T cells/Total CD3+ T cells) in purified TILs and PBMCs were evaluated using flow cytometry analysis. Results: %DP T cells in RCC was significantly higher than that in other cancer types (0 - 49.3% vs. 0 - 4.94%, p< 0.01). Even in RCC patients with higher (> 10%) %DP TILs, %DP T cells in PBMCs was significantly lower than that in TILs (median: 0.43% vs. 22.2%, p< 0.01). Conclusion: In RCC, extremely high %DP TILs were observed in tumor microenvironment.

▲TOP

[P-2246] P14-27 [English/Japanese]
Hepatocellular carcinoma (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-2246

Cytolytic activity (CYT) is a prognostic biomarker reflecting host immune status of hepatocellular carcinoma (HCC)

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Background: CYT calculated from mRNA expression level of *GZMA* and *PRF1* is a new index of cancer immunity and associated with good outcome in pan-cancer (Cell, 2015). We assessed the clinical significance of CYT in HCC.
Methods: First, we analyzed a pan-tissue microarray dataset (GeneAtlas U133A) and performed immunohistochemical analysis (IHC) of GZMA and PRF1 expression using HCC tissues. Then, we evaluated correlation between CYT and *CD8A*, *CD4* and *NCAM1* expression, which are surface protein of CD8 and CD4 T cell and NK cell, respectively, in two datasets (GSE14520 and TCGA). Finally, we analyzed prognostic value of CYT in three cohorts (our institution, GSE14520 and TCGA).
Result: CYT was high in lymphocytes compared with other cells included liver tissue. IHC showed that GZMA and PRF1 were stained only in lymphocytes. There are positive correlations between CYT and *CD8A* or *CD4* expression, whereas there is no correlation between CYT and *NCAM1* expression. Low CYT was an independent poor prognostic factor in HCC.
Conclusion: CYT could be a useful prognostic biomarker in HCC, possibly through reflecting the amount of tumor-infiltrating lymphocytes; host immune status.

▲TOP

P-2247

Analysis of antitumor mechanism of BET inhibition in hepatocellular carcinoma

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Bromodomain and extra terminal (BET) family proteins are the readers of acetyl-lysine in histone and regulate gene transcription. In recent years, anti-tumor effects of BET inhibitors have been reported in various cancers including hepatocellular carcinoma (HCC). In this study, we aimed to clarify the anti-tumor mechanism of BET inhibition in HCC. We first tested the effects a BET inhibitor JQ1 in a series of 10 HCC cell lines, and found that JQ1 strongly suppressed HCC cell proliferation through inducing cell cycle arrest and apoptosis. Microarray analysis revealed that JQ1 treatment induced marked changes in gene expression profiles in HCC cells, and genes associated with cell cycle and apoptosis were significantly enriched among the affected genes. Notably, we found that a number of cancer-related genes including BCAT1, DDR1 and FANCD2 were strongly suppressed by JQ1 in HCC cells. Our results suggest that JQ1 may exert anti-tumor effects via suppressing multiple BET-target genes in HCC. Currently, we are proceeding analysis on these potential BET-target genes in HCC, and we will discuss their biological and therapeutic implications.

P-2248

ANP32B knockdown suppresses apoptosis in hepatocellular carcinoma

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＜Background＞The acidic nuclear phosphoprotein 32 family, member B (ANP32B) is critical for the development of normal tissue. However, its role in the development of hepatocellular carcinoma (HCC) is controversial.＜Aim＞To elucidate the role of ANP32B in HCC cell lines and tissues.＜Methods＞ANP32B expression in HCC cell lines was modulated with siRNA and ANP32B expression plasmids and lentiviruses.＜Results＞ANP32B knockdown reduced the expression of cleaved forms of caspase 3 and caspase 9, but not that of caspase 8, in HCC cells cultured with the pro-apoptotic agent, staurosporine. Phosphorylated Bad was up-regulated, while Bak was down-regulated. Conversely, ANP32B overexpression decreased Bad phosphorylation and up-regulated Bak, but did not induce apoptosis, because Bax expression was down-regulated. In HCC tissues, a low tumor/non-tumor ratio of ANP32B mRNA expression was related to the advanced UICC stage ($p = 0.032$). ＜Conclusions＞ANP32B modulates Bad phosphorylation as well as Bak and Bax expression, resulting in regulation of apoptosis in HCC. These findings indicate the potential value of ANP32B as a therapeutic target for HCC.

P-2249

High FANCD2 Gene Expression is associated with Tumor Progression in Hepatocellular Carcinoma

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Background/Aim:Fanconi anemia complementation group D2 (FANCD2) gene encodes FANCD2 protein which localizes to DNA repair foci and plays central roles as a key component in the DNA damage responses. We investigated the clinical significance of FANCD2 expression in hepatocellular carcinoma (HCC). Patients and Methods: FANCD2 mRNA expression of resected HCC tissues was assessed in two HCC cohorts (our cases: n = 111, The Cancer Genome Atlas (TCGA): n = 371). In vitro proliferation, invasion, and cell cycle assays were performed using Hepatoma cells (HepG2 and PLC/PRF/5) and siRNAs, and the effect of the mTOR inhibitor AZD8055 was evaluated. Results: FANCD2 expression was upregulated in tumor tissues. High FANCD2 expression cases showed poorer prognosis in both cohorts, and was associated with larger tumor sizes and invasive phenotypes. FANCD2 knockdown attenuated proliferation and invasion of HCC cells. Cell cycle assays showed that attenuated FANCD2 expression induced G1/S arrest in HepG2 cells. Moreover, FANCD2 expression was suppressed by AZD8055. Conclusion: High FANCD2 expression in HCC could be a novel biomarker for poor prognosis and associated tumor progression.

▲TOP

P-2250

The Clinical Significance of Alpha-Fetoprotein mRNAs in Patients with Hepatocellular Carcinoma

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AIMS: Alpha-fetoprotein (AFP) mRNA could be a marker of circulating tumor cells of hepatocellular carcinoma (HCC). We analyzed portal and peripheral blood, and peritoneal lavage samples to detect the presence of AFP mRNA-expressing cells, and explored their relationship with metastasis. METHODS: AFP mRNA expression was measured by qPCR in 112 sets of portal and peripheral blood samples and 61 peritoneal lavage samples obtained intraoperatively. In addition, the background factors, and recurrence rate were analyzed. RESULTS: The change in AFP mRNA positivity during hepatectomy was remarkable in the peripheral blood specimens, while that in the portal vein blood and peritoneal lavage samples was similar. Recurrence was more frequent in the patients who were positive for AFP mRNA than in those who were negative 9-24 months after hepatectomy. During this limited period, the recurrence rate was significantly higher in the AFP mRNA-positive cases than in the AFP mRNA-negative cases (p=0.0472). CONCLUSIONS: AFP mRNA positivity might contribute to recurrence-free survival 9-24 months after hepatectomy.

▲TOP

[P-2256] P14-29 [English/Japanese]
Hepatocellular carcinoma (3)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-2256

FABP4 overexpression in intratumoral hepatic stellate cells within hepatocellular carcinoma with metabolic risk factors

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Metabolic syndrome is a newly emerging risk factor for hepatocellular carcinoma (HCC), but tumor-specific biomarkers still remain unclear. We performed cross-species analysis of gene signatures in HCC from human patients and melanocortin 4 receptor-knockout (MC4R-KO) mice, which develop HCC with obesity, insulin resistance and dyslipidemia. Unsupervised hierarchical clustering of 746 differentially expressed orthologous genes classified them into two distinct subgroups, one of which included mouse HCC and was etiologically related to metabolic risk factors. We identified 9 genes commonly overexpressed in human and mouse metabolic disease-associated HCC, and revealed that FABP4 was remarkably enriched in intratumoral activated hepatic stellate cells (HSCs). FABP4 overexpression induced inflammatory chemokines through NF-κB nuclear translocation, resulting in the recruitment of macrophages. A validation study of other human HCC samples indicated that the FABP4-high group consisted of patients with multiple metabolic risk factors. Thus, FABP4 overexpression in HSCs could contribute to hepatocarcinogenesis in patients with metabolic risk factors via modulation of inflammatory pathways.

▲TOP

P-2257

Single cell gene expression profiling in human hepatocellular carcinoma

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Tumor tissues include a diverse population of cells such as cancer cells, stem like cells and infiltrated leukocytes, and become more heterogeneous during the process of disease. The changes of tumor microenvironment could affect the anti-cancer drug sensitivity; therefore, the identification of the infiltrated immune cells and stromal cells in the tumor tissues by single cell gene expression profiling has widely been expected to develop new drugs for immunotherapy.
Recently, we have developed a simple method for identifying single cell from thousands of single cells by transcriptome analysis using a polydimethylsiloxane microwell array with the barcode beads in which oligonucleotide consists of a cell barcode and a poly T tail (Nx1-seq: Next generation 1 cell sequencing).
Here, we show the Nx1-seq results of the samples in human non-B and non-C hepatocellular carcinoma. The obtained libraries by Nx1-seq can be clustered into different cancer cells, infiltrated leukocytes, mast cells, fibroblasts and endothelial cells. The cell population in the microenvironment was different between the samples, and it could be related to the cancer progression.

P-2258

Multi-lesional analysis of TERT promoter mutations in combined hepatocellular-cholangiocarcinoma

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Combined hepatocellular-cholangiocarcinoma (cHCC-CC) is a hepatic tumor with intermingling of two components, HCC and CC. It has not yet been clear whether the origin of these tumors is the same or not. TERT promoter mutations are frequently observed in HCC but not in intrahepatic CC. In this study, we examined TERT promoter mutations in multiple lesions dissected from cHCC-CC and metachronical HCCs to determine whether the mutations are present in the CC of cHCC-CC or not. TERT promoter mutations were analyzed using the Sanger sequencing and TaqMan qPCR. The C228T mutation was detected in all tumors (#1HCC, #2HCC, #3HCC and #3CC in a case with three metachronical liver cancers with three year-intervals) (#1HCC-CC mixture, #2HCC, #3HCC and #4HCC in a case with cHCC-CC). However, the mutation frequency was different between HCC and CC, HCCs within a case. These results are supportive to an idea that the CC containing the TERT promoter mutation is generated from the same origin as HCC. (The same results were obtained from the genomic analysis as shown in the last meeting.) Both HCC and CC develop the genomic micro-diversity within a tumor.

P-2259

New therapy for hepatocellular carcinoma with liver cirrhosis, targeted to hepatic stellate cells

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【Background】Most of the patients with hepatocellular carcinoma (HCC) are reported to be associated with liver cirrhosis (LC). We have hypothesized hepatic stellate cells (HSC) are activated in LC and may be related to HCC progression. We reported CnP suppressed the activation of HSC and hepatic fibrosis (Kubo N, et al. Liver Int. 2014:34:1057-67). The purpose of this study is to clarify the effect of HSC on HCC and to develop a new therapy for HCC targeted to HSC.【Method】(1) The effect of HSC(Lx-2) condition medium (CM) on proliferation and invasion of HCC cell line (HepG2) was examined. (2) The effect of CnP on HSC was examined.【Result】(1) HSC CM significantly promoted the proliferation and invasion of HCC.(2) CnP(0.1 μg/ml) suppressed the expression of alpha smooth muscle actin(α SMA) in HSC. To compared with HSC CM without CnP, HSC CM with CnP significantly suppressed the proliferation and invasion of HCC. In cytokine array of HSC CMs(with or without CnP), CnP suppressed the expression of IL-6, CCL-2, Angiogenin, CXCL-5, CXCL-12, MMP-9 and Osteopontin.【Conclusion】CnP suppressed the activation and cytokines of HSC. CnP may be a new therapy for HCC targeted to HSC.

▲TOP

P-2260

Lysyl oxidase induces EMT and is associated with early recurrence and poor survival in patients with HCC

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Background:Hepatocellular carcinoma (HCC) has high recurrence rates even after curative hepatectomy. Methods:For the gene selection, we used public data base (GSE10141) and searched genes associated with early recurrence. As a validation cohort, 358 patients underwent hepatectomy in our own institution from 2004 to 2014 were enrolled. The expression of target gene was evaluated by IHC staining, and patients were separated the high and low expression groups.Result:We extracted LOX as a gene with high hazard ratio (HR>3) for early recurrence using GSE10141. In IHC staining, the high LOX expression group had a significantly high recurrence rate (p<.0001) and poor survival rate (p<.0001) than those of the low expression group. Multivariate analysis demonstrated that the high LOX expression was an independent risk factor for early recurrence. (HR, 2.52, p<.0001) The correlation between LOX and TWIST expressions was positive (p<.0001); however, the correlation of LOX and E-cadherin expressions was negative (p<.0001).Conclusion:The high LOX expression is associated with EMT markers, and predicts early recurrence and poor survival in patients with HCC after curative hepatectomy.

P-2261

High expression of PFKFB3 in Hepatocellular Carcinoma Relates with Poor Prognosis after Surgery

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【Background】Six-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), a glycolytic activator, was recently reported to have important roles in cancer progression. We assessed the relationship between the expression of PFKFB3 and clinicopathological features and prognosis in Hepatocellular carcinoma (HCC) patients. 【Methods】One hundred one consecutive patients who underwent curative surgery for HCC from January 2010 to December 2015 were studied. The patients were divided into low expression (Low group, n=71) and high expression (High group, n=30) by immunohistochemical analysis of the expression of PFKFB3. 【Results】There was no difference in clinicopathological factors between two groups except high ICG15 value of High group. The 3-year disease-free survival rate was significantly lower in High group (35% vs 68%, p<0.001). The univariate and multivariate analysis revealed that the factors of the high expression of PFKFB3, male, multiple tumor and tumor diameter>3cm were independent prognostic factors with disease-free survival. 【Conclusion】The expression of PFKFB3 might be a novel prognostic factor in HCC.

▲TOP

[P-2269] P14-31 [English/Japanese]
Surgical treatment of hepatobiliary pancreatic cancers (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-2269

Short-term outcomes of laparoscopic liver resection for liver tumors located in the posterosuperior segments

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Background: For laparoscopic liver resection, posterosuperior (PS) segments (S1, S4a, S7, S8) are anatomically and technically more demanding than anterolateral (AL) segments (S2, S3, S4b, S5, S6). **Methods:** 1. We divided into two groups; laparoscopic liver resection in PS (L-PS, n=21), and open liver resection in PS (O-PS, n=19). 2. We divided into two groups; L-PS and laparoscopic liver resection in AL (L-AL, n=54). We compared the short-term outcomes between the two groups. **Results:** 1. For L-PS/O-PS, tumor diameter; 21/25 mm and operative time; 223/242 min were comparable. However, blood loss in L-PS (125 g) was significantly less than that in O-PS (500 g). Hospital stay in L-PS (10 d) was significantly shorter than that in O-PS (11 d). 2. For L-PS/L-AL, tumor diameter; 21/15 mm and blood loss 125/50 g were comparable. However, operative time in L-PS (223 min) was significantly longer than that in L-AL (151 min). Hospital stay in L-PS (10 d) was significantly longer than that in L-AL (8 d). **Conclusions:** L-PS can provide better short-term outcomes than O-PS. However, L-PS is still technically challenging, because it may need longer operative time and hospital stay than L-AL.

▲TOP

P-2270

Study of feasibility and outcome of the radiation therapy against the HCC patients with portal vein tumor thrombus

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[Introduction] The prognosis of the hepatocellular carcinoma (HCC) patients with portal vein tumor thrombus (PVTT) is very poor. We introduced radiation therapy (RT) targeting to PVTT for the prevention of liver failure due to portal vein obstruction. [Subjects/Methods] Twenty HCC patients who were judged to be unresectable and inadequate for TACE were enrolled. A total of 50-60 Gy was irradiated to each patient. [Results] Completion rate of RT was 100% and we experienced no Grade 3 or higher adverse events. One-year survival rate was 53.3%. Champion case indicated extremely good response and survived 5 years without relapse. Curative hepatectomy was performed in one case. Portal vein recanalization occurred in 8 of the 18 (44.4%) patients, allowing implementation of additional TACE. One-year survival rate for the patients with additional TACE (87.5%) was significantly better than that of no additional treatment. No case was dead with liver failure due to portal vein obstruction.[Conclusion] RT was useful for the patients with HCC complicated with PVTT as multidisciplinary therapeutic strategy by enabling the continuation of additional TACE.

P-2271

A case in which residual left lobectomy was effective for multiple metastasis of intrahepatic cholangiocarcinoma

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A 75-year-old man was introduced to our hospital because of a liver tumor. The tumor was diagnosed as hepatocellular carcinoma (HCC) because the tumor showed typical HCC findings of arterial phase enhancement and portal phase washout with enhanced computed tomography (CT). He had hepatectomy of segment 4a+5. The final pathological examination showed that the tumor was intrahepatic cholangiocarcinoma (IHCC). He received a follow-up CT regularly after operation. Two years after, multiple tumors appeared in left lobe of residual liver.They looked like HCC, but the pattern of enhancement was very similar to the tumor of the first operation. So, we diagnosed the tumors were multiple metastasis of IHCC. Fortunately, the tumors were limited in left lobe,we performed left residual lobectomy. The final pathological examination showed metastasis of IHCC. One year after second operation, he has no recurrence and good course.It is necessary to pay attention to the rarely IHCC with arterial phase enhancement and portal phase washout. Even with metastasis of IHCC, if surgical resection is possible, it is worth challenging for long-term survival.

P-2272

An investigation for clinical significance of conversion surgery for initially unresectable pancreatic cancer

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[Introduction]In this study, we retrospectively examined the clinical significance of conversion surgery for initially unresectable pancreatic cancer (PC). [Patients and Methods]Thirty-one patients who underwent conversion surgery following effective initial treatment (conversion surgery group: CS group) from April 2006 to March 2017 were enrolled. The clinical outcomes of CS group were compared with those of 219 cases diagnosed as resectable PC (upfront surgery group: UP group).[Results] Eighteen, three and nine cases in CS group was classified in UR-LA, BR and UR-M respectively. Medial periods of initial treatment was 8 months. Pancreatectomy was performed in 21 cases (84%) in CS group and 190 cases (86.7%) in UP group. Curative resection(R0/1) rate was significantly lower in CS group (CS/UP; 67.7/84.5%, p=0.02). The MST from initial therapy did not show significant difference between two groups (CS / UP; 30/24 months, p=0.23). And also, the MST from operation were equivalent (CS/UP; 21/226 months, p=0.59). [Conclusion] It was suggested that the clinical impact of conversion surgery for initially unresectable PC might be comparable to that of upfront surgery for resectable PC.

▲TOP

P-2273

Reappraisal of overall survival of patients with pancreatic ductal adenocarinoma who underwent surgical resection

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We evaluated overall survival (OS) of PDAC patients who underwent surgical resection followed by adjuvant chemotherapy (AD).Between 2011 and 2015, 120 PDAC patients who had negative CY underwent R0/1 resection . We have used S1 in 67 patients (S1 group) and gemcitabine in 31 patients (GEM group) as AD. 22 patients did not received AD therapy for several reasons (control group). The median OS time in the patients received AD was 59 months which was significantly better than in control group (30 months, p=0.001). There was no significant difference in OS between S1 and GEM groups (3 year OS; S1:76%, GEM: 61%, p=0.112).40 patients (60%) in S1 group and 17 patients (55%) in GEM group completed the AD and OS in these patients were significantly longer than the patients discontinued before completion. (3 year survival rate: S1, 90% vs 53%, p=0.003. GEM, 82% vs 33%, p=0.004)In multivariate analysis, Completion of AD (hazard ratio and range; 0.519 and 0.348-0.756) and R0 resection (0.510 and 0.355-0.740) were significantly independent prognostic factors (p=0.001) Completion of AD and R0 resection were important factors for improving prognosis in patients with PDAC.

▲TOP

[P-2230] P12-4 [Japanese]
Tumor antigens and immunity

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Hiroyuki Kishi / Dept. Immunol., Grad. Sch. Med. Pharm. Sci., Univ. Toyama

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P-2230

Immune response towards mutation-derived tumor antigens in bladder cancer patients treated with CTLA-4 blockade

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Recent studies have suggested that intervention with immune checkpoint inhibitors (ICIs) can prime T cell responses for mutation-derived tumor antigens (MTAs) and mutational load may be associated with clinical responses. We conducted a clinical trial of CTLA-4 blockade against metastatic bladder cancer (NCT01524991, n=36). With the samples of the trial, we aimed to investigate the correlation of mutational load with outcome and to evaluate the T cell immunity elicited by ICIs.
Putative MTAs were identified through whole exome sequencing (WES) with tumor tissue followed by peptide-MHC binding affinity. Mutated peptides were synthesized to cover MTAs and used in in-vitro experiments to address T cell responses for MTAs.
Tumors from 4 patients showed high mutational lord within tumors from 26 patients with WES data. Although 2 patients with complete clinical response had tumors with high mutational lord, mutation lord was not correlated with clinical outcome in all cohort. T cell responses for MTAs were detected in 1 of 6 patients in experiments.
Mutational lord may be associated with clinical responses with CTLA-4 blockade. The experiments for MTA-specific T cells are in progress.

▲TOP

P-2231

TCRs of clonally expanded TILs recognized tumor-associated antigens and showed cytotoxicity to tumors

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TCR gene therapy is a next promising treatment of cancers. In this study, we tried to clone cDNAs of tumor-specific TCR &alpha&beta from primary tumor infiltrating lymphocytes (TILs). To this end, we single-cell sorted CD8+CD137+ TILs from B16F10 melanoma that had been inoculated in C57BL/6 mice. TCR repertoire analysis revealed the clonal expansion of CD8+CD137+ TILs. Thirteen TCRs were obtained from clonally expanded TILs and their reactivity against B16F10 cells was examined. Nine of them induced IFN production and cytotoxicity against B16F10 cells when expressed on splenic T cells. We next investigated in vivo antitumor activity of two TCRs that showed the strongest cytotoxicity in vitro using B16F10 cell lung metastasis assay and found that they suppressed lung metastasis of B16F10 cells. Concerning their antigen-specificity, seven of them were found to react to p15E peptide. The other two did not react to p15E peptide but reacted to the antigen(s) expressed not only in B16F10 melanoma cells but also in EL4 lymphoma cells. These results showed that the majority of TCRs in CD8+CD137+ TILs recognize tumor-associated antigens.

P-2232

Inflammation of the lung enhances antitumor effects of anti-PD-1 immunotherapy

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Immune-checkpoint inhibitors, such as PD-1 / PD-L1 blockade therapies have shown better survival benefits than standard chemotherapies in patients with non-small lung cancer. Better outcomes have been reported in lung cancer patients who developed immune-related interstitial lung diseases (ILD) during anti-PD-1 treatments, suggesting that effector T cells recognized similar antigens presented on both tumors and normal lung tissues. To elucidate the mechanisms underlying the augmentation of antitumor effects in NSCLC patients with ILD, C57BL/6J mice bearing lung metastases were administered with anti-CTLA-4 mAbs, LPS, EGFR-TKIs or naphthalene combined with anti-PD-1 mAbs. Antitumor effects of anti-PD-1 treatment were enhanced in mice which developed lung inflammation. Further analysis revealed that PD-1 treatment increased CD4+ and CD8+ T cells and decreased regulatory T cells and myeloid-derived suppressor cells. These findings help clarify the mechanisms of the enhancement of antitumor effects in patients with ILD.

P-2233

Can PD-L1 expression by biopsy specimen accurately reflect its expression of the entire tumor in gastric cancer?

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Background: Nivolumab is the first effective immune checkpoint inhibitor in advanced gastric cancer (GC) regardless of PD-L1 status. The aim of this study is to investigate whether the evaluation of PD-L1 status by biopsy specimen can reflect its expression of entire tumor under hypothesis that intratumoral heterogeneity of PD-L1 status exist in GC. Materials and methods: A total of 119 patients with advanced GC after radical resection between 2005 and 2014 were eligible for this study. Both paired biopsy and resection samples in the same patients were used for evaluation of PD-L1 status by IHC staining. Results: The sensitivity, specificity, accuracy of evaluation by biopsy samples were 62.1%, 83% and 71.4%, respectively. Furthermore, there was a positive correlation between the number of biopsy fragment and the accordance rate reached 80% when the number of biopsy fragment was 4 or more.Conclusion: The findings in the current study suggested that evaluation of PD-L1 status by biopsy specimen can reflect its expression of entire tumor and that more than 4 biopsy fragments are required to evaluate PD-L1 status in GC more accurately in clinical setting.

▲TOP

P-2234

Identification of therapeutic-specific mutations induced by anti-cancer drug using pancreatic cancer xenograft

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Immune checkpoint inhibitors caused a paradigm shift in cancer treatment and began to be used for several cancers. Especially strong effect was shown in cancer that had a lot of gene mutations. However only 20 to 30% of patients are effective. So it is important to take measures for patients with few gene mutations. The final goal of this study is to find a novel target for solid tumor with few gene mutations for pancreatic cancer. Patient-derived xenograft (PDX) has been used for developing a novel anti-cancer treatment because it maintains patient original tumor structure and character. We had established pancreatic cancer PDXs, and made anti-cancer drug treatment model (gemcitabine and nab-Paclitaxel) using 10 kinds of those PDXs, and analyzed the genome sequence of the tumors by next generation sequencer. In the results, there was no common mutation, but there were 1 to 13 gene mutations for each PDX after anti-cancer drug treatment. These mutations were not detected in no treatment tumors of PDXs, normal tissues and tumor tissues from the original patient. These anti-cancer drug specific mutations can be therapeutic targets for personalized medicine of pancreatic cancer.

P-2235

Low density neutrophils (LDN) in postoperative peripheral blood may assist the recurrence of gastrointestinal cancer

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Background: LDN are known to be increased in peripheral blood of cancer patients, but their role remains unknown. Here, we examined the frequency and function of LDN in patients who received abdominal surgery for gastrointestinal cancer. Method: In 77 patients, peripheral blood was collected before and after operation. After Ficoll centrifugation, mononuclear cell layer was collected and CD66b(+)CD45(+) cells were defined as LDN. Then, LDN was separated from CD66b(-) mononuclear cells by MACS method and examined for their function in vitro. Results: The ratio of the LDN in preoperative samples were low (M=2.04%, 0.04-21.1 %), but significantly increased after surgical procedure (M=10.3%, 0.12-86.2%). The LDN in postoperative samples were immature phenotype and the ratio was positively correlated with operation time and blood loss. Short time culture of the LDN produced massive neutrophil extracellular traps (NETs), which efficiently trapped tumor cells. Finally, LDN strongly suppressed the growth of co-cultured autologous T cells. Conclusion: Many LDN are recruited in peripheral blood by surgical stress and produce NETs which may have supportive roles for tumor recurrence.

P-2236

Relationship between diversity of CD8+ T cell exosomes and destruction of mesenchymal tumor stroma

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Exosomes (Exs) released from single cells have been thought to be diverse populations in membrane structures, membrane charges and bioactive substances. We have reported that CD8+ T cell Exs can deplete mesenchymal tumor stromal cells and suppress tumor invasion and metastasis. In this study, we examined the diversity of CD8+ T cell Exs and destruction of mesenchymal tumor stroma. Murine CD8+ T cell Exs were separated according to the negative charge with ion-exchanged column after concentration with ultrafiltration of culture supernatant. Each fraction of CD8+ T cell Exs was investigated for vesicle number, protein amount, RNA content, expression of tetraspanin molecule, capacity of MSC engulfment, and inhibitory effect of MSC-dependent spheroid formation. By the elution with NaCl gradient, it was clarified that CD8+ T cell Exs in each fraction were different in protein amount, RNA content, and expression of tetraspanin molecule. Interestingly, capacity of MSC engulfment and inhibitory effect of spheroid formation were found only in Exs in specialized fractions with weak negative charge, indicating that a part of CD8+ T cell Exs have capacity for destruction of tumor stroma.

▲TOP

[P-2242] P12-6 [Japanese]
Cancer immunity (3)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Koichi Kawamoto / Kinki Regional Bureau of Health & Welfare

P-2242

The role of tumor-associated neutrophils (TAN) in the progression of hepatocellular carcinoma (HCC)

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[Background]TAN and other immune cells in the progression of HCC is unclear.[Method]Sixty-four patients who underwent curative hepatectomy for HCC in our institution were enrolled. Immunohistchamical staining was performed with CD66b and CD8 antibody to evaluate TAN and CD8+ T lymphocytes, respectively. Expression high or low was determined by cut-off value of each number of immune cells. We counted the number of each immune cells in both tumor (intra-tumor) and the tumor marginal area (peri-tumor). The relationship between the infiltration of each immune cell and clinicopathological factors was examined.[Result]There was no difference in between background clinicopathological factors and the number of TAN and CD8+cells in either intra-tumor or peri-tumor areas.Overall survival was significantly better in the group with high CD8+cells in peri - tumor area compared to the low group ($p = 0.0491$), intriguingly though CD8+cells in intra-tumor area was not associated with prognosis. TAN was not involved in the prognosis either in intra-tumor or peri-tumor areas.[Conclusion]Infiltration of CD8+cells not in intra-tumor but in the peri-tumor area may be involved in the progression of HCC.

▲TOP

P-2243

Differentiation and cloning for anti-tumor immunity of intratumoral B cells in gastric cancer

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[Background] We previously reported that CD20+ B cells are present in the form of Tertiary Lymphoid Structure (TLS) in gastric cancer, that patients with high infiltration CD20+ B cells have a good prognosis and that there is significant correlation between CD20+ B cells and CD8+ T cells infiltration. In this study, we investigated the phenotype and the function of intratumoral B cells.[Methods] We used flow cytometry to examine each B cell subset in tumor based on the expression of IgD and CD38, and examined the expression of HLA-ABC/DR, CD80/CD86 and CD27/CD70. In addition, we analyzed the B cell receptor (BCR) gene by PCR and investigated its clonality.[Results] Compared with other regions, intratumoral B cells showed a decrease in the expression of IgD and an increase in that of CD38, and there was an increase in plasma cells. Moreover, intratumoral B cells expressed CD80/CD86 and CD70 in addition to HLA-ABC/DR. The PCR results showed a trend of cloning in BCR gene expression. [Conclusions] These results suggest that intratumoral B cells in gastric cancer are antigen sensitized and differentiated in tumor and function as antigen-presenting cells and antibody-producing cells.

P-2244

EZH2 inhibitors can restore epigenetically silenced CD58 expression of B-cell lymphomas

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CD58 is an adhesion molecule important for antigen-presenting cells to bind to and form an immunological synapse with T and NK cells. Downregulation of CD58 is one of the popular immune escape mechanisms of lymphoid malignancies, and reported to be caused by both genetic and non-genetic factors. We hypothesized that restoration of epigenetically silenced CD58 expression would facilitate immune recognition of lymphoma cells. We selected CD58-low B-cell lymphoma cell lines with or without CD58 gene mutations, and performed epigenetics compound library screening to seek for agents that can restore their CD58 expression. We found that 3 different EZH2 inhibitors specifically restored CD58 expression of CD58-unmutated lymphoma cells. EZH2 inhibitors could upregulate CD58 expression in various lymphoma cell lines, irrespective of EZH2 mutation status, and enhance IFN-γ production of allogeneic T cells that were co-cultured with these cell lines. Gene expression profiling and quantitative PCR analyses revealed that EZH2 inhibitor can upregulate not only CD58 but also HLA class I gene expression. Our data indicate a potential usefulness of EZH2 inhibitors for immunotherapy of lymphomas.

P-2245

Effects of laughter on a comprehensive immune profile in cancer patients -Initiative On Smile And Cancer (iOSACA)-

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It has been reported that reduction of emotional stress and improvement of immune function are induced by a single event of "laughter" in the patients with cancer and other diseases. We made the iOSACA project, a clinical study to investigate the effect of serial experiences of "laughter" on self-efficacy, quality of life, and a comprehensive immune profile in patients with cancer. The patients enjoyed a series of comical talk shows called "Warotemae gekijyo" by Rakugo storytellers and Manzai comedians twice a month (totally 4-8 times) in our institute. Then, we examined the change of various fractions of circulating immune cells and cytokine producibility in peripheral blood cells stimulated with Con A or LPS. Relative ratio of M2-type monocyte fraction was increased and IL12p40 production was enhanced in cancer patients, and they were positively correlated. These results suggest that M2-type monocytes might shift to IL12-producing M1-type ones by experiences of "laughter". In future, we will confirm the reproducibility. This study was performed with iOSACA project team (Kitasaka, M., Morishima, T., Higeno, A., Idota, A., Sato, A., and Sakon, M).

▲TOP

[P-2251] P14-28 [English/Japanese]
Hepatocellular carcinoma (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-2251

The impact of obtaining sustained virological response on the outcomes in HCC patients with hepatitis C

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Aims: To elucidate the transition of outcomes and the impact of sustained virological response (SVR) on the prognosis in HCC patients with hepatitis C (C-HCC) who underwent hepatecotmy.**Patients and methods:** This study included 316 patients who underwent hepatectomy between 2002 and 2017. The treatment period was divided into two groups; early: 2002-2012, late: 2013-2017. The patients treated with antiviral therapy were divided into SVR and non-SVR groups, and compared including the non-treated group.**Results:** Antiviral therapy was performed significantly in the late group (65% vs. 31%, P<0.001), and the SVR rate was also high in the late group (88% vs. 66, P<0.001). The 3 year recurrence rate was significantly better in the late group (48.4% vs. 67.8%, P=0.012). The survival curve was close in the two groups up to 15 months postoperatively, whereas the two curves were apart after 15 months. There were 185, 100 and 31 patients in the non-treated, SVR and non-SVR groups, respectively. The 3-recurrence rate was 68.1%, 53.9% and 60.8%, respectively, and the SVR group was significantly better (P=0.029). **Conclusion:** The obtaining SVR in C-HCC patients has a significantly better prognosis.

▲TOP

P-2252

Impact of liver cirrhosis on the development of hepatocellular carcinoma in the various liver diseases

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Aim: We examined clinically by the meta-analysis how the incidence of developing HCC is increasing in the cirrhotic state due to various liver diseases, and compred with those in the non-cirrhotic state. Methods: A search of Pub Med database was carried out (1989-2017) in each liver disease to search for English-language studies published concerning the follow up results for the development of HCC. Meta-analysis was performed in earch liver disease. Results: Annual incidence (%) of HCC in the non-cirrhotic stage and cirrhotic stage and the ratio of incidence of HCC in cirrhotic stage / non-cirrhotic stage are as follows. (1)HBV liver disease; 0.32%→3.48%(10.9fold), (2)autoimmune hepatitis(0.09%→0.88%, 9.8fold), (3)primary biliary cholangitis(0.15%→1.71%, 11.4fold), (4)NASH(0.06%→2.39%, 39.8fold). With regard to primary hemochromatosis and alcoholic liver diseases, only follow up studies in cirrhotic stage were presented; 2.47%, 2.10% respectively. Conclusions: Once liver diseases reached to liver cirrhosis, the incidence of developing HCC is markedly elevated. We must survey the development of HCC intensively with US and MRI(CT), irrespective of the kinds of liver diseases.

P-2253

Clinical manifestation and the pathology of spontaneous regression seen in hepatocellular carcinoma (HCC)

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Purpose: Spontaneous regression of cancer has been reported such as Renal cell renal cell carcinoma and lymphomas, however, rare with HCC. Four HCC cases of spontaneous regression are assessed for their clinical feature and their possible mechanism. Patients: Out of 142 HCC, four patients (age 57 to 85, all male) with pathologically proven HCC showed decrease in size without treatment on CT and MRI.Results: Patient 1 with interstitial pneumonia revealed liver tumor of 65 mm in diameter, that diminished to 28 mm on the next year then re-expanded to 43mm half a year later. Biopsy revealed poorly differentiated HCC. Poorly enhancing mass was seen smaller than the previous hospital in Patient 2 and 3. In Patient 3, portal tumor embolism developed three months later. Patient 4 developed tumor necrosis and decrease before the surgery. Surgical specimen revealed aggressive HCC at the edge of the necrotic mass.Discussions and Conclusion: Our cases of spontaneous regression of HCC were considered to be hemorrhagic necrosis resulted from hypervascular feature of HCC. We noted the tumor re-growth and/or residual viable cells which can give rise to recurrence.

P-2254

DEPDC5 deficiency contributes to resistance to leucine starvation via p62 accumulation in hepatocellular carcinoma

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Decrease in leucine is known to promote liver carcinogenesis in patients with chronic liver disease, but the mechanism is unclear. We herein established hepatocellular carcinoma (HCC) cells knocked out for DEPDC5 by using the CRISPR/Cas9 system, and elucidated that cell viability of the DEPDC5-KO cells was higher than that of the DEPDC5-WT under leucine starvation. Considering that autophagy deficiency might be involved in acquired resistance to leucine deprivation, we observed reduction of LC3-II followed by accumulation of p62 in the DEPDC5-KO, which induced ROS tolerance. DEPDC5 overexpression suppressed cell proliferation and tumorigenicity in immunocompromised mice, and triggered p62 degradation with increased ROS susceptibility. In clinical specimens of HCC patients, decreased expression of DEPDC5 was positively correlated with p62 overexpression, and the PFS and OS were worse in the DEPDC5-negative cases than in the DEPDC5-positive. Thus, DEPDC5 inactivation enhanced ROS resistance in HCC under the leucine-depleted conditions of chronic liver disease, contributing to poor patient outcome. It could be a potential target for cancer therapy with oxidative stress control.

▲TOP

P-2255

Anticancer effects of heat shock on liver cancer via autophagic degradation of aquaporin 5

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Background: Previous studies described that the expression of aquaporin 5 (AQP5) was altered in tumors of various organs. In the present study, heat shock-induced changes in AQP5 expression were evaluated by immunofluorescent staining (IF) and western blotting (WB) of liver cancer cells. Methods: AQP5 knockdown experiments or a heat shock treatment were conducted, and their effects on cell volume, proliferation, cell cycle, the activity of apoptosis and migration/invasion were compared. Cycloheximide (CHX) chase experiments and double IF of AQP5 and light chain 3B (LC3B) were performed to investigate the mechanisms. Results: The results showed that IF and WB revealed decrease in AQP5 expression on cellular membranes and in the cytoplasm of heated cells. AQP5 knockdown and heat shock similarly decreased cell volume, suppressed migration/invasion and proliferation, and induced apoptosis and partial G0/G1 arrest. CHX chase experiments revealed that heat shock accelerated the degradation of AQP5, which was rescued under CHX and the autophagy inhibitor, bafilomycin A1 (BafA1). Double IF showed the co-localization of AQP5 and LC3B on BafA1-treated heated cells.

▲TOP

[P-2262] P14-30 [English/Japanese]
Biomarkers for hepatobiliary pancreatic cancers

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Shunsuke Kato / Dept. Clin Oncol, Juntendo Univ. Grad. Sch. Med.

P-2262

Low expression of circRNA-HIPK3 as potential hepatocellular carcinoma biomarker

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Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, with poor prognosis and high risk of recurrence. Circular non-coding RNA (circRNA) is highly conserved and stable covalently closed RNA circles with gene-regulatory potential. Thus, we aim to investigate the clinical relevance of circHIPK3 in HCC.We analyzed clinical specimens from 152 pairs of HCC and corresponding normal liver (NL) tissues. CircHIPK3 expression levels were determined by quantitative real-time PCR (qRT-PCR). CircHIPK3 expression was significantly down-regulated in HCC tissues compared to corresponding NL tissues (P=0.036). In HCC patients, circHIPK3 expression was strongly suppressed in more advanced tumors (P<0.001). Further correlation analysis showed that circHIPK3 expression was significantly associated with T-stage (P<0.001), TNM stage (P<0.001), BCLC stage (P<0.001), and alpha-fetoprotein (AFP) expression (P=0.033).We have determined clinical significances of circHIPK3 expression in HCC, which may provide clinical evidence for the potential of circHIPK3 as novel markers for diagnosis and predicting prognosis in HCC patients.

▲TOP

P-2263

Serum pyruvate dehydrogenase kinase 3 as a prognostic marker for cholangiocarcinoma

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Pyruvate dehydrogenase kinase (PDK) is a Ser/Thr kinase that inactivates mitochondrial pyruvate dehydrogenase and plays a key role in aerobic glycolysis which is a hallmark of cancer. The aims of this research are to determine the PDK in cholangiocarcinoma (CCA) and evaluate whether they could be used as bio-markers for CCA. The expression patterns of PDK isoforms in CCA tissues were examined for 15 CCA cases using immunohistochemistry. The PDK isoforms levels in the sera were measured for 39 CCA, 20 benign biliary diseases (BBD) and healthy groups using dot blot assay. The differential expressions of mitochondrial proteins of CCA tissue were analyzed using mass spectrometry. We found 27-folds overexpression of PDK3 in cancerous tissues compared to the adjacent non-cancerous tissues. Immunohistochemical results showed that PDK1, 2 and 3 were over-expressed in cancerous tissues. When PDKs level in the sera were examined, the PDK3 level was higher in CCA than in both BBD and healthy groups. The sensitivity and specificity of PDK3 as a marker for CCA was 33% and 95%, respectively, and high PDK3 correlated with short survival of CCA. PDK3 can be used as a prognostic marker for CCA.

P-2264

Prediction of intrahepatic recurrence after surgery for hepatocellular carcinoma

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Background: Post-operative intrahepatic recurrence is a significant problem for patients with hepatocellular carcinoma (HCC) in terms of the leading cause of post-operative death. Therefore, it is essential to determine the risk factors of recurrence which can be used for early detection and intervention of recurrence. Method: The gene expression level of inflammation-related genes (IL8, TNF, IL6, IFNG, TGFB1, IL12A, IL12B, IL17, FOXP3) in tumor and background non-tumor liver tissue were extracted from the microarray data composed of 27 cases with surgical resection. Disease free survival (DFS) were evaluated using the Kaplan-Meier method. Result: The DFS period after liver resection for the low IFNG or IL17 group in background non-tumor liver tissue was found to be significantly longer than that for the high IFNG or IL17 group, nevertheless there were no significant differences in the clinical background including the status of virus infection. Conclusion: The differences in gene expression profile of background non-tumor liver tissue may be associated with the risk of intrahepatic recurrence for HCC.

P-2265

High levels of APEX1 in sera from patients with cholangiocarcinoma

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Cholangiocarcinoma (CCA) is a cancer that arises from the cells within the bile ducts. Although several serum markers have been used for aiding of diagnosis, they are still low sensitivity and specificity. We aim to find a new CCA biomarker from our secretome database of CCA cell lines.The candidate proteins were selected from three CCA secretomes, which were not expressed in control immortalized cholangiocyte cell line using In-gel digestion coupled with mass spectrometric analysis (GeLC-MS/MS). The candidate proteins were analyzed by using bioinformatics approaches. To validate the selected protein as a marker, the sera from CCA (n=40), benign biliary disease (BBD)(n=19) and healthy controls (n=20) were collected and the levels of the selected protein were estimated semi quantitatively by using dot blot method. To confirm the APEX function, CCA cell lines were depleted of APEX1.APEX1 was selected as a serum marker for CCA. The APEX1 levels in sera of CCA were significantly higher than that of BBD and healthy controls. The migration and invasion activity of APEX1-depleted cells were lower than those of wild type cells. In conclusion, APEX1 level is a promising marker for CCA.

▲TOP

P-2266

TGF-β 1: A Potential EMT-Biomarker for prediction of cholangiocarcinoma

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Cholangiocarcinoma (CCA) is a cancer arising from bile duct epithelium caused by liver fluke infection via chronic inflammation process. Epithelial mesenchymal transition (EMT) has been proved that plays role in cancer progression. The objective of this study is to investigate the expression of EMT proteins which are E-cadherin, transforming growth factor- β 1 (TGF- β 1) and bone morphogenetic protein-7 (BMP-7) in CCA tissues and to measure the level of candidate protein in sera of CCA patients by ELISA. The immunohistochemistry results demonstrated that high expression of TGF- β 1 and BMP-7 was observed in bile duct cancer whether E-cadherin was expressed low. Only patients showed high TGF- β 1 expression were significantly correlated with poor prognosis, lymph nodes metastasis and recurrence status. Moreover, serum TGF- β 1 level was significantly elevated in CCA patients compared to normal and was effective in distinguishing CCA patients from normal at cut-off was 38.54 ng/mL with high sensitivity (71%) and specificity (69%). In conclusion, our results suggest that TGF- β 1 could be a potential EMT-biomarker for prediction of CCA.

P-2267

A novel cancer stem cell biomarker CD44v9 in liver fluke-related cholangiocarcinoma

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Opisthorchis viverrini (OV) is a major cause for high incidence of cholangiocarcinoma (CCA) in northeastern Thailand. Owing to its poor prognosis, the development of biomarker for early diagnosis is required. One of cancer stem cell biomarkers, CD44 variant (CD44v), was overexpressed in several cancer cells. Here we examined the new role of CD44v9 as a specific biomarker of OV-related CCA (OV-CCA). Immunohistochemistry revealed that intensity of CD44v9 was significantly higher in cancer cells of OV-CCA tissues (n=33) compared to bile duct cells of normal liver (n=21) and Non-OV CCA tissues (n=98). The CD44v9 expression was observed in the half cases in Non-OV CCA and ninety percent cases of OV-CCA tissues, particularly high positive score in OV-CCA patients. To clarify the relation between CCA and inflammation, S100P and COX-2 expressions were analyzed, and those biomarkers showed high expressions in OV-CCA tissues, significantly. In conclusion, CD44v9 is a promising molecule to be served as a potential marker for OV-related CCA. We will confirm the expression levels of the target molecules using human CCA cell lines and further study on CD44v9 function in CCA.

P-2268

Hypoglycemia predicts survival after hepatectomy for hepatocellular carcinoma: A propensity score-based analysis

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Background Whether hypoglycemia affects surgical survival in patients with hepatocellular carcinoma (HCC) is controversial. Here we retrospectively evaluated the impact of hypoglycemia on survival in HCC patients after curative hepatectomy.Methods Patients with Child-Pugh A liver function who underwent curative hepatectomy between 2004 and 2013 were categorized as hypoglycemia (blood-glucose <3.9 mmol/L, n=172) and euglycemia (3.9-6.0mmol/L, n=504). Propensity score-matched analysis was performed in the ratio 1:1 using a caliper width of 0.1. Surgical survival were compared for 172 hypoglycemia and 167 euglycemia patients.Results Estimated overall survival (OS) rates at 1, 3, and 5 years were 82.6%, 58.1% and 53.4% in the hypoglycemia and 83.3%, 65.9%, and 52.1% in the euglycemia group, respectively (P = 0.298). In the propensity-matched cohort, estimated OS rates at 1, 3, and 5 years were 82.6%, 58.1% and 53.4% in the hypoglycemia and 95.2%, 88.6%, and 79.5% in the euglycemia group, respectively (P <0.001).ConclusionOur propensity score-matched analysis strengthens the case that hypoglycemia predicted poor survival after curative hepatectomy for hepatocellular carcinoma.

▲TOP

[P-2274] P14-32 [English/Japanese]
Surgical treatment of hepatobiliary pancreatic cancers (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-2274

Laparoscopic hepatectomy for the elderly patients

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The laparoscopic surgery is less invasive and better cosmetic. The laparoscopic liver resection has been accepted for the insurance in April 2010, increasingly performed in many hospitals, even for the poor liver functional cases or elderly cases. We will report the result of the laparoscopic liver resection for the elderly patients.From June 2010 through December 2017, 443 cases of laparoscopic hepatectomy were undergone for liver cancer in our hospital, and 63 of 443 cases were over 80 years old (the elderly group). Now we compared patient factors and surgical results.The hepatic resection range (Hr 0:S:1:2) in the elderly or the non-elderly group was 41:5:11:6 / 269:13:61:37 (p=0.371), the operation time was 327 minutes : 342 minutes (p=0.493), the blood loss was 301 ml : 217 ml (p=0.582), and the hospital stay after operation was 20.8 days : 14.5 days (p=0.176). There were 4 cases / 16 cases of bile leakage (p=0.507), 9 cases / 28 cases of SSI (p=0.083).The laparoscopic hepatectomy for liver cancer could be performed in safety, even for the 80 or more years old patients.

▲TOP

P-2275

Efficacy and safety of laparoscopic repeat liver resection for the recurrence of hepatocellular carcinoma

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【Introduction】The efficacy and the safety of laparoscopic repeat liver resection for the recurrence of hepatocellular carcinoma are unclear. We investigated about the surgical results of laparoscopic repeat liver resection for the recurrence of hepatocellular carcinoma.【Patients and methods】We performed laparoscopic liver resection for 302 patients with hepatocellular carcinoma from May 2010 to March 2018. The number of patients performed laparoscopic first liver resection was 244, and laparoscopic repeat liver resection was 58. In these patients, we investigated about the patients' backgrounds and surgical results.【Results】There were no significant differences between the first liver resection and the repeat liver resection in Child-Pugh (A/B/C: 227/17/0 vs 56/2/0, p=0.546) and liver damage (A/B/C: 180/63/1 vs 37/20/1, p=0.142). There were no significant differences in blood loss (p=0.513) and postoperative hospital stay (p=0.719). There were no significant differences in the incidence of biliary fistula.【Conclusion】It is conceivable that laparoscopic repeat liver resection is safety and effective surgery for the recurrence of hepatocellular carcinoma.

P-2276

Curability is the only prognostic factors of ampullary carcinoma: analysis of 47 resected cases

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Introduction: The prognostic factors after resection of ampullary carcinoma have not been identified.
Methodology: Between 2000 and 2017, consecutive 47 patients with carcinoma of the papilla of Vater underwent pancreatoduodenectomy (PD) with dissection of regional lymph nodes in Ohta Nishinouchi General Hospital. We retrospectively analyzed surgical procedures, macroscopic and microscopic curability, clinicopathologic variables, adjuvant chemotherapy and survivals.
Results: A total of 47 patients underwent PD aged 44 to 88 years and consisted of 26 males and 21 females. The overall and disease-specific 1-, 3-, 5-, 10-year survival rates were 84.3%, 67.2%, 44.0%, 40.4%, and 90.3%, 59.9%, 51.4%, 51.4%, respectively. Patients were grouped according to Japanese classification of biliary tract carcinoma as Stage 0 (n=4), Stage IA (n=5), Stage IB (n=12), Stage IIA (n=8), Stage IIB (n=17), Stage III (n=1). Log Rank test revealed significant differences only in curability.
Conclusions: Curability is the key of long-survival, therefore, radical surgery seems to confirm long-term survival in patients with ampulla of Vater carcinoma.

P-2277

Prognostic relevance and constitutive alteration of TLO following neoadjuvant chemoradiotherapy in Pancreatic cancer

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In pancreatic cancer having extremely poor prognosis, response to preoperative neoadjuvant chemoradiotherapy (NAC) has been reported as one of the prognostic markers. In this study, we investigated novel biomarkers to reflect prognosis following chemoradiotherapy, focusing on tertiary lymphoid organs (TLO) appeared in the tumor microenvironment. We retrospectively analyzed clinical relevance of TLO, dividing patients into 2 groups, those who underwent NAC and those who performed upfront surgery (Surgery First; SF). Cellular components within TLO were analyzed by immunohistochemistry (IHC). In IHC analysis, a proportion index of CD8+ cytotoxic T lymphocytes, PNAd+ high endothelial venules (HEV), CD163+ macrophages, and Ki-67+ cells within TLO were higher in NAC group than in SF group. In contrast, a proportion of PD-1+ immunosuppressive lymphocytes within TLO were lower in NAC group than in SF group. Our study demonstrated that preoperative chemoradiotherapy could change tumor microenvironment through the TLO in pancreatic cancer and contribute to patients' prognosis.

▲TOP

[P-2278] P14-33 [English/Japanese]
Breast cancer (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Hiroko Yamashita / Breast Surg., Hokkaido Univ. Hosp.

P-2278

Development of a Tri-Specific Antibody Guiding Liposomal Drugs to Breast Cancer Cells and Cancer-Associated Fibroblasts

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Cancer-associated fibroblasts (CAFs) contribute to tumor progression and hinder therapeutic efficacy by secreting growth factors and forming physical barriers, which make CAFs considered to be a potential target for cancer therapy. We developed an anti-HER2/anti-FAP/anti-mPEG tri-specific antibody (TsAb) allowing methoxypolyethylene glycol(mPEG)-coated liposomal drug to target breast cancer cells and CAFs by a simple non-covalent modification. After TsAb was attached mPEG of liposomal doxorubicin (Lipo-Dox), TsAb-Lipo-Dox selectively bound HER2 (breast cancer surface marker) and fibroblast-activated protein (FAP, CAFs' surface marker). Compared to single- or non-targeting Lipo-Dox, TsAb-Lipo-Dox exhibited significant cytotoxicity against each cell line or co-culture of MCF-7/HER2-18 ($HER2^+$ breast cancer cells) and WS-1/FAP (FAP^+ fibroblasts), and also improved inhibition of tumor growth in a xenograft model of co-implanted breast tumor. TsAb was retained on Lipo-Dox in vivo and did not affect PK profile of Lipo-Dox. Our data indicate TsAb will be able to enhance the efficacy of clinical mPEG drug for solid tumor.

▲TOP

P-2279

Critical pathophysiological roles of a potential therapeutic target RHBDL2 in triple negative breast cancer

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Triple negative breast cancer (TNBC) is a highly diverse subtype of breast cancers. We here report the critical roles of a cancer specific RHBDL2 (Rhomboid-like-2), a member of Rhomboid serine protease, in chemoresistance and malignancy of TNBC. RHBDL2 overexpression in TNBC conferred a worse prognosis. RNAi-mediated knockdown of RHBDL2 led to the significant reduction of cell growth of TNBC. Notably, ectopic overexpression of wildtype RHBDL2 in HCC1806 cells that expresses a low level of RHBDL2, but not mutant RHBDL2 which abolishes protease activity resulted in significant enhancement of cell proliferation, invasion and resistance to doxorubicin, docetaxel and paclitaxel, respectively. Furthermore, we demonstrated that RHBDL2 interacted with SLC1A5, a glutamine transporter, thereby promoting glutamine uptake into TNBC cells. More importantly, anti-RNHBDL2 antibody treatment led to significant reduction of glutamine uptake, resulting in significant suppression of TNBC cell growth. These findings suggest that RHBDL2 play critical roles via its Rhomboid protease activity for TNBC cells, and may be a promising therapeutic target for TNBC treatment.

P-2280

Periostin exon17 fragments in breast cancer cells is required for tumor metastasis

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In metastasis, aberrant alternative splicing is increasingly linked to cancer development and progression. Periostin (PN) is a 93 kDa matricellular protein, and which N-terminus is conserved, but the C-terminal exon17-23 regions gives rise to different splice isoforms upon alternative splicing. It has known that PN is highly expressed in cancers, however the report on fragments of PN splice variants is rare in malignancy. In this study, PN overexpression was involved in the proteolytic cleavage of PN exon17. We showed that Ex17ab inhibited growth of primary tumors as well as metastatic tumors in vivo in TNBC. Notably, PN exon17 localized around the periphery of TNBC cells in metastatic lung. In a secreted fraction from fibroblasts, 35-50 kDa fragments were detected and identified by LC-MS/MS. Furthermore, the fragments could not be recognized by anti-full-length PN exon12 ab. In molecular interaction analysis, it was revealed that PN having exon17 specifically bound to Wnt3a. Our conclusion is PN exon17 from fibroblasts selectively attached to cancer cells, and Ex17ab inhibits metastasis of breast cancer.

P-2281

Increased chemosensitivity by the depletion of TREX2 components: R-loop-dependent or independent mechanism?

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DSS1 (deleted in split-hand/split-foot malformation 1) was originally identified as a BRCA2-associated protein, and its downregulation results in the degradation of BRCA2. Previously, we investigated whether altered expression of DSS1 was associated with malignant advancement of sporadic breast cancers and the high DSS1 expression groups in breast cancer patients showed worse prognosis in relapse-free survival. Consistent with the cohort study, we demonstrated that high expression of DSS1 increased resistance of breast cancer cells to cytotoxic chemotherapy in vitro. Conversely, DSS1 knockdown increased the susceptibility to these drugs, though BRCA2 depletion did not affect chemosensitivity. Similar results were observed in PCID2-overexpressed or -depleted cells, suggesting that deregulated TREX2 component including DSS1 and PCID2 may be involved in chemosensitivity. We are currently analyzing whether this mechanism is dependent of R-loop formation or not.

▲TOP

P-2282

Nuclear localization of intracellular domain of LRP1B predicts poor outcome in invasive ductal carcinoma of the breast

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We have been investigating the role of ARID1A in carcinogenesis and screened the downstream genes for ARID1A using cDNA microarray. Among many candidates, we focused on LRP1B (LDL receptor-related protein 1B), which is relocated to nucleus in a γ-secretase dependent fashion. To investigate the expression of LRP1B in human breast cancer in vivo, we performed immunohistochemistory using surgical specimens in Gifu University Hospital. Fifteen of 92 (16.3%) was positive in nucleus, 60of 92 (65.2%) was positive only in surface membrane and cytoplasm, 17 of 92 was negative in both nucleus and cytoplasm. The prognosis of nucleus-positive patients was worse than that of only cytoplasm-positive patients, especially with luminal A type breast cancers. Next, we performed cDNA microarray using tet-on inducible system for LRP1B. One of the most up-regulated molecules was a long noncoding RNA, NEAT1, in MCF-7. We confirmed these overexpression in breast cancer cells using quantitative RT-PCR in vitro. Present finding indicated that nuclear localizes intracellular domain of LRP1B promoted breast cancer progression with a poor prognostic value, possible though NEAT1 pathway.

P-2283

Differential prognostic relevance of promoter DNA methylation of CDO1 gene and HOPX gene in primary breast cancer

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Background and method
We have previously identified that promoter DNA methylation of cysteine dioxygenase type 1 (CDO1) gene and homeobox only protein homeobox (HOPX) gene were both cancer specific, and have clinical potential as prognostic biomarkers in breast cancer (BC). This research is to compare the differential prognostic relevance by the combination analysis.Methylation levels were quantified in 7 BC cell lines and 133 BC patients by TaqMan methylation specific PCR and functional traits were explored for CDO1.
Result
1.The relation of TaqMeth values of CDO1 to HOPX was statistically significant(r^2=0.07, p<0.01).
2.CDO1 hypermethylation was rather accurately representative of poorer prognosis than HOPX in any combined patterns.
3.Multivariate Cox proportional hazards model identified CDO1 hypermethylation and Ki-67 positive were independent prognostic factors related DSS(p=0.02, p<0.01).
4.Overexpression CDO1 was decreased the anchorage-independent growth, but did not affect cancer invasion.
Conclusion
CDO1 was a definitely tumor suppressor gene to suppress anchorage-independent growth, while its prognostic relevance was more than expected in the context of its functional relevance.

▲TOP

[P-2290] P14-35 [English/Japanese]
Breast cancer (3)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Takayuki Kinoshita / Natl. Cancer Ctr. Hosp.

P-2290

Establishment and pre-clinical test of trastzumab resistant HR-/HER2+ breast cancer patient-derived xenograft model

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Patient-derived xenografts (PDXs) are powerful tools for translational and pre-clinical cancer research. Although trastzumab, a humanized monoclonal antibody to target HER-2, is broadly used for the therapy of early and advanced breast cancer patients, many patients either do not respond to treatment with trastzumab or undergo disease progression following therapy. Here, we established the two PDXs of hormonal receptor (HR)-/HER2+ breast cancers with trastzumab resistance and conducted in vivo drug sensitivity test with PX-478 and neratinib for optimizing the therapeutic strategies. The tumor volumes of both PX-478 single and the combination groups were significantly reduced relative to control. However, there was not the effect for the single treatment of neratinib. Staining of Ki-67, cell proliferation marker, was more reduced in both PX-478 and the combination groups than that of control and neratinib. Thus, we suggests that inhibition of HIF-1a by PX-478 is an alternative therapeutic strategy against trastzumab resistant HR-/HER2+ breast cancer tumors through in vivo drug examination for the two PDX models. (This study was supported by National Cancer Center, Korea, 1710450-2)

▲TOP

P-2291

Synergistic antitumor effect of eribulin and HDAC inhibitor for triple negative breast cancer

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To develop a novel therapeutic strategy for triple negative breast cancer (TNBC), we tested the combinational effect of Eribulin(ERI) and HDAC inhibitor for TNBC cells in vitro. Two TNBC cell lines (MDA-MB-231, Hs578T) and their ERI-resistant cells (MDA-MB-231/E, Hs578T/E). Vorinostat (VOR), a pan-HDAC inhibitor, and ricolinostat (RICO), a selective HDAC6 inhibitor were used.Prior treatment with VOR or RICO increased the expression of acetylated α -tubulin in both parental and ERI-resistant cells. When low concentration of VOR (0.5 μM) or RICO (0.5 μM) was added with ERI, the sensitivity to ERI was enhanced in parental MDA-MB-231 and Hs578T. When parental cell lines were treated with VOR (5 μM) or RICO (5 μM) for 48 hrs prior to addition of ERI, the sensitivity to ERI was enhanced in both cells. The enhancement of sensitivity to ERI by both the simultaneous addition with VOR or RICO and the prior treatment with VOR or RICO was observed in the ERI-resistant cells as well. Our study demonstrated the possibility that the combination of ERI with HDAC inhibitor might enhance the antitumor effect of ERI through acetylation of α -tubulin by HDAC6 inhibition in TNBC cells.

P-2292

Downregulation of SALL3 by recurrent genetic and epigenetic alterations is involved in triple negative breast cancers

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Triple negative breast cancers (TNBC), defined by the lack of ER, PgR expression and HER2 amplification, is a biologically heterogeneous and clinically aggressive disease. To characterize somatic alterations in TNBC, we performed whole-exome sequencing and qPCR analyses using 36 TNBC resected from patients, and identified a list of epigenetic-related genes which were recurrently mutated and downregulated. Among them, we focus on a transcriptional suppressor, spalt like transcription factor 3 (SALL3) gene, which were observed two somatic mutations and frequent DNA methylation-mediated silencing in TNBC cases. Ectopic overexpression of the wildtype SALL3, but not the somatically mutated SALL3 into BT549 cells significantly inhibited cell proliferation. Notably, knockdown of SALL3 expression in BT20 cells caused chemoresistance to paclitaxel and docetaxel. Importantly, TCGA database analysis showed that downregulation of SALL3 was significantly correlated with poor prognosis of basal like breast cancers. Our findings provide the evidence of a pathophysiological role for SALL3 as a tumor suppressor which is possibly associated with chemoresistance and carcinogenesis for TNBC.

P-2293

Inhibition of autophagy induced accumulation of soluble prorenin receptor in cultured cancer cells

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Prorenin receptor, which is a specific receptor for renin and prorenin, functions as a subunit of Vacuolar-type H〈SUB〉+〈/SUB〉-ATPase (V-ATPase). V-ATPase is a proton pump, which acidifies the inside of the vesicle and is related to autophagy. We have previously found that inhibition of V-ATPase induced accumulation of soluble PRR in cells. We therefore hypothesized that there might be the functional relationship between the production of soluble PRR and the activity of autophagy. In this study, we aimed to clarify the relationship between soluble PRR production and autophagy. The experiments were done in MCF-7 breast cancer cell line and A549 lung cancer cell line. Bafiromycin A1(Baf) or Chloroquine(CQ) was added as the inhibitor of autophagy to the culture medium and the cells were cultured for 72h. Baf or CQ suppressed the cell proliferation and increased the amount of soluble PRR protein in cells. Moreover, these drugs increased the amount of LC3-I and LC3-II (autophagy-related protein) in cells. Our results suggested that suppression of autophagy may promote the production of soluble PRR and soluble PRR may be a marker of inhibition of autophagy in tumor cells.

▲TOP

P-2294

(Pro) renin receptor stimulated proliferation of cultured breast cancer cells via ERK-independent pathway

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(Pro) renin receptor [(P)RR], a receptor for prorenin and renin, is related to both the renin-angiotensin system and V-ATPase. (P)RR may be involved in the tumor biology via ERK 1/2 activation and Wnt/ β -catenin pathway. ERK is activated by insulin and other growth factors and controls cell proliferation and differentiation. The purpose of this study is to clarify the interaction between insulin and PRR in cell proliferation and ERK signaling. The experiments were done in MCF-7 and T47D breast cancer cell lines. Insulin stimulated the cell proliferation of both cell lines. On the other hand, insulin increased protein expression of (P)RR in MCF-7 but not in T47D. Suppression of (P)RR expression by siRNA decreased cell proliferation in MCF-7 and T47D with or without insulin. By suppressing the expression of (P)RR, phosphorylation of ERK was activated in MCF-7, whereas it was suppressed in T47D. In conclusion, insulin and (P)RR are involved in cell proliferation, but the effect on phosphorylation of ERK is different for each cell line. It is suggested that signaling pathways other than the ERK signaling may be involved in cell proliferation by (P)RR.

P-2295

Effects of anti-cancer agents on soluble prorenin receptor expression in cultured human breast cancer cells

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Prorenin receptor (PRR) is expressed in various organs of the whole body, and in various cancers. PRR is a single-transmembrane receptor, and a soluble form of PRR is produced by proteolytic enzymes, such as furin, site-1 protease (S1P) and ADAM19. The mechanism for regulating the expression of soluble PRR, however, has not been clarified yet. The aim of this study is to clarify the effects of anti-cancer agents on expression of full-length PRR, soluble PRR and proteolytic enzymes. T47D breast cancer cell line was used in this study. Expression of full-length PRR, soluble PRR, furin, S1P and ADAM19 was analyzed by western blot analysis. Anti-cancer agents (mitomycinC, paclitaxel, carboplatin and fluvestrant) increased the amount of soluble PRR protein, whereas the protein expression level of the full-length PRR did not change significantly. Paclitaxel increased expression levels of S1P, whereas other anti-cancer agents did not increase expression levels of S1P, furin or ADAM19. In cultured breast cancer cells, the amount of soluble PRR protein in cells is increased by anti-cancer agents. Increased expression of S1P may partly explain increased soluble PRR by paclitaxel.

P-2296

High ceramide levels in breast cancer are associated with low proliferation potency of cancer cells in patients

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Sphingolipid rheostat, a dynamic balance between sphingosine-1-phosphate and ceramide, influences cancer cell fate. However, the clinical relevance of ceramide in breast cancer patients remains unclear. The aim of this study is to reveal the association between ceramide levels in breast cancer and proliferation potency of the cancer cells in patients. Surgical specimens were collected from 44 breast cancer patients and amount of sphingolipid metabolites in cancer tissue were determined by mass spectrometry. The ceramide levels were compared with Ki-67 labelling index or nuclear grade of the cancer. Patients with high Ki-67 labelling index (>30) showed significantly lower ceramide levels in cancer tissue than those with low Ki-67 labelling index (≤30) (p=0.04). Patients with high nuclear grade were significantly associated with low ceramide levels in cancer tissue (p=0.04). For an individual patient, ceramide levels in cancer tissue showed a significant negative association with nuclear grade (p=0.04). We first demonstrated that high ceramide levels in breast cancer are associated with low proliferation potency of the cancer cells in patients.

▲TOP

[P-2304] P14-37 [English/Japanese]
Urothelial cancer (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Masayuki Takahashi / Dept. Urology, Tokushima Univ. Grad. Sch. Biomed. Sci.

P-2304

GPX2 promotes bladder cancer development with squamous differentiation through the control of apoptosis

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Objective: Herein, we elucidated the molecular mechanisms and therapeutic potential of glutathione peroxidase 2 (GPX2) in bladder cancer. Methods and Results: GPX2 expression gradually increased during progression from normal to urothelial carcinoma (UC) in a rat BBN-induced bladder carcinogenesis model. GPX2 overexpression was more marked in UC with squamous differentiation (SqD) than in pure UC. In addition, prognostic analysis using transurethral specimens revealed that low expression level of GPX2 predicted poor prognosis in patients with pure UC. Further, UC cell lines, BC31 and RT4, cultured in vitro also overexpressed GPX2. Knock-down of GPX2 induced significant inhibition of ROS production, in addition to significant growth inhibition and increased apoptosis with activation of caspase 3 or 7 in both BC31 and RT4 cells. Interestingly, tumor growth of BC31 cells subcutaneously transplanted in nude mice was significantly caused the induction of apoptosis by GPX2 down-regulation. Conclusion: Our findings demonstrated that GPX2 plays an important role in bladder carcinogenesis through the regulation of apoptosis against intracellular ROS.

▲TOP

P-2305

Prostaglandin receptors induce urothelial tumourigenesis via modulating PTEN expression

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Background: Expression of PGE2 is elevated in urothelial carcinomas. PGE2 exerts its effects via PGE2 receptor 2 (EP2), EP3, and EP4. Nonetheless, it remains to unclear how EPs are involved in BC initiation and outgrowth. In this study, we investigated the functional role of EPs in urothelial tumorigenesis and progression. Methods: We performed immunohistochemistry in BC tissue microarrays, in vitro transformation assay in a normal urothelial SVHUC line, and western blot/ cell growth assays in BC lines. Results: Positivity of EP2/4 in non-muscle-invasive tumors strongly correlated with disease progression. In SVHUC cells, exposure to a chemical carcinogen increased and decreased the expression of EP2/4 and phosphatase and tensin homologue (PTEN), respectively. Treatment with selective EP2/4 antagonists or celecoxib also resulted in prevention in chemical carcinogen-induced neoplastic transformation of SVHUC cells. In BC lines, EP2/4 antagonists and celecoxib inhibited cell viability and migration, as well as augmented PTEN expression. Conclusions: EP2/4 have an important role in inducing urothelial tumorigenesis and tumor progression via downregulating PTEN expression.

P-2306

The impact of p53 point mutation on the characteristics of BBN-induced mouse bladder cancer

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Introduction and Objectives: A few previous studies have addressed whether BBN-induced mouse bladder carcinomas can be categorized to any of human MIBC subtypes. The aim of the study was to investigate the impact of p53 status on the characteristics of BBN-induced mouse bladder cancer.Materials and Methods: We induced either of Trp53 mutations corresponding to those frequently observed in human MIBC under the control of Upk2 or Krt5promoter. After intraperitoneal Tamoxifen administration, mice were treated with BBN in the drinking water. Furthermore, we performed RNA expression microarray for clustering each tumor.Results: We analyzed 42 wild-type, 31 Trp53 heterozygous and 47 Trp53 mutant mice. Infiltrating tumors were observed in 90.3% (n = 28) of Trp53+/- mice and 53.2% (n = 25) of Trp53-mut mice, while observed in 50% (n = 21) of wt mice. All tumors induced by BBN showed significant enrichment score in TCGA cluster3.Conclusion: Urothelial cells harboring deletion or point mutation of a Trp53 allele may develop invasive bladder UC more rapidly than those with wild-type Trp53. UC induced by BBN indicates TCGA cluster3 regardless of Trp 53 status.

P-2307

Therapeutic effects of the natural flavonoid "Luteolin" on bladder cancer

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INTRODUCTION AND OBJECTIVES: We discussed the effect of luteolin, which is a natural fravonoid with strong antioxidant properties, on bladder cancer. METHODS: T24 and BC31 (human and rat urothelial carcinoma cell lines) were used. Cell viability and apoptosis were assessed using a WST1 assay and flow cytometry. Further, we evaluated the profiles of the proteins after luteolin treatment using western blot. BC31 subcutaneously transplanted nude mice were treated with luteolin (100 ppm) or control diet. All mice were sacrificed at the 5th week after the experimental diets were given. RESULTS: The WST1 assay and flow cytometry revealed a dose-dependent reduction in the number of viable cells and induction of apoptosis by luteolin. The tumor volume in nude mice was significantly reduced by Luteolin intake with no adverse effects. We found that upregulation of both phosphorylated GSK3 β and Thioredoxin-interacting protein (TXNIP) is the major factor involved in the inhibitory effects of luteolin. CONCLUSIONS: Bladder cancer growth was effectively suppressed with luteolin both, in vitro and in vivo via the regulation of apoptosis, phosphorylated GSK3 β and TNXIP.

▲TOP

P-2308

Germline TP53 codon 72 is associated with somatic mutations in bladder cancer

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Objective: Several studies showed germline variants could affect the somatic mutations in some cancers. We previously reported TP53 codon 72 affected the clinical outcomes in bladder cancer (BC). We conducted this study to elucidate the association between TP53 codon 72 and somatic mutations in BC.Material and Method: One hundred three patients (non-muscle invasive, 59; muscle invasive, 44) were enrolled. Germline TP53 codon72 genotype was analyzed by Taqman genotyping assay. Somatic mutations were analyzed with target sequencing. We compared the TP53 codon 72 genotype with the somatic mutations.Results: The allele frequency of TP53 codon72 was no significant difference between our cohort and healthy Japanese population (Arg/Arg, Arg/Pro, Pro/Pro; 37%, 42%, 21%). The patients with Arg/Arg and Arg/Pro genotype had more frequently FGFR3 and PIK3CA mutation than those with Pro/Pro genotype (p=0.003 and p=0.04, respectively). In non-muscle invasive BC, patients with Arg/Arg and Arg/Pro genotype had more frequently FGFR3 mutation (p=0.04), whereas, patients with Pro/Pro genotype had RAS mutation (p=0.02).Conclusion: TP53 codon72 could affect the somatic mutations in BC.

P-2309

Modified Bricker Has Fewer Stoma and Ureteroileal Anastomosis Related Complications Compared with Conventional Bricker

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Purpose: We aimed to assess the stoma and ureteroileal anastomosis related complications of our modified ileal conduit, in which the ureter and ileal conduit are completely extraperitoneal, compared with conventional Bricker urinary diversion. Material and Methods: We retrospectively evaluated data on patients with bladder cancer who underwent radical cystectomy and ileal conduit in our institution between 2000 and 2016. Ileal conduit was created by conventional Bricker or modified technique. Results: Of 245 patients evaluated 145 underwent modified and 100 conventional Bricker. Modified group showed fewer stoma (0.7% vs. 17.0%, P < 0.001) and ureteroileal anastomosis related complications (4.8% vs. 15.0%, P = 0.001) compared with conventional group. In multivariable analyses, modified group was less likely to develop stoma (odds ratio [OR]: 0.027; P = 0.001) and ureteroileal anastomosis related complications (OR: 0.224; P = 0.002).Conclusion: With our modified surgical techniques, early and late complications related to stoma and ureteroileal anastomosis could be reduced and prevented in patients undergoing ileal conduit.

▲TOP

[P-2317] P14-39 [English/Japanese]
Renal cell carcinoma (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

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P-2317

Pathological diagnosis of renal tumors - Reviews of the 683 consultation cases by WHO 2016 classification

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We have reviewed the 683 consultation cases between 2002 and 2018, using WHO 2016 classification along with additional ancillary tests, including immunohistochemistry and molecular biological studies. The patterns of the diagnostic algorithms were as follows; 1) tumors with clear cytoplasm, including clear cell renal cell carcinoma (RCC), translocation-associated RCC and clear cell papillary RCC, 2) tumors with papillary architectures, including papillary RCC and fumarate hydratase (FH)-deficient RCC, 3) tumors with oncocytic cytoplasm, including chromophobe RCC, oncocytoma, succinate dehydrogenase (SDH)-deficient RCC, 4) infiltrative tumor, including collecting duct carcinoma and invasive pelvic urothelial carcinoma, and 5) tumors other than RCC, including epithelioid angiomyolipoma, and juxtaglomerular cell tumor. Through the review, 10 translocation-associated, 5 FH- and 1 SDH-deficient RCCs have been identified. The previous cases of renal tumors require reevaluation to find cases with “hidden” backgrounds to provide the best therapeutic strategies.

▲TOP

P-2318

Three cases of Xp11.2 translocation renal cell carcinoma

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【はじめに】Xp11.2転座型腎細胞癌は成人発生腎癌の 1%を占める稀な腫瘍である。【材料と方法】2005年4月から2018年3月の間に腎癌にて摘出術を受けた患者から 3例のXp11.2転座型腎細胞癌を抽出し、病理学的検討を行い、治療、予後を調査した。【結果】患者年齢は 73, 68, 39歳で、男性：女性は 1:2であった。手術は2例は全摘、1例は部分切除であった。病理学的にはすべてに偽被膜形成があり、腎内転移、腎線維性被膜浸潤はいずれもなかった。Furman gradeは 2例で 2, 1 例で 4であった。術後の病期はいずれも Stage Iであった。フォローアップ期間は15-111か月、平均 50か月で、治療は手術のみが 2例で、1例で術後、リンパ節転移、肝転移をきたし、インターフェロン、ソラフェニブが施行したが、効果なく、自宅療養へと移行した。組織学的には2例では細胞質の豊富な細胞質を有する腫瘍細胞の胞巣状増殖がみられ、1例では淡明細胞型に非常に類似していた。TFE3の免疫染色は全例中等度から強陽性で、TFE3 遺伝子の break apart FISHでは転座が確認された。【結論】成人Xp11.2転座型腎細胞癌では経過中にリンパ節転移、遠隔転移をきたす症例があり、術後の充分なフォローアップが必要と考える。

P-2319

Clinicopathological Analyses of 17 Cases of Xp11Translocation Renal Cell Carcinomas

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Xp11.2 translocation Renal Cell Carcinoma (RCC) belongs to MiT family translocation RCCs. Xp11.2 translocation RCC preferentially develops in children, but it potentially occurs in all ages. Major fusion partners of TFE3 include ASPSCR1, PRCC, and SFPQ. Miscellaneous molecules that form fusion with TFE3 have also been identified. We investigated 17 Japanese patients with Xp11.2 translocation RCCs. The mean patient age was 38 (11-73). Twelve were females and 5 were men. The most frequent pattern was ASPSCR1-TFE3 (n=4), followed by SFPQ-TFE3 (n=3). There were 3 cases with RBM10-TFE3 fusion in which the inversion involving the short arm of the X chromosome; the cases demonstrated false-negative TFE3 break-apart FISH. All cases showed nuclear staining for TFE3, and 10 cases were also positive for cathepsin K. In addition, glycoprotein non-metastatic B (GPNMB), a downstream molecule of TFE3, was strongly stained in all Xp11.2 translocation RCCs. The findings indicate that Xp11.2 translocation RCCs utilize previously unknown TFE3-mediated cascades involving GPNMB

P-2320

Regulation of hypoxia response pathway by chimeric TFE3s in Xp11.2 translocation renal cell carcinoma

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Xp11.2 translocation renal cell carcinoma (Xp11.2 tRCC) has chromosomal translocations involving the TFE3 transcription factor at chromosome Xp11.2. All translocations seen in tRCC produce chimeric TFE3 proteins. To clarify the molecular mechanisms for Xp11.2 tRCC development, we have established cell lines, which express chimeric TFE3s or wild type TFE3 in a doxycycline dependent manner. Noticeably, we found the significant correlation between chimeric TFE3 target genes and hypoxia responsive genes. VHL gene is mutated or methylated in over 90% of sporadic clear cell RCC, in which HIF α are aberrantly accumulated even under normoxic conditions and transcribe hypoxia responsive genes. Thus, hypoxia signaling pathway may have an essential role in Xp11.2 tRCC development. Luciferase Assays demonstrate that a chimeric TFE3 transcription factor upregulates the HIF α promoter activity. In addition, we have developed Xp11.2 translocation RCC mouse models by expressing PRCC-TFE3 specifically in kidney epithelial cells and analyzed hypoxia response pathway in vivo. We will report and discuss the regulation of hypoxia response pathway by chimeric TFE3 transcription factors.

▲TOP

P-2321

A family case with TSC1 and mtDNA mutations developing bilateral chRCCs without other typical phenotype of TSC

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Most bilateral or familial chRCC patients without other features associated with hereditary tumor syndrome rarely undergo genetic analysis. In this study, we examined genetic alterations in mother and son cases with multiple eosinophilic chRCCs showing no other features. Germline DNA and bilateral RCC DNA were genetically analyzed by whole exome sequencing. Candidate gene alterations in the first patient's germline were investigated in her child's germline and the chRCCs.We detected several germline gene alterations in the mother case. Among the identified alterations, TSC1 and mitochondrial DNA mutations were also confirmed in her son. Regarding somatic alterations in bilateral chRCCs, no common candidate gene alteration was found. To the best of our knowledge, this is the first report of whole-exome sequencing revealing bilateral eosinophilic chRCCs associated with TSC in a family case without classical phenotype. These results suggest that germline TSC1 and mitochondrial DNA gene mutations may be involved in the development of chRCCs in some cases.

P-2322

Analysis of circulating-tumor DNA with next-generation sequencing in renal cell carcinoma patients

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Background: Circulating–tumor DNA (ctDNA) is a promising resource to detect and monitor molecular characteristics of various types of tumor. This study aims to clarify the clinical utility of ctDNA in renal cell carcinoma (RCC) patients.Materials and methods: Fifty–three patients histologically diagnosed as clear cell RCC were enrolled. Mutations in plasma cell-free DNA (cfDNA) were detected by next-generation sequencing (NGS). Positive mutations in cfDNA were defined as ctDNA. Mutations in cfDNA and tumor DNA were also validated using droplet digital PCR (ddPCR).Results: Sixteen (30%) of 53 patients had total 38 mutations in cfDNA including TP53 (n=6) and VHL (n=5). In 16 patients with ctDNA, median number of mutations was 2 (range, 1–7), and median mutation allele frequency of ctDNA was 10% (range, 1.2–54.5%). We designed specific ddPCR probes for 11 mutations and detected the same mutation in both cfDNA and tumor DNA. Clinically, positive detection of ctDNA was significantly associated with poor cancer–specific survival (p=0.031).Conclusions: Analysis of mutational landscape of ctDNA using NGS may allow enhanced molecular monitoring of disease progression in RCC patients.

P-2323

PD-L1 expression analysis of circulating tumor cells in advanced renal cell carcinoma

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The anti-PD-1 antibody, nivolumab, has been applied in Japan for metastatic renal cell carcinoma (mRCC). Treatment options have expanded, but establishment of effective molecular biomarkers for administering to suitable cases is required. Liquid biopsy is a method to detect and analyze circulating circulating tumor cells (CTC), cell-free DNA, exosome from peripheral blood, which enables chronologically the monitoring of genetic profile of cancer with less invasiveness. Although CTC can be detected in various advanced cancers, its usefulness in clinical practice has yet to be established in mRCC. We attempted CTC collection and molecular expression analysis using AdnaTest (Qiagen, Germany) and CytoQuest (Abnova, Taiwan). First, from the establishment of the procedure, we verified expression analysis, such as PD-L1, in CTCs from 25 patients with advanced RCC by using AdnaTest. We also attempted CTC detection in CytoQuest. Expression of PD-L1 was positive in 7 cases (28%). CTCs of mRCC could also be detected in only progressive cases by the collection procedures using EpCAM. CTC analysis is feasible and minimally invasive procedure, and will be a promising modality in mRCC.

▲TOP

[P-2284] P14-34 [English/Japanese]
Breast cancer (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Hitoshi Tsuda / Dept. Basic Pathol., Natl. Def. Med. Coll.

P-2284

BAG2 promotes cancer progression by regulating the dual function of cathepsin B in triple-negative breast cancer cells

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Triple-negative breast cancer (TNBC) is extremely aggressive, heterogeneous, and hormonally unresponsive compared to non-TNBC. Patients with TNBC have a higher risk of recurrence and death despite standard treatment, highlighting the need for novel therapeutic targets and strategies for the treatment of TNBC. Here, we report the unique role of Bcl-2-associated athanogene 2 (BAG2), significantly overexpressed in TNBC and strongly associated with poor clinical outcomes, in regulating the dual functions of cathepsin B as either a pro- or anti-oncogenic enzyme. Silencing BAG2 suppresses tumorigenesis and lung metastasis in vivo and induces apoptosis by increasing the intracellular lysosomal mature form of cathepsin B, whereas BAG2 over-expression induces metastasis by blocking the auto-cleavage processing of pro-cathepsin B. Furthermore, BAG2 regulates pro-cathepsin B/annexin II complex formation and facilitates the trafficking of pro-cathespin B-containing TGN38-positive vesicles, leading to the secretion of pro-cathepsin B that induces metastasis. Thus, targeting the BAG2-driven suppression of pro-cathepsin B secretion represents a novel therapeutic strategy for TNBC patients.

▲TOP

P-2285

Phylogenetic analysis of combined ductal and lobular carcinoma of breast

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Background: In order to elucidate the clonal evolution of breast cancer of combined lobular and ductal carcinoma (CLDC), we analyzed mitochondrial DNA D-loop region (mtDNA DL), cancer genome, and chromosomal polymorphism using FFPE samples.Methods: We performed molecular phylogenetic analysis on 3 CLDC cases (total 20 samples), with direct sequencing of the mtDNA DL, quantitative PCR targeting 1q and 16q and comprehensive cancer panel (CCP). DNA was separately extracted from invasive ductal carcinoma, invasive lobular carcinoma, ductal carcinoma in situ, lobular carcinoma in situ, flat epithelial atypia, non-neoplastic mammary gland and extra-mammary organ using laser assisted microdissection system. Results: We confirmed the polymorphism within a total of 5 samples at 3 sites by the mtDNA DL analysis, copy number variation of 1q amplification in 8 samples, and 16q defect in 9 samples by quantitative PCR and 14 mutations detected by CCP validated by mutation detection assay with RT-PCR.Conclusion: It was speculated that a part of CLDC was diverged from a single cell origin, and accumulated genomic and chromosomal alterations formed unique histopathological features.

P-2286

Mutation in Estrogen Receptor in Metastatic Breast Cancer in Japan

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Introduction: Some studies report that long-term endocrine therapy (ET) to metastatic breast cancer (MBC) with estrogen receptor α (ERα) induces the resistance against this therapy due to the acquired mutation of ligand-binding domain in ERα. However, it has not been reported whether the acquired ERα mutation occur in MBC in Japan. We evaluate a correlation between the period of ET and the incidence of ERα mutation. Material and method: 25 samples of ERα positive MBC patients are applied, followed at Keio University Hospital between January, 2012 and December, 2015. DNA was extracted from paraffin-embedded tissue samples from these patients. Genetic sequencing and analysis was done with the Illumina Miseq and publicly software. Results: ERα mutation was developed in 16 patients [64% (12/25)]. Number of treated endocrine drug is no statistically significant factor for ERα mutation (P = 0.26). Statistical analysis revealed that longer ET tends to develop a mutation of ERα (median (interquartile range) 8 months (0.75-62.25) vs 0 month (0-1) P = 0.07). Conclusion: This study suggests that long-term ET could develop a mutation of ERα in Japanese MBC patients.

P-2287

The role of CPEB3 in the development of breast cancer bone metastasis

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Bone is one of the most common metastatic sites in breast cancer. To determine the molecular basis of bone metastatic conditions, we performed microarray analysis using mouse models and selected 142 genes that were up-regulated in cancer cells during bone microenvironments. Out of these genes, we focused on cytoplasmic polyadenylation element binding protein 3 (CPEB3), an RNA binding protein that mediates mRNA polyadenylation and translation. The overexpression of CPEB3 in MDA-MB-231 breast cancer cells suppressed migration, invasion, and soft agar colony formation in vitro. However, CPEB3 overexpressed cells increased their resistance to cisplatin and 5-fluorouracil, common chemotherapy agents. Interestingly, the expression of CXCR4 mRNA was significantly increased in CPEB3 overexpressed cells. By the RNA immunoprecipitation assay, we detected that CXCR4 mRNA significantly associated with CPEB3 when compared with the control IgG. These results suggest that CPEB3 overexpression in bone metastatic cells may promote a state of fixing and chemoresistance to survive for a long time until their recurrence in cancer patients.

▲TOP

P-2288

Amplicons in breast cancers analyzed by MLPA and FISH

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Gene amplification is a common event in breast cancers, and actual and potential targets of molecular therapy. The amplification status of the 22 genes reportedly frequently amplified in breast cancers were determined. An archive of 322 formalin-fixed and paraffin-embedded invasive ductal cancer tissues was screened by multiple ligation-dependent probe amplification（MLPA）and a total 885 gene loci judged as 'gain' or 'amplified' was further confirmed for the amplification by fluorescence in situ hybridization (FISH). The results showed 111 tumors (35%) displayed gene amplification of at least one of the 22 genes. The frequencies of the amplification of 4 regions with known driver oncogenes were as follows: 8p11 (ZNF703, FGFR1, ADAM9 and IKBKB), 8q24 (MYC), 11q13 (CCND1, C11ORF30), and 17q12-21 (CPD, MED1, ERBB2, CDC6, TOP2A, MAPT) were 9.6%, 9.6%, 12.4%, and 12.7%, respectively. Co-localization of the amplicon on 8p11 and the amplicon on 11q13 in single cells was found in 10 tumors, and in the 6 of them the 2 amplicons constituted single amplification units. Precise and feasible analysis of gene amplification status can be possible with the combination of MLPA and FISH.

P-2289

The ultrastructural features of exosomes derived from mouse mammary carcinomas in vitro and in vivo studies

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Mouse mammary carcinoma cells, BJMC338 cells show a low metastatic propensity, while BJMC3879 cells show a high metastatic propensity, especially to lymph nodes and lungs. Both cell lines secrete exosomes, which demonstrated exosome markers, CD63, CD9, CD81, TSG101 and TGN38 by Western blots. In addition, exosomes contained VEGF-C and miR27b, In this study, we classified exosomes by size into 50 nm-exosomes (50 > nm in diameter) and 20 nm-exosomes (<20 nm in diameter) in the inoculated these tumors under transmission electron microscopy (TEM). Fifty nm-exosomes had double membranes and were sometimes in sac of rough endoplasmic reticulum (r-ER) and clustered beneath the Golgi complex. Twenty nm exosomes were mainly in multi-vesicular body (MVB). In lumen-like structures, both exosomes were observed. To analyze their contents, exosome markers were examined by immuno-electron microscopy;50 nm-exosomes had CD63 and TGN38, while 20 nm-exosome did not. These observations suggest that synthesized protein in r-ER was packed into 50 nm-exosomes and released into extracellular spaces. Further studies are needed to differentiate contents of 20 nm-exosomes.

▲TOP

[P-2297] P14-36 [English/Japanese]
Breast cancer (4)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Goro Kutomi / Dept. Surg., Sapporo Med. Univ.

P-2297

Prevalence of pathogenic variants in hereditary breast/ovarian cancer susceptibility genes detected by multigene panel

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We detected the variants of high and middle penetrance rate genes in hereditary breast/ovarian cancer by using 23 multigene panels. Genetic counseling clinic assessed 612 breast/ovarian cancer patients for BRCA1/2 mutations by direct sequencing. 366(60%) patients who were negative for BRCA1/2 mutations were evaluated for susceptibility gene germline mutations through multigene panel. For variants classification, Sanger sequencing, pedigree, and clinicopathological characteristics of mutations were examined according to the ACMG guidelines. We confirmed 12(3.3%) patients had 7 likely pathogenic and 2 pathogenic variants for 6 genes. The number of mutation careers was evaluated as follows: ATM in 3 patients, BRIP1 in 1 patient, CHEK2 in 4 patients, MLH1 in 1 patient, PALB2 in 2 patients, and PMS2 in 1 patient. All variants were validated by Sanger sequencing. Application of multigene panel will provide significant information for patients with cancer predisposition gene so that early detection and reduction of risk can be achieved. Additional functional studies are required to define clinical impact of mutations. (This study was supported by National Cancer Center, Korea, 1710450-2)

▲TOP

P-2298

The combination of cytokeratin 5/6, vimentin, AXL and androgen receptor as a prognostic factor of TNBC

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[Introduction] Triple-negative breast cancer (TNBC) accounts for 15% of whole breast cancer. It would be clinically useful if the combination of several molecular expression can identify good or poor prognosis group of TNBC patients. [Methods] We immunohistochemically investigated six molecule expression of epithelial-mesenchymal transition markers (vimentin, AXL, ZEB1), basal-like markers (CK5/6, EGFR), and androgen receptor. In 54 surgically resected TNBC tissues between 2002 and 2009, the relationship of molecular expression combination with clinicopathological data and prognosis was analyzed. Positive vimentin, negative AXL, positive CK 5/6, and negative AR were detected in 33%, 67%, 24%, and 61%, respectively, a cluster analysis incorporating data of these four molecule expressions, three subgroups of TNBC were identified. The group with positive AXL, negative CK 5/6 showed the best prognosis. A multivariate analysis revealed that this classification was an independent prognostic predictor. [Conclusion] The combination of CK5/6, vimentin, AR and AXL expression was a useful marker for prognostication of TNBC.

P-2299

A new pathological scoring system to evaluate ductal carcinoma in situ (DCIS)

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<Background>DCIS is considered to have a good prognosis. However, we sometimes see DCIS with high malignant potential. Here, we aimed to develop a simplified evaluation method to identify malignant DCIS.<Methods>Genomic alterations of synchronous DCIS with invasive ductal carcinoma (IDC) is reported to be close to that of IDC and to have high copy number alterations (CNAs) compared to pure DCIS. We selected MYC and PIK3CA as oncogenes, and BRCA2 and PIK3R1 as tumor suppressor genes from genes with CNAs which should have a positive correlation with the expression levels. We evaluated the status of each CNAs of them by immunohistochemical staining (IHC) and scored them (IHC score; low, intermediate and high) in 5 IDC and 38 pure DCIS tissues. Then, we assessed a correlation between IHC score and DCIS prognostic index (VNPI and Ki-67).<Results>The rate of low, intermediate and high score in IDC and DCIS were 20%, 20%, 60% and 50%, 42%, 8%, respectively. IHC score had positive correlation with prognostic index. We had one case with subsequent distant metastasis whose VNPI was intermediate but IHC score was high. <Discussion>Our IHC score may be useful system to identify malignant DCIS.

P-2300

Immunohistochemical analysis of immunopathological phenotype in three subtypes of breast cancer tissues

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Immunotherapy with immune checkpoint blockade (ICB) has become the fourth standard therapy for melanoma, non-small cell lung cancer and renal cell cancer; however, it has not been established in breast cancer treatment. In order to know the characteristics of immunopathological phenotype in breast cancer tissues, we analyzed three types of breast cancer tissues by using a panel of immunohistochemical biomarkers including PD-L1, PD-L2, IDO1, CD8, TIA-1, and HLA class I. It was suggested that triple negative type had an ICB-sensitive T-cell-inflamed phenotype, such as PD-L1-high, PD-L2-high, CD8-high, TIA-1-high, HLA-I-high. In contrast, luminal type had an ICB-resistant T-cell-escaped phenotype, such as PD-L1-low, PD-L2-medium, IDO1-high, CD8-low, TIA-1-low, HLA-I-low, and HER2 type had an ICB-partially sensitive T-cell-exhausted phenotype, such as PD-L1-medium, PD-L2-high, CD8-high, TIA-1-low, HLA-I-high. Our study revealed the distinct immunopathological phenotype in each type of breast cancer tissues and indicated that a panel of immunohistochemical biomarkers might serve as a diagnostic tool for precision therapy of cancer.

▲TOP

P-2301

Lymphocytic infiltration pattern in the lung metastatic lesions of breast cancer

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Background: It is well known that metastatic disease is the major cause of death in breast cancers. It is likely that lung metastasis is relatively favorable prognosis than other organs. Recently tumor infiltrating lymphocyte (TIL) was focused as a favorable prognostic marker. Aim: To compare TILs between primary breast cancers and lung metastatic nests.Material and Methods: Nine metastatic tissue materials, resected as whole tumors by surgery 2011/1-2017/12, were retrospectively analyzed. Intra-tumoral lymphocytes (iTIL) and stromal lymphocytes (sTIL) and invasive margin lymphocytes (imTIL) were scored respectively.Results: Median iTIL and sTIL and imTIL of lung metastatic tumor were 9.0% (0.7-35.0%), 17.4% (4.8-50.0%) and 10.9% (5.6-80.0%), and of primary breast cancer were 8.9% (0.5-2.0%), 20.9% (2.0-40.0%) and 3.8% (0.4-8.5%). imTIL% in lung metastatic nests were more frequently than in primary breast tumors (p=0.0449).Summary: While iTILs% and sTILs% were observed no apparently difference between in the primary and the lung metastasis, imTILs increased in lung metastasis. This tendency would be a clue of resolution of favorable outcome of lung oligo-metastasis.

P-2302

CD44 as an invasion marker for encapsulated papillary carcinioma of the breast

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Encapsulated papillary carcinoma (EPC) is a rare tumor which is a minor variant of papillary carcinoma comprised less than 2 % of breast cancers. EPC is regarded as “a form of carcinoma in transition”, between in situ and invasive carcinoma. However, there are some EPC cases that have clearly invasive lesion called as EPC with invasion. To distinguish between EPC and EPC with invasion by immunohistochemistry, we assembled 28 cases of EPC, including 10 cases of EPC with invasion, which were diagnosed at the Nagoya City University Hospital from 2005 to 2016. We examined the expression of hormone receptors (ER, PgR), invasive factors (MMP2, MMP9, and CD44), vascularization (VEGF), and proliferation (Ki-67) by immunohistochemistry. The expression of ER, PgR, MMP2, MMP9, VEGF and Ki-67 index had no significant differences between in situ and with invasion. Meanwhile, the expression of CD44 was a significantly higher in EPC with invasion than non-invasive EPC ($P<0.01$). The higher CD44 expression in EPC with invasion suggested a cancer-stem like cell feature and CD44 may be a marker which detect the potential of invasion in EPC.

P-2303

Usefulness of correction by proportion of estrogen receptor positive cell components in estrogen receptor imaging

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Introduction Previous study has demonstrated the high correlation between ^{18}F-fluoroestradiol(^{18}F-FES) uptake and estrogen receptor（ER） concentration in vitro. In order to simply evaluate the amount of ER-positive cells, we measured the proportion of cell components of tissues using Azan-Mallory staining and evaluated the degree of correlation between the amount of ER positive cells and ^{18}FES uptake.Methods 13 ER-positive primary breast cancer patients who underwent ^{18}F-FES PET between July 2012 and May 2015 were enrolled. We used the SUVmax as an evaluation of ^{18}F-FES uptake of primary tumor. We stained resected specimens with Azan-Mallory staining reagents and calculated the proportion of the cell component in the entire area by using image analysis software Image J. About each case, we calculated the amount of ER-positive cells using ER-positive rate and the ratio of cell component. Result The correlation coefficient between ER expression and ^{18}F-FES uptake was 0.312, and the correlation coefficient between the amount of ER-positive cells and ^{18}F-FES uptake was 0.605.ConclusionThe better correlation were observed between ^{18}F-FES uptake and the amount of ER-positive cells.

▲TOP

[P-2310] P14-38 [English/Japanese]
Urothelial cancer (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

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P-2310

Establishment of PDX model and analysis of phosphorylation status of micropapillary urothelial carcinoma

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Micropapillary urothelial carcinoma (MPUC) is a rare and aggressive variant of UC. Although comprehensive analysis with next-generation sequencing is being performed in several reports, the mechanism of MPUC is unknown because of it being a rare variant. Here, we report the establishment of Patient Derived Xenograft (PDX) models and analysis of phosphorylation and mutation status of MPUC. To establish the PDX models of MPUC, surgical materials from bladder and lymph node metastasis of UC were subcutaneously grafted into the SCID mice. Primary tumors of MPUC were characterized by small tight nests which are often seen within connective tissue spaces in HE staining. PDX tumors revealed similar findings compared with primary tumors. To examine phosphorylation status of MPUC, we performed phosphorylation analysis in eight cases of MPUC. Highly phosphorylation status of HER3, FGFR1, FGFR3, NTRK1, EphA1, EphB1, EphB4, Axl, Tie2, VEGFR2 and Akt were identified in more than 5 out of 8 MPUC cases. The elucidation of the molecular mechanisms and the development of PDX as a disease model might lead to treatment of MPUC, which is one of the rarer forms of cancer.

▲TOP

P-2311

NACC1 regulates cell proliferation as a target molecule of microRNA-331-3p in urothelial carcinoma cells

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Nucleus accumbens-associated protein 1(NACC1), known as one of molecules related to activation of transcription, is constitutively expressed in urothelium and participated in multiple process including cell growth, senescence, autophagy, epithelial-mesenchymal transition. Functional analysis of miR-331-3p and its target molecule, NACC1, using urothelial carcinoma (UC) cell lines was performed. Furthermore, using quantitative RT-PCR and immunostaining, we examined the expression of miR-331-3p and NACC1 in UC specimens derived from liquid based cytology of urine and TUB-Bt specimens. MTS assay in UC cell lines revealed that cell proliferation was significantly reduced by transient transfection of miR-331-3p precursor and/or NACC1 siRNA. Cell senescence was induced through G1-arrest in the cell cycle by inhibition of NACC1. Immunohistochemistry using TUR-Bt specimen showed that more than 90% of UC including normal urothelial cells were positive for NACC1 independently of the pathological parameters including pT stage. Our results suggest that NACC1 contributes to cell proliferation by targeting miR-331-3p in the UC cell line, and has the potential to be a molecular marker of UC.

P-2312

Withdrawn

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No Abstract

P-2313

Analysis of urinary leukocytes during intravesical immunotherapy with BCG for non-muscle invasive bladder cancer

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The intravesical immunotherapy with bacillus Calmette-Guerin (BCG) for non-muscle invasive bladder cancer is an established cancer immunotherapy to achieve a complete remission rate of approximately 70%. This high therapeutic effect is considered to cause an immune response to cancer cells induced by the local inflammation. However, the character of urinary leukocytes is not yet elucidated. In this study, we analyzed the urinary cells after weekly BCG-intravesical treatment. The number of urinary leukocytes was increased by the BCG-treatment. Most cells were $CD33^{+}GPI\text{-}80^{+}$ neutrophilic cells. GPI-80 is known as a GPI-anchored 80 kD protein and a regulator of neutrophil adhesion. The expression level of GPI-80 per cell increased, and the dispersibility (coefficient variation, CV) of GPI-80 expression tended to decrease depending on the number of repetitions. Previously, we have reported that increase of GPI-80 CV on neutrophilic cells correlates to the activity of myeloid-derived suppressor cells (MDSCs) in blood from renal cell carcinoma patients. Therefore, a reduction of GPI-80 CV may be an indicator of induction of cancer immunity, not MDSCs accumulation.

▲TOP

P-2314

The therapeutic effect of sulfasalazine for metastatic urothelial carcinoma targeting to cancer stem cell

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We evaluated the therapeutic effect of sulfasalazine (SSZ) which has been reported as an inhibitor of cystine transporter in urothelial carcinoma (UC) cells. To evaluate the effects of SSZ, the murine bladder cancer cell line MBT2V, which has a high lung metastatic potential, was used. Cell viability was assessed by luminescence measurement. In an in vivo study, lung metastases were generated by injecting MBT2V cells into the tail vein of C3H/HeN mice on day 0. Intraperitoneal administration of SSZ and/or cisplatin (CDDP) was started on day 3. SSZ was observed to have dose-dependent cytotoxic effects in MBT2V cells. The relative cytotoxicity of CDDP in addition to SSZ was significantly lower than that of SSZ alone or CDDP alone. The number of metastatic lung nodules on the lung surface in mice treated with vehicle control was significantly higher than those treated with SSZ alone or CDDP alone. Furthermore, those treated with combination of SSZ and CDDP were significantly lower than those treated with SSZ alone or CDDP alone. SSZ may be a novel therapeutic agent that directly targets UC cancer stem cells and enhances the cytotoxic effect of CDDP.

P-2315

Epigenetic regulation of miR-200b is associated with cisplatin resistance in bladder cancer

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We aimed to identify microRNAs (miRNAs) involved in cisplatin (CDDP) resistance in bladder cancer (BCa). After establishing CDDP-resistant BCa cell lines (T24RC and EJ138RC), TaqMan arrays revealed that members of the miR-200 family were downregulated in T24RC as compared to parental T24 cells. miR-200b was associated with CDDP sensitivity in BCa cells, and its downregulation was associated with CpG island hypermethylation. Pharmacological demethylation using 5-aza-2'-deoxycytidine (5-aza) restored miR-200b expression, and the combination of 5-aza + CDDP strongly inhibited T24RC cell proliferation. Microarray analysis revealed that miR-200b + CDDP induced genes involved in CDDP sensitivity or cytotoxicity, including IGFBP3, ICAM1 and TNFSF10, in the resistant cells. Expression and DNA methylation of miR-200b were inversely associated in primary BCa, and low expression/high methylation was associated with poor overall survival. These results suggest downregulation of miR-200b is associated with CDDP resistance in BCa. Epigenetic silencing of miR-200b may be a marker of CDDP resistance and a useful therapeutic target for overcoming CDDP resistance in BCa.

P-2316

Adenoviral shRNA vector targeting RRM1 has a antitumor activity and overcomes GEM resistance on bladder carcinomas

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RRM1 is the main enzyme responsible for synthesis of the deoxyribonucleotides. It is also the primary cellular target for gemcitabine (GEM). Therefore, we performed this study using a RRM1-suppressing adenoviral vector (Ad-shRRM1) in order to establish the gene therapy. Two human bladder tumor cell lines RT112 and J82 were used. The gene expression of RRM1 was evaluated by quantitative RT-PCR. The drug sensitivity to GEM was assessed by MTT. A human tumor xenograft model in nude mice was prepared. The infection of Ad-shRRM1 effectively downregulated the RRM1 mRNA in both tumor cells. MTT assays demonstrated to significantly inhibit the growth of both tumor cells. The Ad-shRRM1 treatment was found to have the strongest antitumor effect against the RRM1-overexpressing tumor xenografts. Furthermore, Ad-shRRM1 mediated inhibition of RRM1 specifically increased sensitivity to GEM the both of tumor cells. Our result suggests that cancer gene therapy using the adenoviral vector expressing shRNA against RRM1 has a strong antitumor effect against RRM1-overexpressing tumors. Combination therapy with Ad-shRRM1and GEM may become a new treatment option for patients with GEM-resistant.

▲TOP

[P-2324] P14-40 [English/Japanese]
Renal cell carcinoma (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Masayuki Nakagawa / Dept. Urology, Kagoshima Univ. Grad. Sch. Med. Dent. Sci.

P-2324

miRNA expression profiling in serum exosomes for new diagnostic models of renal cell carcinoma

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Introduction and Objectives: Exosomes are lipid bilayer vesicles containing protein, mRNA and miRNA. Cancer cell-derived exosomes may be diagnostic and therapeutic targets. We focused on miRNA expression profiling in serum exosomes from renal cell carcinoma (RCC) patients. The aim of this study is to develop new diagnostic models of RCC.Methods: Serum exosomes were collected from 93 RCC patients and 46 healthy volunteers using an ultracentrifugation method. We performed miRNA expression profiling using microarrays and developed a random forest model and logistic regression analysis model for the diagnosis of RCC. Results: Receiver Operating Characteristic curve analysis showed that both models were useful for diagnosing RCC (random forest model: sensitivity of 97.8% and specificity of 50.0%, AUC = 0.739, logistic regression model: sensitivity of 80.7% and specificity of 87.0%, AUC = 0.856). The logistic regression analysis model was also useful for the diagnosis of early stage RCC (pT1a).Conclusions: We developed useful new diagnostic models for RCC with miRNA expression profiling in serum exosomes.

▲TOP

P-2325

Functional analysis of microRNA 99a-3p in sunitinib-resistant renal cell carcinoma

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The resistance to sunitinib is a large problem in clinical aspect. In this study, we focused on miR-99a-3p, which showed decreased expression in sunitinib-resistant RCC based on previous screening analyses. The expression levels of miR-99a-3p were downregulated in RCC clinical specimens and cell lines compared to normal kidneys. In addition, its expression in the sunitinib-resistant cell (SU-R-786-o) established in our group was much lower compared to the parent cell. Restoration of miR-99a-3p in RCC cells including SU-R-786-o significantly inhibited proliferation through induction of apoptosis. RRM2, involved in the synthesis of deoxynucleotides necessary for polymerization and repair of DNA, was identified as a direct target by miR-99a-3p based on target analyses. This is the first report demonstrating that miR-99a-3p directly regulate RRM2. The identification of novel target gene regulated by tumor-suppressive miR-99a-3p in sunitinib resistant RCC cells may lead to a better understanding of resistant mechanism.

P-2326

Variation of gene expression profiling caused by adding LDL in renal cell carcinoma cell lines

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Introduction and Objective. We have previously demonstrated that Low-density lipoprotein (LDL) contributes to resistant against multi-kinase inhibitors including sorafenib, sunitinib in renal cell carcinoma cell (RCC) cell lines. We examined mRNA expression profiles in RCC cells after/before adding LDL.Methods. Transcriptome analyses were evaluated by CAGE method before/after adding LDL into RCC cell line A498. The results were utilized to perform Gene Ontology (GO) pathway analyses.Results. The up-regulated 80 GO terms and the down-regulated 86 GO terms were identified. The top three of up-regulated GO terms were "skeletal muscle cell differentiation", "negative regulation of transcription from RNA polymerase", and "muscle structure development". The top three of down-regulated GO terms were "cholesterol biosynthetic process", "sencondary alcohol biosynthetic process", and "cholesterol metabolic process".Conclusions. The results were expected to be utilized for overcoming the resistivity against multi-kinase inhibitors.

P-2327

Critical roles of mitochondrial PRELID2 for renal carcinogenesis

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Renal cell carcinoma (RCC) is the most common malignancy of kidney, yet its molecular pathogenesis is poorly understood. We here report the critical roles of a mitochondrial protein, PRELID2 (Protein of relevant evolutionary and lymphoid interest domain containing 2), a member of the UPS/PRELI family, in renal carcinogenesis. DNA microarray, quantitative RT-PCR and TGCA database analyses showed significant upregulation of PRELID2 in RCC cases. Introduction of PRELID2 into HEK293 cells significantly enhanced cell growth, whereas knockdown of PRELID2 expression drastically suppressed growth in RCC cells. Notably, depletion of PRELID2 led to enhance reactive oxygen species production, thereby causing increased oxidized proteins, in RCC cells. Notably, proteomic analysis revealed a list of upregulation of redox reaction-related pathways in PRELID2-depleted cells. Furthermore, we found the interaction of HA-PRELID2 with endogenous PHB2 (Prohibitin 2), which is essential for mitochondrial morphogenesis and homeostasis, in RCC cells. These findings suggest that PRELID2 is likely to play a crucial role in renal carcinogenesis though its interacting with mitochondrial PHB2.

▲TOP

P-2328

The significance of insulin receptor expression in vascular endothelial cells of clear cell renal cell carcinoma

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VEGFR-TKIs demonstrate the significant efficacy for advanced clear cell renal cell carcinoma (ccRCC), however, it eventually becomes resistant. We previously identified the prognostic gene set of ccRCC, which included insulin receptor (INSR). Here, we report the significance of INSR expression as a biomarker to predict the resistance to the VEGFR-TKIs. Immunohistochemical analysis revealed that low INSR expression in vascular endothelial cells demonstrated poor outcome in terms of progression-free survival and overall survival in RCC patients. In the patient-derived xenograft models that we established, the expression of INSR was decreased in the axitinib-resistant tumors. In co-culture experiment of RCC cell lines with human glomerular endothelial cells treated with si-RNA of INSR, , the migration ability of the RCC cell lines was enhanced. The microarray analysis indicated that the decreased INSR expression may be involved with the several important signaling pathway, including interferon α or γ response, TGF-β signaling, and PI3K-AKT-mTOR signaling in the RCC cell lines.

▲TOP

[P-2329] P14-41 [English/Japanese]
Renal cell carcinoma (3)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

Fumiya Hongo / Dept. Urol., Kyoto Pref. Univ. of Med.

P-2329

Knockdown of PD-L1 in Murine Renal Cell Carcinoma Inhibits Tumor Growth and Enhances the Anti-tumor effect of Sunitinib

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PurposeTo evaluate the combination treatment of PDL-1 knockdown and sunitinib in renal cell carcinoma.MethodsWe establised murine renal cancer cell line with stable PD-L1 knockdown expression by introducting shRNA to Renca (ATCC(R) CRL-294TM). We transplanted wild type Renca and PD-L1 knockdown Renca to BALB/c mice, respectively,thenevaluated tumor growth, cytotoxic sensitivity to Sunitinib and expression of Tumor-infiltrating lymphocytes(TILs)in each cell lines in vitro and vivo.ResulltsWe counld not find any significant difference among wild type and PD-L1 knockdown Renca in tumor growth, cytotoxic sensitivity toSunitinib and protein expressions except for PD-L1 in vitro.Tumor growth in the mice with wild type Renca was significantly faster than that with PD-L1 knockdown cells. The mice with PD-L1 knockdown Renca showed additive anti-tumor effect with Sunitinib. Immunohistochemical staining demonstrated that the proportion of CD8+ TILs were significantly increased in mice with PD-L1 knockdown cells.ConclusionKnockdown of PD-L1 inhibited tumor growth and enhanced the anti-tumor effect of Sunitinib.CD8+ TILs may contribute additive effect of Sunitinib.

▲TOP

P-2330

Catfish (Silurus asotus) Lectin Enhances the Cytotoxic Effects of Sunitinib on Renal Cell Carcinoma

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Catfish (Silurus asotus) egg lectin (SAL) bound to globotriaosylceramide (Gb3) expressed on the surface of cells, and in the previous study we found that treatment of Gb3+ cells with SAL caused an increase of intracellular uptake of Sunitinib. In this study, we therefore investigated whether intracellular uptake of molecular targeted drugs and cytotoxic effects were altered in SAL-treated renal cell carcinoma cells. We analyzed Gb3 expression in renal cell carcinoma cell lines using TOS1, TOS3, TOS3LN and ACHN cells, and expressions of Gb3 were observed in TOS1 and TOS3. In the reaction between TOS1 and a molecular targeted drugs such as Sunitinib, Axitinib, Pazopanib and Everolimus, the cell viability decreased in a concentration-dependent manner in any of the drugs. When SAL and molecular targeted drugs were used in combination, there was a significant decrease in cell survival rate only with Sunitinib. TOS1 treated with Sunitinib in addition to SAL significantly increased apoptotic cells compared with Sunitinib alone treatment, and significantly increased Sunitinib uptake into cells.

P-2331

Pharmacogenetics-based AUC prediction model may determine the optimal initial dose of axitinib in renal cell carcinoma

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We investigated axitinib pharmacogenetics and clinical efficacy/adverse events (AEs) in advanced renal cell carcinoma (RCC) and established a model to predict efficacy/AEs using pharmacokinetic and gene polymorphisms related to drug metabolism and efflux in a phase II trial. We prospectively evaluated AUC of axitinib, objective response rate (ORR), and AEs in 44 advanced RCC treated with axitinib. To establish a model for predicting efficacy/AEs, polymorphisms in genes including ABC transporters, UGT1A, and OR2B11 were analyzed by whole-exome sequencing, Sanger sequencing, and DNA microarray. To validate this prediction model, calculated AUC by 6 gene polymorphisms was compared with actual AUC in 16 additional patients prospectively. Actual AUC significantly correlated with ORR and AEs. Calculated AUC significantly correlated with actual AUC, and correctly predicted ORR as well as AEs. In the validation study, calculated AUC prior to axitinib treatment precisely predicted actual AUC after axitinib treatment. Our pharmacogenetics-based AUC prediction model may determine the optimal initial dose of axitinib, and thus facilitate better treatment of patients with advanced RCC.

P-2332

Ankrd1 as a potential therapeutic target for rapamycin-resistant renal cell carcinoma

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Objective:Our objective was to investigate the effect of Ankrd1 and the potential as a therapeutic target in rapamycin-resistant renal cell carcinoma (RCC).Material and Methods:We generated rapamycin-resistant ACHN (ACHN/RR) and 769P (769P/RR) from RCC cell line ACHN and 769P. We analyzed ACHN and ACHN/RR by RNA-seq, and confirmed increase of expression of 49 genes in ACHN/RR. We focused on Ankrd1 among 49 genes and measured the amount of Ankrd1 in ACHN, ACHN/RR, 769P, and 769P/RR using by real time-qPCR. Then we knocked down Ankrd1 by using siRNA and evaluated cell viability with MTS assay.Results:Increase of expression of Ankrd1 was confirmed in each ACHN/RR and 769P/RR comparing to parental cell line. Knockdown of Ankrd1 suppressed the proliferation both in ACHN/RR and 769P/RR.Conclusion: Ankrd1 is a potential therapeutic target for Rapamycin-resistant renal Cancer.

▲TOP

P-2333

Activation level of the mTORC1/4EBP1/eIF4E pathway is a predictor for recurrence of clear cell renal cell carcinoma

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Objectives: We investigated clinicopathological features relating to recurrence of localized clear cell renal cell carcinoma (ccRCC) after curative nephrectomy.Materials and methods: We retrospectively examined 303 localised ccRCC patients treated surgically at our hospital. The extent of mTORC1/4EBP1/eIF4E pathway activation in ccRCC tissues was evaluated as strong, intermediate, or weak based on immunohistochemical semi-quantitation of 4EBP1, p-4EBP1, and eIF4E expression levels in surgical specimens. Effects of several clinicopathological features on recurrence-free interval (RFI) was statistically analyzed.Results: Forty-seven patients experienced recurrence as distant metastasis during median follow-up of 7.53 years. pT1b or higher stages, pN1, Fuhrman grade 3/4, and higher activation of the pathway were significant factors of a shorter RFI. A multivariate Cox model showed that these factors were independent predictors of recurrence. The similar findings were confirmed in an analysis of published data on ccRCC patients from the Cancer Genome Atlas database.Conclusion: Activation levels of the pathway is a prognostic biomarker for postoperative recurrence of localized ccRCC.

▲TOP

[P-2340] P14-43 [English/Japanese]
Renal cell carcinoma (5)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

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P-2340
Withdrawn

No Abstract

▲TOP

P-2341

Exploration of the biomarkers contributing to prediction of progression in clear cell renal cell carcinoma

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INTRODUCTION: Metabolic changes are observed in most cancer cells and lipid metabolism has characteristic biochemical signatures. In this study, we aimed to analyze a broad range of lipids in clear cell renal cell carcinoma (ccRCC) and to identify biomarkers that can predict the progression.METHODS: We obtained 47 paired samples of cancerous tissues and normal renal cortex tissues from patients with ccRCC and applied desorption electrospray ionization imaging mass spectrometry (DESI-IMS). DESI-IMS was performed in negative ion mode. We picked up the ions showing 1.5 times higher signal intensity in the cancerous compared with the normal.RESULTS: The ions of m/z 187.10 (azelaic acid), 279.23 (linoleic acid), 281.25 (oleic acid), 329.25 (docosapentaenoic acid), 389.24 (not assigned), 391.26 (not assigned), and 773.53 (PG (36:2)) were identified as the candidate biomarkers. Of these biomarkers, low intensity ratio (cancerous/normal) of m/z 289.25, 389.25 and 391.26 were significantly associated with disease progression.CONCLUSIONS: This study showed that seven biomolecules were specifically elevated in the cancerous tissues and three of seven were correlated with PFS in ccRCC.

P-2342

Role of FGFR4 in clear cell renal carinoma and its potential as a new drug target

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Background：A comprehensive genetic analysis of ccRCC in Japanese patients has reported that the tumors have an increased copy number of FGFR4 gene. We analyzed the role of FGFR4-mediated signals in ccRCC, and investigated a possiblity as new therapeutic target.Method：FGFR4 expression was analyzed via IHC. FGFR4 gene copynumber determined using qRT-PCR. Protein expression levels were determined via Western blotting. We determined the effect of FGFR4 knockdown and FGFR4 inhibitor, via MTS assay. Analysis of cell death was conducted using FACS (cell cycle,AnnexinV). Results：IHC revealed an increase in expression of FGFR4 in clinical specimens. FGFR4 expression was also confirmed in WB in RCC cell lines. Suppression of cell proliferation was confirmed via siFGFR4 knockdown, and pAKT, pERK1/2 was also inhibited. Cell proliferation was suppressed also in pharmacological inhibiton of BLU9931. Cell death analysis revealed that apoptosis was induced. Results for verification in the animal model will be described in a future report. Discussion：FGFR4 is involved in proliferative signaling in ccRCC. FGFR4 inhibition may be an effective potential target for new antiRCC drugs.

P-2343

Targeting metabolism re-programming in drug resistant renal cell carcinoma

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Metabolism re-programming occurs in cancer cells. In this study, we aimed to elucidate mechanism and conquest of drug resistant acquisition regarding HIF2α based on metabolism re-programming. First, we established a sunitinib-resistant RCC cell (SU-R-786-o), followed by the establishment of HIF2α knockout cell (HIF2α-KO-SU-R-786-o) by CRISPR/Cas9. Metabolomics analysis showed that metabolites were clearly divided among 786-o, SU-R-786-o, and HIF2α-KO-SU-R-786-o, and that serine synthesis was significantly activated in HIF2α-KO-SU-R-786-o. RNA sequence and quantitative proteome analyses showed that phosphoglycerate dehydrogenase (PHGDH), a key enzyme for serine synthesis, was accelerated in HIF2α-KO-SU-R-786-o. PHGDH inhibitor reduced cell growth in vivo and in vitro by inducing apoptosis in HIF2α-KO-SU-R-786-o more than in parent cells. TCGA database showed that patients with PHGDH amplification (n=24) had poor overall survival (P=0.0003) compared to patients without amplification (n=500). In conclusion, activated serine synthesis can be a therapeutic target for patients with PHGDH amplification as first line therapy as well as patients resistant to HIF2α antagonist.

▲TOP

P-2344

The important role of glycine N-methyltransferase in the proliferation of renal and urothelial carcinoma

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(background) Glycine N-methyltransferase (GNMT) plays a role in the metabolism of methionine as well as in gluconeogenesis. We have recently reported that the GNMT gene acts as an oncogene. However, little is known about the specific function of GNMT in carcinogenesis and tumor progression in urothelial carcinoma (UC) and renal cell carcinoma (RCC). To better our understanding of the function of GNMT in UC and RCC, we used small interfering RNAs (siRNAs) to examine the effects of GNMT knockdown on cell proliferation and the cell cycle. In addition, the expression of GNMT protein in UC and RCC tissues was evaluated. (results) GNMT expression was upregulated in some of these RCC and UC cell lines, especially the high-grade malignant cell line. siRNA-mediated knockdown of GNMT had only a modest effect on the proliferation of cancer and normal cells, but dramatically inhibited the proliferation of high grade UC and RCC cells. (conclusion) This is the first investigation to suggest that GNMT plays an important role in promoting cell growth in UC and RCC, especially high-grade UC and RCC, via the regulation of apoptosis.

▲TOP

[P-2350] P14-45 [English/Japanese]
Prostate cancer (2)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

Takahiro Kojima / Dept. Urology, Univ. of Tsukuba

P-2350

CCL2 induces cabazitaxel resistance in prostate cancer cell line through AKT signaling pathway

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Introduction and Objective: Resistance to cabazitaxel (CBZ) is a major clinical problem for patients with docetaxel(DOC)-resistant. The mechanisms of the CBZ-resistance is remained unclear. We tried to elucidate the mechanisms of resistance to CBZ through linked with CCL2 chemokine. Methods: AR-negative human PCa cell line (DU145), and its DOC-resistant cells (TxR), and DOC-CBZ-resistant cells (TxR/CxR) were used in this study. The comparison cDNA microarray was performed and selected the most potential chemokine gene CCL2. The expression and role of CCL2 in those PCa cells was determined using RT-PCR, ELISA, proliferation, migration and Western blot analysis. The optimal dose of CBZ was identified. The results: CCL2 gene expression in CxR cells was increased by 50 folds than DU145, which by PCR. CCL2 stimulates proliferation and migration of DU145, and a specific for CCR2 receptor antagonist suppressed this effect in TxR and CxR cells. CCL2 reduces effect of CBZ on DU145, and CCR2 antagonist enhances the CBZ effect in TxR and CxR. The knockdown of CCL2 gene inhibits TxR and CxR migration as well. Conclusion: CCL2, of apparent contributor of development of cabazitaxel-resistance.

▲TOP

P-2351

CD44 promotes migration ability of docetaxel-resistant prostate cancer cells via induction of Hippo-Yap signaling

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Docetaxel-based chemotherapy confers higher five-year survival rate for patients with castration-resistant prostate cancer (CRPC). However, docetaxel-resistant prostate cancers are more malignant related to poor prognosis. We determine the cause of the aggressive phenotype of docetaxel-resistant prostate cancer (PCa). In our study, the docetaxel-resistant CRPC- PC/DX25 cells exhibit higher mobility and express more endogenous CD44. CD44 is a multifunctional protein involved in regulation of drug resistance and cell migration. CD44 has been reported to function as upstream regulator of Yes-associated protein (YAP), a major mediator in Hippo-pathway which is involved in human tumorigenesis. Knockdown of CD44 reduces protein expression of YAP but increases protein level of phospho-YAP S127. Phosphorylation of S127 on YAP suppresses the oncogenic activity of YAP as well as decreases protein expression of CTGF and CYR61, the downstream genes of YAP. CTGF and CYR61 are related to cell migration, cell invasion, and cell adhesion. In conclusion, CD44/YAP pathway may be a potential therapeutic target for docetaxel-resistant CRPCs.

P-2352

Targeting SIRT1 impairs chemoresistance of prostate cancer cells by a synthesized anthraquinone derivatives

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SIRT1, one of the Sirtuin family of proteins, is highly expressed in prostate cancer (PCa). ANT (1-Hydroxy-3- [(E)-4- (piperazine-diium)- 2-enyloxy]- 9,10-anthraquinone ditrifluoroactate) has been shown to induce autophagic cell death in cancer cells. However, its anticancer effect remains unknown. This study aimed to investigate the effects of ANT on autophagy and the underlying mechanisms in regulating SIRT1 by two human prostate cancer cell lines PC-3 and DU145. We show that ANT induces caspase-independent cell death and autophagy in concentration- and time-dependent manners in both PC-3 and DU145 cells through inhibiting the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) and p38 mitogen-activated protein kinase (MAPK) pathways. ANT downregulates SIRT1, and inhibition of SIRT1 enhances autophagy in PC-3 and DU145 cells. Our findings indicate that ANT induces autophagyand promotes cell death in prostate cancer cells via SIRT1- and PI3K/Akt/mTOR-mediated pathways with contribution from p38 MAPK-associated pathways.

P-2353

The C5a-C5a receptor system in prostate cancer progression

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Recent studies have shown complement activation in cancer tissues, suggesting that the byproduct anaphylatoxin C5a is a cancer microenvironment factor. We found C5a-receptor (C5aR) expression of various cancers and enhancement of cancer cell invasiveness by C5a via C5aR (Clin. Cancer Res. 2013). C5aR expression was associated with cancer progression and poor prognosis of the patients with stomach, breast or urinary tract cancer. To explore a role of the C5a-C5aR system in prostate cancer progression, prostate cancer cell lines were examined for C5aR expression. C5aR mRNA expression was observed in PC3, LNCap, DU145 and C4-2 cells by qRT-PCR and PC3 cells were the strongest. By flow cytometry cell surface C5aR protein was detected in PC3, LNCap and C4-2 cells but not DU145 cells. Matrigel chamber assay revealed that C5a enhanced PC3 cell invasion in a dose-dependent manner. The prostate cancer tissue samples were immunostained with anti-C5aR antibody and the association of cancer cell C5aR expression with cancer progression and prognosis of the patients was investigated. The results suggested cancer-promoting activity of the C5a-C5aR system in prostate cancer.

▲TOP

P-2354

aPKCλ/ι associates with prostate carcinogenesis by increasing cytokine expression

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Background: We have previously reported the association between aPKCλ/ι expression and prostate cancer progression. aPKCλ/ι overexpressed in prostate cancer tissues. Furthermore, aPKCλ/ι increased interleukin-6 expression in prostate cancer cells. Therefore, the aPKCλ/ι is a biomarker and possibly a molecular target for prostate cancer. In this study, we investigated whether aPKCλ/ι is involved in prostate carcinogenesis or not. Materials and Methods: The molecular mechanism of prostate cancer development was investigated using aPKCλ overexpressed RWPE-1 cells (aPKCλ-RWPE-1 cells). Cytokine was detected using cytokine array. MMP-9 expression and activity were confirmed by qPCR and gelatin zymography. Malignant cells were confirmed by the xenograft model bearing aPKCλ-RWPE-1 cells. Results: The results showed that aPKCλ-RWPE-1 cells were increased MMP-9 expression and some cytokines compared to RWPE-1 cells. Furthermore, xenograft model bearing aPKCλ-RWPE-1 cells showed its outgrowth. Conclusion: These results suggest that the aberrant aPKCλ expression plays crucial role in the development of prostate cancer.

P-2355

SLCO2B1 high expression in Prostate Cancer is Associated with worse disease-free survival after Radical Prostatectomy

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SLCO genes encode transport proteins up-taking number of substrates into cells including androgens. High expression of SLCO2B1 has been shown to be associated with the resistance to androgen deprivation therapy. However, the significance of SLCO2B1 expression in the recurrence after radical prostatectomy has not been elucidated. Clinical and RNA-seq data were all obtained from the Cancer Genome Atlas (TCGA). The patients with high expression of SLCO2B1 were found to have more aggressive cancer characteristics, including high Gleason score. High expression group showed significantly worse disease-free survival after radical prostatectomy (p=0.026). Significant difference in disease-free survival between high and low expression groups were only observed in the patients with GS≧8 (p=0.005). GSEA demonstrated that in the high expression group of SLCO2B1 enriched EMT signaling related genes.High expression of SLCO2B1 associated with the aggressive cancer characteristics and recurrence after radical prostatectomy. Furthermore, the high recurrence rate with high expression of SLCO2B1 may be explained with up-regulated EMT signaling.

P-2356

Phosphatidylinositol phosphate profiles in pre-clinical and clinical prostate cancer

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Phosphatidylinositol phosphates (PIPs) are involved in many cellular processes including cancer progression; however, PIP metabolic features associated with prostate cancer (PCa) are unknown. We investigated PIP profiles in pre-clinical and clinical PCa, using mass spectrometry. We examined PIP profiles in PTEN-knockout prostate cells, murine prostate tissues lacking PTEN, and human prostate tissues obtained from patients with PCa and benign prostate hyperplasia (BPH). In PNT1B prostate cells, PTEN deficiency increased PIP_3 levels. In vivo, PTEN KO mice had significantly higher prostate PIPs levels compared to controls. In human prostate tissues, the PI levels in PCa tissues were significantly higher than BPH tissues. Regarding acyl chain profiles, patients with PCa had significantly lower polyunsaturated fatty-acids in PI, PIP, and PIP_2 acyl chains, compared to patients with BPH. During subgroup analyses, several molecular species were significantly higher in the patient with pT3 than those observed in the patients with pT2. Evaluating PIP PCa profiles may enhance our understanding of novel mechanisms of PCa progression and tissue biomarkers for prognostication.

▲TOP

[P-2334] P14-42 [English/Japanese]
Renal cell carcinoma (4)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

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P-2334

Expression and functional analysis of KIF23 in renal cell carcinoma

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In the past decade, it has been generally accepted that cancer stem cells (CSCs) play an important role in cancer progression. One useful method for characterizing CSCs is spheroid colony formation. Previously we found that Kinesin family member 23 (KIF23) was highly expressed in spheroid-forming gastric cancer cells. Then we supposed that KIF23 also plays an important role in CSCs. However, expression of KIF23 in renal cell carcinoma (RCC) has not been investigated. In this study, we investigated the expression of KIF23 in RCC and examined the biological function. Expression of KIF23 was observed in 60 (44%) out of 136 RCC cases, and that was associated with high grade groups of histological classification ($p<0.0001$), T classification ($p=0.0018$) and stage grouping ($p=0.0002$). The univariate and multivariate analysis indicated that KIF23 expression was an independent prognostic indicator ($p=0.0369$). Proliferation capacities of RCC cell line Caki-1 were significantly reduced following transfection of KIF23-targeting siRNA compared with negative-control siRNA. These results suggest that KIF23 can contribute to the progression of RCC.

▲TOP

P-2335

Expression of claudin 1 and 4 in renal cell carcinoma

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Claudins (CLDNs) are major tight junction proteins associated with cellular polarity and differentiation. We investigated expression of CLDN1 and 4 by immunostaining in tissue array of 202 patients with renal cell carcinoma (RCC). In RCCs, CLDN1 was detected in 91 cases (45%) and the 5 (2.5%) showed overexpression than those in tubular epithelium. Although CLDN1 expression was correlated reversely with pT-factor, there is no correlation with Fuhrman grade and clinical stage. CLDN4 was detected in 127 cases (63%) and the 34 (17%) exhibited overexpression. There is no correlation with grade, pT-factor or stage. The 5 patients showed nuclear expression of CLDN4 without membranous staining. All these patients were pT3 or 4, which was at higher frequency than that in patients without nuclear CLDN4 expression. In human RCC cell lines, a high metastatic SN12L1 but not a low metastatic SN12C showed nuclear CLDN4. In SN12L1 cells, serine phosphorylation of CLDN 4 and overexpression of PKC ε were detected, which was associated with EMT phenotype. It is suggested that nuclear expression of CLDN4 might induce EMT in RCC.

P-2336

c-Ski accelerates renal cancer progression through the attenuation of TGF-β signaling

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Although TGF-β is reported to be involved in many kinds of cancer, its role in clear cell renal cell carcinoma (ccRCC) is not fully investigated. In the present study, the function of TGF-β on ccRCC progression was investigated using histopathological examination and in vivo study. First, immunohistochemical analysis revealed that c-Ski, transcriptional co-repressor in Smad-dependent TGF-β signaling, was elevated in ccRCC tissues, compared with normal tissues. Next, renal orthotopic tumor models with bioluminescence imaging demonstrated that overexpression of c-Ski in human ccRCC cells promoted in vivo tumor formation. Enhancement of tumor formation was also reproduced by the introduction of dominant negative mutant of TGF-β type Ⅱ receptor in ccRCC cells, suggesting that tumor promoting effect of c-Ski might be dependent on inhibition of TGF-β signaling. Finally, molecular mechanism for tumor-suppressive role of TGF-β was assessed. TGF-β moderately inhibited proliferation of ccRCC cells through the induction of apoptosis. These findings suggested that c-Ski suppresses TGF-β signaling in ccRCC cells, which thereby attenuates TGF-β-induced apoptosis.

P-2337

Clinical significance of GPI-80 expression in peripheral blood of metastatic renal cell cancer patients

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Myeloid-derived suppressor cells (MDSCs) are difficult to detect in neutrophil or monocyte populations, because of its diversity. In this study, we investigated the value of Glycosylphosphatidylinositol-anchored 80 kD protein (GPI-80), known as a regulator of Mac-1 (CD11b/CD18), in MDSC detection in peripheral blood among mRCC patients and the relationship between GPI-80 expression and overall survival. The coefficient of variation (CV) of GPI-80 was augmented in the CD16hi neutrophil population, and mean fluorescence intensity (MFI) of GPI-80 MFI was increased in the CD33hi monocyte population in peripheral blood from mRCC patients. The GPI-80 CV in CD16hi was inversely correlated with the proliferative ability of T cells, and the GPI-80 MFI of CD33hi was correlated with reactive oxygen species production. Furthermore, the GPI-80 CV in CD16hi and the GPI-80 MFI of CD33hi was correlated with worse overall survival. These results suggested that the pattern of GPI-80 expression is not only a simple and useful marker for MDSC expression but also a useful prognostic factor for mRCC patients.

▲TOP

P-2338

Phospho-eIF4E prevents tumor recurrence by suppressing epithelial-mesenchymal transition in renal cell carcinoma

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Background: eIF4E, which regulates initiation of mRNA translation, is phosphorylated only by MNK1/2 in vivo. However, significance of eIF4E phosphorylation in translation and cancer prognosis remains inconsistent. We examined oncological roles of p-eIF4E in renal cell carcinoma (RCC). Methods: p-eIF4E expression was evaluated immunohistochemically in FFPE specimens resected from RCC patients (N0M0). The molecular status in the subcellular signaling was examined by immunoblotting in human RCC cell lines. Cell proliferation, migration and invasion were studied with MTS, wound healing and Matrigel assays, respectively. Inhibition of MNK1/2 was achieved by chemicals (CGP57380 or ETP45835), or genetic knockdown with specific siRNAs. Results: Increased p-eIF4E expression in primary RCC tissues was associated with low metastatic recurrence rate after nephrectomy. Suppression of p-eIF4E by the pharmacological inhibitors or knockdown with siRNAs against MNK1 or MNK2 increased vimentin and N-cadherin expression, and enhanced cell migration and invasion, but not cell proliferation in RCC cell lines. Conclusion: p-eIF4E would suppress epithelial-mesenchymal transition and recurrence in RCC.

P-2339

GSK-3 can affect mRNA translation and proliferation via regulation of 4EBP1/eIF4E and MNK1/eIF4E in renal cell carcinoma

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Objective: We investigated roles of glycogen synthase kinase-3 (GSK-3) for proteins regarding translation initiation-4EBP1/eIF4E and MNK1/eIF4E axes, in renal cell carcinoma (RCC).Materials and Methods: The expression and phosphorylation of molecules were examined by immunoblotting in human RCC cell lines including 786-O, 769-P, and ACHN. Cell proliferation was investigated by MTS assay. Inhibition of GSK-3 and MNK1/2 was achieved by pharmacological treatment with AR-A014418, SB216763 or LY2090314, and genetic knockdown with siRNAs, respectively. The direct action of GSK-3 β on its substrates was evaluated by an in vitro kinase assay.Results: Cell proliferation and phosphorylation level of 4EBP1 were strongly suppressed by GSK-3 inhibition (independently of rapamycin-sensitivity). In vitro kinase assays showed direct action of GSK-3 on 4EBP1 phosphorylation. Also GSK-3 inhibition increased phosphorylation level of eIF4E in parental RCC cells and in those pretreated with siMNK2, but not with siMNK1, which means MNK/eIF4E axis.Conclusion: GSK-3, highly expressed in RCC cells, can play important roles in translation initiation by regulating p-4EBP1 directly and MNK1/eIF4E axis.

▲TOP

[P-2345] P14-44 [English/Japanese]
Prostate cancer (1)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

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P-2345

Caffeic Acid Phenethyl Ester (CAPE) Suppresses Protein Expression Level of AR-V7 in Human Prostate Cancer

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Androgen receptor splice variant-7 (AR-V7), an androgen-insensitive transcription factor, plays an essential role in the development of castration-resistant prostate cancer (CRPC). Enzalutamide and abiraterone can not efficiently inhibit CRPC with AR-V7 expression. Caffeic acid phenethyl ester (CAPE) is a bioactive component extracted from honeybee hive propolis. We previously reported that CAPE treatment suppresses cell proliferation and tumor metastasis of prostate cancer cells. We thus examined if CAPE treatment can reduce expression of AR-V7. AR-V7 variant is highly expressed in 22RV1 cell line. We observed that CAPE treatment suppressed both mRNA and protein expression of AR-V7 as well as its downstream ubiquitin-conjugating enzyme 2C (UBE2C) in 22RV1 cell line. Protein stability of AR-V7 is affected by the phosphorylation of Serine 81 on AR, which is regulated by CDK1. Our data showed that CAPE reduced protein expression of CDK1 and accelerated degradation of AR-V7. In xenograft model, we observed CAPE treatment repressed tumor growth and angiogenesis of 22RV1 as well as suppressed expression of AR-V7. Our data suggested that CAPE might be a potential therapy for CRPC.

▲TOP

P-2346

Different Role of Androgen Receptor in Regulation of Prostate Cancer Metastasis with or without Androgen

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Androgen receptor (AR), an androgen-activated transcription factor, plays essential roles in the development, progression, and metastasis of prostate cancer (PCa). Previously, we reported that in the absence of androgen, re-expression of AR in PC-3 cells suppresses cell migration and invasion. In this study, we determine the effect of AR on PCa cell invasion under androgen treatment. Surprisingly, androgen stimulates migration and invasion of PC-3 cells re-expressing AR. Micro-Western Array analysis indicates that androgen increases Slug, Snail, Twist, N-cadherin and YAP, and decrease E-cadherin in PC-3 cell re-expressing AR. Co-IP assay also showed AR interacts with YAP in this PCa cell. Our finding suggests that AR has opposite regulatory role on PCa invasion in the absence vs. presence of androgen.

P-2347

Identification and functional analysis of lncRNAs in prostate cancer bone metastasis

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Bone is the most frequent site for prostate cancer metastasis, with up to 90% of patients with advanced disease having bone metastasis. One prostate cancer cells metastasize to bone, the mortality rate of prostate cancer patients increases significantly, and novel therapeutic approaches are needed to address bone metastatic disease. Growing evidence suggests that cancer cells and bone marrow interaction plays a crucial role in prostate cancer bone tropism. In this work, we compared the genetic profile between VCaP cell line (derived from vertebral metastasis) and LNCaP cell line (derived from lymph node metastasis) to identify lncRNAs differentially expressed in VCaP cells. Several lncRNAs within the HOX clusters were differentially expressed in VCaP cells, which are reporter to be involved in cancer metastasis or are located close to HOX genes known to control bone remodeling. Knockdown experiments by siRNA revealed that HOX lncRNAs may regulate bone regulatory genes. Key transcription factors were identified as upstream regulators of these lncRNAs, highlighting their importance in prostate cancer development.

P-2348

Significance of endocrine fibroblast growth factor subfamily as serum biomarkers in castration-resistant prostate cancer

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Introduction and Objective) The aim of this study was to investigate the impact of eFGFs in castration-resistant prostate cancer (CRPC). Materials and Methods) In 35 CRPC patients, 145 hormone-sensitive prostate cancer (HSPC) patients, and 30 non-cancerous participants, serum concentration of eFGFs were measured using ELISA assay. The expression of eFGFs in cancerous tissues was analyzed in 9 cases with CRPC and 149 cases with HSPC. Clinicopathological features and serum eFGFs level were analyzed. Results) Enhanced expression of both FGF19 and FGF21 were detected in 6 of the 9 cases of biopsy specimens derived from CRPC, while in 39 of the 149 cases ($p=0.0090$) and 40 of the 149 cases ($p=0.0107$) in HSPC cases, respectively. The median of serum FGF19 and FGF21 levels were significantly higher than those in cases with HSPC ($p=0.0004$, and $p<0.0001$) and in non-cancerous participants ($p=0.0089$ and $p=0.0373$). In CRPC patients with disease progression, the rate of cases with increased serum FGF19 levels were significantly higher than that in cases with stable disease ($p=0.0046$). Conclusions) Serum eFGFs might be a candidate as a novel biomarker of CRPC.

▲TOP

P-2349

Search for fusion transcripts in hormone sensitive and castration resistant prostate cancer xenograft model

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Acquisition of castration resistance is still important problem in prostate cancer treatment. We established hormone sensitive prostate cancer xenograft mouse model, JDCaP derived from Japanese men and castration resistant xenograft model, JDCaP-CR2M, JDCaP-CR4M and JDCaP-CR2F derived from JDCaP previously. RNA-Seq analysis was performed on JDCaP, JDCaP-CR2M, JDCaP-CR4M and JDCaP-CR2F to search fusion transcripts especially expressed in JDCaP-CRs. Library preparation was performed with SMARTer Ultra Low Input RNA kit for sequencing v3 (Clontech Laboratories, Mountain View CA). Next generation sequencing analysis was carried out with Ion Chef and Ion Proton system (Life Technologies Corporation, Carlsbad, CA). Fusion search was performed by Tophat-Fusion. Number of detected fusion transcript gene pair was 30, 31, 57 and 21 in JDCaP, JDCaP-CR2M, JDCaP-CR4M and JDCaP-CR2F respectively. Six gene pairs were detected in all cases. Three gene pairs were detected in all castration resistant models. These fusion candidate gene pairs might be involved in progression and acquisition of castration resistance in prostate cancer.

▲TOP

[P-2363] P16-1 [English/Japanese]
Signal transduction inhibitor (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Daizo Koinuma / Dept. Mol. Pathol., Grad. Sch. Med., The Univ. Tokyo

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P-2363

Tris DBA[Tris(dibenzylideneacetone)dipalladium(0)] as a selective STAT3 inhibitor for cancer therapy

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Cancer is complex disease involving complex genetic and epigenetic heterogeneity making it the second leading cause of death globally after heart disease. While conventional treatments like surgery, radiotherapy, chemotherapy are principal strategies, the focus is shifting to therapies that can target multiple pathways to treat cancers. In the present report, we hypothesized that Tris DBA, an organometallic compound, might inhibit proliferation and tumor growth, and induce apoptosis in multiple myeloma (MM) and hepatocellular carcinoma (HCC) cells, thereby potentially exhibiting a broad spectrum of anticancer effects. Our preliminary results have clearly indicated that Tris DBA could substantially inhibit both constitutive and IL-6 inducible STAT3 activation in MM and HCC cells. We have also shown Tris DBA efficacy in orthotopic mouse model of HCC. In future work, we aim to investigate the possible molecular target(s) of Tris DBA in MM and HCC cells and further characterize the molecular mechanism(s) underlying STAT3 inhibitory effects of the drug. A proteomic and phospho proteomic study is underway to further elucidate the direct and indirect effects of tris DBA.

▲TOP

P-2364

TNF-alpha Confers Resistance to Apoptosis in Cholangiocarcinoma Cells by Activating MAPK and AKT Signaling

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The objective of this study is to understand what made cholangiocarcinoma (CCA) an aggressive cancer, and why it is resistant to conventional therapy. Here, the mechanism underlying this tolerance was investigated using KKU-100, a CCA cell line derived from a Thai patient as a model. Treatment with TNF-alpha up to 80 ng/mL did not significantly affect KKU-100 cell viability as determined by MTT assay. However, increasing concentrations of TNF-alpha markedly enhanced pMAPK1/2 and pAKT levels, suggesting that activation of MAPK and/or AKT signaling pathways may underlie tolerance to TNF-alpha-induced cell death. This idea was tested by blocking the MAPK or AKT signaling pathways with U0126 or LY294002, a MEK1/2 or a PI3K inhibitor, respectively. This co-treatment significantly reduced cell viability in KKU-100 compared to single treatments with TNF-alpha or the inhibitor alone. In addition, apoptotic nuclei was significantly induced as shown by nuclear staining with DAPI. Together, results of this study showed that MAPK and AKT signaling pathways were activated by TNF-alpha and these may be an important attribute to TNF-alpha tolerance which underlies the aggressiveness in CCA cells.

P-2365

Aberrant FGF/FGFR signaling as a mechanism of pathogenesis in Cholangiocarcinoma

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Cholangiocarcinoma(CCA) is an aggressive cancer of the bile duct with surgical resection or cytotoxic chemotherapy being the only treatment options. Tumor invasion and metastasis makes treatment complicated. In Thailand, it is predominantly caused by a liver fluke Opisthorchis Viverrini whereas different factors are involved in the UK. We aim to elucidate the underlying mechanism and develop treatment strategies based on their molecular subtypes. Aberrant FGFR expression result in constitutively active FGF signaling in cancer which leads to tumor progression. Phosphorylated FGFR1 expression were determined in surgically resected tissue samples from CCA patients (from Ramathibodi Hospital, Thailand and QMC, Nottingham, UK) by Luminex® ELISA and in cell lines by Western blotting. Increased levels of FGF-1 and FGFR1 was observed Thai samples and decrease in FGF-2 was observed in Thai samples relative to the UK samples. CCA cell lines and patient derived primary cell lines (from PDX) were sensitive to potent FGFR1 inhibitor, PD173034.This provides a rationale in pursuing FGFR signaling as a candidate for immunotherapy and genomic medicine for the effective treatment in CCA.

P-2366

Effectiveness of tazemetostat as a novel epigenetic drug for cholangiocarcinoma

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Cholangiocarcinoma (CCA) is a deadly cancer of the bile duct epithelium. Treatment of patients by chemotherapy or radiotherapy has shown unfavorable effectiveness leading to short survival rate. Thus, novel therapeutic effectiveness for CCA treatment is required. Our IHC study in CCA showed overexpression of polycomb repressive complex 2 (PRC2). PRC2 act as histone methyltransferase by trimethylation of lysine 27 of histone H3 (H3K27me3). Tazemetostat inhibits PRC2 activity by competing with S-adenosylmethionine resulting in decreased H3K27me3 level. We aimed to study the effect of tazemetostat on cell proliferation and apoptosis in CCA cell lines in 3D culture. We found the decrease of H3K27me3 after tazemetostat treatment by time dependent and significant anti-proliferative effect compared to control. The modulation of proliferation-regulated genes and apoptosis-related genes after treatment was also examined. Interestingly, an elevated expression of P21, BAK1, TNFSF10 and TNF was observed after treatment suggesting anti-proliferative and apoptosis effect on CCA cell lines. In summary, tazemetostat may be potentially used as a novel epigenetic drug for CCA treatment.

▲TOP

P-2367

Acquired resistant mechanism to afatinib in HER2 amplified gastric cancer cells

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Background: We recently reported the antitumor effect of pan-HER inhibitor, afatinib, to HER2 amplified gastric cancer cells. The purpose of this study is to identify the acquired afatinib-resistant mechanisms and propose treatment strategies. Materials and Methods: Two afatinib-resistant gastric cancer cell lines, N87-AH and SNU216-AH, were established from HER2 amplified cells. We examined the resistant mechanisms including EMT features, stem cell like features, up-regulation of IGF1R or IGFBPs, oncogenic mutation of HER2 or PIK3CA, and gene amplification of MET or Yes1. Results: The activation of HER2 and down signal molecules were down-regulated in N87-AH and up-regulated in SNU216-AH. N87-AH acquired MET amplification and AXL up-regulation; the combination therapy, afatinib and cabozantinib, showed synergistic effect and overcome the resistance. YES1 was amplified in SNU216-AH, and dasatinib, Src inhibitor, showed strong effect by single agent. Conclusion: We identify the MET amplification with AXL overexpression, or Yes1 amplification as afatinib-resistant mechanisms. We believe this finding contribute to the development of personalized medicine for gastric cancer patients.

P-2368

Gedatolisib (PF-05212384) induces anti-tumor activity against various types of canine tumor in vitro

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The PI3K/mTOR pathway is a therapeutic target in various type of human tumors, and dual PI3K/mTOR inhibitors showed anti-tumor activity in clinical and pre-clinical studies. However, in canine tumors, anti-tumor activity of dual PI3K/mTOR inhibitors is unclear. Here we evaluated the anti-tumor activity of gedatolisib, a dual PI3K/mTOR inhibitor, in vitro as a novel therapeutic approach in canine tumors.
Twelve canine cell lines, including six types of tumors (osteosarcoma, transitional cell carcinoma, melanoma, histiocytic sarcoma, mast cell tumor, and lung adenocarcinoma), were used in this study. We evaluated the anti-proliferative activity of PI3K/mTOR inhibitors in the effects on the PI3K/mTOR pathway and cell cycle distribution.
Gedatolisib showed potent anti-proliferative activity in the canine tumor cell lines compared with those of the other PI3K/mTOR inhibitors. Gedatolisib suppressed the phosphorylation of Akt and mTORC1 substrates, and induced G_0/G_1 cell cycle arrest with p27 accumulation in the nucleus.
These findings support the possibility of using gedatolisib as a therapeutic approach to treat various types of canine .

P-2369

The identification of natural compounds targeting Annexin A2 with an inhibitory effects towards NF- κ B

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AnxA2 promotes NF- κ B activation by interacting with NF- κ B p50 subunit and facilitating its nuclear translocation. Here we demonstrated ginsenoside Rg5 (G-Rg5) and Rk1 (G-Rk1) directly bound to AnxA2 by molecular docking and cellular thermal shift assay. Both G-Rg5 and G-Rk1 inhibited the interaction between AnxA2 and NF- κ B p50 subunit, their translocation to nuclear, and NF- κ B activation. Inhibition of NF- κ B by G-Rg5 and G-Rk1 deceased the expression of inhibitor of apoptosis proteins (IAPs), leading to caspase activation and apoptosis. Over expression of AnxA2 K302A mutant, which fails to interact with G-Rg5 and G-Rk1, effectively reduced the NF- κ B inhibitory effect and apoptosis induced by G-Rg5 and G-Rk1. In addition, knockdown of AnxA2 largely enhanced NF- κ B activation and apoptosis induced by the two molecules, indicating that the effects of G-Rg5 and G-Rk1 on NF- κ B were mainly mediated by AnxA2. This study for the first time demonstrated that G-Rg5 and G-Rk1 inhibit tumor cell growth by targeting AnxA2 and NF- κ B pathway, and G-Rg5 and G-Rk1 might be promising natural compounds for targeted cancer therapy.

▲TOP

[P-2376] P16-3 [English/Japanese]
Lung cancer kinase inhibitor

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Masakiyo Sakaguchi / Dept. Cell Biol., Okayama Univ. Grad. Sch. Med. Dent. Pharm. Sci.

P-2376

Mechanism of resistance to third-generation EGFR-TKI, rociletinib, in lung adenocarcinoma cells with EGFR-T790M mutation

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First- and second-generation EGFR-TKIs (gefitinib, erlotinib, and afatinib) are effective in treating non-small cell lung cancer in (NSCLC) patients harboring the EGFR-activating mutation. However, resistant tumors with *EGFR*-T790M commonly develop. Third-generation EGFR-TKIs (osimertinib and rociletinib) were developed to overcome resistance due to EGFR-T790M, but acquired resistance still occurs. Afatinib-resistant (AfaR) cells, harboring exon 19 deletion and *EGFR*-T790M, are sensitive to third-generation EGFR-TKIs. AfaR cells were developed from PC-9 cells with the EGFR-activating mutation, a 15 bp deletion in exon 19. AfaR cells were exposed to increasing concentrations of rociletinib for 12 months to obtain cells resistant to rociletinib (RocR1 and RocR2). RocR1 and RocR2 exhibited dominant WT alleles for exon 19 deletion and T790M. RocR1 exhibited amplification of KRAS, which a combination of MEK inhibitor and afatinib could overcome. RocR2 exhibited amplification of EGFR, which a combination of anti-EGFR antibody, cetuximab, and afatinib could overcome. These results offer insights into the development of more effective therapeutic strategies to treat resistant NSCLC tumors.

▲TOP

P-2377

Establishment of drug sensitivity evaluating system of RET-rearranged lung cancer cells by 3D culture system

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RET gene rearrangement are reported to be observed in approximately 1 % of non-small cell lung cancer. Currently, multiple RET-TKIs such as vandetanib, lenvatinib, cabozantinib, LOXO-292, or BLU667 have been evaluated in clinical trials. But the limited number of RET-rearranged non-small cell lung cancer cell lines are public available. A lung adenocarcinoma cell line LC2/ad was reported to harbor CCDC6-RET fusion, but are only partially sensitive to RET inhibitors in vitro culture due to the activation of EGFR. Since vandetanib actively inhibit both EGFR and RET, we first tried to establish vandetanib resistant cells in vitro. By establishing the vandetanib resistant cells, we found 2 mutations in RET kinase domain. In addition, we newly developed a 3D culture system that restores the RET fusion oncogene dependency. Using this 3D culture systems, we confirmed that the identified vandetanib resistant mutant harboring LC2/ad cells are resistant to multiple RET inhibitors, and found to be sensitive to several inhibitors. In this study, we newly developed 3D culture systems that is available to perform drug screening in vitro using RET-rearranged lung cancer cell lines.

P-2378

TAS0286/HM05, a novel potent and selective RET inhibitor, induced tumor regression in RET fusion positive model

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Taiho Pharm. co ltd

Background: RET fusions discovered in NSCLC are well known as an oncogenic driver.The development of potent and selective RET inhibitors are eagerly desired for RET gene defect tumor treatment.We discovered a highly potent and selective RET kinase inhibitor, TAS0286/HM05.Methods: RET kinase activity of TAS0286/HM05 was evaluated using HTRF assay. Cell viability was measured by ATP quantitation. In in vivo study, TAS0286/HM05 was orally administered in subcutaneous transplant model using PDX and cells with different RET gene abnormalities.Results: TAS0286/HM05 inhibited RET kinase activity at a sub-nM level, and proliferation in cancer cells with RET gene abnormalities. In in vivo study, TAS0286/HM05 suppressed phosphorylation of RET and its downstream factor after oral administration, and induced regression in RET fusion-positive tumor and growth suppression in RET activating mutation positive tumor without body weight loss.Conclusions: TAS0286/HM05 is expected to provide a promising treatment for cancer patients with RET gene abnormalities.Collaborators: Emanuela Lovati (Helsinn HA)

P-2379

Withdrawn

No Abstract

▲TOP

P-2380

Evaluation of tyrosine kinase inhibitors with the patient derived lung cancer cells with Her2 activating mutation

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ERBB2 insertion mutations in exon20 induces constitutive activation of ERBB2 tyrosine kinase and high transforming ability, and have been found in 1-2 % of non-small cell lung cancer. Until recently, several pan-ERBB kinase inhibitors, such as afatinib or neratinib are tested in clinical trials, and shown the certain extent of efficacy in some patients. Still, further effective therapeutic strategies are needed for treating them. First, we established the patient derived cancer cells from the pleural effusion of ERBB2 insertion mutation detected lung cancer patient under the IRB approved protocol. In addition, we created the Ba/F3 expressing the ERBB2 with same insertion mutation (ERBB2-mt). Then, we performed the inhibitor screening and ENU mutagenesis using Ba/F3-ERBB2-mt cells by selecting with afatinib. As the results, we found a resistance mutation at biding site of afatinib, and a few drug candidates from the inhibitor screening. Collaborators: Noriko Yanagitani, Makoto Nishio (JFCR)

P-2381

Identification of HER4 as an actionable target for cancer therapy by the use of anticancer selectivity of gefitinib

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Thanks to the progress in understanding molecular mechanism of tumorigenesis, novel class of anticancer drugs, called “molecular targeted drugs”, have become available. These drugs are highly selective to cancers harboring aberrant molecular features found in a certain type of cancer; however, there remains unmet needs in the treatment of cancers. To explore unknown actionable targets, we utilized drug sensitivity database across the JFCR39 human cancer cell line panel to screen chemicals that display highly selective antitumor effect. Among these, we found that gefitinib, an EGFR-tyrosine kinase inhibitor (TKI), selectively inhibited the growth of human lung cancer cell line NCI-H522, whereas it does not harbor activating mutation of EGFR gene nor express EGFR protein. Instead, NCI-H522 overexpressed HER4 protein, and it was dephosphorylated upon gefitinib treatment. Actually, HER4 siRNA selectively inhibited the cell growth. Moreover, we identified more potent HER4-TKIs from those displaying more specific efficacy to NCI-H522 than gefitinib. These results suggest that HER4 could be an actionable target and these TKIs could be used for the treatment of HER4-addicted cancers.

▲TOP

[P-2386] P16-5 [English/Japanese]
Signal transduction inhibitor (2)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Siro Simizu / Dept. Applied Chem., Fac. Sci. Tech., Keio Univ.

P-2386

Effects of E7386 on colorectal cancer organoids and/or co-cultured systems with carcinoma tissue-derived fibroblasts

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A novel β-catenin/CBP binding inhibitor E7386 is expected to affect colorectal carcinoma (CRC) cells particularly with aberrant activation of Wnt/β-catenin signaling pathway. The Wnt signaling pathway is recently reported to play important roles for activation of not only CRC cells but also cancer-associated fibroblasts (CAFs). Here, we established CRC-derived, 3D-cultured organoids and 2D-fibroblasts, and effects of E7386 on the growth and phenotypes of co-cultured organoids and fibroblasts were examined. As a result, the number of organoids and fibroblasts appeared to increase to 1-6- and 2-8-folds, respectively, at 72 hrs after beginning of the culture. E7386 inhibited growth of fibroblasts from lower concentrations (10-30 nM) than those to organoids (30-100 nM), and co-cultured organoids appeared to be more sensitive to E7386 as compared to single cultured ones. Microarray analyses revealed E7386 reduced Wnt signaling pathway-related gene expressions in organoids, and those in fibroblasts are now under analysis. From the results E7386 affected not only CRC cells but also CAFs, suggesting its target at least partly to be cancer microenvironment.

▲TOP

P-2387

Pharmacological difference between degrader and inhibitor against oncogenic BCR-ABL kinase

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CML is caused mostly by an oncogenic BCR-ABL protein kinase, against which clinically useful inhibitors have been developed. An alternative approach to treat CML is to degrade the BCR-ABL protein. Recently, we have developed a potent BCR-ABL degrader, SNIPER(ABL)-39, in which dasatinib is conjugated to an IAP ligand. Due to the dasatinib moiety, SNIPER(ABL)-39 also inhibit ABL kinase activity, which complicates our understanding of the impact of BCR-ABL degradation in CML growth inhibition. To address this issue, we developed a structurally related inactive degrader, SNIPER(ABL)-59, which inhibits kinase activity but does not degrade the BCR-ABL protein. SNIPER(ABL)-39 showed slightly weaker activity than SNIPER(ABL)-59 in inhibiting cell growth when CML cells were treated for 48 h. However, SNIPER(ABL)-39 showed sustained growth inhibition and BCR-ABL degradation even when the drug was removed after short-term treatment, whereas CML cell growth rapidly resumed and kinase signaling rapidly recovered following removal of SNIPER(ABL)-59 and dasatinib. These results indicate that BCR-ABL degrader shows more sustained inhibition of CML cell growth than ABL kinase inhibitor.

P-2388

Statins induce apoptosis via inhibition of Ras/ERK and Ras/mTOR signaling pathways in hematopoietic tumor

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<Purpose> Statins have been demonstrated to improve cancer-related mortality or prognosis in patients of various cancers. However, the details of the apoptosis-inducing mechanisms remain unknown. In the present study, we investigated the mechanism of apoptosis induction by statins in hematopoietic tumor cell lines. <Methods> Cell viability was assessed by the trypan blue dye method. Signal molecules were determined by western blots. <Results> This study showed that the induction of apoptosis by statins in hematopoietic tumor cells is mediated by mitochondrial apoptotic signaling pathways. In addition, statins decreased the levels of phosphorylated ERK 1/2 and mTOR through suppressing Ras prenylation. <Discussion> The present results suggested that statins induce apoptosis by decreasing the mitochondrial transmembrane potential, increasing the activation of caspase-9 and caspase-3 through inhibition of Ras/ERK and Ras/mTOR pathways. Therefore, our findings support the use of statins as potential anticancer agents or concomitant drugs of adjuvant therapy.

P-2389

Synergistic cytotoxicity and its mechanisms by dual inhibition of ALK and HDAC in neuroblastoma cell lines

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Neuroblastoma (NB) is the most common brain tumor in childhood and the treatment of high-risk NB is challenging. Anaplastic lymphoma kinase (ALK) is thought to be a promising therapeutic target of NB because of the presence of ALK-activating mutations. From our previous experiments, we found that the combination of the ALK inhibitor alectinib and the histone deacetylase inhibitor vorinostat showed the synergistic growth inhibition of NB cell lines with ALK R1275Q mutation. We studied the cellular pathways involved in this synergistic cytotoxicity. In LA-N-5 cells harboring ALK R1275Q, gene expression profiling revealed that the combined treatment of alectinib and vorinostat down-regulated the expression of NFKB1 and anti-apoptotic genes such as BCL2 and BCL2L1, and up-regulated pro-apoptotic genes such as BCL2L11, BIK, and BAD. In addition, the Rap1 signaling pathway, which might be implicated in the pathogenesis of various tumors including NB, was one of the down-regulated pathways by this combination. Taken together, the combination of alectinib and vorinostat may inhibit NB cell growth by regulating the expression of the apoptosis-related genes and the Rap1 signaling pathway.

▲TOP

P-2390

ZSTK474, a PI3K inhibitor, exerts an antitumor effect against synovial sarcoma in vitro and in vivo

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ZSTK474, a pan-PI3K inhibitor, has been evaluated in clinical trials in the USA and Japan, and showed clinical benefit in some of the sarcoma patients. To investigate the antitumor profile of ZSTK474 against various sarcoma subtypes, we previously examined its antitumor effect on 15 sarcoma cell lines and found that it induced apoptotic cell death selectively in three cell lines; one was alveolar rhabdomyosarcoma cell line harboring PAX3-FOXO1 fusion gene and rest of two were Ewing's sarcoma cell lines harboring EWSR1-FLI1 fusion gene. The result encouraged us to investigate its antitumor efficacy against sarcoma cells harboring other oncogenic chromosomal translocations. To this end, we exploited three synovial sarcoma cell lines, SYO-1, Yamato-SS and Aska-SS, all of which harbor SS18-SSX fusion gene. As a result, ZSTK474 effectively induced apoptosis as determined by cleavage of poly (ADP-ribose) polymerase (PARP). Moreover, daily administration of ZSTK474 suppressed growth of SYO-1 tumors xenografted in nude mice. These results suggest that ZSTK474 could be a promising drug candidate for treatment of sarcoma, especially those harboring oncogenic chromosomal translocations.

P-2391

BCR-ABL controls PERK-ATF4 pathway activation and cell survival during ER stress

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The integrated stress response(ISR) plays an important role in cellular adaptation to various tumor-associated stress conditions. The ISR is characterized by activation of eIF2 α kinases, such as PERK and GCN2 that are activated by ER stress and amino acid starvation, respectively, and subsequent induction of ATF4 that enhances transcription of genes for stress adaptation. Recently, it has been reported that oncogenic KRAS can regulate the ISR activation under stress conditions. In this study, we examined whether BCR-ABL controls ISR, especially PERK-ATF4 pathway during ER stress. We treated BCR-ABL-positive K562 cells with BCR-ABL inhibitors during ER stress and found that BCR-ABL inhibitors suppressed PERK activation and subsequent ATF4 induction. Furthermore, we found that this combination strongly induced apoptosis, as assessed by nuclear staining and cleaved-PARP detection. Consistently, we also confirmed that pharmacological inhibition of PERK suppressed ATF4 induction and enhanced apoptosis during ER stress. Taken together, our present findings indicate that BCR-ABL regulates PERK-mediated ISR activation, thereby contributing to cell survival during ER stress.

▲TOP

[P-2397] P16-7 [English/Japanese]
New targeted therapy (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Kensuke Kojima / Dept. Hematol. Resp. Med. & Oncol., Saga Univ.

P-2397

Identification of intracellular target of azithromycin as an autophagy inhibitor

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Co-author：Masaki Hiramoto、Keisuke Miyazawa
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We have reported that macrolides including azithromycin (AZM) have an autophagy inhibitory effect, and co-administration of macrolides with antitumor drugs such as proteasome inhibitors or EGFR-TKIs resulted in enhanced cell death in various cancer cell lines. Although macrolides are good candidate for “anti-autophagy drug” without cytotoxicity by itself, the detailed molecular mechanisms for autophagy inhibition is still unclear. Here, we report the AZM-targeted proteins for autophagy inhibition by high-throughput affinity purification using AZM-immobilized FG-beads (AZM-beads). We incubated AZM-beads with A549 cell lysates, separated the AZM-beads binding proteins by SDS-PAGE, and finally identified the candidate protein by LC-MS/MS.Next, we examined the effect of AZM on the candidate protein function by monitoring the A549 cells stably expressing GFP fused recombinant candidate protein. Interestingly, AZM treatment dramatically suppressed the intracellular dynamics of the candidate protein along with inhibition of autophagy flux. These data strongly suggest that AZM inhibits autophagy via targeting the identified candidate protein.

▲TOP

P-2398

Antitumor activity of a novel PDK4 inhibitor, cryptotanshinone, in pancreatic cancer by suppressing the KRAS expression

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KRAS is the most frequently mutated oncogene detected in the intractable malignant cancers, such as pancreatic ductal adenocarcinoma and colorectal carcinomas. A recent study showed that siRNA inhibition of pyruvate dehydrogenase kinase 4 (PDK4), a key regulator of glucose metabolism, suppressed expression of KRAS and malignant growth of cancer cells. In this study, we examined the effect of a novel small molecule PDK4 inhibitor, cryptotanshinone (CTN), on KRAS-associated tumor formation of pancreatic cancer cells. CTN significantly suppressed the 3D-spheroid formation and the stemness of KRAS-mutated pancreatic cancer cell lines. Expression of mutant KRAS and phosphorylation of PI3K-Akt-mTOR pathway proteins was inhibited by CTN to suppress cyclin D1 expression and Rb phosphorylation. Two weeks of daily injections of CTN (20 mg/kg i.p.) significantly suppressed the tumor formation of MIAPaCa-2 cells in murine orthotopic pancreatic cancer model without detectable adverse effects. Expression of a stemness marker was suppressed in the tumor of CTN-treated mouse. These results suggest that this novel PDK4 inhibitor can be a potential therapeutic drug for KRAS-driven pancreatic cancer.

P-2399

Tumor growth suppressive oligopeptides which derive from HGS/C protein

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Co-author：Koji Kasahara
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HGF-regulated tyrosine kinase substrate (HGS) is composition protein of ESCRT-0 and is involved in endosome transport. We have reported that HGS promoted the TGF- β -SMAD signal transduction and cancer properties (cancer cell metastasis, angiogenesis ability, and tumor growth ability), but that C-domain of HGS (HGS/C) suppressed them. Therefore, we searched comprehensively for tumor growth suppressive oligopeptides which derive from HGS/C protein and found an oligopeptide OP-A (12 amino acid residue). The OP-A oligopeptide suppressed only spheroid growth of COLO205 colorectal cancer cells, but did not suppress adhesive cell growth of them at all in vitro. The OP-A oligopeptide also suppressed tumor growth of COLO205 colorectal cancer cells in vivo. In this study, we synthesized a cyclized OP-A oligopeptide to avoid degradation by peptidases in vivo. The cyclization of the OP-A oligopeptide extended half-life of it in vitro. The cyclized OP-A oligopeptide suppressed effectively spheroid growth and tumor growth of the COLO205 colorectal cancer cells in vitro and in vivo. These results suggested a possibility of the anti-cancer medicine with the HGS/C oligopeptide.

P-2400

Synthesis and biological evaluation of small peptides for survivin targeting cancer treatment

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Survivin is an antiapoptotic and cell cycle-promoting protein, which is consistently overexpressed in cancer cells. It is associated with poor prognosis and resistance to radiation therapy and chemotherapy. Therefore, inhibition of survivin is regarded as an attractive cancer treatment strategy. In this study, we designed and synthesized 7-19 residues of small peptides as survivin targeting agents for cancer therapy. These peptides had affinity for human survivin protein (K_d= 91-255 nM) and INC-7 showed the highest affinity (K_d= 91 nM) among them. Fluorescence images of INC-7 corresponded with survivin expression level in HeLa cells. The r9-INC-7 that is a conjugate of cell penetrating nonaarginine and INC-7 strongly inhibited cell growth of MIA Paca-2 cells (70% at 10 μM) and MDA-MB-231 (40% at 10 μM) as determined by MTT assays. Exposure of MIA Paca-2 cells to r9-INC-7 at the dose of 40 μM markedly reduced intracellular survivin protein levels. These results indicated that the cytotoxicity of r9-INC-7 may be partly caused by direct survivin degradation. Our results suggest the possibility of INC peptides to be novel therapeutic agents for cancer treatment.

▲TOP

P-2401

Enhanced Therapy via Blockade of Therapy-induced Immune Infiltration using E-selectin Aptamer

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Chemotherapy alters tumor stromal landscape drastically in attempt to repair damage. However, little is known of whether and how therapy-induced stroma affects therapeutic response. We observed that residual breast tumors that had received doxorubicin (DOX)-containing chemotherapy regimens exhibit a substantial increase of immune infiltration proximal to E-selectin expressing inflamed vessels. Similarly, DOX-treated tumors had elevated immune infiltration accompanied by M2 polarization in murine breast cancer models, suggesting pro-tumorgenic and pro-fibrotic changes. Functional blockade of E-selectin using anti-E-selectin aptamer suppressed DOX-induced M2 dominance, in turn, enhancing anti-tumor effect of DOX through facilitating the intratumoral drug distribution. This study underpins a consequence of therapy-induced immune infiltration, and further provides a new therapeutic opportunity to suppress it through blockade of E-selectin for the enhancement of therapy.

P-2402

HERC2 is a master regulator of G-quadruplex suppression and affects sensitivity of cells to G4 stabilizers

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HERC2 is an HECT E3 ligase previously implicated in DNA damage response. Here we report that HERC2 promotes the functions of BLM and WRN, the RecQ DNA helicases, to suppress G-quadruplex DNA (G4). HERC2 interacts with BLM, WRN and replication protein A (RPA) complexes in S-phase nuclear fraction. Depletion of HERC2 dissociates RPA from BLM and WRN complexes and significantly increases G4 formation. Triple depletion demonstrates that HERC2 is epistatic to BLM and WRN in the G4-suppressing function. In vitro, HERC2 releases RPA onto ssDNA, rather than anchors on RPA-coated ssDNA. CRISPR/Cas9-mediated deletion of the catalytic ubiquitin-binding site of HERC2 causes RPA accumulation in the helicase complexes and increases G4, indicating an essential role of the E3 activity on the G4 suppression. Both HERC2 depletion and the E3-inactivation sensitize cells to G4-stabilizers telomestatin and pyridostatin. Overall, HERC2 is a master regulator of G4 suppression and affects sensitivity of cells to G4 stabilizers. Given that the HERC2 expression is frequently reduced in many types of cancers, the G4 accumulation by HERC2 deficiency may provide a therapeutic target for the G4 stabilizers.

P-2403

Derivatization of IAP ligands in SNIPER yields improved protein-knockdown activity and antitumor activity

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Targeted protein degradation based on small molecules is an emerging modality that has significant potential for drug discovery. We have devised a protein-knockdown system that utilizes chimeric molecules termed SNIPERs to induce ubiquitylation and proteasomal degradation of various target proteins, and we have previously reported that SNIPER(ER)-87 efficiently degrades the ERα protein. Here, we report that derivatization of the IAP ligand module yields SNIPER(ER)s with superior protein-knockdown activity. These improved SNIPER(ER)s exhibited higher binding affinities to IAPs and induced more potent degradation of ERα than does SNIPER(ER)-87. Further, they induced simultaneous degradation of cIAP1 and XIAP. Notably, these reengineered SNIPER(ER)s efficiently induced apoptosis in MCF-7 human breast cancer cells that require IAPs for survival. We found that one of these molecules, SNIPER(ER)-110, inhibits the growth of MCF-7 tumor xenografts in mice more potently than SNIPER(ER)-87. Our results suggest that derivatized IAP ligands could facilitate further development of SNIPERs with potent protein-knockdown and cytocidal activities against cancer cells requiring IAPs for survival.

▲TOP

[P-2410] P21-1 [English/Japanese]
Gene therapy and oncolytic virus therapy (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Tomoki Makino / Dept. Gastroenterological Surg., Grad. Sch. of Med., Osaka Univ.

P-2410

An MDM2 inhibitor produces cytotoxicity of oncolytic adenoviruses by increasing NF1 in mesothelioma with wild-type p53

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Whole exome sequences showed that a majority of mesothelioma was functionally defective of p53 due to a frequent deletion in the INK4A/ARF region despite having the wild-type genotype. An inhibitor of MDM2, which blocked p53 ubiquitination, thus achieved p53-dependint cytotoxicity to mesothelioma by augmenting endogenous p53 levels. Oncolytic adenoviruses (Ad) lacking of E1B55kDa molecules also increased p53 levels and induced cell death in mesothelioma. Nevertheless, p53-siRNA treatments showed that the Ad-mediated cytotoxicity was irrelevant to p53 levels. We then examined a possible role of the p53 up-regulation in the Ad-mediated cytotoxicity. A combinatory use of an MDM2 inhibitor and the Ad augmented production of Ad progenies and produced synergistic cytotoxicity in p53-wild-type but not p53-mutated mesothelioma. We also found that the combination increased expression of NF1, a transcriptional factor involved in Ad replications, and enhanced DNA damages which were linked with up-regulated expression of Chk2 but not Chk1. These data indicated that increased p53 levels contributed to Ad-mediated cytotoxicity by augmenting Ad replications and sensitizing cells to DNA damages.

▲TOP

P-2411

HSP90 inhibitors augment endogenous wt p53 but decrease the adenovirally-induced expression by inhibiting proteasome

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Heat shock protein 90 (HSP90) inhibitors suppress MDM4 functions mediating p53 ubiquitination, and block a chaperon function stabilizing expression of the client proteins. We tested cytotoxicity of the inhibitors, 17-AAG and 17-DMAG, on mesothelioma and combination of the inhibitors and adenoviruses expressing the wt p53 gene (Ad-p53). A majority of mesothelioma has functional p53 defects despite bearing the wt p53 gene. The inhibitors increased endogenous p53 expression and induced cell death. They however suppressed Ad-p53-induced p53 expression at a posttranscriptional level and inhibited the Ad-p53-mediated cell death. The inhibitors suppressed ubiquitination processes which induced p53 degradation, but a proteasome inhibitor prevented the inhibitors-mediated p53 down-regulation. In contrast, an inhibitor for HSP70 with a chaperon function did not produce the p53 decrease. The HSP90 inhibitors did not suppress expression of Ad receptors or the infectivity. These data suggest that an HSP90 inhibitor has a divalent action on p53, as an activator for endogenous p53 via inhibited ubiquitination and a negative regulator of exogenously over-expressed p53 through the proteasome.

P-2412

Therapeutic microRNA targeting DCLK1 against colorectal cancer

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Since cancer stem cells (CSCs) are considered as a cause of the recurrence, it is important to establish CSC specific therapy to eradicate the cancer. MicroRNAs (miRNAs) are single stranded, non-coding RNAs of 20-22 nucleotides. MiRNA based therapy is expected as a next generation treatment because it is involved in various biological processes by post-transcriptionally or transcriptionally regulating the expression of multiple target genes. In this study, we searched for miRNAs that may regulate the DCLK1 expression, which is considered as a stem cell marker in colorectal cancer. As a result of in silico analyses such as miRBase and Target Scan, we identified 32 candidate miRNAs. These candidate miRNAs were transfected into HCT116 bearing ornithine-decarboxylase degron system (ODC-degron system). ODC-degron system can enrich the cells with low proteasome activity, which is thought to be one of the characteristics of CSCs. We found that 6 miRNAs reduce the cell viability equal to or greater than miR-34a that display a potent anti-tumor effect for various kinds of cancers. These miRNAs may have the potential to be utilized for the CSC specific therapy against colorectal cancer.

P-2413

Mesenchymal Stem Cells Can Be Used As Carriers Of Retroviral Replicating Vectors For Cancer Gene Therapy

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Retroviral replicating vectors (RRVs) have been shown to achieve tumor-selective replication, efficient tumor transduction and therapeutic benefit in a wide variety of cancer models. However, retrovirus-based vectors are produced at only low titers in vitro and are easily inactivated by complement in the blood. For efficient delivery of RRV to tumors, we evaluated mesenchymal stem cell (MSC)-based RRV producer cells as 'RRV carrier vehicles' which preferentially accumulate and engraft at tumor sites. Human MSCs derived from adipose tissue, bone marrow and umbilical cord were infected efficiently with RRVs but produce virus progenies less efficiently than tumor cells. We then assessed tumor cell tropism of the MSCs, using a Transwell plate in vitro migration assay with mesothelioma cells as the targets. All three types of the MSCs showed significant trans-migration toward the mesothelioma cells. Furthermore, when co-cultured, RRVs were found to be transmitted efficiently from MSCs to mesothelioma cells, thereby achieving high levels of tumor cell transduction. These data indicate the potential utility of MSC-mediated delivery of RRVs for cancer gene therapy.

▲TOP

P-2414

Lentiviral vector-mediated gene transfer in bladder cancer cells

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Non-muscle invasive bladder cancer (NMIBC) is most common symptom of bladder cancer, and it shows high refractory rate and malignancy. Currently, intravesical injection of Bacillus Calmette-Guerin (BCG) is thought to be the most effective therapy against refractory NMIBC. However, BCG therapy cause significant deterioration in a patient's quality of life (QOL), and completion rate of BCG therapy is only about 50%. Therefore, development of novel therapeutic options without the deterioration of QOL is very important. In this study, we focused on the lentiviral vector mediated gene therapy because of their low cytotoxicity. Four typical tumor suppressor genes, p53, p16, phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and TNF-related apoptosis-inducing ligand (TRAIL) were transfected to the cancer cells and normal cells using lentiviral vectors. Results of present study showed that the prospective antitumor effects of lentivirus-mediated gene transfection and high safety of lentiviral vectors against normal cells. Then we though that virotherapy using lentivirus may be a possible solution to effectively treat NMIBC without seriously deteriorating patient's QOL.

P-2415

Therapeutic potential of the topical treatment of miR-634 ointment for skin cancer

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Tumor-suppressive miRNAs can concurrently target multiple oncogenes and are expected to be useful as a therapeutic agent for cancer therapy. We have found that overexpression of miR-634 induced the apoptosis by directly targeting genes associated with cytoprotective processes, including mitochondrial homeostasis, anti-apoptosis, antioxidant ability, and autophagy, in cancer cells. The development of drug delivery system (DDS) is critical for the implementation of miR-based therapeutics. While topical pharmacotherapy represents an option to consider in selected skin cancers, we validated the therapeutic potential of topical treatment of miR-634 ointment in model mice of skin cancer. We confirmed that the expression of miR-634 could induce the apoptosis in A431 cells, a human epidermoid carcinoma cell line. In xenograft tumors of A431 cells in mice, the topical treatment of ointment incorporated miR-634 induced tumor growth inhibition. Furthermore, the topical treatment of miR-634-ointment inhibited tumor growth in carcinogen-induced mouse skin papilloma model. Thus, these findings suggest that topical treatment of miR-634-ointment may be an approach of treatment for skin cancer.

▲TOP

[P-2423] P22 [English/Japanese]
Medical care of progressive cancer

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Shogo Ehata / Dept. Mol. Path., Grad. Sch. Med., Univ. Tokyo

P-2423
Withdrawn

No Abstract

▲TOP

P-2424

The needs of rehabilitation staffs of rehabilitation workshop for the patients with advanced cancer

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【objective】We have run workshop for over five years for rehabilitation staffs with rehabilitation interventions of the patients with advanced cancer. We investigated for participants of 12 workshops with questionnaire at the year of both 2016 and 2017. 【subjective】We took questionnaire over 440 participants with 12 workshops.【results】We had 214 responses from the workshops. Response rate were 44.6% (214/480) and we had eight categories for the need of participants of our workshops. Then we analysed the data. The most are two categories equally such as 1. 23% of How do we improve quality of life of the advanced cancer patient, 2. 23% of What the advanced cancer patients really want to. 3. 17% of What interventions did we do when functions of the patients were worse and worse at terminal care. 【discussion】Generally speaking, the purpose of medical rehabilitation was functional recovery. So the-end-of-life care is not fit. But modern hospice in UK had rehabilitation approach from at the beginning. They focused on comfortable of the patient and they called them needs base approach. And we know that the Japanese needs as same the way of UK in hospice with our workshop.

P-2425

The utility of cell free and concentrated ascites reinfusion therapy in gastroenterological cancer

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In gastroenterological cancer, refractory ascites worsens not only patient symptoms but also their daily activities. It often leads to a patient discontinuing chemotherapy. The purpose of this study is to investigate the utility of cell free and concentrated ascites reinfusion therapy (CART) for refractory ascites in gastroenterological cancer. 56 patients underwent a total of 97 times of CART treatments (the traditional CART: 54, KM-CART: 43) from April 2014 to March 2018. The patients included 36 men and 20 women aged 35-89 years. Primary locations were 20 stomach, 12 liver, eight pancreas, six colon, six bile duct, two breast, one ovary and one appendix. 36 patients had peritoneal dissemination, and seven patients had liver metastasis. The mean times of CART were 1.73. The mean punctures ascites volume/reinfusion ascites volume were 4461/480 and 8056/697 ml, in the traditional CART and in KM-CART, respectively. After CART treatment, performance status, appetite score of patients were significantly improved. Seven patients were fit to undergo chemotherapy after CART. In summary, CART is acceptable and effective procedure for refractory ascites with gastroenterological cancer.

P-2426

The current status of advance care planning of gastroenterological cancer patients in our hospital

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Purpose: Current status of advance care planning of gastroenterological cancer patients in our hospital were analyzed. Material and Results: 1) In the latest 3 years, a total of 158 gastroenterological cancer patients were supported by the palliative care team (PCT) of our hospital. In terms of the timing of consultation, 140 patients were consulted after aggressive treatment, 2 were in the middle of treatment and 16 were before treatment. Eleven out of the 16 patients consulted PCT immediately after diagnosed as advanced cancer, decided the therapy during the end of life by themselves, while 5 patients followed the advice of their family. 2) Forty-one patients passed away at hospice in another hospital after moving from our hospital but 13 out of them were decided their death place by not themselves but their family. Alternatively, 19 patients were able to meet death at home with the help of both family and regional medical staff. Conclusion: Even though advance care planning either at home or in hospice at the end of life is still difficult due to some problems to be resolved, attending physician in the hospital should have a great esteem for living will of cancer patients.

▲TOP

P-2427

A novel strategy for treatment of cancer cachexia targeting for alteration of the purine metabolism in the brain

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Cancer cachexia is a systemic wasting syndrome characterized by anorexia, loss of body weight and skeletal muscle. This syndrome causes poor quality of life in cancer patients, thus, therapeutic strategies for cancer cachexia need to be established. However, the lack of appropriate animal models has resulted in the poor understanding of mechanisms causing and developing cachexic symptoms. We thus have established a novel animal model, which meets clinical criteria for cachexia, by subcutaneously inoculating the human gastric cancer cell line 85As2 into mice. The aim of this study is to explore a novel target for the improvement of cancer cachexia in the brain, which may control an appetite, by using the 85As2-induced cachexia model. Metabolome analysis using the brain of cachexic mice has revealed the decrease of ATP accompanied by the increase of uric acid. Moreover, enzyme activity of xanthine oxidase was increased in the brain of cachexic mice. These results suggest the increased ATP degradation and the modulation of the ATP receptor signaling in the brain with the progress of cachexia. Therefore, effect of culture supernatant of 85As2 on this signaling is being investigated.

P-2428

Human stomach cancer cell line 85As2 induced cancer cachexia associated with cardiac dysfunction

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Cardiovascular disorders in cancer patients with cachexia recently have become a great concern. However, the relationship between cancer cachexia and cardiac function remains unclear, because of few suitable models developing cancer cachexia similar to that of humans. We established a novel cancer cachexia mouse model by implantation of the human stomach cancer cell line 85As2. This model shows anorexia, weight loss and low fat-free mass similar to those observed in human patients. In the present study, we evaluated cardiac functions with the model. The mouse model showed a symptomatic cachexia at 2 weeks after cancer implantation; decreased body, skeletal muscle, and heart weight. With the model, we assigned into two groups as pre-cachexia group of 2 weeks and cachexia group of 8 weeks after implantation. The cachexia group developed greater cardiac atrophy and decreased left ventricle ejection fraction than those of the pre-cachexia group. These results imply that our 85As2-implanted cachexia mice model is suitable for studying cancer cachexia with cardiac dysfunction. Further studies are required to clarify mechanisms how cancer cachexia develops cardiac dysfunction and ongoing.

P-2429

The survey on attitude toward shift to palliative care of the hematological malignancy cases

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In some hematological malignancy cases cannot shift to palliative care easily because of continue treatment and a blood transfusion until the end period. To shift to palliative care in cooperation with other medical institutions smoothly, we investigated about a shift to the palliative care of the hematological malignancy case in a questionnaire form. In both physicians, the majority answer was that a timing of shift to palliative care to young patient is treatment resistance. In the elderly people, 20% degree of the hematological physicians considered it from first or a first time recurrence, but about half of palliative care and home medical care physicians considered it from recurrence and the second recurrence. In both physicians answered that cancellation of the blood transfusions becomes the problem most when the cases shift to palliative care. Making a standard to cancel a blood transfusion and treatment, reporting about a hematological malignancy and the treatment to a physician of palliative care and home medical care, reinforcement of the cooperation are necessary for a smooth shift to palliative care.

▲TOP

[SSP-1] SSP [English/Japanese]
Survivor Scientist Program

2018 / 9 / 28 (Fri) 17:05-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Hiroshi Harada / Lab. of Cancer Cell Biol., Grad. Sch. Biostudies, Kyoto Univ.

SSP-1

Speaker：Yasuko Azuma

腹膜偽粘液腫（以下、PMPと略す）は100万人に1～2人程度の発生率で、低悪性でも放置すれば死に至る疾患だが、以前は医師が疾患名すら知らないことが多く、また、治療方法がないと言われていた。
　このPMPが現在の「指定難病」に相当する「特定疾患」に認定されるよう、2006年に患者遺族が立ち上げたのが腹膜偽粘液腫患者支援の会である。当初は署名活動のための会だったが、会員の多くが患者・家族であり、実質的に患者会になっている。
　2014年成立の難病法や、「がん対策基本法」からの「第2期がん対策推進基本計画」から、会は署名活動を終了。2015年より役員全員が患者となり（私もこの時点で役員）、新たに活動を始めた。
　その間には、腹膜播種治療専門医による、治癒をめざす包括的治療が研究・実践され、PMPの治療が可能になってきた。
　悪性度が低い患者も多いPMPだが、がん対策で希少がんも重点項目とされ、「希少がん医療・支援のあり方に関する検討会」では、対策上は少し広い範囲で考えていくとされたことからも、PMPはがん対策で取り扱われるべきである。
　PMPががん研究の分野で、より研究が進むことを望み、会では日本希少がん患者会ネットワークにも参加。今もPMPで命を落とす患者も多いが、PMPは完治する、そしていずれはPMPを予防できる未来となるようにと願う。

▲TOP

SSP-2

Speaker：Hajime Ito

当会は、1997年、人生の最後まで自分らしくありたいと願い「その人らしい生と死を支える」というホスピスの考えが終末期だけでなく、医療全体の、そして社会の基本となることをめざし設立。2002年、任意団体からNPO法人となる。
がん患者・家族・遺族のサポートとして、「ひまわりサロン（がんサロン）」「がん患者家族のための情報提供支援事業“ちえのわ”」「がん患者遺族会”なのはなの会”」「アピアランスケア“みもざサロン”」「ウィッグレンタルサロン」「北海道AYA世代がん患者会」を、一般市民向けに市民講座や会報・ホームページによる、緩和ケア、がん医療、がん対策などに関する情報提供や啓発のほか、政策提言を行っている。
「ちえのわ」は、患者・家族が自分の力で病気や治療と折り合うことができるよう自身の持つ力をサポートする事業で、単一テーマのセミナーの他、がんになっても安心して暮らせる北海道を目指し、医療者や行政と連携し、札幌市中心街で無料相談やがん予防や早期発見の啓発のための「街なかカフェ」を開催している。
がん研究のゴールがんの克服“は患者のゴール（目標）でもあり、私たちアドボケートは、がん研究の成果を学び、患者・家族に届け、それが治療などの意思決定するための一助となるよう、研究の推進を願い、研究者との協働を深めていくことができるよう、努力を続けたい。
発表者　患者　伊藤元　　共著者　副代表理事　山田　富美子

SSP-3

Speaker：Toshimi Oishi

認定NPO法人乳がん患者友の会きらきらとNPO法人広島がんサポートという、ふたつのがん患者支援団体に所属して、様々ながん種のがんに関する講演会や、がん患者さんを支援する方々へ向けての勉強会などを行ってきました。
がんと闘うにあたり、大切なことは正しい知識を得ることです。インターネットの普及、たくさん出版されるがん関連の書籍などで、情報はたくさん手に入れることが出来ますが、正しい知識を取得するのは難しい状況です。そのような状況の中で、がん患者さんやその家族、またがんに興味のある方々に正しい知識を届けることを目的に活動を行ってきました。また、がん拠点病院のがん相談支援センターと連携をもち、その活動などを紹介する「がん相談支援センターご案内」という冊子を作成しました。
また、これまでは会場をつかってリアルな環境で情報提供を行ってきましたが、あらたに、インターネットを使用しての情報提供として、地元のテレビ局とコラボして、がんサバイバー応援番組「Can　Fre Cafe」の配信も行っています。

SSP-4

Speaker：Hiroyuki Onishi

私たちは、希少がんである肉腫の中でも特に治療法の確立が遅れている、主に整形領域以外の成人軟部肉腫患者とその家族を支援するための特定非営利活動法人です。国内での成人軟部肉腫の年間発症数は2～3千人といわれていますが、いまだに早期発見につながる診断法もなく、再発・転移しても治癒につながるような有効な治療法がありません。研究者の少ない肉腫研究を強化することが、今後のがん治療の発展につながることと信じております。
主な活動は3つ。先ずは、情報提供活動です。具体的には、各種学会への参加・発表、患者シンポジウムの企画運営、ホームページ・SNSによる情報提供、チラシの配布、新聞などのメディアへの広報活動などです。次に、肉腫患者交流会やリレーフォーライフへの参加等の交流場所の提供活動です。最後に、政府、厚生労働省などへのアドボカシー活動です。提言内容としては、拠点病院の整備と診療体制の改善、肉腫専門医の育成、肉腫研究に対する研究費の助成です。
一昨年から、国立がん研究センター内に設置された「希少がん対策ワーキンググループ・四肢軟部肉腫分科会」において、患者団体代表として参画し、患者アンケート報告などをし、診療提供体制・情報提供体制について、意見をいたしました。
さらに昨年設立された希少がん患者会ネットワーク(RCJ)では、希少がん共通の課題解決に取り組んでいます。

▲TOP

SSP-5

Speaker：Satoshi Orimo

NPO法人京都がん医療を考える会は平成18年に京都のがん医療向上を目的に設立され、平成19年1月には特定非営利活動法人となりました。活動の目的は、「がん医療が患者本位になるように医療現場の諸問題をがん患者・家庭および医療スタッフとともに考え、その解決方法を国や地方自治体ならびに医療機関へ提言し、その解決に協力していく」ことです。主な活動として、京都府民公開講座開催、病院外のがんサロン企画･運営、参加者との意見交換や会報・報告書の発行、がん患者・家族/遺族、医療スタッフとの意見交換を行っています。これまで、京都府民公開講座14回、がんサロンでの葉月プラナスの勉強会51回、会報発行61号迄、等々、30名程度の会員と数名の役員を中心に活動しています。
今回、日本癌学会学術総会SSPプログラムでは、がん研究について学び、またがん患者･家族の立場から参画･がん研究支援に協力し、今後期待される協働活動に活かします。これまで京都府中心の活動を行ってきましたが、今後は欧米諸国のリサーチ･アドボケイターの活動等も参考に、広くがん患者･家族の意見を集約し、がん研究者に伝えることによってリサーチ･アドボケートとして活動したいと考えています。
Webサイト：http://thinkgankyoto.jugem.jp/

SSP-6

Speaker：Laureline Gatellier

【JBTA設立の目的】
JBTAは医療関係者を含む全国的なネットワークを作り、その知識と経験を結集することにより、脳腫瘍患者と家族を支援することを目的に設立した。JBTAは、患者へのサポートを提供し、またセミナーを通じて教育を行う。また、一般市民のための啓発キャンペーンを実施する。
【JBTAのビジョン】
脳腫瘍の診断を受けることは非常に辛い出来事であるが、JBTAはそれが患者のみならず、家族や友人にもたらす大きな影響についても理解している。そして脳腫瘍と立ち向かう患者と家族を支援する。
【JBTAの具体的な活動内容】
1. 患者、患者家族へのサポートを行う
a. ホームページでの情報提供および相談
b. 国内での患者サポート活動および交流会
c. 各地の拠点病院への脳腫瘍に関するパンフレットの配布
2. 患者の知識を高める。科学に貢献する
a. 脳腫瘍セミナーおよび勉強会（東京、関西、その他国内）
b. 患者団体会議への参加
c. 臨床試験に関する情報提供
3. アドボカシー、ネットワーク作り、啓蒙活動の推進
a. 国内および海外の他団体とのネットワーク構築・情報交換
【JBTAの今後の活動予定】
・ 定期的に患者交流会などを開催し，脳腫瘍患者とその家族のネットワークを深める。
・ 脳腫瘍を含む希少がんのアンメットニーズ調査
・ 新しい治療法へのアクセス情報の収集
・ 国内外ステークホールダーとのさらなる関係構築

SSP-7

Speaker：Fuminori Kayahara

1 活動の主体
・NPO法人パンキャンジャパン（膵臓がんアクションネットワーク）
2 NPO法人パンキャンジャパンの使命
・早期発見、治癒につながる研究を促進するために研究者・医療者を支援すること。
・患者・家族をサポートすること。
・希望を与えること
3 活動内容
・膵臓がんに対するよりよい診療・治療のためには、科学医療の研究から全てが開始されます。2008年から家族性膵癌登録、2012年からパンキャン賞の授与など、複数の方法で膵癌研究の前進に取り組んでいるほか、ドラッグラグ解消など政策提言も行っています。
・また、患者・家族・医療関係者・一般の方々向けのセミナーの開催、膵臓がん患者サロンの開催、患者や家族の疑問や悩みに電話でサポードできるPALS（電話相談）の実施など、複数の取り組みで患者や家族をサポートしています。
4 九州地区における活動内容
・昨夏、九州地区で初となる福岡支部が立ち上がり、従来から取り組んできた癌診療拠点病院がん相談支援センターへのパンキャンパンフレットの設置を継続するとともに、がん相談支援員との更なるコミニュケーションの実施に取り組んでいます。
さらに、早期発見・予防の観点から、一般企業・団体・個人に対する啓発活動を通じて、研究支援・患者支援に対するご理解ご支援を広くいただけるよう活動を継続していきたいと考えております。

▲TOP

SSP-8

Speaker：Ichiro Kawai

NPO法人大阪がんえええナビ制作委員会では、2011年より、がん情報ポータルサイト『大阪がんえええナビ』の運営をはじめ、公開講座・勉強会等の開催による、がん患者・家族・一般を対象としたがん情報提供活動を行っている。
『大阪がんえええナビ』サイトでは、各種がんについて、エビデンスの確立された予防・検診・治療情報をはじめ、患者支援情報等を発信しているが、今回は、当サイト独自の情報発信システムである「大阪がん診療スピード検索」「病床機能報告データ検索システム」「大阪エリア別がん情報」といった、他県にはないコンテンツの紹介を中心に、併せて、当会において実施した、がん患者・家族・一般・医療者等を対象に実施した「がん情報の収集に関するアンケート調査」の発表を行いたい。
また、免疫療法、ゲノム医療やプレシジョン・メディシンといった、近年注目を集めている治療技術を進歩させるための基礎となるがん研究分野の情報発信を、当サイトにおいても研究者との協働により充実させることで、がん研究への期待とし、今後の展望についても述べさせて頂きたい。

SSP-9

Speaker：Tsuyoshi Shiraiwa

すい臓がんに関して、広く市民に認知していただく為、普段膵がんと闘っている患者や家族、医療関係者、市民などが集う環境の提供の役割を担っている“パープルリボン活動”について御紹介致します。医療機関とパンキャンジャパンにて協働で進めているイベントになります。一つ目は、すい臓がんの最新治療法や副作用の緩和、栄養管理など各分野の厳選された、エビデンスに基づいた最新医療情報を提供する「パープルリボンセミナー」。もう一つは、患者、家族、医療関係者、ボランティア、一般市民が東京日比谷公園に集い、世界すい臓がんデーに当たる11月に開催する「パープルストライドウォーク＆ラン」について、すい臓がんに向き合っている皆様が、希望を持ってまい進出来るよう励まし合える心を一つに出来るイベントを目指し、皇居周辺をウォーク＆ランしていただくイベント活動内容について御紹介致します。また、がん研究への期待として、治るがんへの環境を作っていただきたい希望が第一にありまして、更にはがん研究が目指しているビジョン（方向性を示す）や目標としている内容を知る機会が欲しいと思います。享受する側の患者側の考えも生まれて、議論が活発になると感じております。患者側からの要望（向かって欲しい方向性や具体的なアクションアイテム）を研究者の方々に伝えることも重要と考えます。コミュニケーションが活発になる環境が必要と感じます。

SSP-10

Speaker：Mayumi Terada

2006年末に乳がんが見つかったあと、2009年頃から、本業の傍ら、がん医療について学び始め、微力ながら様々な活動を行ってきた。そのなかで柱と考える4つの活動を紹介する。
1.一般社団法人日本癌医療翻訳アソシエイツ（JAMT）での動画字幕翻訳監修
本業の映像翻訳の知識を生かし、2010年以降、NCI米国国立がん研究所が制作した動画の翻訳を指導・監修してきた。2018年7月現在、82本の日本語版動画が、JAMTの運営する「海外がん医療情報リファレンス」で公開されている。
2.OneWorldプロジェクト
2011年4月、医療者や患者経験者が発起人となって、東日本大震災の被災地にある拠点病院に、がん患者さん向けの医療用ウィッグや帽子を送る活動を開始した。
3.mixi内の乳がん患者会コミュニティの副管理人
乳がん罹患時からSNSのmixi内にある乳がん患者会コミュニティに参加していたが、2011年6月から、その副管理人をつとめ、患者さんへの情報提供などを行っている。
4.科学的根拠にもとづくがん検診を考える会
当初は、受診率アップばかりを謳っているかに思えたピンクリボン運動への違和感から、がん検診について学び始めた。過去のRCTやメタ分析の論文を読み、ガイドライン作成手順の概略も学んで、人様の前で発表を行う機会も何度か頂戴した。がん検診は行政面から検討すべき課題も多く難しいが、過剰診断解決のための治療すべきがんと放置可能ながんの見極めなど、がん研究の進展が期待される部分も大きいと感じている。

▲TOP

SSP-11

Speaker：Hiromi Todoroki

毎年、がん検診を受けながらも、発見が難しいがん種のために進行した状態で告知を受けた夫。治療法の確立が無く、情報が少ないために、孤独と大きな絶望を感じたことが、希望の会設立の動機です。情報を集め、国立がん研究センターをはじめとした医師の方々の協力を仰ぎ、スキルス胃がんの情報冊子を作りました。
厳しい状況にあるからこそ、様々な情報に溺れ、科学的根拠が乏しい療法にも走ってしまう例が多発しています。病気のこと、治療のこと、制度のこと、そして、今、取り組まれている研究を知ることが、自分の置かれた状況を理解し、納得する選択に結びつく唯一の道であると感じています。特に、治療に苦慮する難治性がんほど、患者家族の納得が、尊厳のある日々に繋がると思っています。そのために、患者家族の思いを経験した私たちに出来ることを常に考えていこうというのが、希望の会の活動の柱です。
研究は科学的なものであり、公正に行われるべきであると思っています。それを進めるために患者家族の協力は不可欠です。そして、患者家族には想いと心があります。スキルス胃がんの治療が確立される日を願い、剖検をお願いした夫の思いと、今までの活動、そして、今後に向けて、会として考えていることを発表したいと思います。

SSP-12

Speaker：Kei Nakagawa

私たちは広島を中心に活動する乳がん患者とその家族のための認定NPO団体です。「きらら」は年間を通し、フォーラム(500人規模、過去20回継続開催)や学習会などを開催することによって、正しい情報を発信するように心がけています。設立当初より、医療は医療者だけでなく患者も一緒になって作り上げるものだという考えを元に、広島で乳癌医療に携わっておられる医療者に協力していただきながら、活動を続けてきました。また単に治療に関する情報を発信するだけではなく、患者自身ががん研究に対して理解を深めたうえで自らも参画する重要性を考える機会も持っています。がん研究の発展は、私たち患者の希望でもあり、私たち患者が担う力も大きいと感じています。そのためにも、情報の入手方法や医療制度の理解、薬が私たち患者の元に届くまでの過程などを理解し、啓発する機会を設け、アンケートによる意識調査なども行っています。

SSP-13

Speaker：Mayumi Noda

【はじめに】
1998年、乳がんに罹患し自らインターネットで集めた情報をもとに医療の限界や不確実性を知り、自らの価値観を整理し治療法を選択した。以降20年間がんに関する様々な活動を続けてきた。自身の主な活動紹介と、がん研究への思いと期待を整理した。
【概要】
患者会NPO法人支えあう会「α」副理事長として会を運営。患者・家族の交流支援、がん相談、情報提供、講演活動などの活動を行っている。私自身は講演活動や外部での委員会参加などが主な活動となってきた。緩和ケア研修会では、医療者に緩和医療に対する思いや期待を伝え、医療系大学の多職種連携教育や看護学部などでは、未来の医療を担う若者たちへがん医療や研究に対する期待を伝えている。また、千葉県ピアサポート事業において、サポーターの養成・活動の場の創出、パッケージ化したサロンで活動の場を広げる戦略を考えるなど千葉県独自のスタイルの構築に関わってきた。
【考察】
がんに関する活動を始めた頃、正直臨床にしか興味が無かった。今まさにがんと向き合う患者をどうするのかということばかりを考え、未来の患者や家族にしか届かぬであろうがん研究にはあまり関心を持てずにいた。しかし、近年のがん医療の進歩は目覚ましく、がん研究が今を生きるがん患者へと繋がることを患者仲間の様々な体験から知ることとなった。がん研究に自分自身も大いにに期待し、がん研究を応援し啓発に寄与していきたい。

▲TOP

SSP-14

Speaker：Maki Hamamoto

私達がんと共に生きる会は“非常に進行したがん患者と家族”が全国から集結して設立され、18年余りの活動を重ねてきた。自分には間に合わずともこれからの、特に残された治療選択肢が非常に限られる患者に対して、その質の高さが余命やQOLを左右する方策として、臨床試験を含む抗がん剤治療に着目したことがその出発点である。ここでは当会活動履歴から①薬剤・用法の承認・適用拡大への貢献、②当会が運営にあたる患者目線の情報サイト『大阪がんええナビ』内『臨床試験の推進・情報提供』(臨床試験情報検索システム)の現況、④第1回、第2回SSPプログラムの参加成果を会報や研究会発表・イベント参加等で行った普及啓発活動を中心に紹介したい。更に、⑤普及が進みまたは始まった免疫チェックポイント阻害剤による治療、リキッドバイオプシー等のがん遺伝子検査について、実際に利用を考えた・利用した者の声を紹介し、患者・市民の関心が高まる中、自らに合った的確な治療情報を獲得し活用すること、その課題についても述べたい。

SSP-15

Speaker：Naoko Wakao

がん治療は、がん研究の進展とともに急性期の医療技術等がが大きく進歩し、 医療施設内で入院治療する期間がとても短くなった。これは、患者にとって大 きなメリットとなっているが、多くの患者はこのメリットを十分に理解していない。 医療への依存度が大きいためだと思われる。また、入院期間が短いということ は、裏を返すと、社会生活をおくりながら治療を継続していくことを意味する。社 会生活の中には家庭での生活、集学・就労との共生、関連して、通院・通学・通 勤等が含まれる。これら社会生活は、健康に問題がないときでも突発的な困難
を伴うことが多くあるが、ましてやがん治療中での治療と社会生活の両立は、患 者・家族を含めたサバイバーの十分な理解なしでは満足できる状況にはならな い。外来治療自体は概ね満足できる医療環境にあるのにもかかわらず、多様な 患者の理解度やニーズに対する情報共有と情報提供ができていない。このギ ャップは早急に埋めていきたい。また、社会もサバイバーが思っているほどがん に対する理解は進んでいない。そこで、がんサバイバーシップを浸透させつ つ、治療と社会生活の両立支援を充実させるための具体的な課題をあげ、医 療者と共に解決の方法を探ってみたい。事例としては、自分の体験から、造血 幹細胞移植と移植後のフォローアップにおける問題点の現状把握と課題解決 の方向性を探ってみたい。

SSP-16

Nozomi Nonaka
JAMT・ジャムティ

近年のがん研究や治療の進歩は目覚ましく、日本のみならず、世界から様々な新発見がなされている。抗がん薬の開発試験や承認は欧米が先であることが多いが、英語が壁となり日本の患者さんや一般の人たちには伝わらないことも多い。また日本でも正確ながん情報もたくさん増えたが、信頼できない情報やとんでも医療も後を絶たないという問題もある。私たちの活動は、EBMに基づく最新のがんの情報を日本の方々に公開するため、有志の翻訳者と専門家が集い、『海外がん医療情報リファレンス』にて翻訳情報を提供している。翻訳記事は、米国国立がん研究所（NCI）や米国食品医薬品局（FDA）など公的機関、およびMDアンダーソンがんセンター等のリリースを、許諾を得て翻訳している。協力ボランティア数は専門家含め170名以上（現在）、昨年の記事公開数は400記事を超える。
また、近年、世界の患者団体が国際的なネットワークを構築し始めており、そこに日本の患者会が含まれる機会が少ないことがわかり、そうした状況を踏まえ、がん患者支援活動として、英語と日本語の翻訳で協力していければと考える。

▲TOP

SSP-17

Akiko Igarashi
NPO法人支えあう会「α」

千葉県には10団体で構成される千葉県がん患者団体連絡協議会がある。
現在、行政との関係では、がん対策審議会及びその下にある部会の会員として、協議会のメンバーが参加している。また、がん診療連携拠点病院等で構成されるがん診療連携協議会にも委員を出している。
ここ数年で大きく変わってきたのが、がん診療連携協議会との関係である。2年半ほど前から、がん診療連携協議会とがん患者団体連絡協議会が月１回の定例会議を持つようになった。この会議ががん診療連携協議会のなかの正式な会議として認められるようになり、がん患者と医療者が協働する関係は飛躍的に高まった。委員会に委員を出すことは以前からやっているが、委員以外も含めてほとんどの部会に傍聴参加している。これは、会議でなにが問題になっているのか、どのような議論がなされているのかを患者同士で、または医療者と共有するためである。
また、平成29年度は緩和ケア研修会を見学させていただき、緩和ケア専門部会に意見書を提出した。また、今年からは、「緩和ケア研修会見学受入要綱」を作り、それに基づいて申請することで、緩和ケア研修会見学への道筋を作った。
以前から医療者と患者との意見交換会を企画し、試みてきたが、昨年度は「緩和ケア」をテーマに、事例を医療者・患者側から一例ずつだし、ワークショップを行った。これは、双方に好評で、今年も「インフォームド・コンセント～リスクと希望を語るとき～」というテーマでワークショップを行う予定である。
また、相談支援専門部会で相談支援センターのわかりやすさや職員の認知度を、患者がチェックする企画があり、患者団体連絡協議会で協力する計画が進行中である。
こういった内容を報告したい。

▲TOP

[P-2357] P14-46 [English/Japanese]
Prostate cancer (3)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Masahito Watanabe / Dept. Urol., Aichi Med. Univ., Sch. Med.

P-2357

Tissue biomarkers in patients with high-risk prostate cancer treated with neoadjuvant chemohormonal therapy

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This study investigated tissue biomarkers to predict outcomes in patients with high-risk PCa treated with neoadjuvant chemohormonal therapy (NCHT) followed by radical prostatectomy (RP). A tissue microarray was established with 210 cores from 70 patients with three different neoadjuvant treatments. The expression of 12 candidate biomarkers including steroid receptor-, immune cell-, and Hippo pathway-related proteins were statistically assessed using immunohistochemistry. The mean nuclear GR in cancer cells and the nuclear ER aipha and PR in stromal cells were significantly higher in the NCHT group. The patients with a high nuclear AR and a high YAP in the residual cancer cells had a significantly higher rate of biochemical recurrence (BCR). The numbers of T-lymphocyte and macrophage- infiltrations in the stromal cells were not associated with the type of therapy and rate of BCR. Multivariate analysis revealed that a combination of high nuclear AR and YAP in residual cancer cells were independent prognostic factors for BCR in patients treated with NCHT. Thus, tissue biomarkers may have a potential in predicting BCR in patients with high-risk PCa treated with NCHT followed by RP.

▲TOP

P-2358

Prognostic impact of serum N-glycan profiling as a potential biomarker for castration-resistant prostate cancer

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Objective: To evaluate the diagnostic potential of serum N-glycan profiling for CRPC, we retrospectively investigated serum N-glycan structural analysis by glycoblotting for 286 patients with benign prostatic hyperplasia (BPH), 46 patients with prostate cancer treated with androgen-deprivation therapy without disease progression (PC-ADT), and 63 patients with CRPC. N-glycan profiling was compared between the non-CRPC (BPH and PC-ADT) and CRPC patients. We then obtained the quantitative CRPC N-glycan score by discriminant analysis based on the combination of candidate N-glycans.Results: The CRPC N-glycan score was calculated using age, prostate-specific antigen (PSA) and 5 N-glycans that were significantly associated with PC. The CRPC N-glycan score could correctly classify CRPC patients with a sensitivity, specificity, and area under the curve of 78%, 83%, and 0.89, respectively. The CRPC N-glycan score higher than 1.5 points was significantly associated with poor prognosis in patients with CRPC. Conclusions: The overexpression of specific N-glycans may be associated with their castration-resistant status in prostate cancer and may be a potential biomarker for CRPC.

P-2359

KIFC1 is involved in the regulation of resistance against docetaxel in prostate cancer

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Cancer cells with supernumerary centrosomes reshapes multipolar spindles into pseudo-bipolar structures called centrosome clustering to avoid cell death. Recent works have shown that KIFC1 plays an essential role in centrosome clustering. We previously reported that KIFC1 are involved in cancer progression and docetaxel (DTX) resistance in prostate cancer (PC). In this study, we obtained DTX resistant PC cell lines (DTX-R). Western blot showed that KIFC1 was upregulated in DTX-R cells compared with parental PC cell lines. We performed MTT assay to evaluate IC50 value under DTX treatment and found that downregulation of KIFC1 made DTX-R cells resensitized to DTX treatment. CW069 (KIFC1 inhibitor) inhibited cell growth in PC cell lines. In addition, we analyzed the association with KIFC1 expression and therapeutic outcomes in 25 CRPC patients treated with DTX treatment by using immunohistochemical analysis. Kaplan-Meier analysis showed that the expression of KIFC1 was significantly associated with poor therapeutic outcomes in CRPC patients. These results suggest that a combination of KIFC1 inhibitor and docetaxel could be a potential strategy to overcome docetaxel resistance.

P-2360

The efficacy of bromodomain inhibitor for multiple drug resistant CRPC using new patient-derived ex vivo models

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Overseas collaborators
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A new approach for high-throughput drug screening across different stages and subtypes of cancer is to perform ex vivo cultures of patient-derived xenografts (PDXs). Recently, there has been interest in using bromodomain inhibitors (BET inhibitor) for multiple cancers. In this study, we tested the efficacy of BET inhibitor for multiple drug resistant castration resistance prostate cancer (CRPC) using ex vivo cultures. PDX tissues were placed on gelatin sponges in ex vivo culture media containing drug or vehicle control. Tissues were cultured at 37 ° C for 48 hrs and treated with 1 μ M JQ1, iBET151, or ribociclib as control for BETi. The samples were then examined using IHC and image analysis. Both JQ1 and iBET151 reduced the expression level of Ki67, pHH3 and MYC, while cleaved caspase 3 was elevated. Ribociclib did not show any effect for CRPC-PDXs. In conclusion, we observed that some tumours that are resistant to AR-directed therapies are sensitive to bromodomain inhibitors.

▲TOP

P-2361

A study of pathological characteristics of malignant cribriform prostatic lesions

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Background:Prostatic lesions (PLs) are becoming increasingly complex and showing a wide variety of morphology, in which, the cribriform architecture is particularly common, represents a broad spectrum of entities.
Objectives:To study pathological features of malignant cribriform PLs.
Methods: A cross-sectional descriptive study for 3 years.
Results:The mean age was 74,03 ± 8,79; the majority had Gleason score (GS) 8-9 (59,6%); 93,5% HGPIN and 100% intraductal carcinoma (IDC) associated with prostatic adenocarcinoma (PAC), there was a statistical significance between GS 8-9 and IDC (χ 2 ,P<0,001);between GS 7 and HGPIN (χ 2 , P=0,025); 83,38% cases had detached cribriform fragments and associated with GS 8-9 (χ 2;P=0,027); 6,9% cases had periacinar halo but not statistically related to GS 7 (χ 2 ;P=0,111); 61,61% cases had braching contour; 78,2% cases had slit-like spaces; 21,2% cases had neovascularity resembling glomus body and associated with GS 8-9 (χ 2 ;P=0,008).
Conclusion: High grade PAC was always associated with IDC. New features, such as attaching cribriform fragments, periacinar halo and neovascularity resembling glomus body, are very useful clues to diagnose PAC.

P-2362

Castration induces abberant activation between epithelial and stromal cells through TGF- β 1 signaling

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Androgen is considered to play important role in the development, differentiation and maintenance of the prostate through epithelial-stromal interactions. However, the age-dependent decrease of androgen causes proliferative diseases of the prostate. Hence, we investigated the effects of castration on reconstituted prostatic glandular structure composed of human prostatic epithelial cell line BPH-1 and fetal rat urogenital sinus mesenchyme. Percentage of fibrotic area in castration group was higher than that in sham group. Infiltrating solid epithelial cords in castration group were more frequently than those in sham group. In castration group, tenascin-C positive fibroblasts were increased in stroma surrounding infiltrating solid epithelial cords. In vitro, mRNA expression of TN-C in fibroblasts was not affected by androgen but it was significantly increased by co-culturing with BPH-1 cells. Fibroblasts upregulated protein secretion of TGF- β 1 from BPH-1 cells. Our data showed that castration induced tenascin-C positive fibroblasts surrounding disrupted prostatic glandular structure. This suggests that androgen may be responsible for stabilization of prostatic glandular structure.

▲TOP

[P-2370] P16-2 [English/Japanese]
Cell death / synthetic lethal target

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Shinichiro Hasegawa / Tondabayashi Hosp. Surg. Dept.

P-2370

Lysosome-targeted cytocidal effect of CDK4/6 inhibitor abemaciclib

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Newly developed CDK4/6 inhibitors named abemaciclib, ribociclib, and palbociclib are under clinical trials. Palbociclib is now approved for breast cancer therapy. In this study, we tried to elucidate the precise molecular mechanism of antitumor effect of these CDK4/6 inhibitors.
All three CDK4/6 inhibitors induced not only cell growth arrest at G1 phase but also cell death in A549 lung cancer cells. Of note, treatment with abemaciclib induced formation of a number of large cytoplasmic vacuoles. The cell death phenotype induced by abemaciclib did not agree with apoptosis, necroptosis, and autophagic cell death. Bafilomycin A_1, a V-ATPase inhibitor, suppressed abemaciclib-induced vacuolar formation as well as cell-death induction. The red fluorescence of mCherry-GFP-LC3 (as an autolysosome marker) and the high intensity of LysoSensor-green (indicating acidic) were co-localized in these vacuoles. These data suggest that the cytoplasmic vacuoles are derived from the expanded lysosomes with hyper-acidification. Abemaciclib appears to disrupt the regulation of lysosomal pH, leading to the novel cell death phenotype.

▲TOP

P-2371

Analysis of molecular mechanism of a drug that effectively induces cell death in dormant cancer cells

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Hypoxic and poor nutrient conditions are important factors leading cancer cells to a dormant state. The dormant cancer cells are often resistant to chemotherapy, becoming a hotbed for recurrence. Therefore, a drug that effectively target dormant cancer cells without affecting normal cells has been desired. We screened a chemical library with cancer tissue-originated spheroids in a dormant state and identified one candidate for the drug. In addition, we found a lung cancer cell line (CL-1) that enters a dormant state under hypoxic and poor nutrient conditions and confirmed that the candidate drug preferentially killed CL-1 in a dormant state. In this study, we tried to explore the mechanism of the preferential cytotoxicity for dormant cancer cells at the molecular level. We found that the candidate drug disrupts the mitochondrial membrane potential, reducing the intracellular ATP concentration. In addition, flow cytometric analysis revealed that the candidate drug induced caspase-3-independent cell death to the CL-1 in dormant state. The generality of these drug effects on dormant cells, which may contribute to the prevention of recurrence, is under investigation.

P-2372

Mechanistic study of cell death caused by a potential anticancer agent MO2455, which induces PAR accumulation

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Poly (ADP-ribose) glycohydrolase (PARG) is the main enzyme that degrades poly(ADP-ribose) (PAR). PARG knockdown induced an enhanced sensitivity after ionizing irradiation or treatment with alkylating agent in a particular cancer cells and induces PAR accumulation. Thus, PARG could be considered as a candidate target for anticancer agent. We recently reported a novel compound MO2455 that causes PAR accumulation and cytotoxicity to various cancer cells. Previously, our results suggested that MO2455 treatment in cancer cell lines including human A549 cells and mouse melanoma B16 cells rapidly induced cell death via PAR accumulation and the activation of caspase cascade. The aim of our study is to elucidate the mechanism of MO2455-mediated cell death in cancer cells. Treatment of cancer cell lines with the particular caspase inhibitors decreased rapid cell death caused by MO2455. When the apoptosis inducing factor (AIF) was knocked down, cytotoxicity was attenuated to some extent, suggesting that AIF is involved in MO2455-induced cell death. To further examine the mechanism of MO2455-mediated cell death, relationships of PAR accumulation and cell death pathways are being analyzed.

P-2373

Identification of Synthetic Lethal Targets in SWI/SNF Chromatin Remodeling Deficient Cancers

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Targeting synthetic lethal partners for genes deleteriously mutated in cancer holds a great promise for treating cancer without known oncogene activating mutations. One of the most frequently mutated genes in various human cancers are chromatin regulator genes. Mutations in genes encoding subunits of SWI/SNF chromatin remodeling complexes collectively extend to 20% of all human cancers. We have been proposing “synthetic lethal therapy” targeting vulnerability of cancer cells with loss-of-function mutation of chromatin regulator genes (Ogiwara et al., Cancer Discov, 2016). We have focused on several SWI/SNF chromatin remodeling genes which are frequently mutated in various types of cancers. We established cells knocked-out for those genes by a CRISPR/Cas9 system. To identify synthetic lethal targets, we are carrying out a screening of drugs that show differential sensitivity between cancer cells deficient and proficient for SWI/SNF chromatin remodeling genes. Several candidate drugs showing such a differential sensitivity have been identified. The promise of synthetic lethal therapies targeting SWI/SNF chromatin remodeling complex deficiencies will be discussed.

▲TOP

P-2374

Identification of Synthetic Lethal Targets for Kidney Cancer Deficient in Chromatin Regulators

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Kidney cancer is one of the top 10 most common cancers in the Japan and United States with about 16,000 and 60,000 new cases diagnosed annually. Most cases of renal cell carcinoma are sporadic, and over 70% of them are clear cell renal cell carcinoma (ccRCC). Loss-of-function mutations in tumor suppressor genes have been frequently identified in ccRCC. Mutations in PBRM1 encoding a component of the SWI/SNF chromatin remodeling complex, SETD2 encoding a histone methyltransferase for lysine 36 of histone H3, and BAP1 encoding a deubiquitinating enzyme, are observed in approximately 40%, 20% and 20% of ccRCC, respectively. Targeting synthetic lethal partners for those mutated genes holds a great promise in the treatment of ccRCC. In this study, we established cells knocked out either for the PBRM1, SETD2 or BAP1 gens by a CRISPR/Cas9 system. To identify synthetic lethal targets, we are carrying out a screening of drugs that specifically kill kidney cancer cells deficient in those three genes. We have identified several candidate drugs up to the present. Synthetic lethal therapies targeting chromatin regulator mutations will help establishment of precision medicine of ccRCC.

P-2375

Synthetic lethal genes in MYCN-amplified neuroblastoma cells and their potential for therapeutic application

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MYCN gene amplification clearly correlates with poor prognosis in patients with neuroblastoma. Basically, transcriptional factors, including N-Myc, are thought to be “un-druggable” targets, and therefore, alternative approaches are required to develop new therapies. For instance, synthetic lethal (SL) approaches are emerging as a promising strategy for cancer therapy. It has been reported that aurora kinases and some cyclin-dependent kinases are SL genes in MYCN-amplified neuroblastoma cells. In order to identify new SL genes, we performed a genome-wide shRNA library screening. The commercial library targeting about 16,000 human genes was used. In addition to already known SL genes (e.g. SMC2, CSNK1E), about 130 genes were identified as new candidates. Based on experimental validations using siRNA and chemical compounds, some mitotic kinases and enzymes involved in nucleic acid metabolism were proposed to be new SL targets. In accordance with the previous reports, the importance of mitotic kinases was further confirmed by our results. Elucidation of the molecular mechanisms underlying the lethality and development of molecularly targeted drugs will be required.

▲TOP

[P-2382] P16-4 [English/Japanese]
New target screening

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Jun Koseki / Grad. Sch. Med., Osaka Univ.

P-2382

Cancer drug screening based on refractoriness of cancer cell reprogramming

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Cellular reprogramming is accompanied by dynamic changes of epigenetic modifications, thus it is considered to be a useful tool to induce global epigenetic alternation in genome of various cell types, including cancer cells. However, cancer cells are generally refractory to cellular reprogramming, suggesting that cancer cell identity is stably maintained.　However, we found that blockage of key oncogenic signals by molecular target drug treatment facilitated the early stage of TF-mediated reprogramming of human cancer cell lines. Based on these results, we established high-throughput-RT-qPCR (HT-RT-qPCR) system which can monitor the *NANOG* expression to find compounds and genes which block key oncogenic signals and to identify therapeutic oncogenic signal pathways. To validate this high-throughput-RT-qPCR system, we performed chemical screening in *KRAS* mutant lung cancer cell line, A549. Notably, we found that the expression level of *NANOG* is upregulated by Trametinib, a MEK inhibitor, as well as other compounds which inhibit *KRAS* related pathways. These results raised the possibility that our screening platform can identify unknown key oncogenic signals in diverse cancer types.

▲TOP

P-2383

The screening system based on the polarity switching of cancer cell clusters to investigate metastasis related signals

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Cancer tissue-originated spheroids (CTOSs) prepared from differentiated adenocarcinoma of the colon maintain polarity (2011 PNAS). Apical membrane was formed on the outer surface of the spheroid when cultured in suspension, while on the surface of the lumen inside of the spheroids when cultured in gel. Similar polarity status in suspension culture was pathologically found in the regions of microvessel invasion. We found the polarity switching was critical for establishment of metastasis (2016 Am. J. Path.).
To reveal the metastasis signaling related to the polarity switching, we have been trying to develop a monitoring system of polarity status for a drug screening. We generated a colorectal cancer CTOS expressing GPI linker-fused GFP, which enabled to monitor localization of the apical membrane. We screened 125 compounds and confirmed that the inhibition of Src and FAK suppressed the polarity switching as previously reported. Some of the other compounds significantly promoted or inhibited the polarity switching. These compounds which affected to the polarity switching may lead to the study of novel metastasis related signaling.

P-2384

Evolution of kinase inhibitors sensitivity in three-dimensional suspended spheroid culture platform

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Somatic mutations in the tyrosine kinase domain of epidermal growth factor receptor (EGFR) have been identified in NSCLC and those mutations confer sensitivity to the EGFR-TKIs such as gefitinib. Unfortunately, it is already known that the almost cases are developed to drug resistance after chemotherapy. EGFR-TKI resistant mechanisms have been reported, including T790M gefitinib/erlotinib resistance mutation, c-MET amplification and ErbB3 activation. Generally, developed resistant cell lines and cell culture techniques were used in drug resistance research. However, some resistance mechanisms observed in cultured cells are not reflected the results in an in vivo condition. Recently, some three-dimensional (3D) cell culture techniques are developed and expected to bridge the gap between normal cell culture and experimental animal models. In this study, we evaluated sensitivity of kinase inhibitors for Gefitinib resistant cell line under 3D condition. Phosphorylation levels of cMET, EGFR, Akt, ERK were decreased under 3D compared to 2D conditions. We also found some kinase inhibitors, including Akt inhibitor showed more sensitive in Gefitinib resistant cell lines under 3D conditions.

P-2385

Screening for chemical inhibitors targeting the interaction between tumor and platelets

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Tumor progression is well known to be supported by its microenvironment. Platelets are one component of the tumor microenvironment and their roles in tumor have been indicated. Podoplanin (PDPN), expressed on the surface of tumor cells, functions in the platelet-interaction through CLEC-2, a receptor on platelets and induces the platelet aggregation. PDPN-mediated platelet aggregation promotes tumor progression by increasing metastasis formation through the tumor embolization and by enhancing growth and invasion through the release of platelet-derived growth factors. To identify the effective tools for suppressing tumor, we performed high-throughput screening from chemical compound library deposited in RIKEN Chemical Biology Research Center by detecting the inhibitory activity against PDPN-CLEC-2 interaction. As the next step, picked-up candidates were subjected to secondary screening using PDPN-expressing cells. Further in vitro analyses showed some hits have the potential to suppress PDPN-dependent platelet aggregation. Their availability are on testing in vivo model.
Collaborators: Takao Ukaji (JFCR), Yasumitsu Kondoh (RIKEN)

▲TOP

[P-2392] P16-6 [English/Japanese]
Signal transduction inhibitor (3)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Etsu Tashiro / Fac. of Sci. & Tech., Keio Univ.

P-2392

Mining the novel combination therapy based on molecular profiling analysis in KRAS positive colorectal cancer

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Mutations in KRAS proto-oncogene have been identified in 30% to 40 % of colorectal cancer (CRC). To date, cytotoxic agents combined with blockade antibody against VEGF pathway has been demonstrated to improve the clinical outcome, whereas, the therapeutic options are still limited. Activated KRAS induce the downstream RAFs-MEK-ERK activation resulting in tumorigenesis. However, KRAS mutant cells have shown resistance to MEK inhibitors due to the feedback reactivation of this pathway through ERK and other cell survival pathway activation. In current study, we established over 70 KRAS-mutant patient-derived cell lines (PDCs) under the IRB approved protocol. To identify the effective therapeutic strategies for KRAS-mutant CRC, we then performed combination drug screening, and targeted sequencing analysis focused on 108 cancer related genes. We identified at least three sub-groups, (1) sensitive to combination MEK with ERBB inhibitor, (2) sensitive to combined MEK inhibitor with a specific inhibitor, and (3) others need to find alternative target in the future study. Our results suggest KRAS-mutant CRCs are able to subdivide at least in 3 groups using the combination drug screening.

▲TOP

P-2393

The finding of new subgroups in BRAF V600E mutation positive Colorectal Cancer

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BRAF mutations (mainly V600E) have been found in about 10 % in colorectal cancer (CRC). Different from the BRAF V600E positive melanoma or lung cancer, BRAF inhibitor demonstrated poor treatment outcome to BRAF mutated CRC mainly due to the feedback re-activation of EGFR. The combined therapy of BRAF (with MEK) and EGFR inhibitors have been evaluated in clinic and showed improvement of antitumor effect in BRAF V600E positive CRC. However, the response rate of the combined therapies is still around 20 %. In this study, we selected BRAF V600E mutant CRC by ddPCR from the surgical specimens and established the more than 10 CRC patient-derived cells (PDCs) under the IRB approved protocol. By the cell viability assay of these PDCs treated with the inhibitor library, we found that the BRAF mutant CRCs can be categorized into sub-groups; (a) highly sensitive to BRAF inhibitor monotherapy, (b) sensitive to BRAF with ERBB inhibitor, and (c) sensitive to different type of TKI. Our results suggest that the establishment of biomarker for selecting the patients who will respond to the BRAF targeted therapy, and finding of new treatment strategies are needed in the future.

P-2394

RK-287107, a potent and specific tankyrase inhibitor, blocks colorectal cancer cell growth in a preclinical model

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Aberrant Wnt/β-catenin signaling causes tumorigenesis and promotes proliferation of colorectal cancer cells (CRC). Tankyrase (TNKS) enhances Wnt/β-catenin signaling through PARsylation and subsequent destabilization of Axin, a negative regulator of β-catenin. We have recently reported that tankyrase inhibition exerts a growth inhibitory effect on CRC with “short” APC mutations, which lack all of seven 20-amino acid repeat domains and cause excessive cell dependence on β-catenin. Here we developed a novel, potent and specific TNKS inhibitor, RK-287107. This compound specifically inhibits the PARP activities of TNKS and TNKS2 but not of PARP-1. Consistent with its ability to repress TCF reporter activity, RK-287107 stabilizes Axin, downregulates β-catenin and its target gene expression, and inhibits the growth of CRC with short APC mutations in vitro and in a mouse xenograft model. These observations indicate that RK-287107 has a proof-of-concept antitumor effect in vivo and suggest that TNKS is a therapeutic target for CRC with short APC mutations. Collaborator: RIKEN DMP and CSRS, Masayuki Okue, Takeshi Tsumura, Tsubasa Chikada (Meiji Seika Pharma Co., Ltd Pharm. Res. Ctr.)

P-2395

Combination effect of the Anti-PD-1 antibody and STAT3 inhibitor, STX-0119, using humanized MHC-dKO NOG mouse

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Recently, immunotherapy has been recognized as one of effective therapeutic tools against cancers, and clinical studies using anti-immune checkpoint antibody medicines have been successful for solid tumors, such as melanoma and non-small cell lung cancers. However, further antitumor effect improvement is needed, therefore combination use with molecular targeted drugs is also considered as a therapeutic option. The important thing about combination therapy, immunologically synergistic effects should be expected in vivo. Previously, we developed a novel humanized MHC-double knockout (dKO) NOG mouse model and demonstrated that the injection of anti-PD-1 antibody inhibited PD-L1 positive advanced solid tumor growth and induced tumor-specific immune responses in this model (Clin Cancer Res, 23, 149, 2017). Additionally, we found that the STAT3 inhibitor, STX-0119, showed anti-tumor activity occurring though the promotion of TIL accumulation in the humanized dKO-NOG mouse system (Immuno Lett, 190, 20, 2017). In the current study, using humanized mouse model, we have tried to develop the combination therapy of anti-PD-1 antibody with STX-0119 in vivo against PD-L1 positive solid tumors.

▲TOP

P-2396

A novel NF-kappaB/STAT3 inhibitor, bavachin, induces apoptosis in multiple myeloma cell lines

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<Purpose> Multiple myeloma (MM), the second most frequent haematological malignancy, is characterised by an accumulation of abnormal clonal plasma cells in the bone marrow. It has been shown that in case of MM, the expression of STAT3 is high, and is associated with MM progression and development. Therefore, inhibiting the NF- κ B and/or STAT3 pathway is a potential target for the treatment of MM.. In this study, we investigated the mechanism of bavachin-induced apoptosis in MM cell lines. <Methods> Cell viability was assessed by the trypan blue dye method. Signal molecules were determined by western blots. <Results> We found that bavachin decreased the viability of MM cell lines, but was not cytotoxic towards normal cells. It inhibited the activation of NF- κ B and STAT3. <Discussion> Our results suggest that bavachin induces apoptosis through the inhibition of NF- κ B and STAT3 activation in MM cell lines. Most importantly, few NF- κ B and STAT3 inhibitors with high efficiency, specificity, and safety are currently available for clinical cancer therapy. Hence, bavachin, which targets NF- κ B and STAT3, is a potential anticancer agent for the treatment of MM.

▲TOP

[P-2404] P16-8 [English/Japanese]
New targeted therapy (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Akinobu Hamada / Div. Mol. pharm., Natl. Cancer Ctr. Res. Inst.

P-2404

Targeted silencing of SOX2 by an ATF showed antitumor effect in lung and esophageal squamous cell carcinoma

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SOX2 is a transcription factor essential for mammalian development and for the maintenance of stem cells. Recently, SOX2 was identified as a lineage specific oncogene, recurrently amplified and activated in lung and esophageal squamous cell carcinoma (SCC). In this study, we have developed a zinc finger-based artificial transcription factor (ATF) to suppress SOX2 expression in cancer cells and termed the system ATF/SOX2. We engineered the ATF using six zinc finger arrays designed to target a 19 bp site in the SOX2 distal promoter and a KOX transcriptional repressor domain. A recombinant adenoviral vector Ad-ATF/SOX2 suppressed SOX2 in lung and esophageal SCC cells expressing SOX2. In these kinds of cells, Ad-ATF/SOX2 decreased cell proliferation and colony formation more effectively than the recombinant adenoviral vector Ad-shSOX2, which expresses SOX2 short hairpin RNA (shSOX2). Moreover, Ad-ATF/SOX2 effectively inhibited tumor growth in a lung SCC xenograft mouse model. These results indicate that ATF/SOX2 would lead to the development of an effective molecular-targeted therapy for lung and esophageal SCC.

▲TOP

P-2405

Potential antitumor effects of M-COPA via targeting the Golgi apparatus under the spheroid culture conditions

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The Golgi apparatus plays an essential role in the transport, processing, and sorting of cell surface proteins. We previously demonstrated that M-COPA, a Golgi disruptor, exhibited antitumor effect in human cancer cells in vitro and in vivo; however, in vivo antitumor efficacy of M-COPA could not be simply predicted by its in vitro efficacy, probably due to the lack of the features of microenvironment in solid tumors. To bridge the gap between in vitro and in vivo efficacies, we exploited 3-dimensional (3D) spheroid culture models, which can recapitulate cell-cell/cell-matrix interactions and nutrient/oxygen gradients that is lacking in monolayer culture conditions. Interestingly, human gastric cancer MKN1 cells displayed hypersensitivity to M-COPA under the spheroid culture conditions compared to those in monolayer culture. Especially, we found that M-COPA effectively suppressed sphere formation of MKN1 cells. The results indicate the unique antitumor effect of a Golgi disruptor M-COPA under the spheroid culture conditions. Furthermore, we will demonstrate the potential involvement of cell adhesion molecule downregulation in sphere destruction upon M-COPA treatment.

P-2406

The cooperated effects of steroid structure drug, cucurbitacin D with MEK inhibitor on ATL cells

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We previously reported that the inflammasome inhibitor cucurbitacin D (CuD) induces apoptosis in peripheral blood lymphocytes (PBLs) isolated from an adult T-cell leukemia (ATL) patient. Additionally, at the transcriptional level, cucurbitacin D enhanced LPS-induced IL-1 β mRNA expression through activation of ERK1/2 mitogen-activated protein kinases (MAPKs). Here, we investigated the effects of CuD with MAPK inhibitors and NF- κ B signaling inhibitors on PBLs from ATL patient. The PBLs from ATL patient highly expressed CD4, CD5, and CD45RO but not CD8, CD16, CD19, and CD45RA. MEK1/2 inhibitor also induced cell death and enhanced CuD-induced cell death in PBLs from ATL patient. To the contrary, JNK inhibitor decreased CuD-induced cell death. The IKK inhibitor could induce cell death of PBLs and enhance CuD-induced cell death. The western blot analysis revealed that CuD dramatically inhibited Erk activation in PBLs from ATL patient. Taken together, these results suggest that Erk is a new target for effects of steroid-induced cell death in ATL cells.

P-2407

New mode-of-action of a telomerase inhibitor MST-312 and modifiers of its anticancer efficacy

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Telomeres, the protective caps of chromosome ends, shorten at each cell cycle, resulting in senescence or apoptosis in human somatic cells. Most cancer cells activate telomerase to maintain telomeres and grow infinitely. We previously reported that long-term treatment of cancer cells with a telomerase inhibitor MST-312 at non-acute cytotoxic doses gradually shortens telomeres and induces eventual crisis. Meanwhile, MST-312 exerts an acute anticancer effect in mouse xenograft models when used at maximum-tolerated doses. By using COMPARE analysis, here we found that MST-312 is a dual inhibitor of telomerase and topoisomerase II, and induces non-telomeric DNA damage at acute cytotoxic doses. We also found that cancer cells with shorter telomeres and lower lamin A expression were more sensitive to MST-312. When lamin A was overexpressed and telomeres were elongated in MST-312-sensitive cancer cells, MST-312-induced DNA damage was alleviated. The resulting cells were also resistant to DNA-damaging drugs, such as etoposide, camptothecin and cisplatin, but not paclitaxel. These observations suggest that lamin A and telomeres modify deleterious effects of DNA-damaging drugs on cancer.

▲TOP

P-2408

Synthesis of a Novel Pyrrole Imidazole Polyamide Compound and its Influence to Expression of EMT-related Genes

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Epithelial mesenchymal transition (EMT) plays an important role in various developmental processes, its involvement in cancer invasion and metastasis has long been suggested. Therefore, the expression of MET related genes including CDH1 (E cadherin) could be suppressed by cancer metastasis. Pyrrole imidazole (PI) polyamide, is a class of peptide compounds recognizes base pairs of DNA by a pairwise hetero ring. In addition to such ability , PI polyamide is cell permeable and localized in cell nucleus.Its accumulation and retention in tumor tissues were also observed in invivo models, indicating that PI polyamide can be used as DDS homing target DNA sequence of the cancer genome. For example, PI polyamide SAHA(HADCi) conjugates increased target gene expression, indicating that the SAHA moiety in the conjugate inhibits HDAC activity around the targeting binding sites. In this study, we designed and synthesized PIP drug conjugates to develop a PIP compound which can regulate the expression of MET related genes. We will report on synthesis scheme of these compounds in detail and their influence on the MET and its related genes such as CDH1.

P-2409

Ivermectin suppresses the Wnt/beta-catenin pathway and specifically binds to target proteins

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Wnt/beta-catenin signaling controls cell proliferation, and excessive activation of this signal contributes to tumorigenesis. We identified Ivermectin as an inhibitor of the Wnt/beta-catenin signaling in a chemical suppressor screening in zebrafish embryos. Here, we show that Ivermectin inhibits Wnt/beta-catenin signaling in colorectal cancer cell lines and report the identification of Ivermectin-binding protein (IvBP) as a potential therapeutic target. Ivermectin reduced beta-catenin/TCF reporter luciferase activity and suppressed the expression of target genes in the Wnt/beta-catenin pathway. Ivermectin reduced cytoplasmic beta-catenin levels by a pathway independent of proteasomal degradation. Ivermectin suppressed colorectal tumor growth in an APCMin/+ mouse model. Furthermore, to explore the mechanism of action, we searched for target molecules of Ivermectin using chemically immobilized Ivermectin, and identified IvBP by mass spectrometry. The involvement of IvBP in the regulation of the Wnt/beta-catenin pathway is currently under investigation. IvBP may become a novel therapeutic target for the Wnt pathway-dependent cancers.

▲TOP

[P-2416] P21-2 [English/Japanese]
Gene therapy and oncolytic virus therapy (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Tomoyuki Nishikawa / Gene Therapy Sci., Sch. of Med., Osaka Univ.

P-2416

Potential applicable range of oncolytic virotherapy with a recombinant measles virus in dogs

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Nectin-4, a receptor of measles virus (MV), was identified as a new histological tumor associated marker for various cancers in human. Recently, we have reported that dog nectin-4 (dN4) was expressed in mammary cancers and that an oncolytic recombinant MV (rMV-SLAMblind) uses dN4 to infect them. However, there is limited information available regarding the expression of dN4, and thus applicability of rMV-SLAMblind to other types of cancer is unknown in dogs. In this study, immunohistochemical analysis of dN4 was conducted using clinical specimens of dog cancer patients. We found that, whereas the expression of dN4 in normal tissues was limited, 48% of overall specimens expressed dN4. Positive staining cells were observed in various cancers as in human cases. Histopathological analysis suggested that the frequency of vessel invasion was higher in nectin-4 positive specimens compared to nectin-4 negative ones. These results suggest that dN4 is up-regulated in various solid tumors and may be relevant to cancer cell migration, and thus dN4-targeting virotherapy is applicable more broadly than previously expected in dogs.

▲TOP

P-2417

Reovirus induces down-regulation of HIF-1α in subcutaneous tumors following systemic administration

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<Purpose> Oncolytic reovirus has received much attention as a novel antitumor agent. Reovirus induces antitumor effects via induction of apoptosis of tumor cells and antitumor immunity. In addition, reovirus has been reported to induce down-regulation of HIF-1α protein levels in cultured tumor cells. In this study, we examined whether systemic administration of reovirus led to down-regulation of HIF-1α and its target genes in subcutaneous tumors.
<Results & Discussion> Reovirus induced down-regulation of HIF-1α and HIF-1α target genes in subcutaneous tumors 120 hrs post systemic administration, suggesting that reovirus induced antitumor effects via reduction in HIF-1α target genes expression. At this time point, numbers of TUNEL-positive tumor cells were comparable between PBS-administered and reovirus-administered mice, indicating that reovirus-mediated tumor cell killing did not largely contribute to the down-regulation of HIF-1α in the subcutaneous tumors. On the other hand, UV-inactivated reovirus did not induce HIF-1α down-regulation. These data suggest that reovirus infects and replicates in the tumor cells, leading to down-regulation of HIF-1α and its target genes.

P-2418

Oncolytic effect of HF10 for breast cancer lung metastasis

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Over 40% of all breast cancer patients will develop metastasis involving lung, liver, bone, and brain, which accounts for the majority of death. We have been exploring the oncolytic efficacy of HF10, an attenuated, replication-competent HSV, using various tumor models. Previously, we made a mouse model of lung cancer metastasis by injecting colon cancer cells via the tail vain, and treated with intravenous injection of HF10. In this study, we used a highly-metastatic breast cancer cell line which inevitably makes lung metastasis when inoculated subcutaneously. In vitro experiment, HF10 replicated well, and cytopathic effect and cell death were observed. In vivo experiment, HF10 inoculation reduced subcutaneous tumor and prolonged the survival. When the mice were sacrificed, lung metastasis was observed in all the mice; however, the metastasis was less in the HF10-treated group. When co-cultured with the tumor cells, more IFN-gamma was measured from the supernatant of murine splenocytes in the HF10-treated group. These data showed that HF10 showed oncolytic effect for the mouse model of breast cancer.

P-2419

Therapeutic efficacy of IL-12 expressing oncolytic HSV-1 for neck lymph node metastases in mouse tongue cancer model

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Conventional therapies for oral squamous cell carcinoma (OSCC) often fail to control neck lymph node metastases, resulting in unfavorable outcome of the disease. In this study, we evaluated the antitumor efficacy of an oncolytic HSV-1 armed with murine interleukin 12 (IL-12), termed T-mfIL12, in an immunocompetent orthotopic mouse tongue cancer model using KLN205-MUC1 cells. This model nicely reproduces the clinical course of OSCC progression; cervical lymph node metastases followed by lethal lung metastases. In vitro cytopathic effect of T-mfIL12 in KLN205-MUC1 cells was comparable to that of T-01, a control virus. Intratumoral injection with T-mfIL12 (1×10^6 pfu) 3 days after tumor implantation significantly prolonged the survival and suppressed the growth of metastasized neck lymph nodes compared with mock treatment. In contrast, treatment with T-01 at this dose did not affect the survival nor the size of neck lymph nodes. The results suggest that T-mfIL12 may be a promising therapeutic tool for OSCC, especially for preventing the development of neck lymph node metastases.

▲TOP

P-2420

siRNA-PLGA hybrid micelle-mediated knock-down of Glypican3 inhibits tumor growth in melanoma lung metastasis

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It has been known that Glypican3 (GPC3) was one of the high expression protein in melanoma metastatic cells. In previous study, we prepared self-assembled siRNA-PLGA hybrid conjugate micelles for gene silencing to deliver siRNA into the cell. The purpose of this study was to evaluate the effect of knock-down of GPC3 using siRNA-PLGA hybrid conjugate micelles for melanoma lung metastasis in mice model. Melanoma lung metastasis model mice were treated with siRNA-PLGA hybrid conjugate micelles intraperitoneally after surgical removal of the tumor in the hind limb induced by the subcutaneous injection of B16/BL6. The surgical removal of the tumor in the hind limb significantly increased the number of metastatic tumor cells in the lung with time. An intraperitoneal injection of micelles significantly decreased the number of metastatic tumor cells in the lung compared with control. The anti-metastasis effect of micelles induced by knock-down of GPC3 was more remarkable than conventional standard medications for melanoma such as dacarbazine or anti PD-1 antibody. These results suggested that siRNA-mediated knock-down of GPC3 is useful as a target for therapy of melanoma metastasis.

P-2421

Centrosome related gene introduced dendritic cells (DC), with functional modification of DC, show cytotoxic activity

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Both survivin and Cep55/chromosome 10 open reading frame 3 (Cep55/C10orf3) are centrosome-related genes expressed in various malignancies. Survivin, isolated as an inhibitor of the apoptosis protein family, has anti-apoptotic effects. In this study, we targeted both survivin and Cep55/C10orf3, and modified the function of dendritic cells (DC) by transducing survivin gene to DC.DC were infected with the survivin gene (DC-survivin) and Cep55/C10orf3 gene (DC-Cep55/C10orf3). DC-survivin and DC-Cep55/C10orf3 induced cytotoxic activity against T-24 and LNCaP respectively. However, cytotoxic activity against HLA non match cells (DU145) was very low. DC transduced with survivin maintained cell viability three weeks after culture, while control DC showed deterioration. By combination of two genes, survivin and Cep55/C10orf3, the cytotoxic activity against LNCaP was increased from 48.3 to 64.5%. Against T-24 it was also enhanced from 19.1 to 45.2%.This enhancement effect seems to be due to the use of two genes, especially due to the modification of DC by survivin. This combination might offer a useful approach for the urologic cancers.

P-2422

A possibility of nucleic acid medicine using PD-L1 siRNA

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Tumor cells suppress host T cell anti-tumor activity through the programmed cell death 1 (PD-1) and programmed cell death 1 ligand (PD-L1) immune checkpoint pathway. Immune checkpoint inhibitors (representatively the PD-1 antibody, Nivolumab) have emerged as a promising clinical modality for cancer therapy through enhancement of anti-tumor immune response. However, this treatment also breaks homeostatic immunosuppression, which eventually causes adverse reactions such as hypothyroidism and pancreatitis. In this study, we explored to develop in vivo small interfering RNA (siRNA) treatment which preferentially accumulates at the tumor site. Flow cytometric analysis showed that one of 4 siRNA candidates efficiently knocked down PD-L1 expressing at cell surface of MC38 colon adenocarcinoma. Red fluorescence-tagged siRNA was injected from mouse tail vein, and abundant accumulation of the fluorescent signals was observed in the tumor tissues. In the therapeutic model of MC38 subcutaneous tumors, systemic injection of PD-L1 siRNA significantly inhibited tumor growth. In conclusion, systemic administration of PD-L1 siRNA may be one option for an efficient anti-tumor treatment.

▲TOP

[P-2437] P23-2 [English/Japanese]
Natural products / dietary factors (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Keiji Wakabayashi / Grad. Div. Nutritional & Environmental Sci. Univ. Shizuoka

P-2437

A fish extract has a potential to inhibit metastasis via reduction in cell migration in breast cancer cell lines

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Metastasis, one of the properties of malignant cancers, is a cause of death in breast cancer patients. At the cellular level, the migratory ability is required for metastasis. To establish a strategy to prevent metastasis, we focused on fish extracts, because fish has various physiologically and biologically active substances. This study shows the inhibitory effect of a fish extract on cell migration in breast cancer cells.
To obtain extracts, we sampled fish in an aquaculture ground, and kept frozen. We made various fractions and treated breast cancer cell lines with them. In general, in 2-dimensional cell culture, high migratory cells show elongated morphology, and if they lost the migratory ability, they shrunk. We observed shrunk morphology in cells treated with a fish extract. To determine whether the extract reduces cell migration, we performed wound healing assays in breast cancer cell lines, and observed reduced cell migration in the extract-treated groups. Moreover, we investigated the effects on cell proliferation and survival, and the extract showed no effects on these properties. Our findings may contribute to the development of metastasis prevention.

▲TOP

P-2438

A curcumin-binding protein, ribosomal protein S3, regulates XIAP expression independently of NF-κB in breast cancer

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In breast cancer, the anti-apoptotic protein XIAP is known to be overexpressed. In order to elucidate the mechanisms underlying the overexpression of XIAP protein, we attempted to clarify the mechanisms by which the natural compound curcumin downregulates XIAP in breast cancer cells. In this study, we identified ribosomal protein S3 (RPS3) as a curcumin-binding protein using curcumin-fixed magnetic FG beads. Knockdown of RPS3 inhibited cell growth and induced apoptosis with downregulation of XIAP in breast cancer cells. Although RPS3 is known to directly activate NF-κB, which induces several anti-apoptotic genes such as XIAP, RPS3 knockdown unexpectedly reduced the levels of XIAP protein, but not those of its mRNA and NF-κB activity. These findings suggest that RPS3 upregulates XIAP independently of NF-κB in human breast cancer cells. It is likely that curcumin downregulates XIAP protein by inhibiting this novel function of RPS3.
(Collaborator: Wakana Goi)

P-2439

Development of an animal hepatocarcinogenesis model through non-obese (Asian-type) nonalcoholic steatohepatitis (NASH)

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Background: Obesity is a major risk of non-alcoholic steatohepatitis (NASH), but lean NASH patients with the normal body mass index has become recognized, especially in Asia. The present study tried to develop a mouse hepatocarcinogenesis model through non-obese (Asian-type) NASH, using a modified choline-deficient, methionine-lowered, L-amino acid-defined (mCDAA) diet.
Methods: Male C57BL/6J mice (6 weeks old) were fed a control diet (fat 10 kcal%, methionine 0.6%) or experimental diets; mCDAA-Met0.1 (fat 45 kcal%, methionine 0.1%), or mCDAA-Met0.6 (fat 45 kcal%, methionine 0.6%), for 6 months.
Results: mCDAA-Met0.1 induced fatty and inflammatory changes in the liver, more markedly than mCDAA-Met0.6, but not the body weight increase. In addition, only mCDAA-Met0.1 caused hepatocellular pretumoral and non-pretumoral, poliferative nodular lesions with the marked changes on the liver phosphatidylcholine composition.
Conclusions: The present data indicate that mCDAA-Met0.1 induce non-obese (Asian-type) NASH with pretumoral lesions in the liver of mice, and that liver PC composition may play some roles in the mechanisms underlying this phenomenon.

P-2440

Strain difference on the influence of trans fatty acids on NASH induced by the CDAA diet between Hsd and F344 rats

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Nonalcoholic steatohepatitis (NASH) is a lifestyle-related disease with progressive liver lesions, including cirrhosis and cancer. We have established a rat NASH model featuring the chronic feeding of a choline-deficient, methionine-lowered, amino acid-defined (CDAA) diet, and found the clear strain difference, which may give a hint to control human NASH. The present study was thus conducted to evaluate phenotypical and genotypical strain differences between male Hsd:Sprague Dawley (Hsd) and Fischer 344 (F344) rats fed the CDAA diet on the NASH manifestation and the influence of trans fatty acid (TFA). Both strain rats were fed TFA-containing or TFA-free CDAA diet for 6 weeks. Relative liver weight was increased, and liver fatty change and fibrosis were induced in both strains. Plasma activities of aspartate and alanine aminotransferases, and inflammation and fibrosis related gene expression were increased only in F344 rats. While RNA sequence data obtained by the next-generation sequencing in the livers of both strain rats are now analyzed, TFA had no obvious effects at this moment.

▲TOP

P-2441

Impact of Sterol Regulatory Element-Binding Protein-1c in White Adipose Tissue on Cancer Prevention

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Aging is one of the strong risk factors for carcinogenesis. Sterol regulatory element-binding protein-1c (Srebp-1c), a master transcription factor of fatty acid (FA) biosynthesis, is responsible for the pathogenesis of fatty liver /steatosis. In contrast, caloric restriction (CR) can delay the onset of several age-related pathophysiologies, including cancer, and extend lifespan in various species. We previously showed that CR upregulated expression of Srebp-1c and its downstream target genes in white adipose tissue (WAT). Hence, CR-associated responses in Srebp-1c knockout mice and their embryonic fibroblasts were compared with those of wild-type mice. Our study showed that, under CR conditions, Srebp-1c enhanced mitochondrial biogenesis via increased expression of peroxisome proliferator-activated receptor gamma coactivator-1 α , and upregulated expression of proteins involved in FA biosynthesis within WAT, but not in other tissues. Moreover, Srebp-1c is suggested to play an important role in both CR-associated suppression of oxidative stress, and the prolongevity action of CR. Our data imply that CR-associated Srebp-1c activation in WAT may prevent aging-associated carcinogenesis.

P-2442

Reactive stromal fibroblast contribute to high-fat-associated prostate cancer by the upregulated MIC-1

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Recent studies indicated that the high-fat diet (HFD) plays an important role in prostate cancer (PCa) progression. The prostate stromal microenvironment has emerged as a key factor in growth and development of PCa by induce of proinflammatory cytokine production. In this study, we investigated the role of HFD on PCa stromal microenvironment using the PC-3M-luc-C6 PCa metastatic model mice fed with HFD or control diet. In results, HFD consumption increased the adipocyte infiltration in the tumor microenvironment, activation of PCa stromal fibroblast, and PCa metastatic progression by the upregulation of macrophage inhibitory cytokine-1 (MIC-1) and IL-8 secretion. Clinically, the activated PCa stromal fibroblast was associated with advanced PCa progression, and increased level of IL-8 and IL-6 through the upregulated MIC-1. In addition, the MIC-1 specific receptor GFRAL was highly expressed in the PCa cells and stromal fibroblast, and the expression was decreased by the anti-cancer therapy. Taken together, HFD mediated activation of PCa stromal fibroblast by the upregulated MIC-1 may be one of critical mechanism on HFD or obesity induced PCa progression.

P-2443

Cancer-preventing property of hydroxymethylfurufural as the aglycon in glycoside produced by heating the glucose

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In the previous meeting, we reported that, when glucose has been heated, it becomes to acquire a cancer-preventing function. Namely, the growth of solid cancer from the fibroblast transformant W14 by H-ras oncogene was suppressed by water drinking method of heated glucose. As its glucose didn't directly depress the proliferation of W14, the cancer-preventing action appeared to be an indirect effect by way of intestine. Moreover, we reported that the active size of heated glucose shows some broad molecules around ten thousand, because glucose occurs condensation polymerization by heating. We searched the precursor molecule from heated glucose to unknown condensed compound. As a result, its molecule was detected as the glycoside composing of glucose and hydroxymethylfurufural (HMF) of aglycone by MS and NMR analysis. In this meeting, we report that with rising heat treatment HMF has increased the cancer prevention. And its mechanism had been brought by the stop of cell cycle of W14.

▲TOP

[P-2450] P25-1 [English/Japanese]
Data science / AI (1)

2018 / 9 / 28 (Fri) 16:30-17:15 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Masato Morikawa / Dept. Mol. Pathol., Grad. Sch. Med., Univ. Tokyo

P-2450

Empirical evaluation of variant calling accuracy using ultra-deep whole-genome sequencing data

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In the study design of whole-genome sequence (WGS), sequencing depth is a crucial parameter to define variant calling accuracy and study cost, while standard recommendations for depth setting has not been established. We empirically evaluated variant calling accuracy of the WGS pipeline using ultra-deep WGS data (approximately 410×). By randomly sampling sequence reads, we created a series of simulation WGS datasets with a gradual variety of depths (from 0.05× to 410×). We evaluated genotype concordances of the WGS data with those in the SNP microarray data or the WGS data using all the sequence reads. The WGS data showed a reduction of the depths in the variant calling process, with higher reduction rates for higher depths. In the comparison with the SNP microarray, the WGS data with higher depths showed higher concordance rates, and the >13.7× depth achieved as high as >99% concordance. Comparisons with the whole read WGS data showed that SNV achieved >95% of concordance at 17.6× depth, while indels showed only 60% of concordance. 15× is required for accurate SNV calling and recommended for cost-effective WGS study, while higher depths are required for accurate indel calling.

▲TOP

P-2451

The impact of intratumor heterogeneity to prognosis

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Human cancers accumulates various mutations during the development and consist of highly heterogeneous cell population called intratumor heterogeneity (ITH). ITH is known to be involved in tumor progression and metastasis, and to be obstacles to accurate diagnosis and effective treatments. In order to explore the dynamics of ITH, numerous studies have been performed so far. However, it is still challenging to use the characteristics of ITH as prognostic factor because of difficulties in quantifying the ITH. In this study, we analyzed the relationship between the distribution of variant allele frequencies (VAFs) in each tumor sample and the prognosis of the patient across 16 tumor types registered in TCGA. We calculated six parameters to measure the VAF distributions for survival analysis and found significant relationships with the prognosis and samples having certain VAF distribution in nine tumor types. This result suggests that there are no general association between genomic instability and tumor survival common to all types of cancer. In addition, we observed that tumors with high genomic instability were not necessarily linked to poorer prognosis.

P-2452

New development of cancer-related disease gene / protein interaction database CanceProView

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We developed cancer-related gene / protein interaction database system “CancerProView” for a cancer-related gene and the gene about the outskirts and the elucidation of the disease [Mitsuyama S and Shimizu N, Genomics.100:81(2012)]. CancerProView is made to search it by Web browsers such as Internet Explorer (http://cancerproview.dmb.med. keio.ac.jp/). We implemented the following functions: (1) Graphical indication of a pathway, a domain of the cancer-related gene and protein and the disease to participate, (2) Links from a raster display to OMIM and Pubmed, (3) Search of the protein having a common domain structure. Here, we further collected the data from NCBI Refseq (19305 gene and 43291 protein). Then, we improved the detailed indication of the protein domain and developed the program that could display the details of domains from Pfam and NCBI Refseq. Furthermore, we developed new search program by using deep learning. The improved CancerProView would facilitate various cancer research including basic molecular biology, clinical diagnosis, treatment and prevention.

P-2453

Withdrawn

No Abstract

▲TOP

P-2454

Revealing novel mutation signatures by Latent Dirichlet Allocation with Variational Bayes inference

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Cancer genome includes many mutations derived from various mutagens and mutation processes, leading to specific mutation patterns.It is known that each mutagen leads to characteristic mutation types, and a mutagen with the preference of mutation types is called "mutation signature". To determine mutation signatures is an important task to investigate carcinogenic mechanisms. In previous studies, some statistical methods revealed a number of mutation signatures, but those existing approaches used an ad-hoc method to estimate the number of mutation signatures.In this study, we present a novel method to estimate the plausible number of mutation signatures and its mutation distributions using Latent Dirichlet Allocation with Variational Bayes inference (VB-LDA).In the simulation with artificial data, we confirmed that our method estimated the correct number of mutation signatures and the mutation distributions that exactly correspond to original data.Also, applying our method to real mutation catalog in COSMIC, we extracted almost known signatures.Moreover, our method found some new signatures, which have been seen in multiple primary lesions.

P-2455

Agent-based complex system modeling for cancer research

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Agent-based complex systems are dynamic networks of many interacting agents. In the biological background, the agents include genes, cells, and tissues. For instance, a cell is regarded as a complex system consist of interacting genes whose expression levels are dynamically changing. It is certainaly useful for understanding the basics of disease to model and elucidate the general principle of such complex system. In recent big data biology era, we have been able to construct the agents with completely data-driven approach. It means that many types of machine learning methods, such as Random Forests and deep neutral networks can be regarded as agents in the complex systems. In this study, we will show the preliminary results of computational simulations using this modeling concepts, and discuss about the feasibility for applying to cancer research.

▲TOP

[P-2430] P23-1 [English/Japanese]
Natural products

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Naoki Yoshimi / Path. &Onco., Grad. Sch. Med., Univ. of the Ryukyus

P-2430

The attenuation of epithelial-to-mesenchymal transition by cypripedin in non-small cell lung cancer cells

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Lung cancer became the most common cause of cancer morality worldwide due to high metastasis rate. To accomplish metastasis, cancer cells acquire the transition process from epithelial to mesenchymal (EMT)-like properties that enhance cell motility and invasion. Since lung cancer appears to have this aggressive phenotype, EMT regulators became emphasized as an attractive drug target against cancer. We discovered that cypripedin, a phenanthrenequinone isolated from Thai orchid, *Dendrobium densiflorum*, exhibits significant pharmacological effect on EMT suppression in non-small cell lung cancer cells. The EMT markers including Slug, N-Cadherin, and Vimentin were remarkable decreased as a consequence of cypripedin-inhibiting Akt activation. The decrease active status of Akt by this compound enhances the Slug degradation via ubiquitin-proteasomal degradation mechanism. In addition, the cypripedin was able to attenuate *in vitro* tumorigenesis and tumor spheroid-based cell migration, supporting the profound effect of such compound for further pharmacological research and development.

▲TOP

P-2431

Cypripedin sensitizes non small cell lung cancer H460 cells to cisplatin mediated apoptosis

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Lung cancer treatment by chemotherapeutic drugs makes an increasing of life-threatening frequency due to the acquisition of chemotherapy resistance, particularly, cisplatin. As a reason for this response, it contributes to the research and development of new drug entity especially from natural origin. In this study, we discovered the remarkable effect of cypripedin, a phenanthrenequinone extracted from Dendrobium densiflorum, against non-small cell lung cancer cells. As low as 50 μM of cypripedin was able to induce apoptosis with an appearance of apoptotic body and chromatin fragmentation. Western blot analysis revealed that the active caspase-3 became increasing significantly, in opposite to, the reduction of anti-apoptosis Bcl-2 and Bcl-xL following cypripedin treatment. In addition, non-toxic concentration of cyperipedin synergistically sensitized cisplatin-mediated apoptotic cell death through a Bcl-xL dependent mechanism. Our study provides the scientific information on anti-cancer effect of cypripedin in non-small cell lung cancer that could be a promising alternative approach to improve lung cancer treatment.

P-2432

Comparison of anti-liver cancer activity in vitro between ethanolic and water extracts of Tamarindus indica L. seed husk

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The cytotoxic and antimigratory effects of ethanolic and water extracts of Tamarindus indica L. (T. indica) seed husk on human liver cancer HepG2 cell lines were evaluated by using sulforhodamine B (SRB) and wound healing assays. Oligomeric proanthocyanidins in the crude extracts were identified by using UV-Vis spectrophotometry and HPLC. Very good antioxidant activity and high total phenolics compound of the crude extracts were shown by DPPH assay and Folin-Ciocalteu method. Interestingly, the water extract of T. indica seed husk showed significantly more cytotoxicity of HepG2 cells in a time- and dose-dependent manner with IC50 values of 216.9±14.3, 117.0±7.1 and 44.1±0.9 μg/ml at 24, 48 and 72 h than the ethanolic extract with IC50 values of 915.2±80.2, 419.4±20.7 and 408.3±9.8 μg/ml at 24, 48 and 72 h, respectively. Furthermore, treatment with the water extract caused a significant and dose-dependent decrease in cell migration. These findings suggest that the water extract of T. indica seed husk may be more useful than ethanolic extract for developing an anticancer drug candidate for the treatment of liver cancer.

P-2433

Cucurbitacin B induces apoptosis in human cholangiocarcinoma cells by modulation of apoptotic-related proteins

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Cholangiocarcinoma (CCA) is one of the most deadly cancers. At present, the development of new therapeutic strategies for CCA is a crucial requirement. The aim of this study was to investigate the apoptosis-inducing effect of cucurbitacin B (Cu.B), a triterpenoid derived from Cucurbitaceae family, in human CCA cells, KKU-100. The results showed that Cu.B had a potent cytotoxicity against CCA cells and the compound significantly induced CCA cell apoptosis. To further investigate the mechanism underlying its apoptosis-inducing effect, the effects of Cu.B on caspase activities and the expression of apoptotic-associated proteins were determined. The results demonstrated that Cu.B significantly increased caspase-3 and -9 activities. The expression of pro-apoptotic proteins including apoptosis-inducing factor, Bax, Cytochrome C and p53 was up-regulated after Cu.B treatment. Moreover, Cu.B could down-regulate the expression of anti-apoptotic protein Bcl2 and Bcl-xl. These results indicated that Cu.B exhibits anti-cancer activity against CCA by targeting the apoptosis pathway. This may provide a new approach to CCA therapy. This work was supported by Graduate School, Khon Kaen University

▲TOP

P-2434

The inhibitory effects of ziyuglycoside Ⅱ on AOM/DSS-induced colitis-associated tumorigenesis

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Ziyuglycoside Ⅱ, a major bioactive compound of Sanguisorba officinalis L., had various pharmacological activities. We previously reported the inhibitory effects of ziyuglycoside Ⅱ on angiogenesis at this meeting. However, little information concerning its anticancer properties in vivo is available. The aim of this study was to investigate the inhibitory effects of ziyuglycoside Ⅱ in dextran sulfate sodium (DSS)-induced colitis. BALB/c mice were administered ziyuglycoside Ⅱ (0.5 mg/kg and 2.5 mg/kg) orally for 10 days. Colitis was induced by administering 5% DSS in drinking water for 7 days. The effect of ziyuglycoside Ⅱ in DSS-induced colitis were evaluated by measuring relevant clinical symptoms (fecal blood, diarrhea, and body weight loss), colon length, inflammatory mediators, and histopathology. Mice fed ziyuglycoside Ⅱ with significantly less mean tumour numbers compared to control mice. Also, administration of ziyuglycoside Ⅱ decreased the levels of several inflammatory mediators. Ziyuglycoside Ⅱ treatment provided moderate suppression of inflammation, proliferation, and certain inflammation-related dysbiosis in a murine model of colitis associated-colon cancer.

P-2435

Cucurbitacin-I, a natural triterpenoid of Cucurbitaceae, exerts potent anticancer effect in human ovarian cancer cells

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Cucurbitacin-I is a triterpenoids found in medicinal plants and have diverse pharmacological and biological activities. In this study, the antitumor effects of cucurbitacin-I on human ovarian cancer cells and possible roles in apoptosis and cell cycle arrest were investigated. Treatment of SKOV3 cells with cucurbitacin-I decreased cell viability and cell proliferation in a concentration-dependent manner. Cucurbitacin-I induced increased cleavage of caspase-3, -7, -8, -9, and poly ADP ribose polymerase. When we examined the effects of cucurbitacin-I on cell cycle progression in SKOV3 cells, any significant changes were not observed. In summary, the present study showed that cucurbitacin-I reduced ovarian cancer cell proliferation by enhancing apoptosis.

P-2436

REVEAL CHEMOPREVENTIVE ACTIVITY OF Boesenbergia pandurata (Roxb.) Schlechter ON 4T1 CELLS THROUGH ALDH INHIBITION

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Overexpression of aldehyde dehydrogenase (ALDH), one of antioxidant enzyme, causes the hyper-metabolism of reactive oxygen species (ROS) leading to cancer chemoresistance. Boesenbergia pandurata, one of Indonesian herbs, had been known its cancer chemopreventive activity. The aim of this research is to scrutinize the chemo preventive activity of ethanolic extract of B. pandurata (EEBP) against 4T1 breast cancer cells. EEBP showed cytotoxic effect on 4T1 cells in dose-dependent manner with IC_{50} value of 44 μg/mL based on MTT assay. Cell cycle analysis by PI-staining flow cytometry showed EEBP induced G2/M arrest at the concentration of 40 μg/mL. Under ALDH activity assay, EEBP inhibited activity of ALDH at the concentration of 10 and 20 μg/mL. Treatment of EEBP at the concentration of 60 and 80 μg/mL increased ROS level based on DCFDA staining assay. Overall, EEBP performs chemopreventive activity on 4T1 cells through inhibition of ALDH.

▲TOP

[P-2444] P23-3 [English/Japanese]
Natural products / dietary factors (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Masumi Suzui / Dept. Mol. Toxicol. Nagoya City Univ. Grad. Sch. Med. Sci.

P-2444

Therapeutic effects by immunostimulation of extract from nori (Porphyra yezoensis) for mouse model of melanoma

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Immunostimulation effects of water soluble extract from dried nori (Porphyra yezoensis) in murine spleen cells have been obtained. Single oral administration of nori extract induced the IFN-γ production in the serum of normal mice. In this study, we examined therapeutic effects by immunostimulating activity of nori extract for subcutaneous mouse model of melanoma. The decrease in the tumor weights in mouse model for 14 days after the per os (PO) administration of nori extracts was obtained. Time-dependent increase in IgA and IgG was obtained in serum of mouse model after the multiple PO administration of nori extract. Increase in IgA in supernatant solution that homogenized the ileal tissue of the mouse model was obtained after the PO administration of nori extract. Furthermore, IgA positive cells in ilea tissue sections of mouse model after the PO administration of nori extract were observed using immunostaining. These results suggest that the nori extract could be effective for inhibiting the growth of tumor cells by immunostimulation effects for subcutaneous mouse model of melanoma.

▲TOP

P-2445

Peridinin, a marine carotenoid, induces G1 cell cycle arrest by inhibiting CyclinD1/E1 and ERK in DU145 cells

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Peridinin is a polar carotenoid, distributed widely in the sea, and has a unique butenolide structure and an allene bond. We have previously reported that peridinin inhibited the growth of HepG2 cells by inducing G1 arrest with apoptosis by modulating the expression of gadd45 and MAPK phosphorylation. In the present study, we show that peridinin reduces cyclin proteins and modulates MAPK phosphorylation, and the involvement of MAPK in the induction of G1 arrest by peridinin in DU145 cells.
Materials and methods; Expression of cell cycle-related proteins and MAPK was analyzed by Western blot. Expression of cell cycle-related genes was analyzed by real time RT-PCR. Cell cycle was analyzed by flow-cytometry.
Results; Peridinin reduced the expressions of Cyclin D1 and cyclin E1. Peridinin reduced Cyclin D1 by inducing proteolysis. Peridinin induced the phosphorylation of p38 MAPK and JNK, and slightly reduced that of ERK. Inhibition of MAPK and pim1 kinase modulated G1 arrest and gene expressions induced by peridinin.
Conclusions: Peridinin induces G1 arrest by reducing Cyclin D1/E1. ERK may negatively and Pim1 kinase may positively regulate G1 arrest by peridinin, respectively.

P-2446

New prevention strategy against cancer stem cells through the induction of connexin 43

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Cancer stem cells (CSCs) contribute to oncogenesis and recurrent cancer. Soybean is a cancer-preventive food. The most predominant protease inhibitor in soybeans is Bowman-Birk inhibitor (BBI), a well-established cancer chemo-preventive agent. Our previous study has shown that BBI induces connexin43 (Cx43) which is a tumor suppressor gene and that forms gap junction (GJ). Through the GJ, it can restore normal cell functions. This study was preformed to reduce cancer CSCs characteristics through the induction of Cx43. Androgen-dependent prostate cancer cells, LNCaP were used, and we made spheroid-formed CSCs from LNCaP parental cells. Cell viability was evaluated by a WST-1 assay, mRNA level was determined by RT-Real time PCR and protein level measured Western blotting or Immunohistochemistry. Cx43 mRNA and protein levels were increased by BBI. In parallel with the Cx43 induction, cell viability was inhibited in a dose-dependent manner, and some markers of CSCs and chemo-resistance was decreased. Additionally, BBI reduces resistance to anticancer drugs and had the same effect as normal cancer cells. Herein, we propose a new strategy on how to reduce CSCs characteristics by BBI.

P-2447

Inhibition of intestinal polyp formation by active hexose correlated compound in Min mice (2nd report)

Maiko Takahashi
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It is now known that an inflammation plays a critical role in the promotion of carcinogenesis. Active hexose correlated compound (AHCC) extracted from basidiomycete mycelia is used as an adjuvant for anti-cancer drugs. However, the effect of AHCC on cancer prevention has not been studied in detail. To clarify the anti-inflammatory function of AHCC, we used AHCC to treat epithelial cancer cells, HCT 116, and differentiated 3T3-L1 cells, and measured the expression level of inflammatory cytokines. Treatment by AHCC on 25 ng/ml TNF alpha-stimulated HCT 116 cells showed significant reduction of the expression level of IL-6. In the next study, AHCC (1,000 ppm) was administered to model mice of familial adenomatous polyposis, Min mice, for 8 weeks, and we found that AHCC significantly reduced the total number of intestinal polyps. Next, we examined the IL-6 and MCP-1 mRNA expression levels in intestinal mucosa and polyp tissues, but this only revealed a decreasing trend. However, these results indicate the possibility that AHCC suppresses the inflammatory response and may inhibit the formation of intestinal polyps.

▲TOP

P-2448

Effect of long-term aspirin pretreatment plus nicotine treatment on the transcriptional activities in HCT116 cells

Takahiro Hamoya
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In recent clinical trials, it was reported that treatment of low-dose aspirin in the patient that removed sporadic colorectal tumor endoscopically suppressed recurrence of new colorectal tumor. Interestingly, the current smokers with aspirin increased the risk of colorectal tumor recurrence. However, the mechanisms of increasing risk of tumor recurrence in smoking with aspirin is not clarified yet. Thus, in the present study, we aimed to investigate the effects of aspirin and nicotine, one of cigarette harmful ingredients, on NF- κ B promoter transcriptional activities in human colon adenocarcinoma, HCT116 cells. The cells were treated with 1 mM aspirin for 2 weeks, and then, 1 mM nicotine or combination (1 mM aspirin plus 1 mM nicotine) treated for 24 hours. Twenty-four hours of combination treatment increased 126% ($p < 0.01$) of NF- κ B promoter transcriptional activity, compared to that of 1 mM aspirin treatment alone. Several data using the metabolites of tobacco or aspirin will be shown and discussed in this presentation.

P-2449

Genistein suppresses Src-induced proliferative activity by arresting at G2/M through increasing the p53 and p21 levels

Misaki Ono
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Co-author：Shuji Nakano
Dept. Nutritional Sci., Nakamura Gakuen Univ.,

Src has been strongly implicated in the growth, progression, and metastasis of a variety of human cancers. Although soy isoflavones have potential anticancer activity, the role of isoflavones in oncogenic activity of Src remains unknown. Using HAG-1 human adenocarcinoma cells transfected with v-Src, we investigated here the functional role of Src in anti-proliferative activity of isoflavones including genistein (GEN), daidzein, glycitin and equol. Activation of Src conferred resistance to either daidzein, glycitin or equol, but rendered the cells more sensitive to GEN. GEN arrested HAG/src cells at G2/M phase, while other isoflavones could not arrest HAG/src cells at any phase of cell cycle. The sub-G0/G1 apoptotic cell populations were not increased after 72h exposure with either isoflavones. GEN increased the expression levels of p53 and p21 with decreased phosphorylated p21. The levels of other main cell cycle-related proteins and apoptosis-related proteins were not significantly altered. These data suggest that GEN would be the only isoflavone component that may potentially suppress oncogenic activity driven by Src through increasing p53 and p21 levels.

▲TOP

[P-2456] P25-2 [English/Japanese]
Data science / AI (2)

2018 / 9 / 28 (Fri) 17:15-18:00 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Hidetaka Eguchi / Diagnos. & Ther. Intractable Diseases, Juntendo Univ. Grad. Sch. Med.

P-2456

Identifying genomic biomarkers for immune checkpoint therapy in biliary tract cancer

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Co-author：Hiromi Nakamura[1]、Mihoko Adachi[1]、Molly Schmidt[2]、Yasushi Totoki[1]、Tatsuhiro Shibata[1]、Mamoru Kato[3]
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Biliary tract cancer (BTC) has an increased incidence globally with no approved targeted molecular therapy. Previously, we found that a subgroup of BTC with poorest prognosis has the higher expressions of immune checkpoint (ICP) genes. Patients in this group might get benefit from ICP blockage therapy, of which the clinical results are showing revolutionizing efficacy. There is an urgent need to find molecular biomarkers that are quickly usable in already-build-in assays to select patients, such as clinical sequencing where genomic alterations are measured.We used exome sequencing and RNA-Seq data of BTC to identify potential genomic biomarkers correlating with the high expressions of 9 ICP genes, performing univariate and multivariate analyses. Hyper-mutation status, as well as mutations in several genes, were significantly associated with high ICP gene expressions.We validated the identified biomarkers using TCGA cholangiocarcinoma data. Some biomarkers can be validated using the TCGA cohort while other biomarkers are cohort specific.Using biomarkers, we built a model for the prediction of the expression of ICGs that showed a reasonable performance.

▲TOP

P-2457

Association of GSTA family genes with clinical parameters and overall survival in gastric cancer

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Glutathione S-transferases (GSTs), a superfamily of detoxification enzymes, are involved in the biotransformation of carcinogens, such as polycyclic aromatic hydrocarbons. In the current study, we have examined the mRNA expression of GSTA in gastric cancer patients by using Oncomine analysis and investigated their prognostic value in the Human Protein Atlas and the Kaplan Meier plotter database. We found a downregulation of GSTA1, GSTA2, GSTA3 and GSTA4 in gastric cancer compared with normal tissues. The mRNA level of GSTA4 is higher in early stage. Futhermore, lower expression of GSTA1 was associated with poor overall survival (OS) for patients treated by adjuvant; lower expression of GSTA3 was associated with better OS treated by 5-FU based adjuvant; and lower expression of GSTA4 was associated with better OS by surgery alone. In conclusion, GSTA family members could be potential biomarkers of gastric cancer and provide a new strategy for therapy selection.

P-2458

Bioinformatics of VASH2 in breast cancers

Kazuki Komori
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Co-author：Yasufumi Sato
Dept. Vasc. Biol., IDAC, Tohoku Univ.

Our laboratory has isolated anti-angiogenic Vasohibin-1 (VASH1) encoded in 14q24.3 and its homologue Vasohibin-2 (VASH2) encoded in 1q32.3. Expression of VASH2 is absent in normal tissues, but is elevated in various cancers, and promote not only tumor angiogenesis but also accumulation of cancer associated fibroblasts (CAFs) and epithelial to mesenchymal transition (EMT) of cancer cells. Here we analyzed the influence of VASH2 on breast cancers by using the data sets open to public. The first analysis was performed with the data set from GSE 58812 on triple-negative breast cancers. We found that regardless of the level of VASH1 expression, the higher the expression of VASH2, the poorer prognosis was. The second analysis was performed with the cBioPortal. We noticed that VASH2 gene amplification was common in breast cancer, and this should be due to the double minute around 1q32 during chromothripsis. Form these results, we propose VASH2 to be a useful biomarker that predicts the prognosis of patients with breast cancer.

P-2459

Characterization of survival influential genes in cancer genomes

Chen-Ching Lin
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Co-author：Yu-Lin Chang、Yin-Chen Lin
Inst. of Biomed. Informatics, Natl. Yang-Ming Univ.

Essential genes have been reportedly critical in cancer development. So far, the direct identification of the human essential genes was not ethical. Thus, we proposed an in silico approach adopting the Cox regression model to identify the survival influential genes. We denoted the highly expressed genes associated with poor and better prognosis as harmful and protective respectively. Notably, the cancer-specific survival influential genes were exclusively identified between cancers and enriched with distinct functions across cancers. Moreover, the cancer-specific harmful/ protective genes were significantly down-/up-regulated in the corresponding cancer. These observations showed the particularity of identified survival influential genes in cancers. Additionally, we found that pan-cancer harmful genes were enriched with cancer essential genes and tend to occupy the pivotal positions in the protein interaction network. Furthermore, the pan-cancer harmful and protective genes were associated with the cell cycle and cellular energetic respectively. Our result indicated that the underlying molecular mechanisms of survival influential genes might participate in the cancer hallmarks.

▲TOP

P-2460

Nutritional status of cancer patients seen from nutritionDay oncology 2016 in Japan

Hiroyoshi Takemoto
Dept. Surg., Kinki Central Hosp.

【Object】 The nutritionDay project is an international questionnaire survey that grasps the nutritional status of hospitalized patients. Every year a specific day (November 10, 2016) is set as nutritionDay. In response to the publication of the 2016 national report, I will report on the current status of cancer patients in Japan. 【Method】 In 2016, we conducted an investigation on November 10. In Japan, 1380 patients were registered, of which 301 were cancer patients. We compared with 959 cancer patients registered in the whole world. 【Results】 Body Mass Index (BMI) was 21.5 in Japan and 23.7 in the world. For goal of therapy, curative, palliative, terminal was 57.8%, 35.8%, 3.9% worldwide, compared to 44.5%, 38.3%, 14.5% in Japan, and the proportion of hospitalization not aimed for cure was higher in Japan. Looking at nutritional therapy, "no special diet" was the largest number of people in both the world and Japan. 【Conclusion】 nutiritionDay makes it possible to objectively evaluate the current situation of cancer patients and nutritional therapies in Japan and will be the basic data for improving nutritional therapy in the future.

P-2461

Impact of dietary folate intake on the risk of gastric cancer

Yumiko Kasugai
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Previous studies of the association between folate intake and gastric cancer (GC) risk have been inconsistent. In addition, no studies investigated whether the association differs across H. pylori (HP) infection status. We conducted two case–control studies (HERPACC2 and 3: total 1357 cases and 2017 controls) to examine the association between folate intake and GC risk according to HP infection status. We calculated odds ratios (ORs) by logistic regression model and combined with a fixed effects model. Folate intake was associated with a dose–dependent decrease in overall GC risk (low intake (Q1): reference, moderate (Q2): OR = 0.94, 95%CI = 0.75–1.18; high (Q3): 0.77, 0.61–0.96; very high (Q4): 0.77, 0.61–0.96; P–trend = 0.023). When stratified by HP infection status, this association was apparent for HP–infected GC (Q2: 0.91, 0.72–1.16; Q3: 0.84, 0.66–1.07; Q4: 0.72, 0.55–0.92; P–trend = 0.007), while weak non–significant inverse association was observed in non HP–infected GC. The test for heterogeneity was not significant. Our finding suggested that possible protective effect of folate intake for GC risk regardless of HP.

▲TOP

[AACR2-1] AACR2 [English]
Cancer metabolism

2018 / 9 / 29 (Sat) 9:00-11:30 Room 1/5F Large Hall, Osaka International Convention Center Room 1

Tatsuhiko Furukawa / Dept. Mol. Onc, Kagoshima Univ., Grad. Sch. Med. Dent. Sci.、Alec C. Kimmelman / New York Univ. Langone Health, New York

Metabolic changes of cancer cells are old and new problems in cancer research, as it was described almost a hundred years ago by Dr. Warburg. However we are still on the way of resolving the complicated subjects. Comprehensive metabolic analyses by metabolome techniques as well as observations of genetic and epigenetic changes of cancer are needed for better understanding of cancer metabolic changes. Cancer metabolism reprograming gives various effects to microenvironment of cancer cells including immune systems, microbiota, epigenetic changes and chromosomal stabilities.
In this JCA-AACR joint symposium, we will discuss about not only metabolic reprograming of cancer cells but also from the point of view of cancer therapy and prevention.

AACR2-1

Thymidine catabolism and Cancer

Tatsuhiko Furukawa
Dept. Mol. Onc., Kagoshima Univ. Grad. Sch. Med. & Dent.

Co-author：Sho Tabata[1]、Masatatsu Yamamoto[2]、Kentaro Minami[2]、Kohichi Kawahara[2]
[1]Inst. Adv. Biosci., Keio Univ.、[2]Dept. Mol. Onc., Kagoshima Univ. Grad. Sch. Med. & Dent.

Thymidine phosphorylase (TP) is an enzyme for thymidine catabolism and has several important roles in biological and pharmacological aspects, as an angiogenic factor and one of metabolic enzymes of fluoro-pyrimidine agents. Previously we indicated TP expression is related to the malignant phenotypes and poor prognosis of the patients with several cancers. We recently found that TP-dependent thymidine catabolism contributes to tumor survival in low nutrient conditions and the pathway from thymidine to the glycolysis cascade plays critical role at the condition. In TP expressing cells thymidine was catabolized to glucose 6 phosphate, lactose and so on through the glycoltic pathway. TP and uridine phosphorylase double knockout mice had a distinct thymidine metabolic pattern. In human gastric cancer samples thymidine reduced and deoxyribose phosphate increased. Furthermore TP expressing induces more NADPH production, activates NADPH oxidase and increases ROS production. In turn ROS activate NF-kB cascade and induce IL-6, IL-8, and PDGF beta. These metabolic and cell signaling effects are the causes of malignant phenotypes and microenviroments in TP expressing cancers.

▲TOP

AACR2-2

Identifying Metabolic Dependencies in Pancreatic Cancer

Alec C. Kimmelman
NYU Langone Health

Pancreatic cancers are highly resistant to available therapeutics. We believe that this resistance points toward altered cell metabolic pathways. In this regard we have previously shown that that oncogenic Kras promotes a rewiring of pancreatic cancer metabolism allowing carbon sources to be utilized in a variety of biosynthetic pathways. Ongoing studies from our group are exploring targeting various aspects of metabolism as therapeutic approaches. We have also demonstrated pancreatic cancers have elevated basal autophagy which is required for their continued growth. Importantly, inhibition of autophagy leads to decreased oxidative phosphorylation, a drop in ATP production, and ultimately growth inhibition. These findings have implicated autophagy as a key component of pancreatic cancer metabolism and have motivated the opening of multiple clinical trials. Recently, we have identified an autophagy-dependent metabolic cross-talk that exists between pancreatic tumor cells and the surrounding stroma. Ongoing work from our group seeks to understand the metabolic contributions that autophagy makes in pancreatic tumors. These aspects of pancreatic cancer metabolism will be discussed.

AACR2-3

Revisiting the Warburg effect in cancer: lessons from a Pkm knock-in model

Nobuhiro Tanuma
Div. Cancer Chemother., Miyagi Cancer Ctr. Res. Inst.

Limited glucose oxidation even in the presence of sufficient O2 is known as the Warburg effect. Many reports suggest that metabolic phenotypes resembling the Warburg effect are cancer-abetting in tumor cells, although recent studies present conflicting findings. The glycolytic enzyme PKM exists as two isoforms: PKM1, which is constitutively active and promotes glucose catabolism, and PKM2, which is activated only in response to increased levels of allosteric activator(s). The latter property ensures that PKM2 will maintain a lower rate of glycolysis flux to limit glucose oxidation relative to PKM1, contributing the Warburg effect. One conclusion in the field was that PKM1 expression is not compatible with proliferation and that PKM2 is the only isoform expressed in dividing cells. However, mouse genetic analysis has revealed discrepant findings: PKM2-specific knockout mice, which express compensatory PKM1, develop normally and exhibit enhanced tumorigenesis in several models. As such, a major unanswered question then became, whether PKM2 is cancer-promoting or -suppressing? I will discuss this question by evaluating roles for PKM isoforms using novel mouse models.

AACR2-4

Metabolic Transitions in Cancer: Regulation by Cell-Extrinsic Cues

Heather R. Christofk
Dept. of Biological Chemistry, UCLA

Cellular metabolic state can be determined by cell-extrinsic mechanisms, including nutrient availability and growth factor signaling. Here we present extracellular matrix remodeling as another potent node of cell-extrinsic metabolic regulation and discuss a new pathway by which glycolysis is rapidly stimulated to fuel migration. We elucidate the molecular mechanism by which matrix remodeling acutely stimulates glycolysis and provide evidence that matrix remodeling impacts glucose metabolism through this mechanism in vivo during early neoplastic growth, when there is a high degree of tissue reorganization. Because matrix turnover is a feature of cellular processes such as proliferation and migration, which often require the energetic and biosynthetic support of glycolytic metabolism, we think our elucidated mechanism could help coordinate these interdependent processes both spatially and temporally.

▲TOP

[TYPL] TYPL [Japanese]
The Tomizo Yoshida Prize Lecture

2018 / 9 / 29 (Sat) 12:55-14:25 Room 1/5F Large Hall, Osaka International Convention Center Room 1

Masaki Mori / Dept. Gastroenterological Surg., Osaka Univ.

TYPL

Exploration of TGF-β family signaling and its roles in cancer invasion and metastasis

Kohei Miyazono
Dept. Mol. Path., Grad. Sch. Med., Univ. Tokyo

No Abstract

[MNPL] MNPL [Japanese]
The Mataro Nagayo Prize Lecture

2018 / 9 / 29 (Sat) 12:55-14:25 Room 1/5F Large Hall, Osaka International Convention Center Room 1

Fuyuki Ishikawa / Kyoto Univ., Grad. Sch. of Biostudies

MNPL

Broad range of achievements in cancer research from environmental carcinogenesis to its clinical application

Okio Hino
Dept. Mol. Path., Juntendo Univ. Faculty of Med.

No Abstract

▲TOP

[CS4-1] CS4 [English]
Significance of cancer stem cells as therapeutic target

2018 / 9 / 29 (Sat) 14:35-17:05 Room 1/5F Large Hall, Osaka International Convention Center Room 1

Koichi Akashi / Dept. Med. & Biosystemic Sci., Faculty of Med., Kyushu Univ.、 Nobuyuki Takakura / Dept. Signal Transduction, RIMD, Osaka Univ.

Tumor tissues consist of heterogeneous cancer cells including cancer stem cells (CSCs) that can give rise to cancer cells. Such CSCs have specific abilities such as invasion, metastasis, and drug resistance. Tissue specific stem cells in normal organ maintain the stemness in their specific microenvironment, the stem cell niche; several studies have suggested that there are specific microenvironments that keep stem cells to be of immature phenotype. Therefore, in addition to an intrinsic malignant activity of CSCs, it is possible that niche environment regulates CSCs to control their propagation and/or self-renewal activities. In this session, researchers, who are all specialists in the field of CSC, will get together to discuss our future for fighting against so far incurable nature of cancer.

CS4-1

[Keynote] Targeting vulnerable mechanisms in pancreatic and brain cancers

Giulio F. Draetta
The Univ. of Texas MD Anderson Cancer Ctr.

Our laboratory works at the interface of functional genomics and drug discovery. We are interested in elucidating how tumors naturally evolve as they grow and metastasize and in response to treatment. It has become clear that the genomic heterogeneity of tumor cells results in significant functional heterogeneity, such that in response to treatment, only a fraction of the tumor is often ablated, with the remaining populations of cells being able to expand and reconstitute the tumor mass. In this context, multiple cell populations within a tumor can be considered to have the properties of cancer stem cells, given their ability to be serially transplanted and giving rise to tumors having the original histological characteristics. I will summarize our recent work in pancreatic and brain cancers, in which we have identified several mechanisms that are now being targeted for new drug discovery. I will also provide an update on the clinical development of IACS-10759, a potent and selective inhibitor of oxidative phosphorylation.

▲TOP

CS4-2

Heterogeneous Tumors Composed of Epithelial-type and Mesenchymal-type Breast Cancer Cells

Yoshimi Arima
Gene Regulation, IAMR, Keio Univ. Sch. Med.

Tumor heterogeneity is one of the major reasons for therapeutic resistance. Intratumor heterogeneity is composed of genetic and phenotypic diversity, and it is also induced by tumor microenvironments and therapeutic stress. Heterogeneous cancer cells interact with each other and contribute to cancer progression and therapeutic resistance. We are developing therapeutic strategies targeting cancer promoting events driven by intratumor heterogeneity that are composed of epithelial-type and mesenchymal-type cancer cells. We have established heterogeneous cancer xenograft mice models via co-injections of two different breast cancer cell lines. After performing RNA-seq analysis, we identified the genes that express specifically in heterogeneous breast cancer. We think these genes contribute to cancer promotion. Hence, we are developing new therapies that target our candidate genes. To successfully eradicate tumors, we would like to propose three strategies; develop therapies that do not promote intratumor heterogeneity, develop diagnostic tools to detect early stage intratumor heterogeneity, and determine the most appropriate therapeutic combinations for treating heterogenous components.

CS4-3

Autophagy and Wnt/&beta-catenin pathway promote CD133+ pancreatic cancer stem-like cells chemo-resistant under hypoxia

Ming Wang
Jiangsu Univ.

Understanding the mechanisms responsible for pancreatic cancer chemo-resistance is still of great interesting. In this study, we investigated the role of activated autophagy and Wnt/&beta-catenin pathway in promoting hypoxia cancer stem cells to acquired chemo-resistance ability. The exposure of Pan-1 and BcPC-3 cells to hypoxia induced chemotherapeutic resistant and was accompanied by the increased size of the CD133+ cancer stem-like cell subpopulations. Autophagy and Wnt/&beta-catenin pathway were activated in chemo-resistant pancreatic cancer stem-like cells. We also determined the effect of autophagy and/or Wnt/&beta-catenin on the sensitivity of Panc-1 tumors resistant to gemcitabine treatment in vivo. From the clinic samples, we found co-localization of HIF-1&alpha, Becline, &beta-catenin and CD133 in hypoxic regions of the tumor. Collectively, our findings suggest that cancer stem-like cell population located in hypoxia regions, in association with activation of autophagy and Wnt/&beta-catenin pathway, can be used as a biomarker of a poor prognosis for human pancreatic cancer patients and may offer a new target treatment strategy to improve recurrence-free survival.

CS4-4

Linking biological heterogeneity and genetic complexity of human leukemia using patient-derived xenograft

Fumihiko Ishikawa
RIKEN Ctr. for Integrative Med. Sci.

One of the obstacles in treating malignant diseases is their biological heterogeneity and genetic complexity. Recent reports showed the presence of pre-malignant stem cells that may foster emergence of malignant stem cells, leading to overt disease. To understand the pathophysiology of pre-malignant and malignant cells, we took an approach to link in vivo disease biology with genomic profiling by combining a PDX model with single-cell sequencing, using acute myeloid leukemia (AML) as an example. This integrative approach enabled demarcation of pre-malignant vs. malignant cells and identification of permissive vs. disease-initiating mutations among multiple mutations within patient specimen. Inhibition of pathways activated by disease-initiating mutations but not by mutations permissive of in vivo engraftment of non-malignant cells lead to specific targeting and elimination of human AML cells in vivo. These approaches using PDX and single cell genomics help our understanding of malignant pathogenesis and development of new therapeutic strategies.

▲TOP

CS4-5

Identification of BCAAs metabolism pathway as a critical metabolic machinery for the maintenance of leukemia stem cells

Yoshikane Kikushige
Dept. Med. & Biosystemic Sci., Kyushu Univ.

Co-author：Koichi Akashi
Dept. Med. & Biosystemic Sci., Kyushu Univ.

Recent advances in measuring cellular metabolites have revealed that several specific metabolism pathways actively contribute to the maintenance of stemness in several types of stem cells including ES cells, iPS cells and tissue stem cells. In this study, we comprehensively analyzed cellular metabolites of human undifferentiated CD34+ normal HSPCs and CD34+ acute myeloid leukemia(AML) and acute lymphoblastic leukemia (ALL) cells to test whether specific metabolic pathways or metabolites could govern stem cell properties of malignant stem cells. We found CD34+ AML/ALL cells exhibited the significantly higher cellular contents of BCAAs as compared to normal CD34+ HSPCs. The immature CD34+ AML/ALL cells commonly expressed BCAAs-transporters and BCAT1, a critical molecule for BCAA metabolism. Inhibition of BCAA metabolism pathway significantly attenuated primary AML/ALL progression via targeting malignant stem cells through the alteration of specific epigenetic enzyme activity in vivo. This study provides the novel evidence that human acute leukemia cells commonly utilize BCAA metabolism pathways to maintain the malignant stem cell properties irrespective of their lineage origin.

CS4-6

The understanding of gastrointestinal cancers: cancer stem cells and their niche

Toshiro Sato
Keio Univ. Sch. Med.

Co-author：Yuki Ohta、Mami Matano、Masayuki Fujii、Mariko Shimokawa
Keio Univ. Sch. Med.

The biological understanding of gastrointestinal cancer requires faithful disease modeling that recapitulates the disease heterogeneity and pathobiological traits of original cancers. We optimized stem cell niche factor medium for gastrointestinal tumor organoids and established over 100 patient-derived organoid lines from various tissue origins and histological subtypes including previously uncultured rare tumors. Tumor organoids reproduced the histopathological grade and differentiation capacity of parental tumors in vitro and upon xenografting, which enabled to reconstruct cancer stem cell hierarchy in an experimental setting. Using CRISPR-Cas9 genome editing technology, we succeeded in visualizing dynamic regulation of human LGR5+ cancer stem cells in tissue environments. Furthermore, we developed selective genetic ablation system for LGR5+ cancer stem cells, which led to temporal tumor regression, followed by tumor regrowth driven by de-differentiated cancer stem cells. In this session, we would like to share our recent progress of disease modeling of human cancer stem cell system and discuss potential therapeutic strategy targeting cancer stem cells.

▲TOP

[ML14] ML14 [Japanese]
Morning Lectures 14

2018 / 9 / 29 (Sat) 8:00-8:50 Room 2/5F Small Hall, Osaka International Convention Center Room 2

Satoru Miyano / IMS, the Univ. of Tokyo

ML14

Integrated analysis of myeloid neoplasms

Seishi Ogawa
Dept. Pathology & Tumor Biology, Kyoto Univ.

Discussant：Hirotoshi Akita
Dept. Med. Oncol., Faculty Med., Hokkaido Univ.

Intensive efforts of genome sequencing studies during the past decade identified more than 100 driver genes recurrently mutated one or more subtypes of myeloid neoplasms, which collectively account for the pathogenesis of more than 90% of the cases. However, approximately 10% of the cases have no alterations in known drivers and their pathogenesis is still unclear. A possible explanation might be the presence of alterations in non-coding regions that are not detected by conventional exome/panel sequencing; mutations and complex structural variations (SVs) affecting these regions have been shown to deregulate expression of relevant genes. In addition, cancer cells have more complex abnormalities in gene expression and epigenetic alterations, in addition to alterations to primary genome sequences. To address these issues, an integrated analysis of gene mutations, expressions, and epigenetic analysis is warranted. In this morning lecture, an example of integrated molecular analyses of myeloid neoplasms will be presented, in which hundreds of samples from different myeloid neoplasms are analyzed using whole genome/transcriptome sequencing and Infinium methylation platforms.

▲TOP

[IS9-1] IS9 [English]
Universal health coverage (UHC) and cancer control

2018 / 9 / 29 (Sat) 9:00-11:30 Room 2/5F Small Hall, Osaka International Convention Center Room 2

Tetsuo Noda / Cancer Inst. of JFCR、Thomas Cueni / InterNatl. Federation of Pharm. Manufacturers & Association

The target for global-level health strategy at the World Health Organization (WHO) and other organizations is shifting from communicable diseases to non-communicable diseases (NCD). These NCD include such conditions as cardiovascular disease and diabetes, but despite the fact that Universal Health Coverage (UHC) is a basic principle for international health strategy, UHC for cancer control measures has yet to be discussed in any great depth. It is in view of this current situation that this symposium will invite Andre● Ilbawi, Technical Officer for Cancer Control at the WHO's Department for Management of Noncommunicable Diseases, Disability, Violence, and Injury Prevention, and Thomas Cueni, Director General of the International Federation of Pharmaceutical Manufacturers and Associations (IFPMA), an organization that is expected to play a significant role in UHC, who will join representatives of industry and academia in Japan who are playing central roles in international health and cancer control promotion efforts. Together the speakers will consider what needs to be done to realize affordable cancer care and create delivery systems to ensure effective UHC for cancer in Japan, Asia and the world.

IS9-1
Role of Pharmaceutical Companies in Cancer Control Measures through UHC

Thomas Cueni
Director General, InterNatl. Federation of Pharm. Manufacturers & Associations (IFPMA)

The Access Accelerated is a unique cross-industry collaboration that seeks to reduce barriers to prevention, treatment and care for NCDs in lower- and middle-income countries through health systems strengthening and alignment with Universal Health Coverage objectives and priorities. For the first time, 24 global biopharmaceutical companies have come together to bring their global reach and local expertise in partnership with countries, civil society, international organizations, multilaterals and NGOs to support cross-sectoral practical engagement and drive on-the-ground implementation and sustainable action to address NCDs. AA works towards a future where no one dies prematurely from treatable, preventable diseases and all people living with or at risk of NCDs have access to appropriate, quality, and affordable prevention, treatment and care. AA recognizes that only by putting the needs of people living with NCDs first and acting in collaboration with governments, civil society, multilaterals, NGOs and others can the global community make measurable and sustainable progress to reduce the burden of NCDs in developing countries and the world.

▲TOP

IS9-2

Achieving Health for All: WHO Perspective on where are we now and where we want to be

Andre Ilbawi
World Health Organization

Co-author：Rei Haruyama
World Health Organization

Universal Health Coverage (UHC) is the defining political commitment of this decade. It is founded on the recognition that all people should have access to needed health services of sufficient quality while protecting against financial hardship. While the principle of UHC is clear, its attainment has been challenging, particularly for complex health services such as cancer. In the 2017 Country Capacity Survey of the World Health Organization (WHO), about one-third of countries do not have cancer services generally available and approximately 40% do not have access to radiotherapy. For countries where cancer care is available, there are often large segments of the population who cannot afford it; in select studies from Asia, more than 50% suffer financial catastrophe. In the face of an increasing burden and rapidly rising costs, how can countries achieve UHC? The focus must be on value for money: prioritizing cost-effective, feasible and quality services. WHO works with countries to set national priorities, expand access to essential services, improve efficiency of how money is spent to secure financial protection, and provide population coverage of cancer to ensure health for all.

IS9-3

A strategy on cancer policy of Japanese government

Masahiro Sasaki
Cancer & Disease Control Div., MHLW, Japan

Japan achieved UHC in 1961, when it was still in the early stages of post-war economic development. The Japanese public health insurance program has its root in employees insurance of large corporations, and smaller companies gradually followed and introduced the same scheme. We have challenged cancer control as UHC. We will have presentation some policy on it at the session.

IS9-4

UHC and Asian Cencer

Shinjiro Nozaki
WHO Ctr. for Health Development

Rapid population ageing is having profound implications for transforming and re-aligning health, social, and economic systems to improve health and wellbeing. Expanding and disseminating evidence required to improve decision making leading to more sustainable and inclusive policies and programmes for ageing populations. Most countries have not yet focused on the implications of these demographic changes. According to ageing trends, we have to pay more attention for NCDs. Among NCDs, cancer is one of the top causes of death in many countries including lower middle income countries. It means we, Asian countries have to prepare increasing NCDs and cancer in the trends of rapid ageing in Asia.We expect many technological, social and system innovations for ageing populations will be developed through the research and we have responsibility to show future policy option on UHC, Innovation and Ageing to low/middle income countries which will face ageing problem in near future..

▲TOP

IS9-5

UHC and Cancer Control: The Role of UICC-ARO and Future Outlook

Hideyuki Akaza
Grad. Sch. of Int. Inf. Stud, ITASIA, Univ. Tokyo

BackgroundWith international momentum growing towards the achievement of the SDGs, in the area of cancer care, many stakeholders are now mobilizing efforts to realize the goal of affordable access for all. In response to the WHO's call in 2017 for strengthened collaboration among various stakeholders in order to realize effective medicines and affordable access to cancer care, UICC-ARO held a meeting on public-private partnership at United Nations University in April 2018.ObjectiveTo date there has been a general lack of recognition concerning UHC for cancer care in the context of global health. In this session we aim to raise awareness of this issue among the cancer research community and engage in discussions on the feasible methods of working to achieve UHC for cancer care in Asia.Method Based on recent developments in UHC and the opinions that were first discussed in the April meeting, specific challenges and future outlook will be identified and discussed.ConclusionUICC-ARO will utilize the outcomes of this session to further develop public-private approaches to cancer care, within the framework of the Japanese government's Asia Health and Human Well-being Initiative.

▲TOP

[IS11-1] IS11 [English]
Role of innate immunity and tumor microenvironment in cancer progression

2018 / 9 / 29 (Sat) 13:40-16:10 Room 2/5F Small Hall, Osaka International Convention Center Room 2

Masanobu Oshima / Div. Genetics, Cancer Res. Inst., Kanazawa Univ.、Brendan John Jenkins / Hudson Inst. of Med. Res., Monash Univ.

A unifying feature of many cancers is the well-established causal link with chronic inflammation, which has implicated host immune regulators as candidate genetic factors that can influence disease susceptibility. In this respect, targeting the immune system in cancer thus far has primarily focused on immunotherapies that boost anti-tumor adaptive immunity. However, for numerous cancers, such immunotherapy trials have yielded modest patient outcomes with variable response rates. In contrast, there is a paucity of studies on targeting innate immunity. This is surprising since dysregulated innate immunity is a hallmark of many inflammation-associated cancers, and often promotes pathogenesis. Notably, the pro-tumorigenic actions of innate immune effectors are elicited by both direct intrinsic effects on cancer cell proliferation, survival, invasion, and metastasis, and indirect effects on stromal cells within the tumor microenvironment. Therefore, this session will host a series of presentations discussing the latest developments in the identification of key regulators of host innate immunity and the tumor microenvironment in cancer.

IS11-1

TNF in anti-tumour immunity and resistance to immunotherapy

Jane Oliaro
Peter MacCallum Cancer Ctr.

Co-author：Conor Kearney[1]、Stephin Vervoort[1]、Simon Hogg[1]、Najoua Lalaoui[2]、Kristin Brown[1]、John Silke[2]、Ricky Johnstone[1]
[1]Peter MacCallum Cancer Ctr.、[2]Walter & Eliza Hall Inst. of Med. Res.

Immunotherapies that enhance cytotoxic T cell activity against tumour cells have revolutionised outcomes for cancer patients. However, tumours may evade this form of therapy. To investigate this, we carried out a series of CRISPR screens to identify mechanisms of tumour immune evasion from T cell killing. We found that deletion of key genes within the TNF signalling, IFN-gamma signalling, and antigen presentation pathways provided protection of tumour cells from T cell killing, and blunted anti-tumour immune responses in vivo. Our results also highlighted a role for TNF-mediated bystander killing as a potent T cell effector mechanism that can be enhanced by a class of drugs, called smac-mimetics, that inhibit IAPs and can sensitise tumour cells to TNF-induced cell death. Indeed, our studies showed that the smac-mimetic, birinapant, significantly enhanced tumour cell death in the presence of T cells; an effect that can be amplified upon checkpoint blockade. Taken together, we identify T cell-derived TNF as a potent anti-tumour effector mechanism that can be enhanced with birinapant, and an opportunity for combination therapy through co-inhibition of immune checkpoints.

▲TOP

IS11-2

Cancer cell-derived HMGB1 promotes tumor growth by recruiting myeloid cells into the tumor microenvironment

Hideyuki Yanai
Inst. of Industrial Sci., The Univ. of Tokyo

Co-author：Sho Hangai、Tadatsugu Taniguchi
Inst. of Industrial Sci., The Univ. of Tokyo

High mobility group box 1 (HMGB1) is a DNA-binding nuclear protein, released actively following cytokine stimulation as well as passively during cell death. HMGB1 is the prototypic damage-associated molecular pattern (DAMP) molecule and has been implicated in several inflammatory disorders. However, whether and how HMGB1 regulates the immune system remains unresolved. We found that genetic deletion of HMGB1 in cancer cells results in lower tumor growth in vivo. This is accompanied by a reduced accumulation of $Ly6G^+$ myeloid cells in the tumor microenvironment (TME). We also found that migration of the $Ly6G^+$ myeloid cells by HMGB1 is mediated through the receptor for advanced glycation end products (RAGE) signaling. Consistent with these results, enhanced anti-tumor responses in a mouse tumor model demonstrates the potential of an HMGB1 inhibitor alone and in combination with a checkpoint inhibitor antibody to enhance antitumor immune responses. Our results unveil a hitherto unknown facet of HMGB1-mediated signaling in the modulation of the TME and raise the notion that HMGB1 is an attractive candidate for tumor immunotherapy.

IS11-3

Targeting cancer stem cells and cell states during disease progression

Wai Leong Tam
Genome Inst. of Singapore, A*STAR, Cancer Sci. Inst. of Singapore, Natl. Univ. of Singapore, Dept. Biochem., Natl. Univ. of Singapore, Sch. of Biological Sci., Nanyang Technological Univ.

Cancer cell states such as the epithelial-mesenchymal transition (EMT) and cancer stemness, contribute towards therapy resistance and metastasis. Cancer-specific metabolism represents an area with therapeutic potential. A feature of cancer cells that has regained attention is their altered metabolic state, such as the utilization of glucose and amino acids. Because cancer stem cells (CSCs) are functionally distinct, they exhibit unique metabolic requirements for supporting stemness. Using metabolomic approaches, we uncovered metabolic genes that are essential for CSCs. Likewise, the EMT is important for invasion and metastasis. Associated with changes in cell states are differential metabolic needs. By perturbing metabolic pathways, one may control EMT and disrupt disease progression. Finally, niche cells residing within the tumor microenvironment are key regulators of stemness and metastasis. We generated a resource of patient-derived tumor and stromal cells to discover signaling/ metabolic crosstalks between the interacting cell types. Understanding the niche interactions will enable new methods for disrupting niche-based signals that support stemness and resistance.

IS11-4

Inflammatory microenvironment for malignant progression of colon cancer

Masanobu Oshima
Div. Genet,. CRI, Kanazawa Univ.

Co-author：Mizuho Nakayama[1]、Hiroko Oshima[2]
[1]Div. Genet. Cancer Res. Inst., Kanazawa Univ.、[2]Div. Genet,. CRI, Kanazawa Univ.

It has been established that inflammatory responses play a role in cancer development. We have constructed intestinal tumor mouse models by introduction of driver gene mutations in combinations. Apc and Tgfbr2 mutations in combination induced submucosal invasion of intestinal tumors with generation of inflammatory microenvironment. Notably, inhibition of TGF-β pathway by Tgfbr2 disruption together with inflammatory responses can cause invasion of epithelial cells, while Tgfbr2 mutation alone did not, indicating a role of inflammation in the induction of invasion step. On the other hand, Apc and Trp53 R270H mutations also induced submucosal invasion of tumor cells. Mechanistically, mutant p53 expression resulted in increased promoter accessibility of transcription factors possibly through chromatin modification, and NF-κ signaling was significantly activated. Moreover, we found that mutant p53-induced NF-κB activation caused expression of cytokines and chemokines. These results, taken together, indicate that inflammatory microenvironment promotes malignant invasion process, and such inflammatory reaction is induced by accumulation of driver mutations including Trp53.

▲TOP

IS11-5

Adipocytes enhance tumor growth and cancer stem cell-like properties through the complement activation pathway

Yohei Shimono
Div. Mol. Cell. Biol., Kobe Univ. Grad. Sch. Med., Div. Med. Oncol.

Co-author：Hideaki Goto[1]、Yohei Funakoshi[1]、Masanori Toyoda[1]、Naoki Shibuya[2]、Toru Mukohara[3]、Hironobu Minami[4]
[1]Div. Oncol.

Adipose tissue is a predominant component of stroma in mammary tissues, and secretes various cytokines and growth factors, called 'adipokines'. However, the roles of adipocytes in breast cancers remain to be elucidated. In this study, we found that an adipokine adipsin (complement factor D) enhanced proliferation and cancer stem cell (CSC)-like properties of breast cancer patient-derived xenograft (PDX) cells through the complement activation pathway. Adipsin was predominantly expressed in both adipose tissues of breast cancer patients and adipocyte-derived stem cells (ADSCs) isolated from them. ADSCs enhanced the sphere-forming ability of breast cancer PDX cells; suppression of the adipsin-mediated signaling by a complement receptor C3aR inhibitor or adipsin knockdown in ADSCs significantly decreased the sphere-forming ability and CSC marker expression of breast cancer PDX cells. Growth of breast cancer PDX tumors was enhanced when co-transplanted with ADSCs, but the effect was significantly weakened when co-transplanted with the adipsin knocked-down ADSCs. These results propose important roles of adipocytes as an active player in the tumor microenvironment of human cancers.

IS11-6

Uncovering the pro-tumourigenic role of innate immune pattern recognition receptors in cancer

Brendan J Jenkins
Hudson Inst. of Med. Res.

Pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), are essential regulators of innate immunity, and despite their involvement in chronic inflammatory disorders and autoimmune diseases, their role in inflammation-associated cancers is ill-defined. We have previously revealed that TLR2, as well as the inflammasome adaptor ASC, play critical roles in driving tumour growth in gastric cancer. Here, using the gp130F/F spontaneous and Helicobacter-induced gastric cancer models, we now extend these findings to uncover a key role for the innate immune DNA sensor Absent in melanoma (AIM)2 in gastric cancer. Interestingly, in these preclinical disease models and clinical datasets, AIM2 upregulation associated with hyperactivation of the oncogenic transcription factor STAT3, and high AIM2 expression/STAT3 activity conferred a poor prognosis in patients. Emerging data suggests that some of these innate immune PRRs also promote lung and pancreatic tumourigenesis. Collectively, our findings pave the way forward to therapeutically target these innate immune regulators in various cancers associated with their dysregulated expression and/or activation.

▲TOP

[ML15] ML15 [Japanese]
Morning Lectures 15

2018 / 9 / 29 (Sat) 8:00-8:50 Room 3/10F 1003, Osaka International Convention Center Room 3

Toshirou Nishida / Natl. Cancer Ctr. Hosp., Dept. Surg.

ML15

Rare Cancers, how to improve the clinical treatment outcomes and progress research

Akira Kawai
Dept. Musculoskeltal Oncol, Natl. Cancer Ctr. Hosp.

Discussant：Norifumi Naka
Dept. Orthop., Osaka Int. Cancer Inst.

A rare cancer is defined as a cancer with an approximate morbidity (incidence) of less than 6 per 100 thousand population, which has more unsolved clinical and therapeutic problems compared to other cancers because of the limited number of patients. As for the actual clinical practice for rare cancers in Japan, since a limited number of patients are dispersed across the country and treated at different sites in different clinical areas, the patients have difficulty to receive the newest evidence-based treatment at the right time and clinical studies are difficult to promote. On the other hand, even among more common cancers, rare variants characterized by specific molecular abnormalities have been identified through recent dramatic advances in genomic analysis technology. As a result, cancers that have not previously been considered to be rare, are now regarded as an assembly of rare subtypes of cancer based on different molecular abnormalities. In this morning lecture, we will discuss current obstacles to the rapid development and application of safe and effective treatments for rare cancers, and considers measures required to overcome these challenges.

▲TOP

[S16-1] S16 [English]
Signal transduction analysis for cancer profiling

2018 / 9 / 29 (Sat) 9:00-11:30 Room 3/10F 1003, Osaka International Convention Center Room 3

Makoto Noda / Mol. Oncol., Kyoto Univ. Grad. Sch. Med.、Akira Kikuchi / Dept. Mol. Biol. & Biochem., Grad. Sch. Med., Osaka Univ.

The term “signal transduction” appeared in the biological literature after the 1970s. This term was originally used in the field of physics or electronic engineering to refer to energy or information that is transduced from one form into another. Cells respond to many kinds of extracellular information appropriately, resuting in modulation of various cellular functions. This is called the signal transduction system. Studies of oncogenes and tumor-suppressor genes provided importnat breakthoughs in our understanding on the molecular mechanisms of signal transduction. On the other hand, recent evidence indicates that there is heterogeneity in individual cancer and each cancer shows different profiles. More detailed signal transduction analysis is necessary for the understanding of different cancer properties. We believe that the discovery of novel signal transduction in cancer leads to the development of molecularly targeted therapy. In this symposium, 5 distinguished speakers will present the cutting edge of their researches regarding novel cancer signaling. We look forward to sharing latest information of cancer at the molecular level and enjoying hot and fruitful discussion!

S16-1
[Keynote] Altering the Transcriptome of Cancer Stem Cells with SUMO Inhibitors

Ronald J. Weigel
Dept. Surg., Univ. of Iowa

Cancer diagnosis and therapy has increasingly relied on molecular profiling of cancer cells. In breast cancer, expression of estrogen receptor (ER) and HER2 represent signaling pathways that define molecular breast cancer subtypes, which have characteristic patterns of gene expression that can be used to predict response to therapy. Patterns of gene expression in breast cancer are highly influenced by the TFAP2A and TFAP2C transcription factors. TFAP2C regulates expression of ER and luminal differentiation markers, whereas loss of TFAP2C induces epithelial-mesenchymal transition and an expansion of the cancer stem cell (CSC) population. Though highly homologous, TFAP2A has a distinct repertoire of target gene activation. Recently, we have discovered that the transcriptional activity of TFAP2A is altered by post-translational modification involving sumoylation (SUMO). Inhibiting SUMO conjugation of TFAP2A induces mesenchymal-epithelial transition and elimination of the CSC population in breast and colon cancer cells. Novel CSC-targeted therapy based on SUMO inhibitors is now under intense investigation.

▲TOP

S16-2

The KEAP1-NRF2 Stress Response System in Cancer Biology and Medicine

Masayuki Yamamoto
Med. Biochem., Tohoku Univ. Grad. Sch. Med.

NRF2 is a transcription factor essential for the coordinated induction of cellular defense enzymes and protection of tissues. KEAP1 acts as a subunit of ubiquitin-E3 ligase that degrades NRF2 constitutively and as a sensor for electrophilic and oxidative stresses. Covalent modifications of KEAP1 cysteine residues abrogate the ubiquitin ligase activity and stabilize NRF2, which leads to cellular NRF2 activation. NRF2 suppresses inflammations through repressing pro-inflammatory cytokine gene expression and also suppresses oxidative tissue damage through inducing a set of antioxidant enzyme genes. Many somatic missense mutations have been identified in KEAP1 and NRF2 genes of human cancers. These mutations disrupt the KEAP1-NRF2 complex and result in constitutive activation of NRF2. Subsequently, elevated expression of NRF2 target genes confers advantages on the growth of cancer cells. Thus, NRF2 inhibitor is now important for the chemo-sensitization therapy of cancers. On the other hand, NRF2 activity in the host microenvironment is shown to repress cancer cell growth and NRF2 inducers are also important for cancer treatment.

S16-3

The Dickkopf1-CKAP4 pathway, a novel cancer signaling, represents molecular targets for cancer therapy

Akira Kikuchi
Dept. Mol, Bio. Osaka Univ. Med.

Co-author：Hirokazu Kimura、Katsumi Fumoto
Dept. Mol. Biol. Biochem., Grad. Sch. Med. Osaka Univ.

Dickkopf 1 (DKK1) is a secretory protein and antagonizes oncogenic Wnt signaling by binding to the Wnt coreceptor LRP6. DKK1 is also suggested to regulate its own signaling to promote cancer cell proliferation, however the underlying mechanism of DKK1-induced cell proliferation has remained to be clarified. We found that cytoskeleton-associated protein 4 (CKAP4), which is a type Ⅱ single-span transmembrane protein, functions as a novel DKK1 receptor. The binding of DKK1 to CKAP4 activated AKT through phosphatidylinositol 3-kinase, resulting in normal and cancer cell proliferation. Both DKK1 and CKAP4 were frequently expressed in tumor lesions of pancreatic, lung, and esophageal cancers and their simultaneous expression was correlated with poor prognosis. Knockdown of CKAP4 or DKK1 from cancer cells inhibited AKT activity and decreased their xenograft tumor formation abilities. Furthermore, the anti-CKAP4 antibody inhibited the binding of DKK1 to CKAP4 and AKT activity, thereby suppressing cancer cell-induced xenograft tumor formation. Thus, the DKK1-CKAP4 axis may represent a novel therapeutic target for cancers expressing both DKK1 and CKAP4.

S16-4

Cell Competition between Normal and Transformed Epithelial Cells

Yasuyuki Fujita
Inst. Gen. Med., Hokkaido Univ.

At the initial step of carcinogenesis, transformation occurs in a single cell within an epithelial sheet, and the emerging transformed cells grow while being surrounded by normal epithelial cells. However, it was not clear what happens at the boundary between normal and transformed cells. Using newly established cell culture and mouse model systems, we have shown that various phenomena can occur at the interface between normal and transformed epithelial cells. For example, when Ras- or Src-transformed cells are surrounded by normal epithelial cells, various signaling pathways are activated in the transformed cells and they are often eliminated from the apical surface of the epithelial monolayer. These phenomena are not observed when transformed cells alone are present, suggesting that the presence of surrounding normal cells affects the signaling pathways and fate of transformed cells. Furthermore, we have demonstrated that normal epithelial cells can recognize and actively eliminate neighboring transformed cells and named this process EDAC (Epithelial Defense Against Cancer).

▲TOP

S16-5

Signaling pathways affecting, and affected by, the tumor metastasis suppressor RECK

Makoto Noda
Kyoto Univ. Grad. Sch. Med.

Co-author：Tomoko Matsuzaki、Yoko Yoshida、Hitoshi Kitayama
Kyoto Univ. Grad. Sch. Med.

A membrane-anchored protease regulator RECK was first identified as a transformation suppressor against KRAS. Although RECK mutations are rare in cancer genomes, RECK is downregulated in various cancers, and forced re-expression of RECK in cancer cells results in reduced tumor angiogenesis, invasion, and metastasis in xenograft models, suggesting the potential value of agents re-activating dormant RECK in cancer therapy. RECK expression in fibroblasts is density-dependent, suppressed by serum, and activated by multiple kinase inhibitors. RECK expression is upregulated after EMT in normal epithelial cells but not in carcinoma cells. RECK is also under multiple other regulations, including DNA methylation, microRNAs, and GPI-anchor-cleavage, indicating the delicate nature of RECK regulation in normal cells. Acute RECK expression in carcinoma cells results in cellular senescence via SKP2-downregulation. Reck-deficient mice die in utero with precocious neuronal differentiation due to attenuated NOTCH-signaling and vascular defects due to reduced WNT7 signaling. These findings illuminate the role of RECK to support multiple signaling pathways, probably by regulating proteolytic events

S16-Special_Remarks

Special Remarks

Eigo Otsuji
Dept. Digestive Surg., Kyoto Pref. Univ. Med.

No Abstract

[LS27] LS27 [Japanese]
New Biomarker for Cancer Immunotherapy pioneered by Precision medicine

2018 / 9 / 29 (Sat) 11:50-12:40 Room 3/10F 1003, Osaka International Convention Center Room 3
：Bristol-Myers Squibb K.K./ONO PHARMACEUTICAL CO., LTD.

Tetsuya Mitsudomi / Department of Surgeons, School of Medicine, Kinki University

LS27

New Biomarker for Cancer Immunotherapy pioneered by Precision medicine

Kazuya Tsuchihara
Division of Translational Informatics, Exploratory Oncology Research & Clinical Center, National Cancer Center Japan

No Abstract

▲TOP

[S19-1] S19 [English]
Liquid biopsy paves the way for next-generation medicine

2018 / 9 / 29 (Sat) 13:40-16:10 Room 3/10F 1003, Osaka International Convention Center Room 3

Koshi Mimori / Dept. Surg., Kyushu Univ., Beppu Hosp.、Hidetoshi Eguchi / Dept. Gastroenterol. Surg, Osaka Univ., Grad. Sch. Med.

In State of the Union address in 2015, President Obama launched the Precision Medicine Initiative to revolutionize how we improve health and treat disease. The liquid biopsy is one of the perceptive approaches defined to implement the precision medicine in a minimally invasive manner through the sampling of blood or other bodily fluids. Objectives for analysis as the liquid biopsy are tumor specific materials, such as cells, extracellular vesicles, nucleic acids and proteins. Genetic, epigenetic, and proteomic profiling of bodily fluids might provide the following clinical benefits in oncology; First, we can identify patients with minimal residual disease who are at a high risk of cancer. Second, we could stratify appropriate treatments by molecular profiling of each patient individually. Finally, we can monitor the tumor burden after treatment, replacing current modalities imaging and serum marker procedures. In the current symposium, the congress committee and chair persons selected and invited excellent speakers who will introduce their own approaches deserving future standard diagnostic tools for liquid biopsy. Speakers are experienced in this field and examined adequate number of samples with their own methods to satisfy the above clinical demands. You have my word, it's gonna be incredible and awesome. Please come to study and enjoy together in this symposium!

S19-1

Nationwide Cancer Genome Screening Project Using Circulating Tumor DNA Analysis for Metastatic Colorectal Cancer (mCRC)

Takayuki Yoshino
Dept. Gastrointestinal Oncol., Natl. Cancer Ctr. Hosp. East

Liquid biopsy has emerged as a molecular diagnostic tool for assessing spatial and temporal intratumoral heterogeneity in cancer with minimal invasiveness. Circulating tumor DNA (ctDNA) analysis can identify not only genomic alterations associated with resistance to anti-EGFR therapy, but also predictive biomarkers to immune checkpoint inhibitors. Furthermore, the emergence of alterations in genes, such as RAS, EGFR, HER2, and MET, can be detected as acquired resistance mechanisms in specific genes by longitudinally monitoring ctDNAs during treatment. We launched a ctDNA analysis-based cancer genome screening project as part of the SCRUN-Japan GI-SCREEN since February 2018. Genomic alterations in 73 genes in ctDNA from blood have been analyzed using a next-generation sequencing based method for patients with mCRC. Furthermore, umbrella trials have been conducted for patients with specific genomic alterations as HER2 amplification. Serial monitoring of ctDNA is conducted to explore the resistance biomarkers. Our project can help to realize of cancer precision medicine utilizing ctDNA analysis for mCRC. Updated results will be presented.

▲TOP

S19-2

Liquid autopsy: Assessment of tumor heterogeneity by sequencing analysis of post-mortem plasma cell-free DNA

Erina Takai
Dept. Cancer Genome Info. Grad. Sch. Med., Osaka Univ.

Co-author：Daichi Maeda[1]、Akiteru Goto[2]、Shinichi Yachida[3]
[1]Dept. Clin. Genomics Grad. Sch. Med., Osaka Univ.、[2]Dept. Cell & Organ Path. Grad. Sch. Med., Akita Univ.、[3]Dept. Cancer Genome Info. Grad. Sch. Med., Osaka Univ.

Genomic analyses of multiple samples collected at rapid autopsy have given significant insights to understand cancer progression and heterogeneity of cancer genome. In order to clarify the potential of cfDNA to assess tumor heterogeneity, we investigated how post-mortem plasma cfDNA reflects mutational status of metastatic cancer. Whole-exon sequencing was performed on 8 tumor DNA and post-mortem plasma cfDNA collected from an autopsy case of metastatic prostate cancer. In addition to clonal mutations, significant number of subclonal mutations and a large number of somatic mutations that were not identified in any tumor were also detected in cfDNA, indicating that more comprehensive genomic information of metastatic cancer could be got by cfDNA analysis than analysis of multiple samples. Furthermore, our data suggested that the contributions of tumor DNA to cfDNA might vary by lesions. Some of mutations exclusively detected in cfDNA were localized by Sanger sequencing of FFPE tumor samples sectioned at autopsy. Based on these findings, we propose a novel concept of "liquid autopsy", which utilizes post-mortem cfDNA to understand tumor heterogeneity more comprehensively.

S19-3

Clinical sequencing of circulating tumor DNA by CAPP-seq

Kazuko Sakai
Dept. Genome Biol., Kindai Univ. Faculty of Med.

Co-author：Kazuto Nishio
Dept. Genome Biol., Kindai Univ. Faculty of Med.

NGS-based multiple gene panel tesing is available for precision medicine and has been approved as companion diagnostics (CDx) in Japan as well as other countries. CAncer Personalized Profiling by deep Sequencing (CAPP-Seq) is a capture-based NGS ctDNA detection assay for SNVs, indels, rearrangements, and CNVs. We are investigating the utility of CAPP-seq analysis in the patients with various types of cancers such as gynecologic cancer, non-small cell lung cancer, and pancreatic cancer. CDx for tyrosine kinase inhibitors (TKIs) including a NGS panel are availble for TKI treatment of non-small cell lung cancer patients in Japan. During TKI treatment, multifactorial mechanisms of resistance occurs. Monitoring of resistant mechanisms with CAPP-seq ctDNA analysis will contribute to the adaptive treatment paradigm for these patients. In the real world, mechanisms other than secondary mutations are detected frequently by ctDNA analysis and the clinical relevance of CNV analysis has been recognized. To develop liquid biopsy-based in vitro diagnositics, it is necessary to know the ctDNA contents in cell free DNA of each patient. Our approach for this porpose will be introduced.

S19-4

Feasibility and clinical usefulness of detecting aberrant methylation in cell-free DNA

Genta Nagae
Genome Sci. Div., Res. Cent. Adv. Sci. Tech., Univ. Tokyo

Co-author：Hiroyuki Aburatani
Gen. Sci. Div., RCAS, Univ. of Tokyo

Recent advances in genomic technology have made it possible to analyse nucleic acids from a limited amount of samples, sometimes from a single cell. Cell-free DNA from cancerous tissue has recently been recognized as a useful resource for early detection, "tissue of origin" prediction, disease monitoring and therapeutic decision-making in precision medicine. However, technical limitations remain in terms of accuracy, specificity and sensitivity for practical application. Here we evaluated a series of aberrant promoter/enhancer methylations among the pan-cancer datasets of the TCGA project and screened distinct sets of abnormal methylation with tissue- or subgroup-specificity. We would like to discuss the technical solutions and the potential utility of abnormal methylation compared to genomic mutations for circulating tumour DNA.

▲TOP

S19-5

Clinical relevance of circulating tumor DNA assessed through amplicon-based next-generation sequencing

Hitoshi Zembutsu
Dev. of Liq. Bx, Cancer Pre. Med. Ctr., Cancer Inst.

The development of circulating tumor DNA (ctDNA) panel covering hundreds of mutations is important to establish high sensitive diagnostic system for cancer. We enrolled 101 and 76 patients with metastatic colorectal (CRC) and pancreatic cancer (PC), respectively. The genomic profiling of 14 genes in plasma were performed to evaluate the feasibility of this panel. Somatic mutations were detected in 87.1% and 90.8% of patients with CRC and PC in their plasma, respectively. The mutations in TP53, KRAS and APC genes were detected in 69.3%, 38.6% and 23.7% in CRC, and 64.3%, 60.5%, 13.2% in PC. ctDNA level were significantly associated with metastasis (liver, P<0.00001, lymph node, P=0.008, number of metastatic organs, P<0.0001), tumor markers (CEA and LDH, P<0.0001, CA19-9, P=0.006), and tumor diameter (maximum and sum of diameter, P<0.0001). The overall concordance rate of RAS status between ctDNA and matched CRC tissue was 77.2%. PC patients with lower ctDNA level showed significantly longer overall survival (P=0.00000026). These results suggest that profiling of mutated genes in the panel could be biomarkers to monitor changes in mutational status and tumor burden.

S19-6

Circulating tumor DNA as a tool for monitoring gastrointestinal tumor burden dynamics in the therapeutic context

Satoshi Nishizuka
Iwate Med. Univ. Inst Biomed. Sci.

Co-author：Takeshi Iwaya
Iwate Med. Univ. Dept. Surg. Mol. Ther. Lab.

The greatest challenge to advanced gastrointestinal cancer treatment is to fully prevent post-therapeutic relapse. To monitor post-therapeutic tumor burden, we conducted cancer-related gene panel sequencing of esophageal, stomach, and colorectal primary tumors, followed by the identification of patient-unique mutations that can be used for circulating tumor DNA (ctDNA) detection. A high success rate of ctDNA detection was achieved using primer/probe synthesis with Hypercool technology for digital PCR (dPCR), in which the stable detection level for SNVs were 0.01%. The dynamics of the ctDNA were largely consistent with the tumor burden defined by the CT scan, except that the ctDNA's elevation level had been seen six months earlier than the visual detection of the relapsed lesion. Multi-region tumor sequencing revealed that clonal mutations had a higher chance of being detected as ctDNA, while their functional effects at the protein level remain to be determined. By combining panel sequencing and dPCR, tumor burden dynamics that are monitored using ctDNA can readily be a sensitive indicator for earlier detection of post-the rapeutic relapse of gastrointestinal cancers.

S19-Special_Remarks

Special Remarks

Masaki Kitajima
InterNatl. Univ. of Health & Welfare

No Abstract

▲TOP

[ML16] ML16 [Japanese]
Morning Lectures 16

2018 / 9 / 29 (Sat) 8:00-8:50 Room 4/10F 1001, Osaka International Convention Center Room 4

Keiya Ozawa / Div. Immuno-Gene & Cell Ther

ML16

Finding targets and creating therapeutic compounds for genetically-complex human hematological malignancies

Fumihiko Ishikawa
RIKEN Ctr. for Integrative Med. Sci.

Discussant：Junji Suzumiya
Shimane Univ. Hosp. Innovative Cancer Center

Over the last decade, through genomic analyses, we have come to understand that individual patients with malignancies carry mutations in patient-specific combination of genes. In leukemia patients, hematopoietic cells contain both pre-leukemic and leukemic cells that share same sets of mutations. Such genomic complexity and heterogeneity complicates interpretation of clinical significance of each mutation and makes drug discovery difficult. To find functional significance of multiple mutations in patients, we defined patient-derived hematopoietic subpopulations as pre-leukemic or leukemia by NOD/SCID/Il2rgKO (NSG) xeno-transplantation. Then, by single cell mutational profiling, we determined how mutations are distributed among single cells. By combining functional assessment of patient-derived hematopoietic cells and single cell genomic analyses, we succeeded in identifying mutations that permit stem cells to give rise to normal blood cells from those associated with in vivo leukemogenesis. In the session, I would also like to introduce our approach of creating therapeutic compounds for acute myeloid leukemia through multi-faceted analysis.

▲TOP

[MVA-1] MVA [English]
JCA-Mauvernay Awards Session

2018 / 9 / 29 (Sat) 9:00-11:30 Room 4/10F 1001, Osaka International Convention Center Room 4

Hitoshi Nakagama / Natl. Cancer Ctr.、Takashi Takahashi / Div. Mol. Carcinog., Nagoya Univ. Sch. Med.

MVA-1

Fluctuating Stress in Cancer Cells

Fuyuki Ishikawa
Kyoto Univ., Grad. Sch. Biostudies

Cellular senescence was originally found in cells that exhausted telomere DNA after numerous cell divisions. We previously reported that stress MAPK p38 is activated and induces cellular senescence, suggesting us that weak stress may lead to significant cellular responses in cancer cells. Since no gene had been identified in responding to weak stress, we conducted genetic screening in fission yeast. We identified the histone chaperone HIRA complex plays a pivotal role in the acquired tolerance, where weak stress confers cells stress resistance to the following lethal stress. Moreover, we have shown that skin papillomas, induced in chemical-induced carcinogenic experiments, spontaneously regressed significantly more frequently and progressed to carcinomas less frequently in HIRA knockout mice compared to control mice. These results suggest that fluctuating environments contribute to cancer progression by rendering cells stress-resistance via the acquired tolerance pathway. We propose that the pathway is a potential target for developing anti-tumor therapies, which is based on a unique MOA, namely forestalling clonal evolution.

▲TOP

MVA-2

From genomic alterations to target genes for cancer therapy; miRNA therapeutics has emerged as a promising strategy

Johji Inazawa
Dept. Molec. Cytogenet., Med. Res. Inst., Tokyo Med. Dent. Univ., Bioresource Res. Ctr., Tokyo Med. Dent. Univ.

Using high-throughput technologies originally developed, we screened genomic alterations in over 1000 of cancer cell lines and primary tumors, and identified 50 candidate drivers including GASC1, cIAP1, PPM1D and SKP2 within novel amplifications. Some of their inhibitors are currently ongoing in clinical trials. In 2008, we first reported tumor suppressor miRNAs (TS-miRs) silenced by a tumor-specific DNA-hypermethylation. We then extensively explored novel TS-miRs using miR library-based functional screening, and identified more than 20 novel TS-miRs. Among those, miR-634 activates the apoptotic pathway by directly targeting genes associated with cytoprotection. The enforced expression of miR-634 remarkably enhanced chemotherapy-induced cytotoxicity in cancer cells in vitro and in vivo. Recently, we identified miR-3140, which directly suppresses BRD4. BRD4 mediates transcriptional elongation of MYC and plays a critical role in various cancers including NUT midline carcinoma (NMC) bearing BRD4-NUT fusion gene. Taken together, these findings provide novel insights into the application of miR-based therapeutics in precision cancer medicine (PCM).

MVA-3

Cell cycle regulation in cancer stem cell

Keiichi Nakayama
Dept. Mol. Cell. Biol. Med. Inst. Bioreg., Kyushu Univ.

Fbxw7 is the F-box protein component of an SCF-type ubiquitin ligase, in which it functions as a receptor responsible for the recognition of many targets including c-Myc and Notch. We found that Fbxw7 plays a pivotal role in maintenance of quiescence in leukemia-initiating cells (LICs) of chronic myeloid leukemia (CML). Our findings reveal that ablation of Fbxw7 in a mouse model of CML results in accumulation of c-Myc and disruption of quiescence in LICs. Furthermore, we demonstrate that Fbxw7-deficient LICs are sensitive to currently available anticancer drugs and combination therapy with Fbxw7 depletion and these drugs is able to eradicate LICs, leading to a decreased relapse rate and a significant survival advantage. Finally, we present data that such combination therapy is also effective for human CML LICs, supporting our conclusion that Fbxw7 is a promising target for the treatment of human leukemia. Therefore, combination of Fbxw7 suppression and anti-cancer drugs may provide the basis for new approaches to eradication of cancer stem cells.

MVA-4

Structural variations in the Helicobacter pylori CagA oncoprotein impacts the global landscape of gastric cancer

Masanori Hatakeyama
Dept. Microbiol., Grad. Sch. Med., Univ. Tokyo

East Asian countries such as Japan, Korea and China have the highest incidences of gastric cancer in the world. Helicobacter pylori infection is causally associated with the development of gastric cancer. The CagA protein of H. pylori plays a critical role in the development of gastric cancer. CagA is delivered into gastric epithelial cells by a bacterial syringe termed the type IV secretion system. Once inside the epithelial cells, CagA undergoes tyrosine phosphorylation at the Glu-Pro-Ile-Tyr-Ala (EPIYA) motif, which is present in a variable number in the C-terminal CagA region, by Src family kinases. Tyrosine-phosphorylated CagA then acquires the ability to interact with and thereby aberrantly activate the pro-oncogenic phosphatase SHP2. The magnitude of SHP2 deregulation by individual H. pylori CagA has been linked to the qualitative (sequence) and quantitative (repeat number) polymorphisms in the EPIYA motif-containing region. Recent crystal structure analysis shed light on the molecular mechanism by which the EPIYA polymorphism determines the magnitude for the pathogenic action of individual CagA, which may underlie worldwide variation in the incidence of gastric cancer.

▲TOP

MVA-5

Biological and clinical implications of EGFR mutation in lung cancer

Tetsuya Mitsudomi
Thoracic Surg., Kindai Univ. Fac. Med.

In 2004, the mutation in the EGFR gene in lung cancer was first reported. We started our analysis as soon as we know this great discovery. By the end of 2004, we published our results confirming that the presence of EGFR mutations was strongly associated with histology, gender, non-smoking status and Asian ethnicity. In addition, we were the first that found the mutual exclusionary relationship between EGFR mutations and KRAS mutations. In the next year, we showed for the first time patients with EGFR mutation survived for a longer period of time upon EGFR tyrosine kinase inhibitor treatment compared with those without. It was 2005 when I received JCA-Mauvernay award and this was very encouraging. After that, I had a privilege to lead as a principal investigator of the phase III trial comparing gefitinib with standard chemotherapy for EGFR+ patients (WJTOG3405). The trial was turned out positive in 2009. This was one of the early positive clinical trials for the molecularly-defined subset of cancer patients. The same principle was applied ALK, ROS1, and BRAF mutated lung cancer to date. Our efforts clearly mark the start of the era of "precision medicine".

▲TOP

[LS28] LS28 [English]

The development of future therapies for multiple myeloma in the era of increasing role of immunotherapy

2018 / 9 / 29 (Sat) 11:50-12:40 Room 4/10F 1001, Osaka International Convention Center Room 4
：OncoTherapy Science, Inc.

Yusuke Nakamura / Cancer Precision Medicine Center of JFCR

LS28

The development of future therapies for multiple myeloma in the era of increasing role of immunotherapy

Andrzej Jakubowiak
The University of Chicago, Medicine

No Abstract

▲TOP

[E-3036] E5-2 [English]
MicroRNAs in cancer progression (2)

2018 / 9 / 29 (Sat) 13:40-14:55 Room 4/10F 1001, Osaka International Convention Center Room 4

Hidetoshi Tahara / Dept. Cell & Mol. Biol., Grad. Sch. of Biomed. & Health Sci., Univ. of Hiroshima

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E-3036

Demonstration of 5' Isoform MicroRNA in Lung Adenocarcinoma

Mei F. Hsieh
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Co-author：Ching C. Lin
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The microRNA isoforms (isomiRs) have been demonstrated to be mainly generated by shifting of Drosha and Dicer in cleavage site rather than sequencing artifacts. The isomiRs have been discovered to be different in disease subtypes, so it is important to examine isomiRs and their aberration in cancers. In our study, the isomiRs were defined by 5' nucleotides shifting according to the archetype miRNAs, which theoretically diverse the seed sequence and thus influence the regulation on the target mRNAs. In lung adenocarcinoma (LUAD), we observed that the isomiRs with farther shifting seem to be expressed higher in tumors. To scrutinize the possible function of these isomiRs, we investigated the protein interaction network (PIN) formed by the down-regulated target mRNAs regulated by the up-regulated isomiRs. In LUAD, 18 and 7 down-regulated genes were predicted to be targeted by miR-182-5p and miR-183-5p respectively. Furthermore, the functional enrichment analysis of the PINs formed by these 18 and 7 genes indicated that differentially expressed isomiRs might be involved in LUAD through regulating signal transduction and metabolic process.

▲TOP

E-3037

Tumour-suppressive microRNAs as negative regulators of extracellular vesicle secretion from cancer cells

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Extracellular vesicles (EVs) from cancer cells dictate their surrounding microenvironmental cells or distant cells in the future metastatic organs for the benefit of cancer cells. Although the precise mechanism of EV production from cancer cells remains unclear, revealing these mechanisms would lead to the establishing of EV-targeted therapy against cancer. Here, we hypothesized that microRNAs (miRNAs) are a key regulator of EV production in cancer cells because it is well known that dysregulation of miRNAs leads to oncogenesis. Screenings for more than 2000 miRNAs in different types of cancer cells were performed. Consequently, we found that tumor-suppressive miRNAs attenuated the secretion of EVs from cancer cells. To address the molecular mechanisms mediated by these miRNAs, the target genes of these miRNAs were identified and evaluated for their contribution to EV production in cancer cells. Furthermore, we found that metastasis of the cells with knock-down of target genes was abolished. These findings suggest that the suppression of EV production from cancer cells by tumor-suppressive miRNAs is a part of the anti-cancer activity mediated by the tumor-suppressive miRNAs.

E-3038

Genome-wide miRNA expression analysis for identification of a novel CRC-specific miRNAs using next-generation sequencing

Yoshinaga Okugawa
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Co-author：Yuji Toiyama、Shozo Ide、Takahito Kitajima、Junichiro Hiro、Koji Tanaka、Masato Kusunoki
Dept. Gastrointestinal & Pediatric Surg., Mie Univ.

Background:Next generation sequencing(NGS) has emerged as a powerful tool for discovery of novel microRNA(miRNA)s. Methods:MiRNA profiles were generated by NGS using 8 pairs of matched CRC and normal mucosa(NM). We compared these results with an independent microarray-based profiles from 74 colorectal tissues(54 CRCs and 20 NMs). We performed qRT-PCR analysis for clinical validation in 239 colorectal tissues from two independent cohorts. Knockdown or overexpression of miR-549 was performed for functional validation using series of in vitro and in vivo analysis. Results:NGS analysis combined with array-based profiles identified 7 CRC-specific miRNAs. Among these, miR-549 expression was robustly discriminated CRC tissues from NM in testing(AUC:0.92) and validation set(AUC:0.94). Furthermore, increased miR-549 was significantly correlated with poor survival. Series of in vitro and in vivo analysis revealed that miR-549 was involved in proliferation, migration, invasion, anoikis resistance, and tumorigenesis. We explored array-based profiling to identify downstream target genes. Conclusions:MiR-549 plays an important role in CRC development and might be novel prognostic marker in CRC.

E-3039

Exploring novel tumor suppressive microRNAs in OSCC

Yuki Takagawa
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MicroRNA (miR), endogeneous small non-coding RNA, decompose or inhibit protein translation of mRNA by binding to complementary sequences of their target gene mRNAs. miRs play crucial roles in the modulation of various biological processes. Tumor suppressive miR (TS-miR) mimics could be applied to cancer therapy in nucleic acid medicine. Oral cancer, predominantly oral squamous cell carcinoma (OSCC), is the most common head and neck neoplasm. To investigate the novel candidate TS-miRs for the development of miR-based cancer therapeutics, we performed function-based screening with 2565 human miRNAs in OSCC cell lines. miR-X and miR-Y (lab names) were identified as the novel TS-miRs in this screening. Overexpression of miR-X and miR-Y significantly inhibited tumor cell growth in various OSCC cell lines in vitro. To investigate target genes of miR-X and miR-Y, gene expression array analysis was performed in OSCC cell lines after transfection of each miR or negative control miR. The expression levels of 327 genes and 307 genes were decreased by more than 1.5-fold in miR-X- and miR-Y- transfected cells, respectively.

▲TOP

E-3040

Nucleic acid medicine for KRAS mutant colon cancer

Sho Ishikawa
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Co-author：Hidekazu Takahashi[1]、Yuhki Yokoyama[2]、Yamin Qian[2]、Haruka Hirose[2]、Norikatsu Miyoshi[1]、Naotsugu Haraguchi[1]、Taishi Hata[1]、Chu Matsuda[1]、Tsunekazu Mizushima[1]、Yuichiro Doki[3]、Masaki Mori[3]、Hirofumi Yamamoto[4]
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We previously demonstrated that miR–29b–3p is a hopeful miRNA–based therapy against colorectal cancer. In this study, we aimed to clarify a value of miR–29b–1–5p as a next–generation treatment. Mimic–miR–29b–1–5p significantly inhibited proliferation of KRAS–mutant colorectal cancer cell lines DLD1 and SW480. Considering that miR–29b–1–5p is a passenger miRNA and may have no physiologic function, we found that completely opposite complementary strand to miR–29b–1–5p, rather than miR–29b–1–5p possessed a potent antitumor effect and named this byproduct miRNA sequence "MIRTX". MIRTX directly targeted the 3′–UTR of CXCR2 and PIK3R1 mRNA and suppressed the NF–κB signaling pathway in KRAS–mutated colorectal cancer cells. MIRTX induced apoptosis in DLD1 with downregulation of antiapoptotic BCL2, BCL–xL, and MCL1 and upregulation of cleaved caspase–3 and cleaved PARP. In mouse xenograft models, systemic administration of MIRTX using a super carbonate apatite significantly inhibited tumor growth of DLD1 cells. These findings indicate that inhibition of NF–κB signaling by this novel miRNA–based therapeutic could be a promising treatment against refractory KRAS–mutant colorectal cancer.

E-3041

Relevancy of organ-specific miRNAs and PKM gene expression in the carcinogenesis

Kohei Taniguchi
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PKM has 2 isoforms, i.e., PKM1 and PKM2. These genes have organ-specific distribution. Recently, we found that increment of PKM2/PKM1 ratio has been essential phenomenon for the carcinogenesis. In this presentation, we show that several organ-specific miRNAs regulate PKM isoform expression through the direct targeting of PTBP1 (one of the crucial splicers for PKM2-dominant expression) or PKM2 in each specific organ. Namely, both brain-specific MIR124 and 137 and muscle-specific MIR1 and 133b targeted PTBP1 directly. On the other hand, liver-specific MIR122 targeted PKM directly to enable the expression of PKLR in liver tissue. Also, the up-regulation of PTBP1 and PKM2 expressions were detected in various clinical cancer samples through the dysregulation of these microRNAs. Our results suggest that the organ-specific distribution of miRNAs is one of the principal means by which miRNA establishes characteristics of a tissue and that dysregulation of these miRNAs results in cancer development through a change in the ratio of PKM isoform expression.

▲TOP

[E-3042] E5-3 [English]
Transcriptional regulation

2018 / 9 / 29 (Sat) 14:55-16:10 Room 4/10F 1001, Osaka International Convention Center Room 4

Taro Yamashita / Dept. Gen. Med., Kanazawa Univ. Hosp.

E-3042

A RNA splicing factor drives prostate cancer progression

Keisuke Nimura
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RNA splicing creates multiple transcript variants from one pre-mRNA via removing introns/exons. Although an impediment in Androgen-Androgen receptor (AR) axis effectively represses the growth of prostate cancer, most of all cases eventually become castration-resistant prostate cancers (CRPCs). One of the mechanisms to obtain the abilities as CRPCs is AR-V7 expression, a constitutive active form of AR, generated as a result of RNA splicing. However, the molecular mechanisms to generate AR-V7 are still unknown. Here we show that a RNA splicing factor (SFx) that was identified by *in silico* and CRISPR/Cas9 analyses, promotes AR-V7 expression and malignancy in prostate cancer. Genome wide RNA splicing and photoactivatavle ribonucleoside-enhanced cross-linking immunoprecipitation(PAR-CLIP) analyses reveal that SFx directly control RNA splicing in AR. SFx promotes tumor growth *in vivo* and is correlated to biochemical recurrence in prostate cancer patients. Moreover, SFx inhibition suppresses the growth of tumors addicted to high SFx expression. Our data suggest that SFx is a potential therapeutic target.

▲TOP

E-3043

Regulation of HAT induces apoptotic cell death through regulating hypoxia mechanism in RCC and osteosarcoma cells

Saho Takasaki
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Hypoxia is critically important for carcinogenesis including renal cell carcinoma (RCC) and osteosarcoma. Hypoxia-inducible factors (HIFs) are associated with tumor progression and often accumulate in RCC lacking Von Hippel-Lindau (VHL) and osteosarcoma. Although role of HIFs is well clarified in a variety of solid tumors, therapeutic strategy targeting HIFs have not been investigated yet. Herein, we approached to regulate histone acetyltransferase, transcription co-activator (HAT) to decrease the expression of HIFs, the suppression of the tumor progression. siRNA-mediated silencing HAT efficiently decreased HIFs expression and its downstream factors such as VEGF and Glut1. This result indicated that the suppression of HAT is effective method toward the suppression of the tumor growth through HIFs down-regulation. Therefore, we next conducted small molecule library screening targeting HAT to identify effective inhibitor. HIT compounds had killing effect on RCC and osteosarcoma cells through apoptotic cell death. These data suggest that inhibition of HAT could be a potential therapeutic approach in RCC and osteosarcoma.

E-3044

Long noncoding RNAs contribute to the epigenetic regulation of epithelial-mesenchymal transition (EMT) in cancer cells

Takeshi Suzuki
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Div. Func. Genom., Cancer Res. Inst., Kanazawa Univ., Mol. Therap. Target Res. Unit, InFIniti, Kanazawa Univ.

EMT is a paradigm of cell plasticity characterized by the reversible changes in epithelial and mesenchymal gene expression. Previously we showed that Polycomb repressive complex-2 (PRC2) and its accessory component, JARID2, which regulate the methylation of K27 of histone H3, were essential for gene expression program in EMT of cancer cells. During the investigation of molecular mechanism for proper recruitment of PRC2 to the target genes, we discovered the involvement of long noncoding RNAs (lncRNAs). MEG3 lncRNA was shown to be required for TGF-beta-induced EMT in cancer cells. Gene expression program during EMT was disturbed by MEG3 knockdown and potentiated by MEG3 overexpression. MEG3 was involved in epigenetic regulation of several EMT-related genes through the recruitment of JARID2 and PRC2 to the target chromatin. However, MEG3 overexpression itself was not sufficient for EMT. Recently we found that another lncRNA regulated the expression of a different subset of EMT-related genes and could induce EMT phenotype with co-expression of MEG3 in the absence of TGF-beta. This study demonstrated a crucial role of lncRNAs in the epigenetic regulation of EMT process in cancer cells.

E-3045

Multi-omics characterization of lung cancer cells based on gene co-expression modules

Ayako Suzuki
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[1]Grad. Sch. of Front. Sci., Univ. of Tokyo、[2]EPOC, Natl. Cancer Ctr.

In this study, to stratify lung adenocarcinomas using multi-omics information, we defined transcriptome network modules which were associated with genomic and epigenomic aberrations and would be essential for survival of cancer cells. By conducting gene co-expression network analysis with in-depth omics information of 26 lung cancer cell lines, we identified 51 modules which were aberrantly activated or repressed in some of the cells. We found that combinations of the modules classified cells at various cellular states including cell lineage, differentiation status, and activities of stress response pathways. These cell line-based modules were also applicable to characterization of TCGA clinical samples. We further generated a map of transcriptome and epigenome changes using 3240 RNA-seq and 3393 ATAC-seq datasets which were obtained from cells with drug-perturbed conditions. Using the data of basal transcriptome modules and their perturbations, we investigated drug combinations could control transcriptome patterns at a module level and consequently regulate cellular survival of cancers. Here we demonstrated novel module-based stratification and regulation of lung cancer cells.

▲TOP

E-3046

Phosphoproteomics analysis of nuclear protein kinase complexes associating with growth-related gene expression

Miwako K. Homma
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Co-author：Shiho Saito、Yoshimi Homma
Dept. Biomol. Sci., Fukushima Med. Univ., Sch. Med.

Protein kinase CK2 is a conserved serine/threonine kinase that functions in multiple cellular processes. CK2 protein is invariably elevated in highly proliferating tissues and tumors, and its elevated nuclear expression has been observed associating with human carcinomas. In this study, given the importance of nuclear CK2 in cell proliferation, we have elucidated its precise role for gene activation by revealing enrichment of transcriptional machinery and chromatin binding proteins during the progression of the cell cycle. Phosphoproteomics analysis demonstrates multiple proteins interacting with nuclear CK2 upon cell proliferation, including DNA topoisomerase 2, retinoblastoma binding protein RBBP4, thyroid hormone receptor associating protein 3, and zinc finger proteins. Identification of other proteins implicates important functions for CK2 on triggering chromatin remodeling and gene expression. Precisely, ChIP-qPCR analysis reveals CK2 binding to the active gene locus for expression of growth-associated genes, supporting the role of CK2 as an important mediator of transcription that enable cells to progress through the cell cycle.

E-3047

Molecular dissection of ASPSCR1-TFE3, the fusion gene associated with alveolar soft part sarcoma

Miwa Tanaka
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Alveolar soft part sarcoma (ASPS) is a rare soft tissue sarcoma affecting predominantly young adults. It is characterized by blood vessel-rich alveolar structure, frequent hematogenous metastasis, and invariable ASPSCR1-TFE3 (AT3) fusion as a causative mutation. We have generated a mouse model for ASPS that well recapitulates above characteristics. In the model we observed significant upregulation of Gpnmb, Syngr1 and genes involved in the lysosome pathway that are important for vasculogenesis and metastasis of ASPS. A ChIP-seq analysis showed frequent overlapping between AT3-binding peaks and histone H3K27ac as well as H3K4me3 peaks including known target loci. GO analysis showed enrichment of the lysosome pathway. The model is also utilized for in vivo sarcomagenic assay for the MiT/TFE family to identify the important domains within TFE3 and to reveal the function of AT3. In the presentation, epigenetic landscaping of ASPS clarified by ChIP-seq and the novel functions of AT3 in target gene regulation important for sarcomagenesis will be discussed.

▲TOP

[ML17] ML17 [Japanese]
Morning Lectures 17

2018 / 9 / 29 (Sat) 8:00-8:50 Room 5/10F 1002, Osaka International Convention Center Room 5

Yutaka Kondo / Div. Cancer Biol, Nagoya Univ., Grad. Sch. Med.

ML17

T cell reprograming for cancer immunotherapy

Akihiko Yoshimura
Dept. Micobiol. Immunol., Keio Univ. Sch. Med.

Discussant：Hideki Ohdan
Depart. Gastro

Generation of effective memory T cells is important not only for adoptive T-cell immunotherapy but also for promoting anti-tumor immunity. The persistence and resistance to exhaustion of transferred T cells are critical for improvement in patient outcomes. Stem cell memory T (Tscm) cells have been proposed as a new class of memory T cells which have longevity and proliferative potential. We established a new strategy of generating Tscm-like cells in vitro (designated as "iTscm" cells) from activated CD4+ and CD8+ T cells by coculturing with stromal cells expressing a Notch ligand. These iTscm cells lost PD-1 and CTLA-4 expression, were resistant to cell cycle arrest and apoptosis, and produced a large number of effector cells after restimulation, therefore exhibiting a strong antitumor activity. We also found a key factor, NR4a for regulatory T cell generation and T cell exhaustion. I will discuss about therapeutic potentials of Tscm and Nr4a for cancer immunotherapy.

▲TOP

[E-3001] E12-3 [English]
Tumor antigens and immunity

2018 / 9 / 29 (Sat) 9:00-10:15 Room 5/10F 1002, Osaka International Convention Center Room 5

Yasuharu Nishimura / Dept. Immunogenet., Grad. Sch. Med. Sci., Kumamoto Univ.

E-3001

Development of a new comprehensive method determining neoantigens with next-generation sequencing for immunomonitoring

Hidetoshi Sumimoto
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Whole exome sequence (WES) of tumor/normal tissues and web-based epitope prediction have made it easier to find candidate neoantigen epitopes of cancers. However, immunogenicity of the candidate epitopes has to be confirmed by functional assays. An ELISPOT assay is a gold standard, but is poorly sensitive and time consuming. To develop a more sensitive and faster method, we selected candidate neoantigen epitopes using WES from tumor/normal tissues of non-small cell lung cancer patients in combination with their RNA-seq and NetMHC epitope prediction. We stimulated HLA-matched PBMCs from a healthy donor with 8 candidate epitopes individually, and extracted RNA from CD8 T cells for TCRβsequencing. We determined T cell clones with peptide-specific expansion showing odds ratio > 10 (test peptide odds/no peptide odds). Nine to 15 T cell clones (median 10) showed specific expansion with each peptide. Our results suggest that this functional assay based on T cell clonal expansion stimulated with candidate epitopes is more sensitive, faster, and easier for repetition than a conventional ELISPOT assay for immunomonitoring and is useful for developing new personalized immunotherapy.

▲TOP

E-3002

Integrated Omics Analysis on Temporal Changes of Neoantigen and Tumor Microenvironment in Primary and Recurrent Gliomas

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Limited success of immune-based therapies in glioma might be in part due to the lack of enough neoantigens (neoAgs) that can be targeted by immune effector cells. In this study, we evaluated neoAg expression change and its relation to immune microenvironment using matched primary and recurrent tumor samples from 25 glioma patients. Predicted neoAgs (p-neoAgs) deriving from missense mutations were identified by whole-exome sequencing (WES) analysis and netMHCpan prediction algorism. Expressed neoAgs (e-neoAgs) among the p-neoAgs were determined by incorporating RNA sequencing (RNA-seq) data. The ratio of e-neoAgs to p-neoAgs ("neoAg expression ratio") on each sample significantly decreased at recurrence (p=0.003). RNA-seq analyses on gene expression pattern changes illustrated that the cases with strongly reduced neoAg expression ratio compared to primary counterpart (top 8), but not those without (bottom 8), retained gene expression related to antigen presentation machinery and immune effector cells at recurrence. These data may suggest that the tumor cells with reduced neoAg expression survived at recurrence as a result of persistent anti-tumor immune responses in some gliomas.

E-3003

The investigation of personalized immunotherapy targeting neoantigen for Liver, Pancreas, and Biliary tract cancer

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In recent, some clinical studies with immune checkpoint inhibitors suggested that neoantigens derived from passenger gene mutation might cause anti-cancer immune response in vivo. Passenger mutations are naturally individual in each patient; therefore, personalized immunotherapy is needed to achieve the maximum response by cancer immunotherapy. Here, to explore the possibility of personalized immunotherapy against liver, pancreas, and biliary tract cancers, we evaluate the correlation between the mutation burden, the gain of neoantigens, and the immunological phenotype of these cancers. Total 105 patients who were received radical resection in NCCE until Dec. 2016 to Jan. 2018 were enrolled to this study, and, in 59 of patients, the reactivity of TILs against autologous tumor were assessed by CD107a assay in vitro. We detected the CD107a+ fraction of CD8+ TILs in half of these patients, however, its were usually less than 1 %. This result was supported by the analysis of TCR repertoire in primary TILs. Furthermore, the candidates of neoantigen were predicted from omics analysis with tumor tissues. Now, we try to determine the CTL epitopes that stimulate tumor reactive TILs.

E-3004

Immuno-genomic subtypes of stage II colon cancers related with prognosis following surgery

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Immune response has been suggested to influence prognosis & responses to various cancer therapies, including colon cancer (CC). However, molecular and cellular mechanisms for different immune status in CC remain to be investigated.We defined 6 immunological subtypes (S1-S6) of 300 stage II CC patients, with distinct post-surgery prognosis. S1, an MSI subtype, has highly activated CD8+ T cells & best post-surgery prognosis, S2 & S5 also have relatively high immune responses. S3 has a benign adenoma like feature, S4 is a highly proliferative CC with less immune reaction, S6 has mesenchymal features with worst prognosis. Combination immunotherapy may be applied to S2 & S5 in addition to S1 MSI subtype for which PD-1/PD-L1 blockade may work. High infiltration of CD8+ T cells, FOXP3+ T cells, & CD20+ B cells were correlated with favorable prognosis even in MSS CC for which PD-1/PD-L1 blockade alone does not work. We successfully classified immunological subtypes of stage II CC which indicated that immune status is significantly involved in stage II CC prognosis. Furthermore, appropriate immunotherapies combination may be developed & applied on particular subtype of stage II CC.

▲TOP

E-3005

TCR sequencing analysis of cancer tissues and lymph nodes in colorectal cancer patients

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Tumor draining lymph nodes (TDLNs) are located in the routes of lymphatic drainage from a primary tumor to the most probable sites of metastasis in various types of solid tumor. TDLNs are considered as a first tissue to initiate the antitumor immunity, where antigen-specific effector T cells are generated and released to the blood stream. However, T cell receptor (TCR) repertoire in TDLNs has not been well characterized. We collected 23 colorectal cancer patients with lymph node metastasis and performed TCR sequencing of colorectal cancer tissues, corresponding normal mucosa tissues and a total of 203 regional lymph nodes, including 67 metastasis-positive lymph nodes. Metastasis-positive lymph nodes showed a significantly lower TCR diversity and more abundant TCR shared with primary tumor tissues compared to metastasis-negative lymph nodes. Hierarchical clustering and principal component analyses also supported that TCR repertoires in metastasis-positive lymph nodes were more similar to primary tumor tissues than metastasis-negative lymph nodes. These finding suggest that certain cancer-reactive T cell clones might expand in the metastasis-positive lymph nodes.

E-3006

Snail upregulates CXCL1/2 and induces immune escape through migration of MDSCs in ovarian cancer microenvironment

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Snail is a major transcriptional factor that induces epithelial-mesenchymal transition (EMT). Snail expression is high in mesenchymal subtype of ovarian cancer in the four subtypes of TCGA microarray dataset. We explored the effect of Snail on tumor immunity.
Snail knockdown in mouse ovarian cancer cells suppressed tumor growth in immunocompetent mice, associated with an increase of CD8+ tumor-infiltrating lymphocytes and a decrease of myeloid-derived suppressor cells (MDSCs). Snail knockdown reduced the expression of CXCR2 ligands (CXCL1 and CXCL2), chemokines that attract MDSCs to the tumor through CXCR2. Snail upregulates CXCR ligands through NF-kB pathway, and most likely, through direct binding to the promoters. A CXCR2 antagonist suppressed MDSC infiltration and delayed tumor growth in Snail-expressing mouse tumors. Ovarian cancer patients showed elevated serum CXCL1/2, which correlated with Snail expression, MDSC infiltration, and short overall survival. Thus, Snail induces cancer progression via upregulation of CXCR2 ligands and recruitment of MDSCs. Blocking CXCR2 represents an immunological therapeutic approach to inhibit progression of Snail-high tumors undergoing EMT.

▲TOP

[E-3007] E20 [English]
Regenerative medicine

2018 / 9 / 29 (Sat) 10:15-11:30 Room 5/10F 1002, Osaka International Convention Center Room 5

Akira Shimamoto / Field of Regenerative Med. Res. Faculty of pharm. Sci. Sanyo-Onoda City Univ.

E-3007

Immunophenotype and antitumor ability of cytokine-induced killer (CIK) cells from patients with hepatocellular carcinoma

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Cytokine-induced killer (CIK) cells are a heterogeneous subset of ex-vivo expanded lymphocytes cultured with a cytokine cocktail. The purpose of this study is to evaluate the functional characteristics and anticancer activity of CIK cells from hepatocellular carcinoma (HCC) patients. HCC patients were enrolled for collecting PBMCs. CIK cells were ex vivo activated and expanded from PBMCs cultured with IFN-γ, IL-2, anti-CD3, and IL-1. We found most cells were CD3+CD8+, including CD3+CD56+. These cells express high level of NK receptors (NKG2D and DNAM1), but low level of inhibitory receptors (CTLA-4, PD-1 and LAG-3). CD62L and CXCR3 were enhanced in CD8+ T cells, representing the trafficking potential to inflamed sites. CIK cells possessed the ex vivo killing ability. Human CIK cells treating J7 tumor-bearing NOD/SCID mice could significantly suppress the tumor growth. Human immune cells could be detected in the peripheral blood and tumors of NOD/SCID mice after CIK treatment. The present study found that CIK cells from HCC patients expressed effector NK receptors and chemokine molecules, but less suppressive checkpoint molecules. They could suppress human HCC ex vivo and in vivo.

▲TOP

E-3008

Hlf expression marks the developmental pathway for hematopoietic stem cells but not for erythroid-myeloid progenitors

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Within the adult bone marrow, hematopoietic stem cells (HSCs) give rise to lineage-restricted progenitors to produce various types of mature blood cells. However, some lineage-restricted progenitors, such as erythroid-myeloid progenitors (EMPs), are detected in the mouse embryo or in pluripotent stem cell cultures in vitro before the emergence of HSCs. Although both HSCs and HSC-independent EMPs are derived from hemogenic endothelium through endothelial-to-hematopoietic transition (EHT), it remains unclear how and when these two developmental programs are segregated during ontogeny. Here, we show that hepatic leukemia factor (Hlf) expression preferentially marks a developmental continuum between HSC precursors and HSCs. Using the Hlf-tdTomato reporter mouse, we found that Hlf is expressed in aortic hematopoietic clusters and fetal liver HSCs. In contrast, EMPs and hematopoietic clusters in the yolk sac before embryonic day 9.5 do not express Hlf. These results strongly suggest that HSCs and EMPs are generated from distinct hemogenic endothelium. Selective induction of the Hlf+ lineage pathway may lead to the in vitro generation of HSCs from pluripotent stem cells.

E-3009

Essential role of Arid1a in intestinal stem cell maintenance and homeostasis through Sox9 regulation

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Inactivating mutations of Arid1a, a subunit of the SWI/SNF chromatin remodeling complex, have been reported in multiple human cancers. Intestinal deletion of Arid1a has been reported to induce colorectal cancer in mice; however, its functional role in intestinal homeostasis remains unclear. Our study revealed that intestinal deletion of Arid1a results in loss of intestinal stem cells (ISCs), decreased Paneth and goblet cells, disorganized crypt-villous structures, and increased apoptosis in adult mice. Spheroids did not develop from intestinal epithelial cells deficient for Arid1a. Lineage tracing experiments revealed that Arid1a deletion in Lgr5+ ISCs leads to impaired self-renewal of Lgr5+ ISCs. The Wnt signaling pathway was strikingly downregulated in Arid1a-deficient intestines. We found that Arid1a directly binds to the Sox9 promoter to support its expression. Remarkably, overexpression of Sox9 in intestinal epithelial cells abrogated the above phenotypes. These results indicated that Arid1a is indispensable for the maintenance of ISCs and intestinal homeostasis through regulation of Sox9 in mice.

E-3010

The stemness of Jag2 in the small intestine

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[Background]The Notch ligand Jag2 has recently been linked to human pancreatic and breast carcinoma. The purpose of this study is to evaluate the role of Jag2 in intestinal carcinogenesis, which is currently unknown. [Results]We have generated a new line of Jag2 knock out (KO) mice and performed phenotypic analysis of these mice. Using x-gal staining, we focused on the intestines due to the selective presence of Jag2 in the intestinal crypts. To evaluate Jag2 function in the "stem cell niche", we have established organoid cultures. We found that organoids derived from Jag2 heterozygous mice could not be maintained beyond one passage. This suggests reduced stemness of Jag2 heterozygous organoids. Additionally, we used dextran sulfate sodium (DSS) to induce intestinal mucosa inflammation in Jag2 heterozygous and wild type mice. We found that the weight of Jag2 heterozygous mice at day 8 was significantly lower compared to wild type mice. Histology showed that Jag2 heterozygous intestines have more severe inflammation than wild type at day8. In sum, these results are consistent with a functional role of Jag2 in the intestine, particularly in the maintenance of crypt stemness.

▲TOP

E-3011

A human brown adipocyte-specific monoclonal antibody for an evaluation of brown adipose tissue mass in humans

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The type-Ⅱ diabetes is one of the major health problems caused by overweight and dysregulated metabolism. Moreover, clinical studies have shown a positive correlation between the incidence of type-Ⅱ diabetes and cancer, suggesting that discovery of new molecular targets to treat metabolic disorders will contribute to preventing cancer development.Brown adipocyte tissue (BAT) has been attracting attention as a new therapeutic target for the treatment of metabolic disorders. Not only by its high thermogenic capacity to promote energy expenditure, it improves metabolism via various secreted factors. Currently, 18F-FDG-PET/CT examination is the only measure to assess the volume of BAT. However, there is a problem in view of cost and labor and development of more feasible methods are required. Here we have generated a monoclonal antibody that specifically recognizes human Brown adipocyte (BA). The antigen molecule is a secreted peptide of small molecular weight (< 5 kDa). We also determined the presence of the antigen molecule in animal sera. Currently, we are trying to identify this molecule, which we believe to provide an excellent serum marker to assess the volume of human BAT.

▲TOP

[E-3048] E17-2 [English]
Drug delivery system (1)

2018 / 9 / 29 (Sat) 13:40-14:55 Room 5/10F 1002, Osaka International Convention Center Room 5

Yasuhiro Matsumura / Div. Dvelopmental Therap., EPOC, Natl Cancer Ctr

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E-3048

Amphiphilic polymeric micelles from structurally-modified chitosan for cancer therapy

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Chemotherapy has an important role in cancer treatment. Most anticancer drugs have low water solubility and life–threatening side effects. Therefore, there are drug administration problems and application concern. We aimed to develop polymeric micelles to increase water solubility and decrease toxicity of anticancer drugs. Chitosan–grafted poly (ethylene glycol) (CS–PEG) was fabricated with high yield. Degree of substitution was 21–38%. Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) were used to confirm structural changes. The critical micelle concentration was 1.1μg/mL. The size of blank and curcumin (a model of poorly water–soluble anticancer drug) loaded micelles were 275–371 nm with positive surface charge. The percentage of entrapment efficiency of curcumin was 61. These micelles were able to increase water solubility of curcumin without using organic solvent. Over 6 hours, curcumin was released from micelles more than 24%. Therefore, CS–PEG micelles may be a potential drug delivery system. The targetabiliy of this micelles will be augmented using targeting peptide. The cytotoxicity and efficacy of this system will be further determined.

▲TOP

E-3049

A novel approach of boron capture neutron therapy-BNCT using polymer conjugated carbohydrate moiety based on EPR effect

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BNCT has been developed for more than 50 years ago. However, its clinical use is still limited probably due to lack of selective accumulation of boron in tumor tissue. Recently we developed a polymer conjugate of styrene-maleic anhydride copolymer (SMA) with glucosamine, which forms complex with boric acid (SGB), and will selectively accumulate in tumor based on the EPR effect. Dynamic light scattering showed that SGB complex having hydrodynamic diameter of 90 ± 5 nm and surface charge of -37 ± 2 mV. Apparent molecular size of this complex was about 10kDa. It can show better EPR effect by binding with albumin during circulation. At physiological pH it is very stable, but in weakly acidic pH (tumor tissue pH) it releases borate. We found that SGB complex given iv at dose of 20 mg/kg did not show toxicity in mice. We hypothesize that SGB complex might play dual roles to kill the cancer cells upon neutron irradiation and by inhibiting glycolysis of cancer cells. This finding may resolve the drawback of existing BNCT using low-molecular-weight boron derivatives, and will bring about a new insight in BNCT. （共同研究者：澤智裕　熊本大学医学部、櫻井和朗 北九州市立大学）

E-3050

Enhancement of ERP effect in drug delivery by the combination of lipid bubbles and ultrasound

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We have developed a new lipid bubble (LB) suitable for sonoporation. New LB with 1-3 μm in size was stable in vivo for long time. New LB was freeze-dried and kept under perfluoropropane gas atmosphere in vial bottle until use. Freeze-dried LB formulation is good for storage stability and ease of handling. We investigated ultrasound imaging of neovasculature, and enhancement of ERP effect by the opening of neovasculature in the combination of new LB and ultrasound. Oscillation and cavitation of LB induced by therapeutic ultrasound exposure showed transiently open the neovasculature of tumor tissue and allowing DOX co-injected with LB was delivered into deep area in the tumor tissue. This system achieved an equivalent antitumor effect at about 1/5 the dose in monotherapy of Doxorubicin. DOXIL (liposomal formulation of Doxorubicin) and LB were administered hemangioperi-cytoma dog and ultrasound treatment was done two times. Tumor volume was decreased clearly after treatment. Thus, new approach by the combination of newly developed LB and ultrasound could help get medicines into tumor tissue and work better.

E-3051

Development of antibody-drug conjugates (ADC) for treating steroid-resistant lymphoid malignancy

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Although steroids have been widely used for the 1st line treatment of lymphoid malignancy (leukemia and lymphoma), steroid resistance remains an unsolved problem, and therapeutic alternatives are strongly required. IL-7R signaling, which is involved in the regulation of lymphocyte growth and survival, has been implicated in the development of lymphoid malignancy. Steroids can bind the enhancer of IL-7R gene. Amplified IL-7R signaling permit the IL-7R-positive cells to escape the steroid-induced apoptosis. We further demonstrated that an anti-IL-7R antibody conjugated with SN-38 (A7R-ADC-SN-38) has strong anti-tumor effects against both parent and steroid-resistant lymphoid malignant cells. About the host toxicity, there was no clear body weight loss. Moreover, although A7R-ADC-SN-38 efficiently eliminated some IL-7R-positive cells, IL-7R-negative mature lymphocytes were preserved. Thus, A7R-ADC may be a promising strategy for treating lymphoid malignancy especially with steroid resistance. Moreover, it would be effective as first line treatment combined with steroid treatment. We will present the progression of A7R-ADC development and its unique MOA (mode of action).

▲TOP

E-3052

Development of Paclitaxel Glycoside Liposomes Conjugated with Anti-CD44 Antibody Targeting Ovarian Cancer Cells

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Paclitaxel, PTX, is one of the frontline drugs on the treatment of ovarian cancer. However, the application is limited due to its significant hydrophobicity. Cremophore EL is currently approved as an efficient solvent for the clinical application despite the consequences of several side effects associated with its immunogenicity. Liposome encapsulation is considered efficient to disperse the hydrophobic compounds in the aqueous condition and to deliver the encapsulated materials into cytoplasm without side effects. Recently, we have demonstrated efficient encapsulation of paclitaxel glycoside, gPTX which is a PTX modified with a glucose moiety reducing hydrophobicity, into liposomes, CD44 expression in cancer cells is associated with cancer stem cells marker and prognosis including ovarian cancer. Therefore, CD44 is considered as a sufficient target for the therapy of ovarian cancer. In this study liposomes conjugated with anti CD44 antibody and loading gPTX are assessed for the efficacy of targeting CD44 positive ovarian cancer cells. Drug delivery by liposomes should be a promising approach because of the specific targeting potential and enhanced therapeutic efficacy of the drug.

E-3053

Polymeric pyropheophorbide-a, a promising tumor-targeted theranostic probe for photodynamic therapy and imaging

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We synthesized an HPMA polymer-conjugated pyropheophorbide-a (P-PyF) as a cancer theranostic agent for PDT and photodynamic diagnostics (PDD). In aqueous solutions, P-PyF showed a mean particle size of ~200 nm as it forms micelle which exhibited fluorescence quenching and very little singlet-oxygen (1O2) production. Whereas upon disruption of micelle strong fluorescence and 1O2 production were observed, indicating P-PyF is inactive and safe during circulation as micelle, but fulfill effect of PDT after accumulating in tumor by EPR-effect, where the micelles would be disrupted. In vitro and in vivo studies clearly showed that PDT effect of P-PyF. Much potent 1O2 production and PDT-effect were observed upon irradiation at ~420 nm. However, irradiation using long wavelength light (~ 680 nm) obtained remarkable tumor imaging effect with little background autofluorescence and perhaps deeper penetration of light. These findings strongly suggested P-PyF may be a potential candidate drug for cancer PDT/PDD. (This work is in collaboration with V. Subr, T. Etrych, and K. Ulbrich of Inst. Macromol. Chem., Academy of Sciences of Czech Republic, and S. Hackbarth of Humboldt Univ. of Berlin).

▲TOP

[E-3054] E15-3 [English]
Radiation / photodynamic therapy and novel cancer diagnostic tool

2018 / 9 / 29 (Sat) 14:55-16:10 Room 5/10F 1002, Osaka International Convention Center Room 5

Masahiko Koizumi / Dept. Med. Phys. Eng., Osaka Univ., Sch. Med. Health Sci.

E-3054

Sparse coding-based tumor-immune characterization based on chromogenic multiplex immunohistochemistry

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Chromogenic sequential multiplexed immunohistochemistry has been developed to evaluate 11-different protein biomarkers in a single formalin-fixed paraffin-embedded tissue section (Tsujikawa et al. Cell Reports 2017). This method enables profiling of tumor-immune characteristics with preserved tissue architecture, providing longitudinal biomarker assessments related to therapeutic response/resistance. To improve objectively and explorative ability in identification of distinct immune cell subsets, we adopted sparse coding-based learning approaches enabling modeling of data vectors as sparse linear combinations of basic elements with unbiased assessments. Comparative analyses between manual gating (ground truth) and sparse coding showed comparable results as validation of our method. This approach was further adopted for analysis of CD8+ T cell exhaustion/activation biomarkers such as CD45, CD3, CD4, CD8, T-bet, Eomes, PD-1, Ki67, Granzyme B, ICOS and IDO, revealing the presence of unique subset of CD8+ T cells related to treatment outcome and spatial patterns of immune infiltrates. Our results demonstrate robustness and objectivity of this novel bioinformatics approach.

▲TOP

E-3055

Effect of a combined treatment with iPSC derived DCs and proton beam irradiation in a murine subcutaneous melanoma model

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Backgrounds:Our previous studies showed that injection of bone marrow derived DCs (BM-DCs) after X-ray therapy (XRT) significantly delayed tumor growth. As compared to X-ray, the unique biological and physical benefits of proton beam therapy (PBT) may prove superior in the systemic immune effect. In addition, usage of DCs induced from iPS cells (iPS-DCs) may overcome practical problems of BM-DCs such as a limited number of applicable cells.Methods:DCs were induced from autologous bone marrow cells or iPS cells of C57BL/6 mice. Syngeneic B16 melanoma cells subcutaneously implanted at the thighs were treated with XRT or PBT 5 days after inoculation. After 1, 3, 5, 7 days from irradiation, induced DCs were injected directly into the tumor site.Results:PBT induced superior immunogenicity of cancer cell comparing to XRT. Also, iPS-DCs showed an excellent ability to incorporate antigens in vitro comparing to BM-DCs. The combination treatment of PBT and iPS-DCs significantly delayed tumor growth in vivo.Conclusions:iPS-DCs should overcome the practical problems of BM-DCs in cancer treatment. The combination therapy of PBT and iPS-DCs can offer a promising novel cancer therapy.

E-3056

Development of Radiation Therapy by Using Gold Nanoparticles as Radiation Sensitizer

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Radiation therapy is one of major modalities in cancer therapy. Radiation causes side effects on normal tissues around the cancer and such a situation remains an important clinical problem. Hence new methods which can reduce radiation dose are strongly required. Previous studies have shown that gold nanoparticles (AuNPs) improve the effect of radiation therapy as radiation sensitizer. However, in these previous studies, following two points were not quantitatively-understood: (1) localization of AuNPs involving in radiation therapy in cancer cells and (2) relationship between the particle numbers of AuNPs and cell cytotoxicity under X-ray radiation. Here we made AuNPs conjugated with anti-human epidermal growth factor receptor type2 (HER2) antibody via polyethylene glycol chains on their surface (Au-PEG-anti-HER2ab). By using these particles, we could estimate above two points quantitatively. In vitro and in vivo experiments, when the cancer cells exposed to Au-PEG-anti-HER2ab are irradiated with X-ray, their survival rate was significantly decreased compared with the case of the cancer cells exposed to only Au-PEG. We are now analyzing above two points in detail.

E-3057

Metronomic photodynamic therapy exerts excellent antitumor effects on remote tumor as well as local tumor

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Low-intensity (<0.1mW/cm2 yet long-term (>2-3 days) photodynamic therapy (PDT), termed metronomic PDT (mPDT), is attracting an attention since it effectively induces cell death of cancer cells. As mPDT is required only a very weak light, the light source can be miniaturized and thus fully implantable in the human body by using the technology of wireless electric power supply. These advantages suggest that mPDT is applied to deeply located tumors. We recently found that mPDT not only exerts an antitumor effect on local tumor but also suppresses the growth of remote tumors. Hence we hypothesized that mPDT activates systemic antitumor immunity through the tumor-specific apoptotic effect, and we conducted several experiments regarding mPDT using a combination of photosensitizer (Photofrin) and implantable light sources. The results showed that long-lasting cell death occurs accompanied by CD8 upregulation followed by HMGB1 expression over the time in mPDT (> 3 days). These findings strongly support our hypothesis and suggest that mPDT has a potential in the systemic treatment of advanced cancers in deeply located organs.

▲TOP

E-3058

Withdrawn

No Abstract

▲TOP

[ML18] ML18 [Japanese]
Morning Lectures 18

2018 / 9 / 29 (Sat) 8:00-8:50 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Kenkichi Masutomi / Natl. Cancer Ctr.

ML18

Long non-coding RNA and cancer

Tetsu Akiyama
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Discussant：Naohiro Nishida
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lncRNAs and cancerRecent advances in genomic research have revealed that only 1.2% of the mammalian genome encodes proteins, while the remainder is transcribed to generate an enormous number of non-coding RNAs (ncRNAs). These ncRNAs are currently divided into two main classes based on their transcript size: small ncRNAs (20-200 nucleotides) and long ncRNAs (lncRNAs, >200 nucleotides). These newly discovered lncRNAs have emerged as a major class of regulatory molecules associated with a broad range of biological processes. Furthermore, lncRNAs are aberrantly expressed in many diseases conditions, including cancer. Accumulating evidence indicates that some lncRNAs play critical roles in cancer progression and metastasis. lncRNAs have been proposed to regulate gene expression by various mechanisms, including acting as scaffolds for chromatin modifiers, transcriptional regulators, microRNA sponges, antisense RNAs, protein decoys and enhancers. Often, these functions of lncRNAs are mediated through complex formation with protein partners. Here I will review recent progress in our understanding of the roles of lncRNAs in cancer.

▲TOP

[E-3012] E4-1 [English]
Novel oncogenes / tumor suppressor genes (1)

2018 / 9 / 29 (Sat) 9:00-10:15 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Hirofumi Arakawa / Div. Cancer Biol., Natl. Cancer Ctr. Res. Inst.

E-3012

Withdrawn

No Abstract

▲TOP

E-3013

DGC-specific RHOA mutations maintained cancer cell survival and promoted cell migration via ROCK inactivation

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RHOA missense mutations exist specifically in diffuse type gastric cancers (DGC) and are considered one of the DGC driver genes, but it is not fully understood how RHOA mutations contribute to DGC development. Here we examined how RHOA mutations affect cancer cell survival and cell motility. We revealed that cell survival was maintained by specific mutation sites, namely G17, Y42, and L57. Because these functional mutations suppressed MLC2 phosphorylation and actin stress fiber formation, we realized they act in a dominant-negative fashion against the ROCK pathway. Through the same inactivating mechanism that maintained cell survival, RHOA mutations also increased cell migration activity. Cell survival and migration studies on CLDN18-ARHGAP (CLG) fusions, which are known to be mutually exclusive to RHOA mutations, showed that CLG fusions complemented cell survival under RHOA knockdown condition and also induced cell migration. Site-directed mutagenesis analysis revealed the importance of the GAP domain and indicated that CLG fusions maintained RHOA in the inactive form. Taken together, these findings show that the inactivation of ROCK would be a key step in DGC development.

E-3014

Oxysterol binding protein-like 3 (OSBPL3) is a novel driver gene stimulating R-Ras/Akt signaling in gastric cancer

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INTRODUCTIONGastric cancer (GC) is one of the most lethal human malignant tumors. Our aim of this study is to identify novel driver genes that can be applied as therapeutic targets in GC. MATERIALS AND METHODSWe identified novel driver genes of GC by in silico analysis with the TCGA dataset. Next, we performed in vitro and in vivo (xenograft mouse model) experiments using GC cells (MKN45 and MKN74). Furthermore, we assessed the clinical significance of the novel driver gene in GC using two public datasets.RESULTSOxysterol binding protein-like 3 (OSBPL3) was identified as a novel driver gene, which was remarkably overexpressed in tumor tissues with DNA copy number gain and promoter hypomethylation. OSBPL3 knockdown reduced the cell growth in vitro and in vivo by inhibiting cell cycle progression in GC cells. Moreover, pull-down assay demonstrated that OSBPL3 activates R-Ras/Akt signaling pathway in GC cells. In clinical analysis, high OSBPL3 expression significantly correlated with advanced stages and was a poor prognostic factor both in the Kmplot and the GSE15459 datasets of GC. CONCLUSIONOSBPL3 is a novel driver gene stimulating R-Ras/Akt signaling pathway in GC.

E-3015

DAXX acts as a tumor suppressor through histone H3.3/H3K9me3 pathway in pancreatic neuroendocrine tumors

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Frequent somatic mutations and loss of DAXX protein expression have been found in pancreatic neuroendocrine tumors (PanNETs). Although DAXX is known as a transcriptional repressor, molecular functions underlying DAXX loss remain unclear in PanNETs. We evaluated DAXX expression by immunohistochemistry in 44 PanNETs. DAXX-knockdown (KD) and -knockout (KO) PanNET cells were used for the functional analysis. Low DAXX expression was found in 12 cases (27.3%). By microarray and chromatin-immunoprecipitation assays of DAXX-KD/KO, we identified 12 genes as the direct targets of DAXX transcriptional repressor, five of which expression including STC2 were suppressed by DAXX/H3.3/H3K9me3 pathway. DAXX-KD/KO cells enhanced sphere forming activity, but its effect was suppressed by knockdown of STC2. In xenograft models, tumorigenicity and tumor vessel density were increased in DAXX-KO cells with high STC2 expression. Clinically, higher recurrence rate was recognized in PanNETs with low expression of DAXX and high expression of STC2 than others (p=0.018). Our data suggest that DAXX plays as a tumor suppressor and DAXX/H3.3 complex suppresses target genes by promoting H3K9me3 in PanNETs.

▲TOP

E-3016

Haploinsufficiency of SNX13 contributes to leukemogenesis as a responsive gene for monosomy 7 in EVI1 high AML

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Monosomy 7 is frequently accompanied by chromosome 3q26 abnormalities in AML with EVI1 high expression (EVI1hgh AML) and is an additional unfavorable marker for AML. When we precisely analyzed genetic changes of EVI1hgh AML with chromosome 7 abnormalities, sorting nexin-13 (SNX13) was identified as a candidate responsive gene from a region of homozygous deletion in chromosome 7. Reduced expression of SNX13 was observed in most of EVI1high AML by genomic deletions with epigenetic abnormalities. SNX13 is a member of both the regulator of G protein signaling and sorting nexin protein families. When we knockdown SNX13 expression in EVI1high AML cell lines, the growth of leukemia cells increased with enhanced cAMP production through increased phosphorylation of CREB. Since systemic Snx13-null mice were embryonic lethal, we established Snx13-conditional knockout mice. Although hematological abnormalities were not observed in the Snx13-conditional deficient mice, the colony forming capacity was significantly enhanced in Snx13-deficient BM cells with MLL-AF9 expression. We are currently studying the development of leukemogenesis by the SNX13-deficiency with EVI1 high expression.

E-3017

Genetic Predispositions to Sporadic Myeloid Neoplasms Mediated by DDX41 variants

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Germline mutations of DDX41 have recently been implicated in late-onset myeloid neoplasms including AML and MDS. However, the prevalence of different DDX41 variants in Japanese and their risk for AML/MDS have not been fully investigated. To address this, we genotyped 1,609 Japanese patients with AML/MDS and 15,000 controls all from eastern Asia using next-generation sequencing. We identified a total of 3 germline truncating variants in 35 (2.61%) AML/MDS patients, compared to 27 in controls (0.19%). Among these, p.A500fs (OR=14.1; 95%CI: 8.2-24.4) and p.E7X (OR=21.3; 95%CI: 3.9-116) showed a significant enrichment in MDS/AML. In addition, we identified two non-truncating variants, p.Y259C (OR=18.6; 95%CI: 5.5-63.8) and p.S363del (OR=10.6; 95%CI: 3.4-33), which were also significantly enriched in MDS/AML. Overall, 3.9% of AML/MDS patients harbored putative risk alleles with a median age of 60 years, which were more enriched in older patients (>60 y.o.) (P=0.02) and most commonly accompanied by ASXL1 mutations (34%) and somatic DDX41 mutations (32%). In conclusions, germline DDX41 mutations account for a major congenital risk for late onset, sporadic MDS/AML in Japanese population.

▲TOP

[E-3018] E4-2 [English]
Oncogenes and tumor-suppressor genes (2)

2018 / 9 / 29 (Sat) 10:15-11:30 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Ryoji Yao / Dept. Cell Biology., JFCR-Cancer Inst.

E-3018

Potential drug-targetable driver oncogenes resulting from amplification and overexpression in 4,000 solid tumors

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Four years have passed since Shizuoka Cancer Center started the Project HOPE (High-tech Omics-based Patient Evaluation) that features whole exome sequencing (WES) and gene expression profiling (GEP) in fresh surgical specimens and blood samples from cancer patients. Identification of driver genes contributes to the understanding of cancer etiology and is imperative for the development of individualized therapies. Here, we searched potential driver oncogenes as well as drug-targetable genes in approximately 4,000 solid tumors, across more than 15 cancer types. Comprehensive integrative analyses of copy number and gene expression identified genes with amplification-dependent overexpression. Excluding tumor samples harboring known driver mutations with oncogenic and tumor suppressive roles and/or fusion genes, we extracted samples with potential oncogenic driver genes. Furthermore, predicting protein cellular localization of these genes to plasma membrane and extracellular domains identify potential drug-targetable genes. Our in-house multiomics studies provide invaluable information to understanding cancer etiology and development of molecular target drugs in individual tumors.

▲TOP

E-3019

Prohibitin-2 regulates p21 expression induced by depleting gamma-glutamylcyclotransferase in breast cancer cells

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Gamma-glutamylcyclotransferase (GGCT) is highly expressed in various cancer tissues and its knockdown inhibits MCF7 breast cancer cell growth via upregulation of p21. However, the precise mechanism has been unclear. Here we report Prohibitin-2 (PHB2) as a novel interacting protein of GGCT, identified with a yeast two-hybrid screening and co-immunoprecipitation method. We also show that nuclear expression of PHB2 is reduced by GGCT knockdown, and that overexpression of PHB2 inhibits p21 upregulation in MCF7 cells. A ChIP assay showed that nuclear PHB2 proteins bind to the p21 promoter, and that this interaction is eliminated by GGCT knockdown. Furthermore, knockdown of PHB2 alone significantly upregulated p21 and induced cellular events observed when depleting GGCT, including G0/G1 arrest, cellular senescence, and growth inhibition, in a p21-dependent manner. Our results indicate that PHB2 plays an important role in p21 upregulation by GGCT knockdown and may promote deregulated proliferation of cancer cells via p21 suppression.

E-3020

Dissecting the transcription-independent function of RUNX proteins in maintaining genome stability

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The Fanconi Anemia, FA pathway, is a pivotal genome maintenance network that orchestrates the repair of DNA Interstrand crosslinks, ICLs. Independent of its transcriptional activity RUNX functions in regulating FANCD2 chromatin association in the presence of ICL induced DNA damage. Here, we provide biochemical evidence that in the presence of ICL induced DNA damage, RUNX3 is modified by PARP dependent Poly ADP Ribosylation, PARylation. Using in vitro reconstitution assays in a transcription free system and isolation of Proteins Enriched on Nascent DNA ,iPOND technique, we show RUNX binds onto different DNA replication intermediates in the presence of DNA damage. SILAC based mass spectrometric analysis revealed pervasive association of RUNX3 with many DNA repair complexes including PARP1 and BLM helicase and this interaction is required for efficient FANCD2 chromatin localization. RUNX1 mutations in breast cancers were impaired for DNA damage induced PARylation, unveiling an alternative mechanism for FA pathway inactivation in human cancers. Our results reinforce the emerging paradigm that RUNX proteins are tumor suppressors with genome maintenance function.

E-3021

Protein kinase A inhibits tumor mutator APOBEC3B through phosphorylation

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Hematology & Oncol., Kyoto Univ.

APOBEC3B cytidine deaminase (A3B) induces genomic DNA mutations in various types of tumors. Accumulation of APOBEC signature mutations is correlated with a worse prognosis for patients with breast cancer or multiple myeloma, suggesting that A3B activity might be a cause of the unfavorable DNA mutations and clonal evolution in these tumors. Phosphorylation of conserved threonine residues of other cytidine deaminases, activation induced deaminase (AID) and APOBEC3G, inhibits their activity. Here we show that protein kinase A (PKA) physically binds to A3B and phosphorylates Thr214. Phosphomimetic mutants of the Thr214 completely abrogates deaminase activity of A3B both in vitro and in vivo. Molecular dynamics simulation reveals that Thr214 phosphorylation disrupts binding between phosphor-A3B catalytic core and ssDNA. These mutants still inhibit retroviral infectivity at least partially, and also retain full anti-retrotransposition activity. These results imply that PKA-mediated phosphorylation inhibits A3B mutagenic activity without destructing its innate immune functions. Therefore, PKA activation could be a novel therapeutic option for A3B overexpressing tumors.

▲TOP

E-3022

Inverse Control of Transcription Co-Activator Function of YAP and TAZ by Tyrosine Phosphorylation Status of Parafibromin

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YAP and TAZ, the Hippo signal-regulated transcriptional co-activators, play crucial roles in morphogenesis and tumorigenesis. Here we report that the YAP/TAZ activities are stimulated upon complex formation with Parafibromin, which undergoes tyrosine phosphorylation and dephosphorylation by kinases such as PTK6 and phosphatases such as SHP2, respectively. Furthermore, TAZ and the Wnt effector β-catenin interact cooperatively with tyrosine-dephosphorylated Parafibromin, which synergistically stimulates the co-activator functions of TAZ and β-catenin. On the other hand, YAP is selectively activated through binding with tyrosine-phosphorylated Parafibromin, which does not interact with β-catenin and thus cannot co-activate YAP and β-catenin. These findings demonstrate that Parafibromin inversely regulates the activities of YAP and TAZ depending on its tyrosine phosphorylation status, suggesting YAP and TAZ exert their redundant and non-redundant biological actions through mutually exclusive interaction with Parafibromin. The current study may help pinpoint the mechanisms underlying the development of various human disorders, especially malignant diseases.

E-3023

Differential oncogenic activities of alternatively spliced human YAP isoforms

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YAP is a pro-oncogenic transcriptional coactivator regulated by the tumor-suppressive Hippo signaling pathway that plays a key role in cell proliferation and tissue homeostasis. Exon 6 of YAP is an evolutionarily conserved cassette exon, which encodes the γ-segment that disrupts the leucine-zipper motif (LZM). To elucidate the biological role of alternatively spliced YAP, we established NIH3T3 cells that ectopically overexpress a YAP isoform containing or lacking the γ-segment, denoted as YAP γ or YAP α, respectively. Xenograft assay in nude mice revealed that YAP α conferred greater in vivo tumorigenicity on NIH3T3 than YAP γ did. YAP regulates pro-oncogenic SHP2 phosphatase activity via complex formation. We therefore examined the SHP2-YAP interaction and found that YAP γ lost SHP2-binding activity. A LZM-mutated YAP α or TAZ knockdown also diminished the YAP-SHP2 complex, suggesting the interaction requires LZM-mediated YAP/TAZ heterodimerization. We also identified a splicing regulator involved in the alternative splicing of exon 6 in YAP. These results suggest differential biological roles for distinct YAP isoforms, deregulation of which may contribute to oncogenesis via SHP2.

▲TOP

[LS29] LS29 [English]
Uncovering the complexity of RNA with NGS

2018 / 9 / 29 (Sat) 11:50-12:40 Room 6/10F 1004+1005, Osaka International Convention Center Room 6
：QIAGEN K.K.

Toshiyoshi Fujiwara / Gastroenterological Surgery at Okayama University

LS29

Uncovering the complexity of RNA with NGS

Song Tian
QIAGEN Sciences Inc. NGS Assay Technologies III

No Abstract

▲TOP

[E-3059] E4-3 [English]
Oncogenes and tumor-suppressor genes (3)

2018 / 9 / 29 (Sat) 13:40-14:55 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Takashi Tokino / Sapporo Med. Univ., Med. Genome Sci.

E-3059

TMEPAI inhibits Wnt signaling by regulating β-catenin stability and nuclear accumulation

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TMEPAI (Transmembrane prostate androgen-induced protein) is a type I transmembrane protein which is induced by many intracellular signaling pathways such as androgen, TGF-β, EGF, and Wnt signaling. It has been reported that TMEPAI down-regulates TGF-β and androgen signaling and enhances PI3K/AKT signaling. Here, we investigate TMEPAI's function on Wnt signaling. First, we showed that TMEPAI significantly suppressed TOP-flash reporter activities which were induced by Wnt3A, LiCl, and β-catenin overexpression. To understand the mechanism, we addressed the effect of TMEPAI on β-catenin stability and localization. TMEPAI overexpression prevented accumulation of β-catenin in the nucleus and TMEPAI knockout in breast cancer cell lines promoted β-catenin stability and nuclear accumulation. Furthermore, TMEPAI knockout in breast cancer cell lines increased the mRNA level of Wnt target genes, AXIN2 and C-MYC. The presence of TGF-β type I receptor kinase inhibitor did not change the mRNA level of AXIN2 in TMEPAI knockout cells. These data suggested that TMEPAI suppressed Wnt signaling by interfering with β-catenin stability and nuclear translocation in a TGF-β signaling independent manner.

▲TOP

E-3060

Role of Mieap-regulated non-canonical mitophagy in p53 tumor suppression via iron-dependent cell death

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Parkin/Pink1-mediated mitophagy plays a critical role in mitochondrial quality control. Here, we report Mieap-regulated non-canonical mitophagy as a new function of tumor suppressor p53. Mieap was originally identified as a p53-target gene. Overexpression of exogenous Mieap induces large vacuoles in cancer cells. Mieap-induced vacuoles directly eat and degrade cancer mitochondria. UVRAG mediates the Mieap-mediated non-canonical mitophagy. Surprisingly, Mieap-mediated non-canonical mitophagy induces cell death via the iron-dependent production of reactive oxygen species (ROS). Introduction of exogenous Mieap strikingly suppresses in vivo tumor growth of Mieap-deficient colorectal cancer cells. Deficiency of endogenous Mieap promotes cancer development, malignancy, and aggressiveness in colorectal and gastric cancer model mice. The cell death induced by Mieap-regulated non-canonical mitophagy occurs via the ROS generation in in vivo hypoxic tumor microenvironment. These results suggest that Mieap-mediated non-canonical mitophagy plays a critical role in p53 tumor suppression via iron-dependent cell death.

E-3061

Digital MLPA identifies frequent homozygous deletion at CDK2A gene in cultured cells and malignant mesotheliomas

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Co-author：Yoshie Yoshikawa、Masaki Ohmuraya
Hyogo College Med.

We previously reported that sporadic malignant mesothelioma (MM) undergoes gross structural alterations in the region of chromosome 3p21 of tumor genome. Here we performed copy number analysis of MM tumors by digital MLPA (Multiplex Location-dependent Probe Amplification) method that combine conventional MLPA with NGS analytical tools. Copy number of total 230 genes could be simultaneously analyzed. Through digital MLPA analysis, we found genome regions with frequent deletion in MM tumor genomes, such as 3p21, 9p,21 and 22q. Digital MLPA will be useful in genetic diagnosis of otherwise hidden genome abnormalities in critical genes for pathogenesis of MM. CDK2A (p16) gene displayed homozygous deletion in 95% of MM cell lines or MM primary cultures by digital MLPA analysis. However, deletion of this gene was detected in only 33% of tumor tissues. There was a large difference of frequency of deletion among the cultured MMs and tissue specimens in CDKN2A, but not in BAP1. We discuss about the association between the copy-number status of each gene and the prognosis.

E-3062

Regulatory mechanism of p53 transcriptional activity by androgen regulated G3BP2 in prostate cancer

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The androgen receptor (AR) has a central role in prostate cancer progression. Loss of the p53 tumor suppressor contributes to malignancy. We previously identified G3BP2 as a novel AR target involved in p53 signals by integrative sequence analysis (Oncogene, 2017). In the present study, we explored how G3BP2 modulates p53 activity and revealed that RanBP2, SUMO-E3 ligase, and TRIM25 are promising G3BP2-associating proteins in addition to known USP10. Mechanistically, translocation of p53 to cytoplasm was promoted by androgen-dependent sumoylation mediated by RanBP2. TRIM25 was indispensable for this complex formation and translocation of p53 to cytoplasm. Furthermore, G3BP2 expression is regulated at protein level through USP10-mediated inhibition of G3BP2 polyubiquitylation. Tumor/cell growth retardation as well as enhanced p53 activity was observed by TRIM25/G3BP2/USP10 knockdown in line with blocking nuclear export of p53. Clinically, we demonstrated that high levels of USP10/G3BP2/TRIM25 expression were correlated with cytoplasmic p53 in prostate cancer tissues. Thus, G3BP2 has a repressive effect on p53 signaling through systematic interaction with RanBP2/TRIM25/USP10.

▲TOP

E-3063

Novel Wnt/beta-catenin inhibitor Tegavivint attenuates high-risk osteosarcoma by blocking beta-catenin/ALDH1 axis

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Wnt/β-catenin pathway is closely associated with osteosarcoma(OS) development and metastatic progression. In this study, we investigated the antitumor effect of Tegavivint on metastatic OS using PDX models. Tegavivint effectively inhibited cell survival in many types of OS cells in vitro. We next examined in vivo activity of Tegavivint using a pair of PDX models derived from the same OS patient: PDX63 was derived from the untreated primary tumor, and PDX84 was from the metastatic lung lesion after chemotherapy. PDX63 tumors in NSG mice and ALDH1 expression were significantly suppressed by Tegavivint. A lung metastasis model made by i.v. injection of PDX84 tumor-derived cells demonstrated Tegavivint markedly suppressed lung metastases. Furthermore, PDX84-derived cells were sorted into ALDH1-high/low populations. ALDH1-high cells showed higher β-catenin expression and higher sensitivity to Tegavivint in vitro. A lung metastasis model showed significantly more lung lesions in ALDH1-high group, and Tegavivint effectively suppressed ALDH1-high-derived lung metastases. Collectively, Tegavivint has promising therapeutic potential for high risk OS via blockade of β-catenin/ALDH1 axis.

E-3064

AF10 links histone chaperones Supt6h and FACT complex to MLL-fusion leukemia

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The chromosomal translocations between MLL and partner genes generate oncogenic MLL-fusion protein such as MLL-ENL, MLL-AF4 and MLL-AF10, developing AML and ALL by activating the expressions of specific genes such as HOXA9 and MEIS1 genes. AF10, also known as a partner of MLL-fusion, contributes to MLL-ENL leukemogenesis by recruiting epigenetic regulators hDot1L and Tip60 to MLL-fusion target genes as a co-factor for MLL-ENL fusion. Here, we have identified histone chaperones Supt6h and FACT complex as a novel interaction factors with AF10. Although both hDot1L and Tip60 interact with C-terminal region of AF10, Supt6h and FACT complex interact with N-terminal region of AF10. AF10 mutant that could not interact with either Supt6h or FACT complex could not compensate for the defects in cellular immortalization ability caused by AF10 conditional KO in MLL-ENL leukemia cells. These results strongly suggest that Supt6h and FACT complex have critical roles in MLL-ENL-induced leukemia in collaboration with epigenetic regulators via the interaction with AF10.

▲TOP

[E-3065] E14-13 [English]
Pediatric cancer (1)

2018 / 9 / 29 (Sat) 14:55-16:10 Room 6/10F 1004+1005, Osaka International Convention Center Room 6

Junko Takita / Dept. Pediatrics, Univ. Tokyo

E-3065

Single-cell transcriptomic analysis reveals the early separation of neuroblastoma fate in Th-MYCN mice

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The study aim is to understand the enigmatic phenomena of spontaneous regression in neuroblastoma. To this end, we performed single-cell transcriptomic analysis to reveal the early fate determination of neuroblastoma observed in Th-MYCN mice. Survival analyses showed that 80% of the hemizygous mice died of tumor until 20 weeks of age, while 20% of them never developed neuroblastoma. However, all the mice had clusters of neuroblastoma cells until 3 weeks of age. Therefore, although neuroblastoma cells appeared in all cases, whether they developed further into a tumor or disappeared, was likely determined during early age, and latter fate was maybe due to a spontaneous regression-like phenomenon. We obtained single-cell transcriptomes of early neuroblastoma cells from the mice. Within MYCN+ neuroblastoma cells, there were distinct sub-populations and the proportion of cells was different between individuals, suggesting that different fates were captured at the single-cell level. Early-stage subpopulation of neuroblastoma cells can be discriminated by the differential expressions of several genes, which possibly explains the mechanisms of spontaneous regression in neuroblastoma.

▲TOP

E-3066

Genomic characterization of ultra-high-risk neuroblastoma

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Neuroblastoma (NB) is known to exhibit a wide range of clinical behavior from spontaneous regression to chemotherapy resistance. Although recent multidisciplinary treatment for NB improved patient outcomes, there still exists ultra-high-risk subset. To characterize such subgroup of tumors, we statistically analyzed 92 tumors with ultra-high-risk phenotype (died within 2 years) among the 610 NB data obtained by array CGH, target sequencing and methylome analysis. Chi-square test indicated that MYCN amplification, diploidy, high levels of ferritin and LDH, metastasis to bone, bone marrow or liver, and CGH pattern 1p loss and 17q gain were significantly correlated with ultra-high-risk phenotype ($p<0.05$). Among the MYCN-non-amplified tumors (n=41 in 462 NBs), 1p loss was still a powerful indicator ($p=0.018$) and, intriguingly, methylome Cluster-2 (CpG hyper-methylation and enriched with MYCN-amplified tumors) showed strong correlation with their ultra-high-risk phenotype. In addition, several remarkable genome alterations (cf. 12q amplification, chromothripsis and mutations) were observed. These genomic characters will be useful for the early prediction of ultra-high-risk prognosis.

E-3067

Genome-wide mistargeting of oncogenic SWI/SNF complexes in SMARCB1-deficient cancers

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Objective SMARCB1 is a core subunit of the SWI/SNF family of ATP-dependent chromatin remodeling complexes. Loss of SMARCB1 has been identified in several cancer types, strongly implicating this event as the oncogenic driver. However, the precise mechanism underpinning the tumor suppressive function of SMARCB1 remains unclear. MethodSMARCB1-deficient cancer cell lines were modified with a constitutive SMARCB1 expression system by lentiviral infection and their effects were evaluated with respect to global chromatin structure, and gene regulation.ResultsReintroduced SMARCB1 significantly suppressed the proliferation of nearly all SMARCB1-deficient cancer cell lines. SMARCB1 loss destabilizes SWI/SNF complexes on chromatin, with little change in complex assembly or integrity. Rescue of SMARCB1 in SMARCB1-deficient sarcoma cell lines results in increased genome-wide SWI/SNF complex occupancy, facilitating widespread enhancer activation and opposition of Polycomb-mediated repression at bivalent promoters.ConclusionThese results provide us with a fundamental insight into the mechanisms of other SWI/SNF complex associated diseases as well as the oncogenesis of SMARCB1-deficient cancer.

E-3068

Detection of minimal residual disease in high-risk neuroblastoma patients by digital PCR

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To overcome extreme heterogeneity of neuroblastoma, several minimal residual disease (MRD) assays using different sets of MRD markers have been reported. However, it remains to be determined whether these MRD assays detect the same neuroblastoma MRDs or not. In the present study, we determined mRNA expression of 7 MRD markers (CRMP1, DBH, DDC, GAP43, ISL1, PHOX2B, TH) in 420 (307 BM and 113 PB) samples from 23 high-risk neuroblastoma patients by digital PCR (dPCR). BM and PB samples were sampled as frequently as possible during the entire course of treatment. During follow-up period between the completion of induction therapy and the diagnosis of relapse, the total expression of 7 MRD markers was significantly larger in BM from relapsed patients than non-relapsed patients (17 and 56 samples from 7 relapsed and 8 non-relapsed patients, respectively). It also tended to be larger in PB from relapsed patients than non-relapsed patients (4 and 17 samples from 2 relapsed and 6 non-relapsed patients, respectively). These results suggest that our 7 MRD marker expression in BM may represent the relapse-causing MRDs in high-risk neuroblastoma patients.

▲TOP

E-3069

MYCN-mediated purine biosynthesis enhances cancer metabolism via MTHFD2 and PAICS in neuroblastoma

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Amplification of the MYCN is associated with poor prognosis and aggressive neuroblastoma (NB) while the underlying mechanism still remains elusive. Integrating MYCN ChIP-Seq and GEO profiles of NB patients revealed the metabolic enzymes MTHFD2 and PAICS required for one-carbon metabolism and purine biosynthesis were differentially up-regulated. Moreover, MTHFD2 and PAICS were positively correlated with MYCN in both tissue samples from NB patients and cell lines; whereas, the promoter luciferase assay further indicated MTHFD2 and PAICS were the MYCN direct target genes. Dual knockdown of MTHFD2 and PAICS in MYCN-amplified cells significantly reduced cell proliferation, colony formation, migration ability, and DNA synthesis comparing to MTHFD2 or PAICS alone. Targeted metabolomics was also performed to investigate whether MYCN could mediate the folate cycle via MTHFD2, which contributes one-carbon units to enhance purine synthesis, and further regulate nucleotide production by PAICS in response to cancer progression. Combining targeted therapies specifically to MTHFD2 and PAICS showed a synergic effect in MYCN amplified cells which might provide a better treatment for advanced NB.

E-3070

Site-directed DNA damage of the amplified MYCN gene promotes neuroblastoma cell death

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Gene amplification of MYCN is involved in neuroblastoma development, although targeting amplified genomic sequences has been a therapeutic challenge. We have previously developed a novel and specific minor groove-binding inhibitor of MYCN expression, MYCN-A3, using DNA-alkylating pyrrole-imidazole polyamide. Since the site-specific DNA alkylation by MYCN-A3 led to the suppression of MYCN expression and cell death, MYCN-amplified cells could be vulnerable to site-specific DNA breaks within its amplified region of the genome. To test this, we employed the CRISPR/Cas9 system targeting MYCN as a site-specific DNA damage agent. crRNAs targeting MYCN significantly increased the number of apoptotic cells compared with control crRNA in MYCN-amplified cells, while no induction of apoptosis was observed in MYCN non-amplified cells. crRNAs targeting MYCN also demonstrated the knockdown of MYCN expression and the reduced signals from FISH and Southern blot probes for MYCN gene in a similar manner to MYCN-A3 treatment. According to these results, site-specific DNA damage within the amplified MYCN gene is an attractive therapeutic approach for MYCN-amplified neuroblastoma.

▲TOP

[ML19] ML19 [Japanese]
Morning Lectures 19

2018 / 9 / 29 (Sat) 8:00-8:50 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Masakazu Yashiro / Mol. Onc. & Therap., Osaka City Univ.

ML19

Nanomedicines for delivering nucleic acid drugs to cancer

Kazunori Kataoka
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Discussant：Osamu Ogawa
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Engineered polymeric nanosystems with smart functions play a key role in nanomedicine as drug carriers, gene vectors, and imaging probes. This presentation focuses present status and future trends of supramolecular nanosystems self-assembled from designed block copolymers for therapy and non-invasive diagnosis of intractable diseases. Most typical example of such nanosystems is polymeric micelle (PM) with distinctive core-shell architecture. Smart functionalities, such as pH- and/or redox potential responding properties, can be integrated into the PM structure. These smart PMs loaded with various chemotherapy reagents as well as oligonucletotides, including ASO and siRNA, were evidenced to have a significant utility in the treatment of intractable and metastatic cancers, such as pancreatic cancer, glioblastoma, and tumors harboring recalcitrant cancer stem cells (CSCs).

▲TOP

[E-3024] E12-4 [English]
Antitumor effector cells and their induction

2018 / 9 / 29 (Sat) 9:00-10:15 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Hitoshi Kiyoi / Dept. Hematol. & Oncol. Nagoya Univ., Sch. Med.

E-3024

Establishment of T-cell receptor-engineered T cells: Implications for head and neck squamous carcinoma

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To establish effective adoptive T cell immunotherapy, we attempted an approach to rapidly identify neoantigen-specific T cell receptors (TCRs) and establish T-cell receptor-engineered (TCR-engineered) T cells. Through whole exome and transcriptome analysis from 20 head and neck cancers, we selected 64 potential neoantigen peptides and applied them to induce of neoantigen-reactive cytotoxic T cells *in vitro* using patient-derived dendritic cells, peripheral blood and/or expanded TILs isolated from corresponding tumors. Neoantigen-specific T cells were screened by 4-1BB expression levels as well as ELISPOT assay, and TCR sequences were determined using isolated T cell clones. We then cloned TCR cDNAs into T lymphocytes and generated the neoantigen-reactive TCR-engineered T cells. We have so far confirmed 11 neoantigen-reactive TCR&alpha/TCR&beta pairs and established 6 TCR-engineered T cells which showed HLA-restricted neoantigen-reactive cytotoxic activity *in vitro*. Our approach is effective and requires only 3 months from cancer tissues to generate neoantigen-specific T cells and establish TCR-engineered T cells and should be applicable in clinical settings.

▲TOP

E-3025

Myeloid-restricted ablation of Shp2 restrains melanoma growth by amplifying the promotion of CXCL9 and IFN-&gamma

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Y Guo*,P Xiao*, H Zhang, Z Xu, X Zhang, H Cheng, Q Cao, Y Ke.
*These authors contributed equally to this work.
The Src homology 2 domain-containing protein tyrosine phosphatase 2 (Shp2) is generally considered to be an oncogene owing to its ability in enhancing the malignancy of multiple types of tumor cells; however, its role in modulating tumor immunity remains largely elusive. Here, we reported that myeloid-restricted ablation of Shp2 suppressed melanoma growth and the formation of lung premetastatic niche. Mechanistically, loss of Shp2 potentiates macrophage production of CXCL9 in response to IFN-&gamma and tumor cell-derived cytokines, thereby facilitating the tumor infiltration of IFN-&gamma-producing T cells that could in turn support CXCL9 production within tumor microenvironment. Collectively, our findings highlight a causative role of myeloid Shp2 in dampening T cell-mediated antitumor immunity by restraining the macrophage/CXCL9-T cell/IFN-&gamma feedback loop. Thus, targeting macrophage Shp2 may help to create a Th1-dominant tumor immune microenvironment.

E-3026

Deoxy-hexose-rich N-glycan induces hyper-active anti-tumor T cell differentiation

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Adaptive T cell immunotherapy has achieved significant progress. However, major barriers are insufficient induction of cytotoxic T cells and exhaustion of tumor-infiltrating lymphocytes. We discovered a new role for 2-deoxy-glucose (2DG) in enhancing anti-tumor T cell cytotoxicity and preventing T cells from binding to galectin-3, one of potent tumor-antigens for T cell anergy. CD8+ T cells treated with 2DG also expressed NK surface markers with more perforin/granzyme. Together with quantitative glycomics showing deoxy-hexose rich N-glycan, 2DG effects on T cells were alleviated by D-mannose, the sugar involved in N-glycosylation, suggesting that the deoxy-hexose rich N-glycan plays a role in 2DG-mediated enhancement of anti-tumor T cell immunity. Moreover, T cells treated with 2DG restricted tumor growth and prolonged the survival of tumor-bearing mice. Besides that decreasing glycolytic flux by 2DG drives CD8+ T cells toward long-lived memory CD8+ T cells, our results demonstrated that modified N-glycosylation of tumor-reactive T cells by 2DG can improve the efficacy of T cell-based immunotherapies against cancer.

E-3027

Effect of abiraterone therapy on anti-tumor immunity in a mouse Pten-deficient prostate cancer model

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Androgen deprivation therapies (ADTs) can influence tumor immune responses via androgen receptor (AR) regulation. Abiraterone (Abi) is a steroidal CYP17 inhibitor approved for the treatment of late-stage advanced prostate cancer. Here, we use a mouse Pten-deficient prostate cancer model to study the antitumor activity of Abi and its influence on tumor immunity. Treatment with Abi (for 8 weeks) reduced prostate tumor burden by 30.5% (P=0.0275). Downregulation of classical mouse Ar-responsive genes confirmed reduced transcriptional activity of AR. qRT-PCR-based analysis of a panel of 54 immune-responsive genes, revealed distinct expression signatures in Abi-treated tumors compared to orchidectomized (Orc) mice. Abi-treated mice demonstrated reduced mRNA levels of the T regulatory cell gene markers Cd4, Foxp3, Ctla4, Tgfb1 and Il10 and immune checkpoint genes Cd274, Pdcd1lg2, Pdcd1 and Ctla4. A 1.8-fold increase of tumor infiltrating granzyme B-positive cells was seen cells in Abi-treated tumors versus Orc. Our findings highlight the complexity of immune modulation by AR and shed light into differences between complete androgen withdrawal and androgen synthesis inhibition.

▲TOP

E-3028

The therapeutic efficacy of new cancer vaccine using NY-ESO-1 expressing artificial adjuvant vector cells

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DCs play a key role of linking innate and adaptive immunity. We have been developing the new type of vaccine system targeting in vivo DCs, artificial adjuvant vector cell aAVC for one decade. This vaccine is composed of CD1d+ cells that were loaded with NKT ligand and transfected with antigen encoding mRNA, thus acting as a cellular adjuvant with delivery of antigen to endogenous DCs in vivo. The aAVC vaccine has three key components of successful vaccine. First, it can link innate and adaptive immunity efficiently by harnessing cross-presenting DCs to tumor-specific CTL systemically. Next, even a single dose of injection of aAVC can generates robust and long lived memory CTL. Furthermore, it can overcome the local immunosuppressive circuit in the tumor therapeutic models by recruiting a number of tumor specific CTL into tumor sites prominently. NY-ESO-1 is one of the representative tumor cancer testis antigens and expressed on several types of cancers. We recently developed the NY-ESO-1 expressing aAVC, aAVC-NY-ESO-1 and investigated its immune response and anti-tumor effect in murine model. We will present the evidence of the efficacy of aAVC-NY-ESO-1.

E-3029

ERK activation in NK cells is required for killing the target cells

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NK cells are presumed to be key effectors in cancer immunosurveillance, and now widely employed in adoptive transfer therapy. However, it is still unknown what is sufficient to kill cancer cells. To address this question, we performed live cell imaging of NK cells in the context of its cytotoxicity. When NK cells bound to mouse melanoma B16 cells, calcium surges were sometimes, but not always, observed in B16 cells. Importantly, the calcium surges were always followed by apoptosis. This result suggested that NK cells may be heterogeneous and require an additional signal input for their cytotoxicity. To further explore this question, we examined the activity of extracellular signal-regulated kinase (ERK), which plays a pivotal role in cytotoxic activity of NK cells. We used mouse NK cells expressing ERK biosensor based on the principle of Forster resonance energy transfer (FRET). Interestingly, NK cells that induced apoptosis of target cells exhibited stronger ERK activation than did the other NK cells. These observations imply that the strength of ERK activation in NK cells determines the fate of the target cells.

▲TOP

[E-3030] E12-5 [English]
Antitumor effector cells and their inhibition

2018 / 9 / 29 (Sat) 10:15-11:30 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Yoshiki Akatsuka / Dept. Immunol/Cell. Immunol, Nagoya Univ. Grad. Sch. Med.

E-3030

Novel myeloid-derived adherent cells promote immunosuppressive tumor microenvironment

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Myeloid-derived suppressor cells (MDSCs) are one of the key drivers of immunosuppressive tumor microenvironment that is a hallmark of cancer. Recently we isolated strongly adherent cells from $CD45^+$ myeloid-derived cells from subcutaneous tumors of Lewis lung carcinoma (LLC) cells. The adherent phenotype is a unique property of macrophages but they did not express macrophage marker F4/80. Analysis of myeloid-derived marker gene expression revealed that these cells are novel MDSC subpopulations and were named MDSC-like adherent cells (MLAC). Both MLACs and MDSCs significantly promoted tumor growth when they were subcutaneously injected with LLC cells to syngeneic B6 mice. Co-transplantation of MLAC with cancer cells significantly increased tumor-infiltration of MDSCs, resulting in an increase in TAM and a decrease in activated T lymphocytes. In vitro migration assay revealed that MLACs attract MDSCs by secretion of CCL2 and CCL3. Since MLACs were constantly present in tumors regardless of size and type of tumor, MLACs may infiltrate the early stage of tumorigenesis and initiate immunosuppressive tumor microenvironment by promoting recruitment of MDSCs.

▲TOP

E-3031

Cancer-associated fibroblasts affect the intra-tumoral infiltration of CD8+ and FoxP3+ T cells via IL-6

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Cancer-associated fibroblasts (CAFs) have been considered to have a central role for tumor progression in tumor microenvironment. We identified that intra-tumoral CD8+ and FoxP3+ tumor-infiltrating lymphocytes (TILs) are independent prognostic factors in esophageal cancer tissue of clinical samples. In addition, CAFs had a significant correlation of those TILs in intra-tumoral, but not peri-tumoral sites, so that CAFs regulated the infiltration of CD8+ and FoxP3+ TILs into intra-tumor tissues. In vitro, CAFs activated by cancer cells secreted high levels of Interleukin 6 (IL-6). In vivo, CAFs accelerated tumor growth obviously in immune-competent mice, along with phenotypic change in T cell populations, decreased CD8+ and increased FoxP3+ TILs. Moreover, the accelerated tumor growth in CAFs co-cultures was significantly reduced by IL-6 blockade, demonstrating that immunosuppressive TILs population was improved. Thus, CAFs may be one of biomarkers as immunosuppressive status in intra-tumoral site and IL-6 blockade, or targeting CAFs, may improve pre-existing tumor immunity and enhance the efficacy of conventional immunotherapies.

E-3032

An upstream RUNX3 enhancer regulates the development of gut-associated anti-tumorigenic CD8+ cytotoxic T lymphocytes

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The RUNX3 gene is frequently involved in a variety of cancers and immunological diseases. Despite such widespread association with human diseases, the transcriptional regulation of RUNX3 remains elusive. Here we report the identification of an enhancer for Runx3, eR3, by employing a combination of in silico prediction and in vivo verification using zebrafish and mouse models. eR3 is active in CD8+CD103 (integrin αE)+ cytotoxic T lymphocytes (CTLs) that reside in the intestinal epithelium via their interaction with the CD103 ligand, E-cadherin, on epithelial cells. Removal of eR3 specifically in CD8 T cells compromised the suppression of tumorigenesis in murine cancer models. In human, single nucleotide polymorphisms (SNPs) in eR3 were overrepresented and were associated with weakened CTL activity in colorectal cancer patients. Together, our results indicate that eR3 plays a role in immune surveillance against gut-associated tumors by upregulating RUNX3 expression in specific CTLs.

E-3033

Resistance of CD44+ subpopulation to CTL though high production a protease inhibitor in colorectal cancer

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Colorectal carcinoma (CRC) is relatively resistant to immunotherapies. We screened 30 CD (cluster of differentiation) antigens which are heterogeneously expressed in human CRC cell lines, and found that $CD44^+$ subpopulation were relatively resistant to CTL lysis. cDNA microarray analysis showed that the $CD44^+$ cells expressed protease inhibitor X (PI-X) higher than $CD44^-$ cells. PI-X showed the highest expression in CRC among17 human cancer tissues in meta-analysis using open-access gene expression data. The PI-X overexpression or knockdown experiments demonstrated that PI-X inhibited in vitro CTL lysis of CRC cell lines, and PI-X overexpressed murine cancer cell lines were resistant to anti PD-1 Ab therapies. Immunohistochemical study and TCGA RNA-seq data showed the best prognosis of the patients with low PI-X expression and high $CD8^+$ T cell infiltration. These results indicate that CD44 and PI-X may be potential biomarkers for prognosis and responses of CRC patients to cancer therapies including PD-1 blockade, and also be attractive therapeutic targets for combination immunotherapies.

▲TOP

E-3034

Crucial role for CD69 in anti-tumor immunity

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The introduction of immune checkpoint inhibitors in cancer treatment highlights the negative-regulation of anti-tumor immunity such as effector T cell exhaustion in the tumor microenvironment. However, the mechanisms underlying the induction and prevention of T cell exhaustion remain largely unknown. We show herein that CD69, a type II glycoprotein known to regulate inflammation through T cell migration and retention in tissues, plays an important role in induction of exhaustion of tumor infiltrating T cells. $Cd69^{-/-}$ mice showed reduced tumor growth and metastasis in a 4T1-luc2 murine breast cancer model, in which increased numbers of tumor infiltrating lymphocytes, less T cell exhaustion and enhanced IFN γ production were observed. Anti-CD69 mAb treatment of tumor-bearing mice attenuated T cell exhaustion and tumor progression. Thus, this study demonstrates a novel role for CD69 to control tumor immune escape mediated by T cell exhaustion, and indicates CD69 as a novel target for cancer immunotherapy.

E-3035

Immune suppressive mechanism of corticosteroids used for immune-related adverse events

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Immune checkpoint blockade therapy augments tumor-specific T-cell responses. Yet, a fraction of the treated patients experiences irAE, suggesting that normal tissue-specific immune responses are accompanied with antitumor immune responses. As a consequence, patients need to receive immuno-suppressive drugs, such as corticosteroids, to control the adverse reactions, though they may impair antitumor effects of immune checkpoint blockade. Here, we investigate the impact of corticosteroids on antitumor immune responses elicited by immune checkpoint blockade such as anti-CTLA-4 mAb. Corticosteroids decreased low-, but not high-affinity memory T cells specific to self-tumor antigens via suppressing fatty acid metabolism essential for memory T-cell survival by nuclear translocation of phosphorylated glucocorticoid receptors. Overall survival was significantly shorter in anti-CTLA-4 mAb-treated patients receiving early corticosteroids or whose tumor mutation burden was low. Together, we propose a novel T-cell suppressive mechanism by corticosteroids that inhibit self-antigen, but not non-selfantigen-specific memory T cells, requiring the proper use of corticosteroids.

▲TOP

[LS30] LS30 [Japanese]
CAT (Cancer-associated thrombosis)

2018 / 9 / 29 (Sat) 11:50-12:40 Room 7/10F 1006+1007, Osaka International Convention Center Room 7
：Daiichi Sankyo Co.,Ltd.

Shoji Natsugoe / Department of Digestive Surgery, Breast and Thyroid Surgery, Kagoshima University Graduate School of Medicine

LS30

1) Cancer Therapeutics-Related Cardiovascular Dysfunction

Taro Shiga
Department of Onco-Cardiology

No Abstract

LS30

2) Cancer-associated thromboembolism- Real World Data in Japanese gastrointestinal cancer patients receiving chemotherapy -

Michio Nakamura
Department of Gastroenterology, Sapporo City General Hospital

No Abstract

▲TOP

[J-3049] J15-3 [Japanese]
Liquid biopsy and pathology

2018 / 9 / 29 (Sat) 13:40-14:55 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Akashi Ooi / Dept. Mol. Cell. Pathol., Kanazawa Univ.

J-3049

Development of a three-dimensional deformable microfilter with with a DNA aptamer for capturing cancer cells

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Background:Tumor recurrence is often observed even after a curative resection, suggesting that circulating tumor cell (CTC) exist at the time of operation. Although detection of CTC is challenging, optimal methodology remains unclear. To capture cancer cells effectively and inexpensively, we developed a three-dimensional deformable microfilter with a DNA aptamer. Methods:A deformable microfilter modified the DNA aptamer is fabricated by photolithography. We prepared a DNA aptamer for EpCAM and scrambled DNA. The cell suspension is passed through the microfilter by pump. Captured cells are fixed by paraformaldehyde and observed and counted by fluorescence microscope. Results:The cancer cells (breast and stomach) were effectively captured on the aptamer-modified substrate (42, 21 and 53 cells/mm2), whereas few normal cell (HEK) was observed. To evaluate the specificity using the cell suspension with several mixing ratio between cancer and normal cell, cancer cells successfully were captured, depending on mixing ratio.Conclusions:We developed a novel devise to capture the cancer cell effectively and inexpensively. Further clinical study is required to be adapted to clinical setting.

▲TOP

J-3050

Single-cell separation of cancer cells from a cell microwellarray chip using a nano tweezers

Kazuaki Kajimoto
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Circulating tumor cells (CTCs) are cells that have shed into the bloodstream from a primary tumor. Thus, counting of CTCs in the bloodstream has attracted a broad interest as potential markers of tumor progression, metastasis and treatment response. In the previous study, we have developed a cell microwellarray chip for rapid and quantitative detection of CTCs, and succeeded to single cell detection of cancer cell spiked in 1,800,000 leukocytes. However, the lack of functional characterization of the detected cells on a microwellarray chip is the next fundamental issue to be solved. In the current study, we established a powerful technique for single-cell isolation of cancer cells from a cell microwellarray chip using a nano tweezers fabricated by using the Micro Electro Mechanical System (MEMS) technology. Furthermore, a single-cell mRNA analysis using whole cDNA amplification coupled with qPCR could quantitatively assess the expression of several marker genes such as EpCAM and cytokeratin. Taken together, our system would be an effective tool to understand metastatic mechanism through single-cell analysis of CTCs. This work was performed in collaboration with AOI Electronics.

J-3051

A novel method to capture circulating tumor cell using 4 antibodies

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Background: The presence of circulating tumor cells (CTCs) in patients with metastatic carcinoma is associated with short survival. However, the yield of CTCs is too small to permit molecular biological analysis. Therfore, to detect mutation, circulating tumor DNA (ctDNA) is more favorable compared with CTCs. However, mRNA or protein, which cannot be analyzed using ctDNA, can be analyzed using CTCs. In this study, we tried to extract CTCs using multi-antibodies. Methods: Ten patients with colorectal cancer were included. Ten mL whole blood was collected. CTCs were captured by Ion Torrent Liquid Biopsy Instrument. We used 3 antibodies (EpCAM, HER2 and Trop2) in Method 1 and 4 antibodies in Method 2 (EpCAM, HER2, Trop2 and EGFR). We defined cytokeratin positive, DAPI positive and CD45 negative cells as CTCs. Results: CTCs were detected in all patients. The median amount of collected CTCs was 27 cells in Method 1 and 33 cells in Method 2. There was no statistical difference but the amount in Method 2 was intended to be larger. Conclusion: We were able to capture a large number of CTCs, by the novel method using multiple antibodies.

J-3052

Artificial intelligence-based colorectal cancer screening using urinary polyamines

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Development of minimally or non-invasive screening tests is necessary for efficient screening of colorectal cancers (CRC). Urinary polyamines have been reported as potential markers to detect CRC. Here, we utilized liquid chromatography triple quadrupole mass spectrometry to profile seven kinds of polyamines, such as spermine and spermidine with their acetylated forms. Totally 242 urinary samples from CRCs and non-CRCs were analyzed. First, we evaluated the reproducibility of quantified concentrations, acquired by collecting three times on three days each from each healthy control, and confirmed the stability of the observed quantified values. Second, in comparison of three groups, N_1,N_{12}-diacetylspermine showed the highest area under the receiver operating characteristic curve (AUC), 0.794 (95% CI: 0.704-0.885, $p<0.0001$), to differentiate CRC from the benign and healthy controls. Alternative decision tree (ADTree) combined polyamine concentrations, yielded a higher AUC value of 0.961 (95% CI: 0.937-0.984, $p<0.0001$). The generalization ability of the models was confirmed computationally. The demonstrated method showed potential as a screening tool of CRC.

▲TOP

J-3053

An experimental study of new technique based on a filter method using non-woven silica fiber sheets for liquid cytology

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When liquid specimens are used for cytology, cell components are collected mainly by using the centrifugation method or the filter method. Silica fiber non-woven fabrics (Cellbed) used as 3D culture scaffolds have been found to be useful as a cell collection filter. Furthermore, Cellbed can be made transparent by using a mounting-media with a refractive index similar to that of silica fibers. We used a thickness adjusted to approximately 72% of that of the commercially available one in this study. We manufactured liquid samples experimentally by using gastric cancer cell lines (Kato Ⅲ and SNU-1), and evaluated the stainability using a staining kit of Cellbed. Floating gastric cancer cells were fixed with the solution (Muto Pure Chemicals, Co., Ltd.), after which the cells were stained using Papanicolaou's staining. Both samples using Kato Ⅲ and SNU-1 cells were clearly visible. In addition, immunostaining using the these cells was feasible. While cell fixation and staining conditions will need to be further examined using human material, our findings suggested that the application of the use of Cellbed in the cytology of fluid samples could be greatly promising in the future.

J-3054

Correlation between tumor heterogeneity and cfDNA

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Introduction: The circulating cell-free DNA (cfDNA) contains tumor-derived DNA in malignant patients. We hypothesized that cfDNA has a great potency to alternate the whole state and heterogeneity of malignant cancer. Methods: The patients with primary lung cancer who approved post-mortem autopsy, with stocked blood sample within one month before the patient death were included our study. The gene alterations detected from each lesion including metastases using next generation sequencing (NGS) were compared with those derived from cfDNA. Results: The seven patients were enrolled. The mean number that was detectable gene alterations from per patient is 253 (range 99-1918) from all tumor DNA samples and 218 (range 56-362) from cfDNA sample, respectively. The truncal gene alterations that deeply involved in carcinogenesis could be apprehended to analyze from cfDNA. Furthermore, the gene alterations detected in cfDNA tended to be high variant allele frequency in tumor DNA. Conclusions: cfDNA have a great potency because it includes the essential gene alterations which were characterized as the broad range among the tumor extent and the deep variant frequency in certain lesion.

▲TOP

[J-3055] J4-3 [Japanese]
Novel oncogenes / tumor suppressor genes (2)

2018 / 9 / 29 (Sat) 14:55-16:10 Room 7/10F 1006+1007, Osaka International Convention Center Room 7

Masatoshi Kitagawa / Dept. Mol. Biol. Hamamatsu Univ. Sch. Med.

J-3055

Homeobox C10 correlates with the malignant phenotype of gastric cancer and its recurrence and poor survival

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Background:The detection of molecules affecting the malignant phenotype of gastric cancer (GC) may contribute to identification of biomarkers, and such molecules may become targets of therapy. We performed transcriptome analysis using surgically resected GC tissues with synchronous metastasis and identified HOXC10 as the most highly expressed gene. Methods:We established stable HOXC10 knockout (KO) GC cell lines using CRISPER-Cas9 system. Cell functions, apoptosis and cell cycle were analyzed. Xenograft subcutaneous tumor model analysis was also conducted. Association with clinicopathological parameters, prognosis and HOXC10 expression levels were examined. Results:HOXC10 knockout significantly suppressed proliferation by increasing apoptosis and reducing the migration and invasiveness. Mouse xenograft models revealed that the tumorigenicity of HOXC10 knockout cells was attenuated. The high expression levels of HOXC10 were significantly associated with hepatic and peritoneal recurrence as well as worse prognosis. Conclusion:The expression levels of HOXC10 may therefore serve as a prognostic biomarker and the products of HOXC10 expression may provide targets of therapy.

▲TOP

J-3056

Pattern-specific Transcriptomics Identifies ASGR2 as a Predictor of Hematogenous Recurrence of Gastric Cancer

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Hematogenous recurrence is a challenging clinical finding that often leads to fatalities of patients with gastric cancer. Here, transcriptome and bioinformatics analyses were conducted to uncover candidate molecules differentially expressed in patients with hematogenous recurrence of gastric cancer. One potential candidate identified was asialoglycoprotein receptor 2 (ASGR2) and small interfering RNA (siRNA) experiments were conducted to determine the effect of manipulating ASGR2 expression has on cell phenotypes. ASGR2 mRNA expression analysis using quantitative real-time reverse-transcription PCR was conducted with stage Ⅱ/Ⅲ gastric cancer clinical specimens (n=95). Transcript levels were increased in gastric cancer cells compared with a control non-tumorigenic epithelial cell line. Knockdown of ASGR2 decreased the adhesion and migration potential. Thus, while gastric cancer cell invasive activity was significantly decreased by knockdown, forced expression of ASGR2 promoted invasive activity. Finally, patients with high ASGR2 expression were more likely to have a high cumulative rate of hematogenous recurrence but not peritoneal or nodal recurrence.

J-3057

Functional analysis of a novel tumor suppressor candidate gene in colorectal cancer

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Background: We have shown the evidence that DEAH (Asp-Glu-Ala-His) box helicase 15 (Dhx15) acts as a tumor suppressor gene in glioma by dysregulating NF- κ B pathway and splicing machinery. Transposon-based insertional mutagenesis identified Dhx15 as a candidate tumor suppressor gene in colorectal cancer.Results: We examined the effects of DHX15 overexpression in colorectal cancer cell lines (SW480, HCT-116). Retroviral-mediated transduction of DHX15 into colorectal cancer cell lines suppressed their proliferation and foci formation in vitro. Dhx15 overexpression resulted in suppressed proliferation of the colorectal cancer cell lines, supporting that DHX15 is a tumor suppressor. Moreover, Actin expression was revealed using Phalloidin antibody by immunostaining. By immunostaining of Phalloidin, overexpression of DHX15 significantly promoted the abnormal cell ratio increasing compared with that in control colorectal cancer cells. These data suggest that DHX15 may induce cancer cell to apoptosis or necrosis. Conclusions: Taken together, these findings provide evidence that DHX15 is potentially a tumor suppressor in colorectal cancer like glioma.

J-3058

FRAS1 expression reflects the malignancy potential of gastric cancer

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【Background】Identification of recurrence-associated biomarkers is necessary to improve the management of gastric cancer (GC). The recurrent pattern specific transcriptome analysis was performed and fraser extracellular matrix complex subunit 1 (FRAS1) was identified as a hepatic recurrence specific molecule.【Methods】We established FRAS1 knockout (KO) GC cell lines by a CRISPER-Cas9 mediated genome editing. Cell functions, apoptosis and cell cycle were analyzed. In vivo xenograft subcutaneous tumor model analysis was also conducted. FRAS1 expression levels of resected GC clinical samples were determined and association with clinicopathological parameters were examined.【Results】Cell functions of FRAS1 KO GC cell line were decreased compared to parent cell line. Apoptosis and cell cycle aberration were detected in FRAS1 KO GC cell line. Proliferation of FRAS1 KO GC cell line was also suppressed in vivo assay. Analysis of clinical sample revealed that the high expression of FRAS1 in GC tissue was associated higher cumulative recurrence of liver metastasis.【Conclusion】FRAS1 is associated with malignancy potential of GC cells and hepatic recurrences after curative surgery.

▲TOP

J-3059

Oncogenic Runx3 downregulates C/ebp α in Osteosarcomagenesis

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Osteosarcoma (OS) is the most common primary malignant tumors of bone and inactivation of p53 has been frequently reported in sporadic OS in human. In the absence of p53, oncogenic Runx3 was found to upregulate a series of potent oncogenes indispensable for osteosarcomagenesis. In this study, we report that Runx3 downregulates C/ebp α in human and mouse OS cells. C/ebp α has a tumor suppressive function to bring about cell-growth arrest through a direct inhibition of Cyclin/Cdk activity as reported recently. In OS or mesenchymal stem cells, disruption of C/ebp α resulted in acceleration of Rb-phosphorylation, conversely, knockdown of Runx3 upregulated C/ebp α , resulted in inhibition of Rb-phosphorylation. An osteoblast-specific p53-knockout mouse line, Sp7/Osx-Cre; $p53^{f/f}$ mouse (OS mouse) is widely used as an animal model of human OS. Osteoblast-specific disruption of C/ebp α (Sp7/Osx-Cre; $p53^{f/f}$C/ebp $\alpha^{f/f}$) promoted tumorigenicity of the OS mice showing increased Rb-phosphorylation. These results show that oncogenic Runx3 promotes Rb-phosphorylation via downregulation of C/ebp α to promote p53-dificient osteosarcomagenesis.

J-3060

OEGC1 identified by in silico analysis is a novel tumor-promoting gene

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Gastric Cancer (GC) is a leading cause of global cancer mortality, with high incidence rates in Asia including Japan. Although recent studies have provided valuable insights into identification of major driver genes, there are still little therapeutic targets of GC. Here, we previously reported a novel driver gene, OverExpressed in Gastric Cancer 1 (OEGC1), identified by analysis of gene expression and relevant clinical data in TCGA and microarray datasets. OEGC1 is expressed especially in intestinal type GC. Log-rank survival and cox proportional hazards regression analysis indicated that OEGC1 high expressed intestinal type GC had an equally poor prognosis as diffuse type. By silencing endogenous OEGC1 using siRNAs, cell proliferation and cell migration were significantly suppressed in NUGC3 and AGS cells with relatively higher OEGC1 expression. Western-blotting analysis showed p21 and p27 protein expression were up-regulated, and SKP2 and YAP1 protein was down-regulated by knockdown of OEGC1. Our findings suggested that aberrant expression of OEGC1 might play an important role in tumorigenesis of GC, and that OEGC1 might be a possible therapeutic target in GC.

▲TOP

[IAL] IAL [English]
JCA International Award Lecture

2018 / 9 / 29 (Sat) 8:00-8:50 Room 8/10F 1008, Osaka International Convention Center Room 8

Yasuhito Yuasa / URA Div., Tokyo Med. & Dent. Univ.

IAL

Histone modifications in controlling genome stability and cancer cell survival

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Histone modifications control a wide spectrum of cellular functions and have been demonstrated to be extensively dysregulated in cancer cells. Our group has a long-standing interest in exploring the roles of histone modifications and their enzymes in regulating DNA damage repair, genome stability and cell survival in cancer. Previously, we found that histone deacetylase SIRT2 regulates autophagy through deacetylation of FoxO1. We also identified the roles of SIRT1, SIRT6 and SIRT7 as critical regulators of transcription, gluconeogenesis and cancer chemosensitivity. Besides, we found that histone ubiquitination is a functional link between autophagy and DNA damage. In addition, we identified a set of substrates of lysine methyltransferase (KMT) Set7/9, including SIRT1, SUV39H1 and beta-catenin. Moreover, we identified that G9a and GLP, as KMTs in regulating H3K9 methylation and gene expession, function differently in DNA repair. Recently, we reported that linker histone and its modifications are also critical regulators of genome stability. Together, our reports established a regulatory network of histone modifications in controlling DNA repair, genome stability and cell survival.

▲TOP

[J-3001] J7-1 [Japanese]
Genomic analysis in gastroenterological disease

2018 / 9 / 29 (Sat) 9:00-10:15 Room 8/10F 1008, Osaka International Convention Center Room 8

Kohichiroh Yasui / Dept. Gastroenterol. Hepatol., Kyoto. Pref. Univ. Med.

J-3001

Diffuse-type gastric cancers are classified into two clusters, which may be formed via different carcinogenic pathways

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We have found that patient-derived xenograft (PDX) cells in primary culture are tumor-initiating cells, and that they are more resistant to anti-tumor drugs than established cell lines. We identified genes that are differentially expressed between these two groups. Using the gene list, hierarchical cluster analysis was performed on primary diffuse-type GCs from TCGA and diffuse-type GC-initiating cells. We found that diffuse-type GCs are classified into two clusters, and that the number of mutations in primary diffuse-type GCs in cluster II (DGC-II) is significantly greater than that in cluster I (DGC-I). The number of mutations in intestinal-type GCs (IGC) was also significantly greater than that in DGC-I, and was similar to that in DGC-II. This indicates that some DGC-II cells may develop from IGC cells, but that DGC-I cells may develop directly from normal gastric epithelial cells. Similar results were obtained by four subtype (EBV, MSI, CIN and GS) analysis of diffuse- and intestinal-type GCs. We thus conclude that diffuse-type GCs may be formed via two carcinogenic pathways; some directly from normal gastric epithelial cells, and some indirectly from intestinal-type GCs.

▲TOP

J-3002

The oncogenic potential of regenerative nodules in cirrhotic liver confirmed by total transcriptome analysis

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Background: Hepatocellular carcinoma (HCC) recurrently develops in the cirrhotic liver. While liver cirrhosis (LC) tissue is composed of numerous regenerative nodules (RNs), their biologic basis for hepatocarcinogenesis remains unclear. Thus, we investigated the oncogenic potential of RNs using total transcriptome analysis.Method: Of 97 recipients who underwent living donor liver transplantation from 2014 to 2015, we selected 10 cases whose fresh liver samples were available. Four HCCs and 15 RNs were manually collected from the explanted liver sections and they were subjected to RNA sequencing together with 6 control normal liver samples.Results: A total of 520 genes were differentially expressed between RNs and normal livers. In RNs, gene sets of cytokine-response or enzymatic antioxidants were enriched and some oncogenic pathways were activated. In contrast, 25 oncogenes including TERT were elevated in only HCCs, but not in RNs and normal livers.Conclusion: RNs in LC tissue possess pre-malignant potential despite they are not neoplastic cell population. These findings give us a clue to a better understanding of the multistep hepatocarcinogenesis of the cirrhotic liver.

J-3003

Genetic Analysis of Pancreatic Neuroendocrine Neoplasms Grade 3

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Pancreatic neuroendocrine neoplasm grade 3 (PanNEN G3) is a rare subtype of PanNEN, which is further divided into well-differentiated neuroendocrine tumor G3 (PanNET G3) and poorly differentiated neuroendocrine carcinoma G3 (PanNEC G3). To understand the genetic basis of PanNEN G3, we performed whole exome sequencing of paired tumor/normal DNA from 25 PanNEN G3 patients. In total, 1688 somatic mutations were detected with a median of 45 (range: 21-450)/sample. Combined with copy number changes, most frequently altered genes were TP53 (38%), SMAD4 (29%), KRAS (25%), and MEN1 (25%). Frequently mutated in PanNET grade 1 and 2 (G1/2), MEN1 and DAXX mutations were associated with well-differentiated morphology (n=4/4), while all tumors without these mutations exhibited poorly differentiated histology (n=6/6). Both mutations frequently co-occurred with mutations in two mTOR pathway genes, NF1 and TSC2 (71%), suggesting that some PanNET G3 might evolve from PanNET G1/2 by acquiring mutations that activate the mTOR pathway. Our results help understand a molecular basis of PanNEN G3, contributing to a better classification of PanNET G3 and PanNEC G3.

J-3004

Genetic and epigenetic analyses of colorectal tumors in a patient with the loss of polymerase proofreading

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Polymerase Proofreading-Associated Polyposis (PPAP) is a hereditary disease associated with multiple polyps in the colon and tumors in other organs such as endometrium. Germline mutations in the exonuclease domains of DNA polymerases, POLE and POLD1, are responsible for this disease. We have identified a germline mutation in the POLE gene (c.4191_4192delCT, p.L1397fs) in a case with three synchronous colorectal cancers and multiple polyps. Interestingly, one of the three tumors in the patient exhibited microsatellite instability-high (MSI-H) status. We found that the MSI-H tumor had hypermethylation of MLH1 promoter, and accumulated a great number of somatic mutations as high as 81.5 per million bases. Additional bisulfite sequencing revealed different patterns of aberrant DNA methylation between the MSI-H tumor and the other tumor. These data will advance the understanding and treatment of colorectal tumors with the loss of polymerase proofreading.

▲TOP

J-3005

A constitutional synonymous variant of PALB2 gene in colorectal cancer case causes exon skipping

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【Background & Aim】 Alterations of PALB2 gene causes various cancer through loss or declining in homologous recombination repair function. We found a synonymous variant of PALB2, and revealed that it was involved in exon skipping.【Method】PALB2 was analyzed by DNA sequencing for 197 cases of colorectal cancer. Detected variants were examined using annotation to various database and algorithms for prediction (Mutationt@ster, Human Splice Finder, etc.) were performed.【Results & Discussion】A synonymous variant (c. 288 C > T p. L96L) was detected in gDNA from cancer and non-cancerous tissues in 2 cases. Mutationtaster and Human Splice Finder were evaluated as "Disease induced", "Alternation of ESE, Creation of new ESS" respectively. Analyses of the transcript from cancer and non-cancerous tissues showed the skipping of exon 9-10. This is a new finding of PALB2 and shows that synonymous mutations should be carefully handled.

J-3006

Difference of methylation and tumorigenesis pathway between familial adenomatous polyposis and colorectal cancer

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Familial adenomatous polyposis (FAP) is an inherited disorder with predisposition to colorectal cancer (CRC) manifested with development of numerous colorectal adenomatous polyps. Here we conducted comprehensive DNA methylation analysis of FAP tumors, including seven cancer and 16 adenoma samples, using Infinium 450K Array, and compared with Infinium data of 297 CRC, 45 adenoma, and 37 normal samples supplied by TCGA. Hierarchical clustering analysis stratified CRC into four subtypes: high, intermediate, low, and normal-like methylation epigenotypes. Five FAP tumors were clustered with intermediate methylation CRC, whereas 18 FAP tumors were clustered with normal-like CRC and normal mucosa. Intermediate methylation FAP cases significantly correlated with KRAS mutation. Within intermediate methylation epigenotype, however, aberrant elevation of DNA methylation was significantly smaller in FAP tumors than CRC or adenoma, and these lower methylated genes included WNT family and some oncogenic genes. There are at least two methylation epigenotypes in FAP tumors, and contribution of aberrant DNA methylation to tumorigenesis might be different in FAP tumors from CRC.

▲TOP

[J-3007] J7-2 [Japanese]
Familial tumor related genes

2018 / 9 / 29 (Sat) 10:15-11:30 Room 8/10F 1008, Osaka International Convention Center Room 8

Yoichi Furukawa / Inst. Med. Sci., Tokyo Univ.

J-3007

c.320T>C, p.Leu107Pro germline mutation of SDHD gene is a pathogenic mutation causing familial paraganglioma

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A 58-year-old woman with a past medical history of a carotid body tumor, resected 4 months prior to presentation, was admitted to the hospital for treatment of a cardiac tumor that was identified on post-operative echocardiography and chest computed tomography. The cardiac tumor was surgically removed and identified pathologically as a paraganglioma, similarly to the carotid body tumor. Family history strongly suggested accumulation of paraganglioma in the sibllings and her father. Genetic analysis identified a non-synonymous DNA variant in the succinate dehydrogenase gene D (*SDHD*) , Exon4, c.320T>C, p.Leu107Pro showing co-segregation with paternal transmission and maternal imprinting among family members. In analysis using multigene panel test (NCC-Oncopanel-FC) showed no other DNA variants in the other paraganglioma related genes such as *RET*, *VHL* and *SDHB*. Protein structure analysis predicted disappearence of α-helix in the downstream of the heme binding domain of the SDHD protein, implying this novel mutation appears to be a pathogenic DNA variant causing familial paraganglioma.

▲TOP

J-3008

Pathogenicity of BRCA Variants for Familial Breast and/or Ovarian Cancer

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Aim; Variant of uncertain significance (VUS) is a major problem of genetic testing and counseling for the client of Hereditary Breast and/or Ovarian Cancer (HBOC), because the high frequency of VUS makes genetic counseling being confusing and unconfident. We pursued pathogenicity of BRCA variants both of confirmed deleterious and minor variants in developing HBOC.Materials and Methods; 1 We analyzed BRCA 1/2 germline mutations for 325 patients with BC and/or OC (BC 193, OC 114 BC and OC 18). Minor Variants (MV) were selected by minor allele frequency (MAF) ≦0.01, and their pathogenicity was classified by C-score of CADD （Combined Annotation Dependent Depletion） and ClinVar data base. 2; We tried further validation using selected 80 genetic variants of BRCA1/2 that had been already classified by history weighting algorithm (HWA) in Myriad database.Results; Confirmed deleterious mutations and VUS (classified conflicting in ClinVar data base) had high C-score (≧10) by CADD in both of our cohort and validation set of 80 variants. Conclusions; We should know that MV had possibility to have pathogenicity for HBOC, and CADD might be useful for their classification.

J-3009

Examination of genotype and phenotype of individual HBOC using the Japanese HBOC consortium database

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Background: In BRCA, the correlation between phenotype and genotype has been previously reported and in some mutated locations, breast and ovarian cancer-related cluster regions have been shown. Purpose/Methods: We examined whether the BRCA genotype in the Japanese population correlates with the phenotype (prevalence, age of onset, bilateral breast cancer, breast cancer subtype and the number of affected family members) of breast cancer / ovarian cancer, using the Japanese HBOC consortium registration database. Results: 152 probands had BRCA1 (likely) pathogenic variants and 134 with BRCA2 had (likely) pathogenic variants. With reference to previous overseas reports, we classified BRCA1 as three cluster regions and BRCA2 as three cluster regions (narrow definition and wide definition). As a result, no statistically significant difference regarding phenotype was found between groups. Discussion: Owing to ethnic differences in the phenotype of hereditary diseases, it is important to increase the number of Asian study cases in order to establish an optimized medical management plan for the treatment of HBOC in the Japanese population.

J-3010

Analyzing pathogenic variants in Lynch syndrome by DNA and RNA sequencing

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Lynch syndrome is a hereditary neoplastic syndrome caused by a pathogenic variant in one of the mismatch repair genes (MMR genes; MLH1, MSH2, MSH6 or PMS2). In recent years, according to the advent of the next generation sequencer (NGS) and the progression of the analysis technique, many variants affecting function of MMR were revealed. However, to detect the large structural aberration and evaluate the variants affecting splicing are difficult by standard NGS method. To solve these problems, DNA sequencing was performed by molecular barcodes and anchored-PCR methods. In parallel, full coding regions of MMR genes were amplified from cDNA and NEXTERA XT DNA library prep kit was used for library construction for RNA sequencing. As a result, genomic deletion or amplification over exon regions was detected from the DNA sequencing comparable to the MLPA method equally. RNA sequencing revealed many splicing events undetectable in DNA sequencing. In conclusion, DNA sequencing with molecular barcode and RNA sequencing contributed to definition of pathogenic variants in genetic testing.

▲TOP

J-3011

Effects of VEGFA amplification on the localizaion of macrophage and lymphocyte in gastric cancer

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Tumors are encircled by the tumor microenvironment composed of a lot of components including fibroblasts, myofibroblasts, adipose cells, immune and inflammatory cells, the blood and lymphatic vascular networks, and extracellular matrix. The interactions between tumor cells and cells in the tumor microenvironment are suggested to be necessary for almost all stages of tumorigenesis. For the mediator of this interactions, the vascular endothelial growth factor (VEGF) and its receptor VEGFR signaling is suggested to be involved via tumor angiogenesis. It remains to be elucidated which cells in the tumor microenvironment are responsible for the interaction by VEGF signaling pathway. In this study, we investigated the relationship between gene amplification of VEGFA or expression level of VEGFA and localization of macrophages and lymphocyte in the tumor microenvironment in surgically resected human gastric cancer specimens by fluorescence in situ hybridization and immunohistochemical analyses.

J-3012

Analysis of the function of estrogen-mediated BRCA2

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BRCA2 has essential functions in all cell types; however, germline mutations in the BRCA2 gene are mainly correlated with an increased risk of breast and ovarian cancers. The reason for this higher risk of cancer development in estrogen-regulated tissues remains unclear, but recent studies have reported that estrogen activates BRCA2 transcription in an estrogen receptor (ER)-dependent manner, thereby promoting BRCA2 expression. Therefore, in the present study, we investigated the function of estrogen-mediated BRCA2 in MCF-7 cells by inserting the estrogen-dependent BRCA2 expression promoter region and the BRCA2 gene into a plasmid vector. Using mass spectrometry, we identified the presence of BRCA2-interactive proteins in estrogen-treated MCF-7 cells, some of which have been reported as regulating factors of estrogen-ER transcriptional activity. Using a promoter reporter assay, we also verified that the transcriptional activity of estrogen-ER was enhanced in the presence of BRCA2. These findings suggest that the greater effect of BRCA2 in estrogen-treated MCF-7 cells is associated with increased estrogen-ER transcriptional activity.

▲TOP

[LS31] LS31 [Japanese]
Pharmacokinetic analysis to predict the clinical effect of new anticancer agents in preclinical study

2018 / 9 / 29 (Sat) 11:50-12:40 Room 8/10F 1008, Osaka International Convention Center Room 8
：Konica Minolta Inc.

Yasuyuki Seto / University of Tokyo, Graduate School of Medicine, Gastrointestinal Surgery

LS31
Pharmacokinetic analysis to predict the clinical effect of new anticancer agents in preclinical study

Akinobu Hamada
National Cancer Center

No Abstract

▲TOP

[J-3061] J14-19 [Japanese]
Pediatric cancer (2)

2018 / 9 / 29 (Sat) 13:40-14:55 Room 8/10F 1008, Osaka International Convention Center Room 8

Tatsuro Tajiri / Dept. Pediatric Surg., Grad. Sch. of Med. Sci., Kyoto Pref. Univ. of Med.

J-3061

Different co-expression patterns of GD2 and GD3 in pediatric neuroblastic tumors

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Pediatric neuroblastic tumors (pNTs) are common solid tumors in childhood. As overexpression of disialoganglioside GD2 were shown in pNTs, targeted therapy against GD2 has expected to provide benefits for the patients with pNTs. We aimed to clarify the correlation among GD2 and precursor GD3 expression, clinicopathological features and glicosyltransferase in pNTs. Thirty-four pNTs were investigated the co-expression of GD2 and GD3 by immunohistochemistry and immunofluorescent staining. mRNA expression levels of glicosyltransferases (ST3GAL5, ST8SIA1, B4GALNT1 and B3GALT4) measured by qRT-PCR using TaqMan probes. Multivariate analysis showed the significant correlation between GD2 nucleic and cytoplasmic staining, and high risk (P=0.020, 0.033). Co-expression analysis showed that the significant correlation between the percentage of tumor cells with only GD3 cytoplasmic staining and low risk (P=0.013). And in the cases with more GD3 positive tumor cells, ST3GAL5 mRNA expression level was significantly high (P=0.026), and ST8SIA1 mRNA expression level tend to be low (P=0.051). In conclusion, These results may provide information for the mechanism of higher expression of GD2 in pNTs.

▲TOP

J-3062

The clinical application of pERK immunohistochemistry predicting MEK inhibitor sensitivity for neuroblastoma treatment

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BackgroundMEK inhibitors have been reported to be effective against neuroblastoma (NB) with MAPK activation. Selecting appropriate MEK inhibitor-sensitive patients is necessary for the clinical application. We evaluated therapeutic effects of two MEK inhibitors (Trametinib and CH5126766) and analyzed the ERK phosphorylation in vitro and in vivo. We also analyzed the phosphorylated-ERK (pERK) immunohistochemistry (IHC) in clinical NB samples as a candidate biomarker.Materials & MethodsThe in vitro study included a cell viability assay, Western blotting, and immunocytochemistry. The in vivo study was performed using NB xenograft mice. Clinical NB samples were stained with pERK and analyzed for the correlation with the prognosis.ResultsBoth MEK inhibitors showed anti-tumor effects against pERK(+) NBs in vitro and in vivo. Regarding the clinical samples, 6 of 16 (38%) post-chemo tumors were pERK(+) and 4 of 6 (67%) these pERK(+) tumors relapsed. DiscussionMEK inhibitors were preclinically effective against MAPK-activated NBs identified by pERK IHC. pERK(+) post-chemo NB samples had a relatively high frequency of relapse. MEK inhibitors may be useful for treating pERK(+) post-chemo NBs.

J-3063

Genetic and chromosomal characterization defines favorable or unfavorable outcomes in Wilms tumor patients

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Survival rate in Wilms tumor (WT) patients has reached to approximately 90%. However, the mortality rate is 10% in favorable WT, and 30% in high-risk WT. The high cure rate for WT leads to a new problem that 25% of survivors have serious chronic health conditions. We require new prognostic factor(s) . We analyzed array CGH (aCGH) patterns, and mutations of WT1 and 7 other genes in 129 unilateral WTs. Thirty-one patients had WT1 alterations in tumors (RFS 83%; WT1 type), 20 had no aCGH aberrations (RFS 95%; silent type), and 78 had aCGH changes without WT1 alterations (non-WT1/non-silent type). When 78 patients were classified into those with presence or absence of +12, 11q-, 16q-, or HACE1 loss, RFS was better for those with +12 than those without (P=.027), and worse for those with 11q-, 16q-, or HACE1 loss than those without (RFS: P=.008, .038, or 3.7E-04). These findings suggest that WTs could be classified into 3 types and 8 subtypes; outcomes are favorable for silent type and +12 subtype, and unfavorable for 11q-, 16q-, or HACE1 loss subtype, intermediate for WT1 type and 3 other (no 11q-, 16q-, and HACE1 loss) subtypes, and unclassifiable for no +12 subtype.

J-3064

The blockade of DNA damage response increase the sensitivity of CHK1 inhibitor in neuroblastoma

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Checkpoint kinase 1(CHK1) plays a central role of S/G2/M phase of cell cycle checkpoints. A series of small-molecule kinase inhibitors targeting CHK1(CHK1is) has been developed. Because most cancer cells possess genomic instability, cell cycle checkpoints blockade(checkpoint abrogation) by the CHK1is has been expected to exert a cytotoxic effect specifically in cancer cells. However, only several CHK1is still have been in early phase clinical trial developments. In this study, we aim to identify the effective clinical usage of CHK1is for an unfavorable neuroblastoma(NB) therapy. Micro array analysis using MYCN-amplified NB cell lines revealed that CHK1i induced CDKN1A(p21) in NB-39-nu cell line, which is relatively low sensitivity to the CHK1i. Further analysis showed that ATM-p53-p21 axis was activated by CHK1i in the NB-39-nu cell. Interestingly, the combined treatment of the CHK1i and the ATM inhibitor significantly increased the sensitivity with an inducing apoptotic cell death. Thus, these results might provide the important aspect that the blockade of DNA damage response could enhance a checkpoint abrogation strategy in cancer therapeutics.

▲TOP

J-3065

Targeting anaplastic lymphoma kinase gene alterations by using alkylating pyrrole-imidazole polyamides in neuroblastoma

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Aberrant status of anaplastic lymphoma kinase (ALK) and MYCN are two major causes in the development of aggressive neuroblastoma. Activating mutations and the overexpression of ALK associated with its gene amplification have been described in both familial and sporadic neuroblastoma disease. In this study, to overcome resistance to ALK inhibitors we examined growth inhibition effects of newly developed ALK-targeting pyrrole-imidazole (PI) polyamides, CCC-002 and CCC-003, which directly bind to and alkylate DNA within the amplified ALK and F1174L-mutated ALK gene transcripts, respectively. The IC_{50} of CCC-002 in ALK-amplified NB-39-nu cells was lower than that of ALK inhibitors, such as crizotinib and alectinib. CCC-002 treatment suppressed ALK expression in ALK-amplified cells. CCC-003 also showed the reduced expression of ALK and cell proliferation with lower IC_{50} values compared to crizotinib and alectinib in ALK-mutated Kelly and SH-SY5Y cell lines. Our data suggest that alkylating PI polyamide conjugates can be promising and innovative therapeutic drugs designed for ALK-amplified or mutated neuroblastoma.

J-3066

Near-infrared photoimmunotherapy using anti-GD2 antibody-photosensitizer conjugate for neuroblastoma

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Neuroblastoma (NB) is a childhood malignancy of the sympathetic nervous system. Despite the advances of treatment strategy, high-risk NB patients still have poor prognosis. Although immunotherapy using anti-GD2 monoclonal antibody (mAb) has been recently developed as a novel antitumor therapy for high-risk NB, the therapeutic efficacy of anti-GD2 mAb is insufficient in many clinical trials. Therefore, the enhancement of therapeutic potential of anti-GD2 immunotherapy is needed. Near-infrared photoimmunotherapy (NIR-PIT) is a promising antitumor strategy to enhance the therapeutic potential of immunotherapy using an antibody-photosensitizer conjugate (APC). In this study, we investigated the efficacy of anti-GD2 APC-based NIR-PIT in human NB cells. NIR-PIT with anti-GD2-APC suppressed the cell viability in GD2-positive NB cells (IMR-32, CHP-134, LA-N-5) more strongly compared to anti-GD2 mAb. Moreover, NIR-PIT significantly increased the immunogenic cell death with the release of extracellular ATP compared to anti-GD2 mAb. These results suggest that NIR-PIT with anti-GD2 APC is a promising antitumor strategy to promote the therapeutic efficacy of anti-GD2 immunotherapy for NB.

▲TOP

[J-3067] J7-3 [Japanese]
Genomic analysis in Japanese population

2018 / 9 / 29 (Sat) 14:55-16:10 Room 8/10F 1008, Osaka International Convention Center Room 8

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J-3067

Next generation sequencing approach for detecting 491 fusion genes in human cancer - Project HOPE

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Next-generation DNA sequencing (NGS) of the genomes of cancer cells is contributing to new discoveries that are illuminating the mechanisms of tumorigenesis. These outcomes contribute to the development of innovative pharmaceuticals and are unveiling genome sequencing as a component of personalized medicine. In particular, chromosomal translocations and fusion genes serve as important pharmaceutical targets and diagnostic markers given their association with tumorigenesis. Although an increasing number of fusion genes are being discovered using NGS, the methodology remains complicated, expensive, and requires relatively large amounts of sample. Here, to address these problems, we demonstrate the successful design and development of a panel of 491 fusion genes in the analysis of 4,033 clinical tumor specimens for Project High-tech Omics-based Patient Evaluation (HOPE).

▲TOP

J-3068

Clinical sequencing using the NCC Oncopanel system in the 2nd term of TOP-GEAR

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We developed an original next-generation sequencing (NGS)-based genomic testing system (NCC Oncopanel system) to identify genetic alterations of 114 cancer-related genes from formalin-fixed paraffin-embedded (FFPE) tumor tissues. We have been using this system to identify therapeutically actionable genetic alterations for patients considering entry into a phase I clinical trial at the National Cancer Center Hospital (TOP-GEAR project). In the 2nd term of the TOP-GEAR project (May 2016 to March 2018), a laboratory dedicated to NGS analysis compliant with CLIA certification was established in the hospital, and the NCC Oncopanel system was operated in this laboratory. Through the 2nd term, 665 patients suffering various type of cancer were enrolled more than 550 genetic alteration profiles were obtained. In this presentation, we will summarize observed genetic alterations and problems in this term. This system has received designation under the "Sakigake Designation System" for in vitro diagnostic pharmaceuticals and medical devices, and is now operating as an "Advanced Medical Care" after April 2018.

J-3069

Molecular profiling of hypermutator in 4,000 Japanese cancer patients

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Tumor mutational burden (TMB) is an emerging characteristic in cancer, and tumors harboring feature of high TMB are known to be hypermutator. This feature has been associated with microsatellite instability (MSI), defective DNA replication/repair, and response to PD-1 and PD-L1 blockade immunotherapy. In the present study, we investigated TMB using whole exome and targeted panel sequencing in 4,000 solid tumors that were obtained from Japanese patients. All samples collected at our single hospital were composed of multiple tissues, among which colorectal, lung, and stomach cancers occupied over 60% of the whole content. The median TMB was 3.1 mutations/Mb, and 6.6% of cases had 20 or more mutations/Mb. In hypermutator tumors (TMB ≥ 20 mutations/Mb), PD-L1 expression was upregulated, and 9.1% of cases were categorized as POLE mutant without MSI or defective homologous recombination repair. However, TMB derived from whole exome sequencing partially correlated with the estimated TMB based on panel sequencing. These results may provide helpful information for interpreting TMB results based on clinical sequencing using a targeted gene panel.

J-3070

Identification of fourteen new susceptibility loci for prostate cancer in the Japanese population

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Recent genome-wide association studies (GWAS) have identified common variants at multiple loci that have moderate effects on Prostate cancer (PC) risk. However, GWAS for PC have been undertaken exclusively among European populations. To identify genetic factors that confer risk of PC in the Japanese population, we carried out extensive GWAS using Japanese PC.
We carried out a GWAS of 7,521,072 SNPs using 5,088 Japanese PC patients and 10,682 controls. In addition, we conducted replication study in an independent set of 4,818 Japanese PC patients and 73,261 controls.
From the 108 associated SNPs reported in previous GWAS, we confirmed the association of 49 SNPs in the Japanese population. In addition, we performed replication study for 101 SNPs which indicated $P < 1.0x10^{-5}$ in the GWAS. As a result, we identified 14 new loci for PC susceptibility. Moreover, the polymorphisms of SNP and the expression of the surrounding gene showed a significant correlation with QTL analysis in the 2 associated SNPs.These findings advance our understanding of the genetic basis of prostate carcinogenesis and highlight the genetic heterogeneity of PC susceptibility among different ethnic populations.

▲TOP

J-3071

Establishment of a catalog of somatic genetic alterations in Japanese cancer patients across multiple tumor types

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Background: Tumor molecular profiling enables efficient identification of somatic genetic alterations as potential therapeutic targets. In January 2014, the Shizuoka Cancer Center launched Project HOPE, which is the first prospective tumor molecular profiling study of multiple tumor types in Japan.
Methods: Until January 2017, 3,174 tumor samples were collected from 3,022 patients with informed consent and subjected to whole-exome sequencing and gene expression profiling. Among them, data derived from 2,820 primary tumor samples across 17 tumor types were used for this analysis.
Results: We identified distributions of the tumor mutation burden, frequency of genetic alterations, and mutational signature across tumor types, and clarified their associations with tissue of origin, clinicopathlogical characteristics, environmental/carcinogenic factors, and ethnicity.
Conclusions: This established catalog of somatic genetic alterations across multiple tumor types in Japanese cancer patients can contribute as benchmark to clinical-trial design with the aim of expanding the number of patients suitable for molecular-targeted therapies in not only Japan, but also the rest of East Asia.

J-3072

Identification and evaluation of novel susceptibility genes in Japanese familial breast cancer by whole exome sequencing

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The major portion of familial breast cancer is still largely unknown, and many susceptibility genes yet to be identified. In this study, we performed whole exome sequencing of 50 affected and 34 non-affected individuals from 24 high-risk Japanese breast cancer families to identify potential predisposing genes. We identified 8 families having germline variants in BRCA1/2, TP53 or PTEN genes. Notably, we evaluated the pathogenic effects of BRCA1/BRCA2 mutations by homology-directed recombination (HDR) assay. In the remaining 16 families which have BRCA1/2/TP53/PTEN-mutation negative, we identified 19 candidate genes as rare Indel variants (allele frequency < 0.01 or unknown) that segregated with all affected members or between affected and non-affected members by using multi-risk prediction tools (SIFT, PolyPhen2, and MutationTester). Finally, we selected two potential predisposing genes which are significantly downregulated in a group of breast cancer cases with poor prognosis by Kaplan-Meier plot analysis. We are examining the effects of these mutations on their gene functions and cancer cell proliferation and invasion, and will report these results at this meeting.

▲TOP

[ML20] ML20 [Japanese]
Morning Lectures 20

2018 / 9 / 29 (Sat) 8:00-8:50 Room 9/10F 1009, Osaka International Convention Center Room 9

Michiaki Unno / Dept. Surg., Tohoku Univ.

ML20

Sugar Chains and Cancer

Naoyuki Taniguchi
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Discussant：Yuichi Ando
Clin. Oncol. Chemother., Nagoya Univ. Hosp.

Sugar chains (Glycans) are components of glycoproteins, glycolipids and proteoglycans. Glycosylation is catalyzed by glycosyltransferases and 80-90 % proteins in cell surface and nuclei are glycosylated. On the other hand glycans are highly implicated in various biological phenomena such as cancer. Actually glycoscience or glycomics are expected to clarify unknown biological functions that genome or proteome is unable to clarify. It is well known that most of cancer biomarkers are glycoproteins or glycolipids. Typical examples are AFP, CEA, CA19-9, CA-125 and PSA etc. Among them fucosylated AFP designated as L3 fraction is a biomarker for hepatocarcinoma. This lecture will focus on glycosyltransferases and their products (name in parenthesis) such as Fut8 (core fucose), GnT-III(bisecting GlcNAc), and GnT-V(beta1,6 branch) in relation to cancer. Glycosylation of cell surface receptors and adhesion molecules such as E-cadherin and integrin will be also discussed. These changes confer the unique characteristic phenotypes associated with cancer cells. In conclusion glycans play pivotal roles in cancer biomarker, epithelial-mesenchymal transition, cancer progression and metastasis.

▲TOP

[IS10-1] IS10 [English]
Molecular basis of therapy resistance for development of next generation drugs

2018 / 9 / 29 (Sat) 9:00-11:30 Room 9/10F 1009, Osaka International Convention Center Room 9

Ryohei Katayama / Div. Exp. Chemother., Cancer Chemother. Ctr., JFCR、Pieter Eichhorn / Cancer Sci. Inst. of Singapore

Anticancer drug treatment has been dramatically improved due to the fundamental understanding of tumor by the analysis with cell-biology and biochemistry. Additionally, Identification the biomarkers accompanied with the anticancer drug largely contributed the improvement of prognosis.
A number of drugs have been developed based on these finding and used in clinic based on the clinical trial results with the marked tumor shrinkage. However, even if a remarkable tumor shrinkage is observed, it is inevitable to relapse the tumor due to the acquired resistance in most patients. In addition, primary and adaptive resistance observed in some patients are needs to be solved to improve the clinical outcome by anticancer drug therapy.
In this session, we aim to share a deep understanding of biological background related to the drug resistance in cancer cells and tissue. Therefore, we would like to share the new finding and discuss based on the multilayer point of view such as “adaptive response” when cancer cells exposed to the therapeutic drugs, acquisition of malignancy or resistance through the interaction between cancer cell and host cells (stromal cells, immune cells etc…) in the tumor tissue, and the regulatory mechanisms of cancer-related proteins by protein modification or degradation. From the various perspectives, we will share the latest research findings of the molecular basis regarding anti-cancer therapy resistance and would like to discuss the possibility of future therapeutic strategies.

IS10-1

c-Met activation leads to the establishment of a TGF-B receptor regulatory network required for bladder cancer invasion

Pieter Eichhorn
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Treatment of muscle-invasive bladder cancer remains a major clinical challenge. Aberrant HGF/c-MET upregulation and activation is frequently observed in bladder cancer correlating with cancer progression and invasion. As part of a negative feedback loop SMAD7 binds to the E3 ligase SMURF2 targeting the TGF-B receptor for degradation. Under these conditions SMAD7 acts as an agonist disrupting the intermolecular interactions within SMURF2, permitting SMURF2 activation. We demonstrate that HGF stimulates TGF-B signaling by inducing c-SRC mediated phosphorylation of SMURF2 at two tyrosine residues impeding SMAD7 binding and enhancing SMURF2 C2-HECT domain interaction, resulting in SMURF2 inhibition and TGF-B receptor stabilization. This upregulation of the TGF-B pathway by HGF leads to TGF-B-mediated EMT and invasion. Using clinically relevant orthotopic mouse models we show that inhibition of TGF-B signaling completely prevents HGF induced bladder cancer invasion. Furthermore, we make a rationale for the use of TGF-B receptor and MEK inhibitors in the treatment of high grade non-muscle-invasive bladder cancers or early stage muscle invasive bladder cancers.

▲TOP

IS10-2

Hypoxia-induced changes in chromatin landscape regulates tumor microenvironment

Sudhakar Jha
Cancer Science Institute of Singapore

Hypoxia is a common feature of solid tumors and adaptation to low oxygen conditions is important in tumorigenesis. Triggered by hypoxia, cells undergo broad changes in the transcriptome, stimulating metabolic adaptation, angiogenesis, metastasis and resistance to radiotherapy as well as chemotherapy. Carbonic anhydrase IX (CAIX), an important hypoxia target gene, is important due to its contribution in tumor microenvironment acidification and metastasis. Breast cancer which occurs in the form of solid tumors is also subjected to intratumoral hypoxia. Using an in vitro breast cancer cell line model, we demonstrate the mechanism of hypoxia-activated CAIX transcription, and show that inhibition of CAIX expression sensitizes breast cancer cells to doxorubicin. In this meeting, I will discuss our efforts to elucidate the transcriptional machinery involved in regulating hypoxia-induced gene expression. Furthermore, I will provide evidence which suggests that targeting epigenetic readers may be coupled with the conventional chemotherapy as a promising therapeutic approach in addressing hypoxia-driven resistance to chemotherapy.

IS10-3

Intrinsic and acquired resistance to tumors aberrant MAPK signaling

Hiromichi Ebi
Div. Mol. Ther. Aichi Cancer Ctr. Res. Ins.

Components of MAPK signal are frequently mutated in various types of cancers. While suppression of aberrant MAPK activation caused by these mutations has been an attractive target, molecular targeted drugs against MAPK signaling have shown variable responses. For example, inhibition of mutant BRAF is enough for MAPK suppression and clinical responses in BRAF V600E mutant melanoma and lung cancer. In contrast, in BRAF V600E mutant colorectal cancer, BRAF inhibition induces feedback activation of EGFR, which leads to reactivation of MAPK signal and resistance to BRAF inhibitor monotherapy. Combination of EGFR inhibitor with BRAF inhibitor can induce complete suppression of MAPK signal and response in clinic, indicating the dependency of these tumors on MAPK signal. In contrast, loss of MAPK dependency is also observed in a subset of KRAS mutant cancers. These results clearly indicate heterogeneity between tissues, mutations, and even in same tumors. I would discuss how we analyze these heterogeneity and translate these intro clinic in cancers with aberrant MAPK signal.

IS10-4

Mutant Kras co-opts an inflammation-induced transcriptional program to drive pancreatic tumorigenesis

Charles J. David
Tsinghua Univ. Sch. Med.

Co-author：Mo Chen
Tsinghua Univ. Sch. Med.

Chronic pancreatitis is a major risk factor for the development of pancreatic ductal adenocarcinoma (PDA). This is supported by work in mouse models, in which tumorigenesis driven by mutant Kras expression is accelerated by experimentally induced pancreatitis. The mechanisms connecting inflammation and mutant Kras-driven tumorigenesis are poorly understood. We previously demonstrated that lineage-restricted transcription factors (TFs) induced in the inflamed pancreas are essential for KrasG12D-mediated tumorigenesis. This indicates that the role of inflammation in transformation may be to promote the emergence of cells transcriptionally permissive to Kras-driven transformation. To investigate this idea, we have systematically characterized the TF dependencies of PDA cells using sgRNA screening. In addition, we used H3K27 acetyl ChIP-seq to identify Kras-dependent enhancers in PDA. Using these approaches, we have found that a lineage-specific transcriptional network that emerges in the inflamed pancreas is the major determinant of the Kras-dependent transcriptome in PDA. These results point to a potential molecular explanation for the link between inflammation and tumorigenesis.

▲TOP

IS10-5

Prediction of TKI resistance in lung cancer through the experimental models and in silico simulations

Ryohei Katayama
Div. Experiment. Chemother., Cancer Chemother. Ctr., JFCR

Co-author：Naoya Fujita
Cancer Chemother. Ctr., JFCR

Identification of the driver oncogene such as EGFR activating mutation, ALK- or ROS1-rearrangements in non-small-cell lung cancers (NSCLCs), and the development of the targeted agents, mainly tyrosine kinase inhibitors (TKIs), largely improved the prognosis of advanced NSCLCs. Many TKIs have been developed and shown marked tumor shrinkage in patients, however, cancers inevitably relapse due to the acquired resistance by multiple mechanisms such as secondary mutations or bypass pathway activations. Recently, some of the resistances, mainly by the secondary mutations, have been successfully overcome by potent second- or third- generation TKIs. However, it is becoming problem that cancers again acquire the resistance to the next generation TKIs. We have examined the resistance mechanisms using cell lines and patient derived cells under IRB approved protocol. And we have explored the therapeutic strategies to overcome the resistance. Here we would like to present about the latest findings of new resistance mechanisms to molecular target drugs and the possibility of the prediction of resistance mutation in silico, and the therapeutic strategies to overcome the resistance.

IS10-6

URST1 is a novel prognostic biomarker and therapeutic target for breast cancers

Masako Nakamura
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To identify and characterize diagnostic and therapeutic targets for breast cancer, we screened genes that were highly expressed in the majority of breast cancers by our gene expression profile database. During this process, we identified URST1 as a good candidate. Immunohistochemical analysis revealed that URST1 was expressed in 195 of 257 (75%) breast cancer cases (69% in luminal type, 88% in HER2-positive cancer and 86% in TNBC) that had undergone curative surgery, whereas no staining was observed in normal breast tissues. URST1 expression was significantly correlated with poor clinical outcome for breast cancer patients (P = 0.0126, Log-rank test) and multivariate analysis showed its independent prognostic value. Suppression of endogenous URST1 expression by siRNAs against URST1 or treatment with URST1 inhibitor significantly inhibited the breast cancer cell growth through cell cycle arrest at G2/M phase and mitotic cell death. URST1 appears to play an important role at the central spindle in mitosis of cancer cells. URST1 is a prognostic biomarker and therapeutic target for breast cancers.

▲TOP

[LS32] LS32 [Japanese]
Optimal treatment Strategy based on Pan-Asian Adapted ESMO Consensus Guidelines for mCRC

2018 / 9 / 29 (Sat) 11:50-12:40 Room 9/10F 1009, Osaka International Convention Center Room 9
：Takeda Pharmaceutical Company Limited

Taroh Satoh / Department of Frontier Science for Cancer and Chemotherapy, Osaka University Graduate School of Medicine

LS32
Optimal treatment Strategy based on Pan-Asian Adapted ESMO Consensus Guidelines for mCRC

Takayuki Yoshino
National Cancer Center Hospital East

No Abstract

▲TOP

[IS12-1] IS12 [English]
Novel therapeutic strategy for primary and metastatic tumors in the central nervous system

2018 / 9 / 29 (Sat) 13:40-16:10 Room 9/10F 1009, Osaka International Convention Center Room 9

Seiji Yano / Div. Med. Oncology, Cancer Res. Inst., Kanazawa Univ.、Byoung Chul Cho / Div. Med. Oncology, Yonsei Cancer Ctr.

Tumors in the central nervous system (CNS), including primary brain tumors, brain metastasis, and leptomeningeal carcinomatosis, are generally refractory to cytotoxic chemotherapy. Though targeted drugs are initially effective to CNS tumors with target driver alterations, acquired resistance eventually occurs almost without exception. The efficacy of immune checkpoint inhibitors is not well documented so far. Therefore, establishment of efficient drug therapy against CNS tumors is urgent unmet need.
In this symposium, we invite 5 distinguished speakers and the cutting edge of their researches regarding pathogenesis, diagnosis and therapeutics of CNS tumors will be present. We look forward to sharing latest information of CNS tumors and enjoy hot and fruitful discussion!

IS12-1
Management of Brain Metastasis in EGFR mutant NSCLC

Byoung Chul Cho
Div. Med. Oncol., Yonsei Cancer Ctr.

Brain metastases are common in patients with non–small-cell lung cancer (NSCLC). Because of associated poor prognosis and limited specific treatment options, there is a real need for the development of medical therapies and strategies for affected patients. Novel compounds for EGFR mutant NSCLC have demonstrated blood-brain barrier permeability and have led to important improvements in central nervous system outcomes. Studies of targeted therapies for oncogene-driven tumors and of immunotherapies in patients with brain metastases have shown promise and, allied with novel radiation techniques, are driving a rapid evolution in treatment and prognosis for NSCLC brain metastases.

▲TOP

IS12-2

Whole-organ quantitative analysis of cancer metastasis by tissue clearing

Shimpei Kubota
Mol. Path., The Univ. Tokyo, Sch. Med.

Co-author：Kei Takahashi、Jun Nishida、Shogo Ehata、Kohei Miyazono
Mol. Path., The Univ. Tokyo, Sch. Med.

Stochastic and proliferative events initiating from a single cell can disrupt homeostatic balance and lead to fatal disease such as cancer metastasis. To overcome metastasis, it is necessary to detect and quantify sparsely-distributed metastatic cells throughout the body in the early stages. Here we demonstrate that CUBIC (clear, unobstructed brain/body imaging cocktails and computational analysis)-based cancer (CUBIC-Cancer) analysis with a refractive-indices optimized protocol enables comprehensive cancer cell profiling in whole body and organs. CUBIC-Cancer analysis is applicable to spatio-temporal quantification of metastatic cancer progression at single-cell resolution. CUBIC-Cancer analysis is applicable to pharmacotherapeutic profiling of anti-tumor drugs. Our methods detected tiny foci consisting of even a single cancer cell in the whole organ of anti-tumor drug treated mouse. CUBIC-Cancer analysis is compatible with in vivo bioluminescence imaging and 2D histology. The scalable analytical pipeline with these three modalities would contribute to overcome incurable metastatic diseases. This work is collaborated with Prof. Hiroki R. Ueda (Department of Systems Pharmacology).

IS12-3

Unravel the mysteries of cancer cell dormancy in brain metastasis

Eishu Hirata
Dept. Onco. Path., Kanazawa Med. Univ.

Recent advances in cancer genomics revealed that brain metastasis can occur in the early stage of cancer progression and these "early disseminated cancer cells" can be the source of recurrence after months or years of dormancy. Although the period of dormancy differs between cancer types and cases, one of the common and critical problems is that diagnosis and treatment are extremely difficult due to their silent nature. Brain metastasis is a functionally devastating complication with a very poor prognosis and development of effective treatment/prevention strategies is one of the most urgent issues, however, little is known about how cancer cells metastasize to the brain, survive, keep dormant, and start regrowth. To tackle these problems, we have established mouse models of brain metastasis and quantitatively delineated the stages of brain metastasis progression. Single cell transcriptome analysis clearly extracted dormant cancer cells with some related molecules/signaling pathways, among which we identified DNMT1 as a key regulator of cancer cell survival and dormancy in brain metastasis.

IS12-4

Asian precision neuro-oncology based on tumor evolution and gene-drug map

Kyeung Min Joo
Dept. Anatomy & Cell Biol.

Outcomes of anticancer therapy vary dramatically among patients. The fundamental tenet of precision oncology defines molecular characterization of tumors to guide optimal patient-tailored therapy. Towards this goal, we have established a compilation of pharmacological landscape of patient-derived tumor cells (PDCs) across cancer types including glioblastoma (GBM), together with genomic and transcriptomic profiling. The results provided unprecedented insights into dynamic pharmacology and genomic associations. The associations provide potential implementation of PDC-derived drug sensitivities for prediction of clinical response. However, this prediction is complicated by spatial and temporal heterogeneity. We further studied genomic and expression profiles across longitudinal specimens from GBMs. Samples from the same tumor mass share genomic and expression signatures, whereas geographically separated tumors are seeded from different clones. Chemical screening of PDCs shows that multifocal tumors have a heterogeneous drug-response pattern. The results demonstrate that evolutionary inference in multisector biopsies can inform more precise targeted therapeutic interventions.

▲TOP

IS12-5

Artificial Intelligence: Understanding the Development of Chemoresistance in Glioma Patients, a Case Study (STAT3)

Carol SL Tang
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The revised WHO classification supports that molecular characterization supersedes histology. Our Brain Tumor Resource serves as a platform to spearhead precision medicine goals with molecular data acquired from clinical material. We recently established a STAT3 gene signature. We showed that the STAT3-signature had prognostic relevance and patients with active STAT3-signature pattern were enriched for the mesenchymal subtype, typically associated with poor survival outcome. Using cellular and animal models, STAT3-high GBM tumors showed favorable response to AZD1480 while STAT3-low tumors developed resistance. As kinases represent dominant therapeutic targets in major pharmaceutical pipelines, we used biological evidence to substantiate our computational predictions, by measuring phosphorylation levels of 144 kinases in STAT3 signature-stratified GBM cells using the PamChip technology. We developed a novel computational pipeline on kinome assay data by integrating phospho-chemical interactions with functional genomics data through kinase-substrate databases. We identified IGF-1R as a top-ranking tyrosine kinase uniquely elevated in STAT3-low tumors upon treatment with AZD1480.

IS12-6

Nanomedicine to Target Glioblastoma

Sabina Quader
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Glioblastoma multiforme (GBM) is the most deadly and prevalent form of brain tumor, less than 5% of patients with GBM surviving longer than 3 years. The current standard treatment of GBM consists of maximal surgical resection followed by radiotherapy (RT) with concomitant and adjuvant chemotherapy with temozolomide (TMZ). While TMZ has made an impact on survival (median survival improved from 12.1 months with only RT to 14.6 months with RT plus TMZ); tumor recurrence and TMZ resistance remain main challenges. Consequently, GBM represents a major unmet medical challenge for the development of a novel therapeutic strategy. The most dominant factors for poor prognosis of GBM are its high invasive and angiogenic character and the presence of the blood-brain tumor barrier (BBTB) in the vasculature of GBM, which limits the penetration and accumulation of drugs. With an attempt to overcome these issues, we utilized polymer micelle-based nanomedicines to transport potent anti-GBM agents into the intracranial GBM tumors. We confirmed that by appropriate surface modification and tuning the drug-release kinetics of pH-responsive nanomedicines we could achieve better therapy outcome in GBM.

▲TOP

[ML21] ML21 [Japanese]
Morning Lectures 21

2018 / 9 / 29 (Sat) 8:00-8:50 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Mitsugu Sekimoto / Dept. Surg., Osaka Natl. Hosp.

ML21

Current status of carbon ion radiotherapyCurrent status of carbon ion radiotherapy

Kazuhiko Ogawa
Dept. Rad. Oncol., Grad. Sch. Med., Osaka Univ.

Co-author：Osamu Suzuki、Masashi Yagi、Yutaka Takahashi
Dept. Radiat Oncol. Osaka Univ. Sch. Med.

Discussant：Masafumi Inomata
Dept. Gatroenterol. & Pediat. Surg. Oita Univ. Faculty of Med.

Radiotherapy has recognized as one of the major treatments of cancer therapy for various cancers, and many cancer patients now receive radiotherapy in Japan. Patients with some types of cancers can be cured without surgical resection. However, several types of cancers, such as pancreatic cancer, sarcomas, are radioresistant, and it is difficult to treat effectively with conventional X-ray radiotherapy for these tumors. Therefore, new approaches of not only more intensive and effective but also less toxic radiotherapy are needed. Recently, particle therapy, including carbon ion radiotherapy and proton beam radiotherapy, has recognized as an attractive therapies for refractory cancers. Particle therapy is more effective and less toxic than conventional X-ray radiotherapy because of more damages to cancer cells and also less damage to surrounding normal tissues. Moreover, carbon ion therapy requires less durations of the treatments compared to conventional X-ray therapy therapy. From this year, carbon ion therapy center has just started in Osaka district. In this session, general aspects of particle radiotherapy, especially carbon ion radiotherapy, are shown and discussed.

▲TOP

[J-3013] J14-14 [Japanese]
Colorectal cancer: cancer immunity

2018 / 9 / 29 (Sat) 9:00-10:15 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Masahiko Shibata / Dept. Advanced Cancer Immunotherapy, Fukushima Med. Univ.

J-3013

Tumor PTGS2 (cyclooxygenase-2) expression status and immune response to colorectal cancer in two prospective cohorts

Keisuke Kosumi
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Prostaglandin-endoperoxide synthase 2 (PTGS2, cyclooxygenase-2) contributes to colorectal cancer development through production of the inflammatory mediator prostaglandin E2 (PGE2). Experimental evidence indicates a crucial role of the PTGS2-PGE2 pathway in forming an immunosuppressive tumor microenvironment. Hence, we hypothesized that tumor PTGS2 expression levels might be inversely associated with immune response to colorectal carcinoma. Using 746 colorectal cancer cases in two U.S. prospective cohort studies, we examined tumor PTGS2 expression status by immunohistochemistry. We found an inverse association of tumor PTGS2 expression with CD45RO+ cell density (Ptrend = 0.004). For each unit increase in ordinal outcome categories of CD45RO+ cell density, the multivariable odds ratios were 0.61 [95% confidence interval (CI), 0.37-1.00] for low-level PTGS2 expression, 0.59 (95% CI, 0.38-0.91) for intermediate-level PTGS2 expression, and 0.37 (95% CI, 0.19-0.71) for high-level PTGS2 expression, compared with absent PTGS2 expression. Our population-data suggests a possible role of the PTGS2-PGE2 pathway in suppressing T cell-mediated anti-tumor immune reaction to colorectal cancer.

▲TOP

J-3014

Study of immune-related prognostic factors in patients with resectable colorectal cancer

Taichi Kuwahara
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Introduction: Previous reports suggest that tumor infiltrating lymphocytes (TILs) and microsatellite instability (MSI) are prognostic factors in colorectal cancer (CRC). In this study, we analyzed TILs and MSI Status for prognostic factors in pts with CRC performed curative resection.Patients and Method: 342 pts with CRC (I: 88, II: 142, III: 112) received surgery from 1993-2012. We studied the number of TILs per mm2 in tumor specimens by IHC. We determined MSI status with MSI Analysis System (Promega Corp). We investigated the relationship between quantity of TILs (CD3+, CD8+, CD4+, Foxp3+) or MSI status with prognosis (relapse free survival: RFS, disease specific survival: DSS).Result: Quantity of TILs was associated with improved RFS (CD3+; P=0.0222, CD4+; P=0.0005, Foxp3+; P=0.0001) and DSS (CD8+; P=0.0219, CD4+; P=0.0001, Foxp3+; P=0.0003). No association was observed between MSI status and RFS/DSS (P=0.1905, P=0.7887). Cox multivariate analysis supports the advantage of TILs (CD4+; P=0.0008) compared with clinicopathologic features in predicting survival.Conclusion: In CRC pts performed curative resection, high TILs, especially CD4+, associated with good prognosis.

J-3015

Evolution of primary colorectal cancers to metastasis might be affected by tumor immune responses

Satoshi Nagayama
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Co-author：Shotaro Sakimura[1]、Dai Shimizu[1]、Atsushi Niida[2]、Hidetoshi Eguchi[1]、Takaaki Masuda[1]、Satoru Miyano[2]、Tatsuhiro Shibata[3]、Koshi Mimori[1]
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We compared single nucleotide variants (SNVs), neoantigens (NAGs), and copy number aberrations (CNAs) between primary tumors from 8 early colorectal cancers (CRCs) with postoperative relapse (CRCR) and from 8 early CRCs without any recurrences (ECRC) by whole-exome sequencing. NAGs were fewer in CRCR than in ECRC, whereas CNAs at the arm level were significantly more frequent in CRCR than in ECRC. Tumor immune responses were diminished in CRCR than ECRC. CNAs were the selective pressure to purify capable clones to metastasize in primary tumor by preventing tumor immune response. In 8 CRCR cases, we compared SNVs, NAGs, CNAs, cytolytic activity (CYT) with immune-related genes and TCR repertoire between 8 primary and 12 metastatic lesions. Although there was no difference in NAG or CNA, 12 metastatic lesions showed not only lower CD8 expression but lower TCR repertoire diversity compared to 8 primary lesions. In the evolution towards metastasis, clones in primary tumor are selected by CNA which works as a driver event as Darwinian evolution manner, while clones in metastatic tumor might be selected not by any genomic drivers but by tumor immune responses as neutral evolution manner.

J-3016

Division of Gastrointestinal Surgery, Department of Surgery, Kobe University Graduate School of Medicine

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Background; The relationship between tumor immunological microenvironment and irradiation has already been studied. Based on these findings, we examined a tumor microenvironment of rectal cancer performed by neoadjuvant chemoradiotherapy(NACRT) with immunohistochemical analysis of surgical specimens.Methods; Locally advanced rectal cancer were surgically treated in our institution and enrolled and 68 were eligible for this study. Immunohistochemical staining of programmed cell death- lignad1(PD-L1), CD8, and CD163 was performed on specimens of all cases. Results; There were 44 cases in which NACRT was performed (NA group) and 24 cases in which surgery alone was performed (SA group). In the SA group, there was no significant change in the infiltration of PD-L1+immune cells(PD-L1+IC) nor CD8 +cells before and after treatment, but in the NA group, the infiltrations of them were significantly increased. In multivariate analysis, low infiltration of CD8 +cells were identified as poor prognostic factors in disease-free survival.Conclusion; The infiltration of PD-L1+IC and CD8 +cells was induced by NACRT. In NACRT for rectal cancer, PD-1 inhibitor combination therapy should be introduced.

▲TOP

J-3017

Impact of primary tumor location as a predictive factor in cytotoxic anti-cancer agent for colorectal cancer

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Recently, primary tumor location in colorectal cancer (CRC) as a predictive factor have attracted attention. Better outcomes for left-sided colon cancer (CC) compared with right-sided CC has been reported. However, in those reports, the chemotherapy regimens always included molecularly-targeted agents. The purpose of this study was to clarify the impact of primary tumor location as a predictive factor in cytotoxic anti-cancer agent regimens alone (FOLFOX/FOLFIRI) using drug sensitivity test.Between Mar. 2008 and Apr. 2017, tumor specimens were obtained from 133 patients without preoperative chemotherapy. In all patients, there was no significant difference in the growth inhibition rate of the FOLFOX and FOLFIRI regimens for right-sided vs left-sided tumors. Moreover, in 87 CC patients and 42 patients received palliative chemotherapy after surgery, there was also no significant difference. In conclusion, there was no impact of primary tumor location in cytotoxic anti-cancer agent regimens for CRC in this study. Therefore, our findings underscore the fact that molecularly-targeted agents rather than cytotoxic anti-cancer agents may result in better outcomes for left-sided tumors.

J-3018

Differential prognostic significance of mesothelin expression in Stage II colorectal cancer according to tumor location

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Introduction
Clinical significance of mesothelin (MSLN) expression has not been fully clarified in colorectal cancer (CRC). Increasing evidence suggests that left- and right-sided colon cancers are two distinct disease entities. We investigated the prognostic value of MSLN expression in Stage II CRC according to tumor sidedness.
Methods
314 Stage II CRC patients with R0 resection were included in this study. MSLN expression in cancer cells was evaluated immunohistochemically and was classified into positive and negative with 30% cutoff value of positive cells.
Results
According to MSLN expression, 16% and 84% patients were judged as positive and negative, respectively. Five-year cancer-specific survival (CSS) rate was 87.9% in MSLN-positive group, whereas it was 96.0% in MSLN-negative cases (P=0.023). Similarly, MSLN expression was associated with decreased CSS rate in 106 right-sided colon cancer patients: i.e., 5-yr CSS was 87% and 99% in MSLN-positive and -negative groups, respectively (P=0.017). However, MSLN expression had no prognostic value in left-sided colon cancer and rectal cancer.
Conclusion
MSLN expression had a prognostic value in Stage II CRC, but only in right-sided ones.

▲TOP

[J-3019] J14-15 [Japanese]
Colorectal cancer

2018 / 9 / 29 (Sat) 10:15-11:30 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Masayuki Ohue / Dept. Gastroenterological Surg., Osaka InterNatl. Cancer Inst.

J-3019

Epigenetic silencing of SMOC1 is associated with development of colorectal traditional serrated adenomas

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We aimed to clarify the molecular mechanism underlying the development of colorectal traditional serrated adenomas (TSAs). Genome-wide DNA methylation analysis in TSAs having both protruding and flat components identified 11 genes, including SMOC1, in which methylation levels were progressively increased during the TSA development. SMOC1 was prevalently methylated in TSAs, but was rarely methylated in sessile serrated adenoma/polyps (SSA/Ps) ($p<0.001$). SMOC1 was abundantly expressed in normal colon and SSA/Ps, but was significantly downregulated in TSAs. Ectopic expression of SMOC1 suppressed colorectal cancer (CRC) cell proliferation and tumor formation. Analysis of 847 colorectal lesions and 61 normal colonic samples revealed that SMOC1 is frequently methylated in TSAs, high-grade adenomas and CRCs, and that SMOC1 methylation is strongly associated with KRAS mutation and CIMP-low. These results suggest that epigenetic silencing of SMOC1 is associated with TSA development. SMOC1 expression could be a diagnostic marker of serrated lesions, and SMOC1 methylation could play a role in neoplastic pathways arising in TSAs and conventional adenomas.

▲TOP

J-3020

Integrative analysis of gene mutations, copy number alterations and DNA methylation in colorectal serrated lesions

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Aim: To clarify the molecular and clinicopathological characteristics of colorectal serrated lesions, we assessed mutations, copy number alterations and DNA methylation of cancer-associated genes in these lesions. Methods: Seventy-eight serrated lesions, including 36 traditional serrated adenomas (TSAs) and 18 sessile serrated adenomas (SSAs), were investigated. We performed sequence analyses of 39 cancer-associated genes. We also assessed the methylation status of a number of cancer-associated genes using bisulfite pyrosequencing. Results: BRAF mutations were observed in 64% of TSAs and 78% of SSAs, while KRAS mutations were observed in 31% of TSAs and 6% of SSAs, respectively. Mutations in WNT pathway-associated genes were significantly higher in TSAs than SSAs (58% and 28%, respectively, p=0.046). Additionally, frequency of methylation-positive cases of SMOC1 was significantly higher in TSAs than in SSAs (53% and 0%, respectively, $p < 0.01$). Conclusions: Significance of mutations in WNT signaling pathway genes was emphasized in carcinogenesis of colorectal serrated lesions. It was confirmed that epigenetic silencing of SMOC1 was significantly associated with development of TSAs.

J-3021

The clinical significance of oxysterol binding protein like 3 (OSBPL3) in colorectal cancer

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Amplification of chromosome 7p(Ch.7p) is observed as common in colorectal cancer (CRC), suggesting that Ch.7p possess driver genes for tumorigenesis or tumor development of CRC. Here, we investigated the clinical significance of OSBPL3 on Ch.7p in CRC. Methods: We assessed the expression levels of OSBPL3 mRNA in 623 tumor tissue and 51 normal tissue using public database (PD) (The Cancer Genome Atlas: TCGA), Next, we observed the localization of OSBPL3 using a PD of immunohistochemical staining (THE HUMAN PROTEIN ATLAS) and the DNA copy number alteration using TCGA and cell line PD (Cancer Cell Line Encyclopedia). Finally, the clinocopathological and prognostic analysis of OSBPL3 expression was conducted using TCGA. Results: 1. OSBPL3 was significantly higher in tumor tissues than in normal tissues. 2. OSBPL3 was highly expressed in cancer cells compared to non-cancer cells. 3. The DNA copy number of OSBPL3 was amplified in 89% cases and 80% cell lines of CRC. 4. In TCGA, high expression of OSBPL3 positively correlated with distant metastasis and was an independent prognostic factor. Conclusion: OSBPL3 could be a novel prognostic biomarker with malignant potential on Ch.7p in CRC.

J-3022

DNA methylation of DYRK2 promoter regulates progression of human colorectal cancer

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Dual-specificity tyrosine-regulated kinase 2 (DYRK2) is known to be a novel tumor suppressor. Recent studies have revealed the downregulation of DYRK2 transcription in colorectal cancer. However, molecular mechanisms by which DYRK2 expression is reduced remains unclear. In this study, we found that promoter of DYRK2 was highly methylated in human cancer calls. Using clinical samples, we demonstrated the DYRK2 promoter was highly methylated in colorectal cancer tissue compared to that in normal tissue. Furthermore, we found that the treatment with 5-azacytidine (Aza), a demethylating agent, inhibited the methylation of DYRK2 promoter, resulting in the increase of DYRK2 expression. In turn, knockdown of DNA methyltransferase (DNMT) 1 enhanced the expression of DYRK2 in colorectal cancer cell lines. In addition, enhanced expression of DYRK2 by both Aza treatment and overexpression experiment significantly inhibited growth of colorectal cancer cells. These findings indicated that DYRK2 expression was regulated by DNMT1 in colorectal cancer. We conclude that DNA methyltransferase inhibitor could be a candidate agent for the effective epigenetic therapy against colorectal cancer.

▲TOP

J-3023

Clinical characteristics and significance of SMAD4 alteration in colorectal cancer

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SMAD4, which is phosphorylated and activated by transmembrane serine-threonine receptor kinase, is an important mediator of the TGF-β pathway. The aim of this study was to evaluate the clinical significance of SMAD4 alteration in colorectal cancer (CRC). We retrospectively investigated 201 patients with Stage I-IV CRC, using a 415-gene panel. Fifty-six patients (28%) had SMAD4 alteration: 24 and 32 patients had SMAD4 mutation and deletion, respectively. SMAD4 alteration was significantly associated with T category (P = 0.027), N category (P = 0.037), M category (P = 0.028), and prognostic invasive-front pathological markers, such as poorly differentiated cluster grade 3 (P = 0.020) and absence of Crohn-like lymphoid reaction (P = 0.004). In 90 patients with stage I-III disease, SMAD4 alteration was significantly associated with poor prognosis on relapse-free and overall survivals (P = 0.047; P = 0.022, respectively). Conversely, in 111 patients with stage IV disease, SMAD4 alteration was not significantly associated with overall survival. In conclusion, SMAD4 alteration was associated with invasive-front pathological markers and poor prognosis in Stage I-III CRC.

J-3024

Estrogen receptor-beta gene cytosine-adenine repeat polymorphism in postmenopausal colon cancer

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Estrogen receptor-β gene cytosine-adenine (ESR2 CA) repeat is a microsatellite sequence, and we have previously shown the association between its germ-line polymorphism and the risk of postmenopausal colon cancer, though the risk converted according to age: longer allele increased colon cancer risk in younger women, but the opposite was true in older women. Microsatellite instability (MSI) is an important carcinogenic mechanism in colorectal cancer especially in older women; however, ESR2 CA repeat has never been examined in surgically removed colorectal tissues. Here, it was examined in cancerous and non-cancerous colon tissue pairs from postmenopausal women, and comparisons were made considering tissue types, age, and immunohistochemically determined MSI status (MLH1/MSH2/MSH6/PMS2). Shorter alleles tended to be frequent in older women than in younger women irrespective of tissue types. MSI positivity was significantly associated with shortening of CA repeat in cancerous tissue than its non-cancerous counterpart. The association among older age, MSI, and shorter ESR2 CA repeat has been suggested in postmenopausal colon cancer.

▲TOP

[LS33] LS33 [English]
The dog as a human cancer model

2018 / 9 / 29 (Sat) 11:50-12:40 Room 10/11F 1101+1102, Osaka International Convention Center Room 10
：TRAC (Translational Research Unit for Small Animal Cancer), Core Clusters for Research Initiatives of Yamaguchi University

Shimpei Nishikawa / TRAC (Translational Research Unit for Small Animal Cancer), Core Clusters for Research Initiatives of Yamaguchi University

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LS33
The dog as a human cancer model

Chand Khanna
Ethos Veterinary Health

No Abstract

[J-3073] J13 [Japanese]
Growth factors / cytokines

2018 / 9 / 29 (Sat) 13:40-14:55 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Kunio Matsumoto / Cancer Res. Inst., Kanazawa Univ.

J-3073

Maintenance of breast cancer stem-like cells by cancer associated fibroblast-derived soluble factors

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Accumulating evidence indicates the presence of cancer stem cells (CSCs) in many types of tumors. They are defined as cell populations which have self-renewal ability and multi-differentiation capacity, and have been thought to contribute to tumor initiation and recurrence. Therefore, developing CSC targeting therapy is in urgent need. Stem-cell properties of CSCs are thought to be maintained in the CSC niche that is the tumor microenvironment surrounding CSCs. It is important to identify factors mediating the interaction between CSCs and their niche. In this study, we focused on cancer-associated fibroblasts (CAFs) that form the great part of tumor microenvironment including CSC niche and investigated how CAFs influence the functions of CSCs. By sphere forming assay, we found that some soluble factors secreted by CAFs induced growth of CSCs. Then, to identify which factors are upregulated when fibroblasts get CAF-like properties, we compared transcriptomes of fibroblasts which were cultured with or without breast cancer cells. We found CSF2 and CSF3 were highly upregulated in co-cultured cells and they could induce sphere forming ability of CSCs.

▲TOP

J-3074

Analysis of disruption mechanism of the breast duct and basement membrane by estradiol

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With the progress of breast cancer from DCIS to ductal invasive carcinoma, tumor cells induce disruption of the breast duct and basement membrane, and invade surrounding tissues, eventually leading to metastasis.Several studies have investigated the relationship between estrogen and breast carcinoma, and the evidence was provided that concentration of estrogen in the tissues of breast cancer patients increased more than 10 times in the blood. However, the molecular mechanisms underlying estrogen effects are not well understood. Here we report that the action of estrogen is involved in the collapse of breast duct formation. The estradiol (E2) increased the secretion of IL-1 β and also increased the expression levels of JNK and p38 phosphorylation and cleaved caspase 3. Furthermore, activation of MMP-3 was assessed after stimulation with E2. We used the most common MCF10A acini as 3D cell model for human breast glands and verified that E2 induced apoptosis in MCF10A cells and disrupted the basement membrane using immunofluorescence confocal microscopy. Our results suggest that E2 promotes the secretion of IL1 β and induces apoptosis via the p38/JNK pathway to disrupt the breast duct.

J-3075

Distinct roles of VEGF isoforms as negative regulator of extracellular matrix deposition for tumor progression

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Christian Stockmann, Randall S. Johnson
Angiogenic inhibitors sometimes show resistance in cancer therapy. In our preceding study, deletion of vascular endothelial growth factor (VEGF) in myeloid cells accelerated breast tumor advance. It increased fibrotic damage in lung fibrosis model. Autocrine VEGF isoforms differentially regulated endothelial cell behavior. Based on them, we hypothesized that VEGF isoforms distinctively regulate fibroblast activation leading to tumor progression. Mouse embryonic fibroblasts (MEFs) were isolated from transgenic embryos expressing each VEGF isoform (VEGF120 or VEGF164) only or with exon 3 of Vegf flanked by loxP sites. VEGF nullizygous up-regulated arginase activity, which is involved in collagen synthesis. MEFs expressing single VEGF164, a matrix-bound type of isoform, reduced collagen deposition and arginase activity, whereas MEFs with only VEGF120, a diffusible type of isoform, showed no significant changes. Treatment with VEGF164 suppressed arginase activity. These results suggest that VEGF164 may play unique roles for microenvironment rearrangement by inhibiting arginase activity in fibroblasts via autocrine and/or paracrine manner.

J-3076

HGF produced by smooth muscle cells promotes lung metastasis as a metastatic niche component

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Hepatocyte growth factor (HGF) is secreted mainly by stromal cells as a single-chain pro-HGF incapable of activating MET receptor. Because activation of MET receptor in cancer cells participates in malignant progression, the processing of pro-HGF to active two-chain HGF (tcHGF) plays an important role in cancer progression, but the biological mechanism has remained unclear. In this study, we investigated distribution of tcHGF in lung metastasis model. Neutralization of systemic HGF suppressed lung metastasis of B16-F10 melanoma cells, indicating intrinsic HGF participates in lung metastasis. In the normal lung, pro-HGF was expressed by α-smooth muscle actin-positive cells, whereas tcHGF was not detected. When B16-F10 cells were injected intravenously, pro-HGF was activated by epithelial cells in the early stage of metastatic colonization, and tumor cells metastasize to the site in which active tcHGF was co-localized. In vitro assay, tcHGF as well as total HGF secreted by primary smooth muscle cells in culture were increased by the culture supernatants of B16-F10 cells. These results indicate that tcHGF may play a role in pulmonary colonization as a metastatic niche factor.

▲TOP

J-3077

Image-based phenotypic profiling using a pharmacologically active compound library identify novel druggable targets

Kenji Tanabe
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Epidermal growth factor receptor (EGFR) is one of major driver gene in cancer, and many clinically effective drugs that target EGFR-associated molecules have been developed. Recently, I developed image-based phenotypic profiling consisting of high-content analysis and unsupervised machine learning to identify mechanism of action of drugs (Tanabe, Sci. Rep. 2016, Tanabe et al., SLAS Discov. 2018). In this study, I combined the image-based phenotypic profiling with a pharmacologically active compound library to identify novel druggable regulators involved in the EGFR pathway. Inhibitors whose targets are associated with EGFR pathway have been discriminated based on the phenotypic classification. Of these, several irrelevant inhibitors also showed similar phenotypes to well known EGFR-associated inhibitors. Biochemical assay revealed that these compounds also have inhibitory activity against targets associated with EGFR pathway. Importantly, some inhibitors targeting ROCK or proteasome also showed distinct phenotypes in the EGFR pathway. These results indicate that the method employed in the present study is effective for identifying druggable novel regulatory molecules.

J-3078

lncRNA NORAD regulates transforming growth factor - β signaling and epithelial-to-mesenchymal transition-like phenotype

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Long noncoding RNAs are involved in a variety of cellular functions. In particular, an increasing number of studies have revealed the functions of long noncoding RNAs in various cancers; however, their precise roles and mechanisms of action remain to be elucidated. NORAD, a cytoplasmic long noncoding RNA, is upregulated by irradiation and functions as a potential oncogenic factor by binding and inhibiting Pumilio proteins (PUM1/PUM2). Here, we show that NORAD upregulates transforming growth factor- β (TGF- β) signaling and regulates TGF- β -induced epithelial-to-mesenchymal transition (EMT)-like phenotype, which is a critical step in the progression of lung adenocarcinoma, A549 cells. However, PUM1 does not appear to be involved in this process. We thus focused on importin β 1 as a binding partner of NORAD and found that knock down of NORAD partially inhibits the physical interaction of importin β 1 with Smad3, inhibiting the nuclear accumulation of Smad complexes in response to TGF- β . Our findings may provide a new mechanism underlying the function of NORAD in cancer cells.

▲TOP

[J-3079] J17-3 [Japanese]
Drug delivery system (2)

2018 / 9 / 29 (Sat) 14:55-16:10 Room 10/11F 1101+1102, Osaka International Convention Center Room 10

Eishi Ashihara / Dept. Clin. & Translational Physiol. Kyoto Pharm. Univ.

J-3079

Efficient synthesis of pyrrole-imidazole polyamides by using the tag

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While typical inhibitors need to bind directly with targets to interfere with cell signaling pathway, pyrrole-imidazole (PI) polyamides can recognize the genomic sites targeting undruggable targets such as KRAS which lack pockets for ligand binding. PI polyamide is a class of artificial peptides containing N-methylpyrrole and N-methylimidazole and can bind with DNA in a DNA-sequence specific manner. The target sequence of PI polyamide s can be programmed by changing the order of these hetero rings by organic synthesis. Nonetheless, current synthesis method have not completely fulfilled a requirement for PIP-based drug development such as time, cost and quality of synthesis. To overcome these problems, we have tried to develop a novel synthetic methodology by which desired PI polyamide can rapidly and easily be enriched from a mixture containing several by-products that are difficult to be eliminated via typical purification methods depending on molecular polarity. In this presentation, we report a novel approach that can provide pure PI polyamide by using the “tag” that selectively alter a chemical property of desired PI polyamide product in a mixture after organic reactions.

▲TOP

J-3080

Hydrophobic primary structure of hairpin-form pyrrole-imidazole polyamide enhances tumor accumulation/retention in vivo

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Pyrrole-imidazole polyamide (PIP), which is a class of compounds consisting of N-methylpyrrole and N-methylimidazole (Im), has been considered as a promising novel anti-tumor drug. PIPs have an intrinsic tumor accumulating property; however, its physiochemical principles remain largely unknown. In this study, we have asked the possible impact of the hydrophobicity of PIP, which is partly influenced by the Im unit numbers, on its tumor accumulation. For this purpose, we synthesized a series of PIP-fluorescein conjugates with the distinct hydrophobicility owing to the different Im unit numbers and monitored their biodistribution in tumor-bearing mice. The hydrophobic PIP showed massive accumulation and long-term retention in tumor as well as liver. The hydrophilic PIP accumulated in tumor, but to a lesser extent, while it also showed the “kidney-homing” property. Additionally, its blood circulating level was lower than the hydrophobic PIP. Collectively, our present results strongly suggest that the hydrophobicity influences the pharmacokinetic property of PIPs in tumor-bearing mice as the balance of its preferential incorporation into tumor tissue and the excretion from the body.

J-3081

Novel pH-Sensitive Nanomedicine conjugating (+)-JQ-1 Homolog Inhibits the Tumor Growth of c-Myc High-Expressing Tumor

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(+)-JQ-1 has received widespread attention as an epigenetic-targeted BRD inhibitor for cancer therapy. However, its short half-life and poor water solubility have limited its translation into human clinical trials. In this research, we succeeded in preparing a (+)-JQ-1 homolog encapsulated in polymeric micelles via a pH sensitive linker. This nanomedicine has a diameter of about 38 nm (PDI = 0.151). The (+)-JQ-1 homolog is released from the micelles at mild acidic pH environment (pH = 5-6.6). The released drug was a (+)-JQ-1 homolog which has more binding potency to BDR4 than the original (+)-JQ-1. Then, we screened the efficacy of the nanomedicine against various cancer cell lines, and found the high efficacy against NUT midline carcinoma (Ty-82) and glioblastoma (GL-261). These cell lines overexpressed c-Myc, which is the downstream signal of the BRD4. We also confirmed that this nanomedicine inhibits tumor growth more effectively than the free drug in GL261 glioma subcutaneous model. These results indicate that this nanomedicine improves the efficacy of the BET inhibitor in relevant tumor models.

J-3082

Staurosporine/Epirubicin-loaded Nanomedicines Induce Immunogenic Cell Death and Suppress Lung Metastasis /Tumor Regrowth

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Nanomedicine has demonstrated advantages for combining drugs within single platforms for regulated pharmacokinetics and concentration of drugs in tumors. We have demonstrated this concept by using polymeric micelles co-incorporating the cytotoxic agent Epirubicin (Epi) and the pan-kinase inhibitor Staurosporine (STS), which was identified as a potent CSC inhibitor. (ACS Nano (2016), JCR (2017)). In this study, we found that the combination therapy of Epi and STS though the micelles induced immunogenic cell death (ICD). Thus, the micelles stimulated dendritic cells by exposure of several proteins, such as calreticulin and HSP70,90 on the tumor cell membrane and accelerated the release of ATP and HMGB1. We challenged the vaccine effect by using murine renal carcinoma (Renca) orthotopic and lung metastatic models. Subcutaneous injection of the cells exposed to STS/Epi/m for 24 h inhibited the metastases to lung and tumor regrowth by the vaccine effect. We concluded that the Epi/STS-loaded nanomedicine could directly kill the tumor and simultaneously induce cancer immunity, to inhibit metastasis and recurrence.

▲TOP

J-3083

HPLC analysis of nuclide-ligand complex formation for efficient radionuclide encapsulation in liposomes

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Radionuclide-carrying liposomes are promising platform for radiotheranostics. Each nuclide must form stable complexes with chelating ligands in liposomes. In this study, we developed an analysis method to evaluate their formation and stability in liposomes by using HPLC, and examined efficient encapsulation of therapeutic nuclides in liposomes.
NOTA, Cu^{2+} and Cu-NOTA were successfully separated and quantified by combination of cation exchange and unique ODS column with HPLC system equipped with an aerosol-based detector, although each of them had no UV absorption. Using this method, optimal condition to load Cu^{2+} in liposomes was determined, and ^{64}Cu was successfully loaded in liposomes with high efficiency (>90%). DOTA, Y^{3+} and Y-DOTA were also separated under similar conditions, and ^{88}Y was efficiently encapsulated in liposomes. Both liposomes containing ^{64}Cu-NOTA and ^{88}Y-DOTA prepared as mentioned above were stable under serum incubation for 24 h, and showed good tumor accumulation (ca. 5 %AD/g) in FaDu-bearing mice after iv injection.
In conclusion, our HPLC analysis of nuclide-ligand complex formation was useful for effective encapsulation of radionuclides in liposomes.

J-3084

Accumulation of sonosensitizer-loaded nanoparticle in cancer tissue in sonodynamic therapy

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Background: Sonodynamic therapy (SDT) using low-intensity ultrasound and a sonosensitizer is a promising approach for cancer treatment. We recently reported that SDT together with microbubble (cSDT) showed the significant anticancer effect. This effect was, however, brought by the intratumoral injection. In this study, we investigate the accumulation of the venously injected sensitizer-loaded nanoparticles with the different size.
Methods: MKN-74 cells, a human gastric cancer cell line, were used in the mouse xenograft model assay. DEG and indocyanine green (ICG) were used as a sonosensitizer without light reactivity and a maker of DEG-loaded nanoparticle. The nanoparticles were characterized by the Nanosight system. The distribution of the nanoparticle is observed by using BZ-7000 or IVIS system.
Results: The nanoparticles with different size, approximately 100 or 200 nm, were prepared by the sonication or the vigorously shaking. We are now investigating the distribution of DEG-loaded nanoparticle in vivo. The data might give some important information to determine the optimal size of nanoparticle for cSDT.

▲TOP

[ML22] ML22 [Japanese]
Morning Lectures 22

2018 / 9 / 29 (Sat) 8:00-8:50 Room 11/12F Conference Hall, Osaka International Convention Center Room 11

Hidenori Inohara / Dept. Otolaryngol-Head & Neck Surg., Osaka Univ. Sch. Med.

ML22

Advances in bio-imaging technology in cancer research

Takeshi Imamura
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Molecular imaging including fluorescence imaging is a promising technique, and has already been applied for in vitro experiments in cancer research. Recently, there has been a growing interest in applying the fluorescence imaging technique to study complex biology of cancer in vivo. Particularly, intravital fluorescence imaging using various functional fluorescent proteins and dyes, in conjunction with appropriate fluorescence microscopy, allows us visualization of cell behavior as well as cell function in vivo. In this lecture, I will talk about applications of the fluorescence imaging technology to monitor cancer cell behavior and function in vivo. In addition, I will demonstrate interaction between cancer cells and tumor microenvironment by fluorescence imaging technique in vivo. Moreover, I would like to talk about a technological development of fluorescence imaging equipment including two-photon microscope and light-sheet microscope, and the application of the fluorescent imaging approaches to cancer research. These techniques will be useful to investigate the cancer biology and test the effectiveness of therapeutic agents.

▲TOP

[J-3025] J14-16 [Japanese]
The pathogenesis and the development of novel therapies for bone and soft tissue tumors

2018 / 9 / 29 (Sat) 9:00-10:15 Room 11/12F Conference Hall, Osaka International Convention Center Room 11

Makoto Endo / Dept. Orthop. Surg., Kyushu Univ.

J-3025

Novel therapeutic strategy with anti-PD-1 antibody and telomerase-specific oncolytic virotherapy in osteosarcoma

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Immunotherapy with immune checkpoint inhibitors, including anti-programmed death protein 1 (PD-1) antibody, has improved the clinical outcome in cancer patients; however, the therapeutic efficacy of anti-PD-1 antibody is limited to certain cancer types. Recently, it has been suggested that combination with immunogenic antitumor therapy including oncolytic virotherapy augments PD-1 blockade immunotherapy. In this study, we assessed the combined effect of anti-PD-1 antibody and telomerase-specific oncolytic adenovirus OBP-502 in murine osteosarcoma. We used 2 murine osteosarcoma cell lines, K7M2 and NHOS with PD-L1 expression. OBP-502 dose-dependently suppressed the viability of K7M2 and NHOS cells. OBP-502 significantly induced the immunogenic cell death, the secretion of ATP and high-mobility group box protein B1, in K7M2 and NHOS cells. Intratumoral injection of OBP-502 significantly suppressed the tumor growth in combination with PD-1 blockade via induction of tumor-infiltrating CD8+ T cells. Our results suggest that telomerase-specific oncolytic virotherapy is a promising antitumor strategy to promote the therapeutic efficacy of PD-1 blockade immunotherapy in osteosarcoma.

▲TOP

J-3026

Clinical genomic sequencing of osteosarcomas reveals distinct molecular subsets with potentially targetable alterations

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Osteosarcoma is the most common primary malignant bone tumor. In this study, to identify potentially actionable alterations and novel targetable alterations, we conducted the clinical sequencing of osteosarcoma using MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets). The tumor and germline DNA in 72 high-grade osteosarcomas (67 cases) were sequenced using MSK-IMPACT (341 to 468 cancer-associated genes) with the cBioPortal. Potentially actionable genetic events were categorized according to OncoKB.15 of the 67 cases (17.9%) had at least one potentially actionable alterations including CDK4 and MDM2 amplification, BRCA2 truncating mutation, GULP1-PTCH1 fusion) were defined according to OncoKB. 4q11-12 amplifications including KIT, KDR and PDGFRA were identified in 13 of 67 cases (19.4%) and these genes co-occurred with PDGFRA, KIT and KDR. These amplifications might be targetable by some multi-kinase inhibitors. VEGFA amplification, which co-occurred with CCDN3 at 6p12 was identified as a targetable dependency (affecting angiogenesis) in 14 of 67 osteosarcoma cases (20.9%) and was mutually exclusive with KIT,KDR and PDGFRA amplification.

J-3027

Identification of genomic alterations in metastatic pediatric osteosarcoma

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Osteosarcoma (OS) is a primary malignant bone tumor in children and adolescents. Frequent alterations in cancer drivers, such as RB1 and TP53, are found in primary OS. Although pulmonary metastasis (PM) is the main cause of tumor-related deaths in patients with OS, genomic alterations associated with PM are unclear. Thus, we conducted whole exome sequencing (WES) and array-CGH analysis using primary OS samples with PM (n=11) and with no-PM (n=7). WES showed that a higher number of somatic single nucleotide variations (SNVs) in primary tumor were significantly correlated with PM (Yes=31 SNVs, No=15 SNVs, P<0.04). In addition, a higher frequency of T>C mutation in primary tumor was also associated with PM (Yes=27%, No=14%, P<0.002). Functional annotation clustering highlighted enrichment of genes with damaging mutation of calcium-phosphatidylinositol (Ca-PI) signals in primary OS with PM. Particularly, 91% of OS patients with PM harbors a mutation and/or loss of heterozygosity within 8 genes corresponding to Ca-PI signals. Collectively, these genomic alterations characteristic of metastatic primary OS will become a new biomarker and treatment target for improving survival with PM.

J-3028

True Neoplastic Cells in Giant Cell Tumor of Bone are not Osteoclast but Osteoblast Lineage Cells

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Denosumab, which is a human monoclonal antibody against RANKL, has recently been introduced into the therapy of giant cell tumor of bone (GCTB). The histology after denosumab shows dramatic changes including disappearance of osteoclast-like giant cells and formation of woven bone, whereas the actual mechanism remains unclear. We reviewed 12 GCTB patients treated with denosumab. Immunohistochemically, NFATc1(an osteoclast marker)-positive cells mostly disappeared after the therapy. However, RUNX2(an osteoblast marker)-positive cells and H3.3 G34W(a mutation specific marker)-positive cells abundantly existed through the therapy. Moreover, RUNX2 and H3.3 G34W constantly co-expressed by immunofluorescent study. H3F3A G34W mutation was also detected through the therapy. We revealed that true tumor cells (G34W+ cells) in GCTB are not osteoclast but osteoblast lineage cells. The tumor cells survive denosumab therapy and unveil their osteogenic histology which is hidden before denosumab therapy. Although osteoclast-like giant cells are not neoplastic, they may have influence to suppress osteogenic nature of the tumor cells, and help expand the lesion with their osteolytic function.

▲TOP

J-3029

Predictive value of CD 34 positivity in myxofibrosarcoma and undifferentiated pleomorphic sarcoma

Yoshiya Sugiura
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Background Recently previous MFH was divided into myxofibrosarcoma (MFS) and undifferentiated pleomorphic sarcoma (UPS), but the validity of this classification is still controversial. Materials and methods Firstly we compared the overall survival between MFS (n=60) and UPS (n=141), and performed a survival analysis. Then we performed an immunohistochemistry on 18 and 65 cases of MFS and UPS, respectively and performed a survival analysis to evaluate the prognostic value of CD34 positivity. Results Five year survival rates of MFS (n=60) and UPS (n=141) were 81.3% and 74.8%, respectively and there was no significant difference between them (p=0.161). On immunohistochemistry, twenty-one cases (25.1%) out of 83 cases were positive for CD34 and five year survival rates of CD34 positive and negative cases were 85.4% and 56.7%, respectively and there was significant difference between them (p=0.024). CD34 positivity was an independent predictor also in multivariate analysis (Hazard ratio=3.65, p=0.048). Conclusion From a viewpoint of prognosis, there was no evidence to divide previous MFH into MFS and UPS. It was better to divide previous MFH into CD34 positive and negative cases.

J-3030

Sleeping Beauty transposon mutagenesis screen of uterine leiomyosarcoma identifies driver genes of sarcomagenesis

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Uterine leiomyosarcoma (ut-LMS) is an aggressive malignancy with extremely poor prognosis, and its molecular mechanisms remain largely unknown. To solve this, we conducted sleeping beauty (SB) transposon mutagenesis screen for ut-LMS. We generated 59 experimental Amhr2-CreTg/+;T2Onc2Tg/+;SBaseKI/+;Ptenfl/fl;KrasLSL-G12D/+ mice, and 14 Amhr2-CreTg/+; Ptenfl/fl;KrasLSL-G12D/+ mice. The experimental mice have homozygous deletion of Pten, constitutive activation of Kras and SB transposon mobilization in uterine smooth muscle cells. All of the experimental mice died of ut-LMS until 2 months of age, while no control mice developed ut-LMS, clearly indicating transposon mutagenesis was required for sarcomagenesis. Eighty primary ut-LMS and 24 normal uterus of mice with ut-LMS were sequenced. Gene-centric common insertion site analysis (gCIS) identified 19 CIS genes in ut-LMS but no genes in normal uterus. To further identify the metastatic drivers of ut-LMS, we established lung metastatic model, by tail vein injection of 9 cell lines established from SB ut-LMS, then obtained 50 lung tumors in immunocompetent mice. gCIS analysis identified 3 potential driver genes of lung metastases.

▲TOP

[J-3031] J9-1 [Japanese]
DNA methylation

2018 / 9 / 29 (Sat) 10:15-11:30 Room 11/12F Conference Hall, Osaka International Convention Center Room 11

Hiromu Suzuki / Dept. Mol. Biol., Sapporo Med. Univ., Sch. Med.

J-3031

Comprehensive analysis to identify aberrant DNA methylation for predicting colitis associated cancer

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Several molecular alterations may be promising as markers for identifying patients at high risk of developing colitis associated cancer (CAC). In this study, we conducted comprehensive methylation array to identify novel DNA methylation markers for predicting the risk of neoplasia in UC patients. We collected 23 rectal samples from UC patients with CRC and 24 from those without CRC in collaborative hospital (Cohort1). We also collected 8 rectal samples from UC patients with CRC and 8 from those without in our hospital (Cohort2). As the results, we identified 486 differentially methylated regions (DMRs) with absolute delta beta-value ＞ 0.1 in rectum from UC patients with CAC compared with rectum from those without. Next, pathway enrichment analysis was performed to select coordinately methylated DMRs, and 180 DMRs were extracted. Finally, optimal 11 DMRs were selected by the Elastic Net classification algorithm. In the ROC analysis for the training set (Cohort 1), the AUC was 0.96 (95 % CI: 0.90, 1.00). For the test set (Cohort 2), the AUC was 0.81 (95 % CI: 0.55, 1.00). In conclusion, we identified 11 DMRs for identifying UC patients with high risk of developing CAC.

▲TOP

J-3032

IRX4, a hypermethylated gene in pancreatic cancer, regulates expression of a subset of cancer-related genes

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Epigenetic gene silencing by aberrant DNA methylation is one of the important mechanisms leading to the loss of key cellular pathways in tumorigenesis. We have previously reported that IRX4 (Iroquois homeobox 4) was highly downregulated by promoter hypermethylation in pancreatic cancer cell lines as well as in resected primary pancreatic cancers. Based on these data, we have constructed a tetracycline-inducible IRX4 expressing system using pancreatic cancer cell lines PK-1 and PK-9. IRX4 induction significantly suppressed cell growth and caused apoptosis in both PK-1 and PK-9. Because IRX4 is a sequence-specific transcription factor, we tried to analyze IRX4 downstream events by performing microarray analyses using IRX4 inducible PK-1 cells with or without tetracycline. We found that IRX4 induction upregulated several genes that had tumor suppressive functions such as PRDM1, CRYAB, and IL32, and downregulated several genes that had oncogenic functions such as PTCH1, CD36, TFAP2C, and MUC4. These results suggest that DNA methylation-mediated silencing of IRX4 may contribute to pancreatic tumorigenesis through aberrant transcriptional regulation of cancer-related genes.

J-3033

Pancreatic cancer cell fraction estimation in a DNA sample

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Pancreatic cancer is characterized by dense stroma. To avoid the interference by contaminating non-cancerous cells in molecular analyses of pancreatic cancer, we aimed to develop a DNA methylation marker that can be used to assess a fraction of cancer cells in DNA samples. First, we screened genes unmethylated in nine non-cancerous tissues and methylated in 22 cancerous tissues, and isolated 42 genes. Among these, four genes, SIM1, MIR129-2, NR1I2, and HOXB-AS4, were identified as candidate markers with a broad coverage across patients. Next, methylation levels of the four genes were validated using bisulfite pyrosequencing and 56 independent pancreatic surgical specimens, including 28 non-tumorous and 28 tumor samples. One or more of three genes, SIM1, MIR129-2, and NR1I2, were highly methylated in 20 tumor samples (71.4%), and all had little methylation in 24 non-tumorous samples (85.7%). The cancer cell fraction measured by the marker of the three genes was highly correlated with that estimated using the KRAS mutant allele frequency (R = 0.81). In conclusion, the DNA methylation marker is useful for the measurement of a pancreatic cancer cell fraction in DNA samples.

J-3034

Cancer-specific DNA methylation of CDO1 gene as an prognostic marker of gastric cancer and colorectal cancer

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In the tissue of gastric cancer / colon cancer with surgery alone, the CDO1 hypermethylation was an independent prognostic factor. In this study, we will quantify methylation in advanced gastric cancer / colorectal cancer, which is an indication for adjuvant chemotherapy, and clarify the significance of clinicopathological traits. (1) In the 321 patients with pStage Ⅱ / Ⅲ advanced gastric cancer, cases with postoperative adjuvant therapy in CDO1 hypermethylated group had a significantly better prognosis (P=0.0037) than those with surgery alone. (2) Hypermethylated cases in pStage Ⅲ advanced colon cancer cases were correlated with good prognosis in postoperative adjuvant chemotherapy group (P=0.0339). (3) In the colon cancer cells transfected with the CDO1 gene, the proliferation activity was augmented in anaerobic environment (0.1%), the mitochondrial membrane potential increased and the susceptibility to 5-FU was decreased. Hypermethylation of the CDO1 gene showed opposite features with regard to cancer aggressiveness and chemosensitivity in primary gastric / colon cancer, which were verified in functional experiments.

▲TOP

J-3035

ZNF750 gene promoter is aberrantly methylated in prostate cancer

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Epigenetic gene silencing by aberrant DNA methylation plays a key role in tumorigenesis. In order to search for hypermethylated genes in prostate cancer, we applied a novel MeTA method to three prostate cancer cell lines, DU145, LNCaP and PC-3, as well as one normal prostatic epithelial cell line RWPE-1. A set of 189 genes were selected as candidate tumor-specific hypermethylated genes. Among these, we focused on ZNF750 because it has recently been identified as a tumor suppressor in squamous cell carcinoma. Analysis of MethHC database revealed that there was an inverse correlation between ZNF750 expression and promoter methylation in 23.5% (8/34) of prostate cancer tissues. We performed qRT-PCR and sodium bisulfite sequencing analyses and found that ZNF750 was hypermethylated and silenced in all three prostate cancer cell lines but not in normal RWPE-1. We further analyzed DNA methylation statuses of ZNF750 using 51 paired DNAs from microdissected primary prostate cancer tissues and found that aberrant methylation was increased in a cancer-specific manner in 43.1% for ZNF750. These results suggest that aberrant methylation of ZNF750 may be involved in prostatic tumorigenesis.

J-3036

Abnormal CpG methylation around the transcription start sites as therapeutic target in adult-T cell leukemia-lymphoma

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Adult T-cell leukemia/ lymphoma (ATL) is an aggressive hematological malignancy derived from CD4 (+) T-cells, which are transformed by human T-cell lymphotropic virus-1 (HTLV-1). In this study, we aimed to reveal the contribution of DNA methylation abnormalities in the carcinogenesis of ATL. Unsupervised hierarchical clustering analysis revealed that the amounts of methylated CpGs around transcription start sites (TSSs) in ATL cells (or HTLV-1 infected cells) isolated from patients with ATL, had a tendency to be correlated with disease progression of ATL. Therefore, we next focused on anti-ATL effects of a DNA demethylating agent OR-21, which was a novel decitabine prodrug with the potential for oral administration. OR-21 inhibited cell growth and decreased DNA methylation at the LINE-1 repeat region in ATL cell lines. Intraperitoneal injection of OR-21 suppressed tumor growth of MT-2 cells, which were subcutaneously implanted in immunodeficient Balb/c Rag-2 $^{-/-}$ Jak3 $^{-/-}$ mice. Based on these findings, we think abnormal CpG hypermethylation around TSSs as novel therapeutic target in ATL.

▲TOP

[LS39] LS39 [English]

Biological considerations in Colorectal Cancer~Primary Tumor location and Consensus Molecular Subtype~

2018 / 9 / 29 (Sat) 11:50-12:40 Room 11/12F Conference Hall, Osaka International Convention Center Room 11
：Merck Serono., Ltd.

Xundi Xu / Department of Gastroenterology, Central South University, China

LS39

Biological considerations in Colorectal Cancer~Primary Tumor location and Consensus Molecular Subtype~

Dan Aderka
Sheba Medical Center, Israel

No Abstract

[J-3085] J9-2 [Japanese]
Histone modification

2018 / 9 / 29 (Sat) 13:40-14:55 Room 11/12F Conference Hall, Osaka International Convention Center Room 11

Keiko Shinjo / Div. Cancer Biol., Nagoya Univ Grad. Sch. Med.

J-3085

Synthetic Lethality by ATR inhibition in Aggressive Prostate Cancer Deficient in Y-linked Histone Demethylase KDM5D

Kazumasa Komura
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Epigenetic modifications control cancer development. Here, we show that loss of KDM5D encoded on the Y chromosome epigenetically modifies histone methylation marks and alters gene expression resulting in aggressive prostate cancer. Fluorescent in situ hybridization demonstrated segmental or total deletion of the Y chromosome in prostate cancer cells. The result of chromatin immunoprecipitation sequencing (ChIP-seq) analysis revealed that KDM5D preferably binds to promoter regions with co-enrichment of the motifs of crucial transcription factors that regulate the cell cycle. Loss of KDM5D expression with dysregulated H3K4me3 transcriptional marks was associated with acceleration of the cell cycle and mitotic entry leading to increased DNA replication stress. Notably, we also found stress-induced DNA damage and reliance on ATR signaling with loss of KDM5D. In KDM5D deficient cells, blocking ATR activity with an ATR inhibitor enhanced DNA damage, which led to subsequent apoptosis. These data start to elucidate the biological characteristics resulting from loss of KDM5D and also provides clues for a potential novel therapeutic approach for this subset of aggressive prostate cancer.

▲TOP

J-3086

Toward precision medicine: developing new toolbox to study the epigenetics applied for cancer treatment

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Over the past few years, precision medicine is an emerging approach for cancer treatment and prevention that takes into account individual variabilities on genomic information. Although the analyses of genomic alterations in some patients successively identify driver mutations that cause cancer, and help the prediction of cancer prognosis and the development of treatments, it has been a challenging to identify them in the vast majority of patients. Accordingly, epigenetic alterations have been considered to play pivotal roles in cancer. Here, we tackle this issue by analyzing histone modifications and chromatin-binding proteins, key molecules to control epigenetics. To this end, we developed novel approach to perform ChIP-seq using clinical samples. We also introduced LabDroid “Mahoro” that reproducibly performs laborious tissue-ChIP experiments. We would demonstrate how we integrate genetic and epigenetic datasets as well as clinical images and information, and analyze them by using machine-learning algorisms. We believe this might be ultimate approach to treat cancer patients.

J-3087

Withdrawn

No Abstract

J-3088

Study of Novel Inhibitor of EZH2/PRC2 in Cancer Cells

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EZH2 is an enzymatic subunit of polycomb repressive complex 2 (PRC2), which mediates trimethylation of histone H3 lysine 27 (H3K27me3). Since the upregulation of EZH2/PRC2 has been linked to tumor progression and aggressiveness, targeting EZH2/PRC2 has been challenged for cancer treatment. We established a chemical screening system based on the measurement of reactivation of promoter activity, which is silenced by H3K27me3 at the base-line level. We screened more than 300,000 small chemicals and identified N47 as a potential reactivator of silenced promoter activity by H3K27me3. Treatment of prostate and ovarian cancer cells with N47 showed inhibition of cell growth together with H3K27me3 depletion on the target genes. Although N47 minimally inhibited the methyltransferase activity of EZH2 in vitro, the genome wide expression analysis revealed that almost half of the upregulated genes after N47 treatment were overlapped with the upregulated genes after EZH2 inhibitor, GSK126, treatment. Together, our data indicate that a novel EZH2/PRC2 inhibitor suppress the tumor cell growth via upregulation of silenced genes by H3K27me3 and that N47 may be a potent drug for cancer treatment.

▲TOP

J-3089

Identification of molecules that regulate the sensitivity of EZH2 inhibitor in neuroblastoma

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EZH2 is a H3K27 methylase and a target of cancer epigenetic treatments. We recently reported the oncogenic roles in the MYCN-amplified aggressive neuroblastomas (NB) (Li et al., ONCOGENE, 2018). Here, we investigated the effects and function of small molecule EZH2 inhibitor (EZH2i) on aggressive NB model cell lines. We examined the antitumor effect of EZH2i using WST assay and colony formation assay. Suppression of proliferation was observed in NB-A, B (sensitive cells) dose-dependently, whereas it was not observed in NB-C, D (resistant cells). FACS analysis showed apoptosis and G1 cell cycle arrest in sensitive cells. Transcriptome analysis and GSEA indicated significant changes were observed in the gene set related to cell cycle arrest and differentiation in sensitive cells. We selected genes induced at mRNA level by EZH2i only in the sensitive cells and confirmed the tumor suppressor function in NB patient database. Gene-A, which is involved in cell growth suppression and differentiation in stem cells, and one of the EZH2i target gene CDKN1C are detected. We studied the functional roles of Gene-A by over-expression and knockdown experiments by lentiviral systems in NB cells.

J-3090

Lineage-specific RUNX2 super-enhancer activates MYC via a chromosomal translocation and promotes the BPDCN

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Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is an aggressive subtype of acute myeloid leukemia. BPDCN cells show high frequencies of mutations of TET2 and TP53; however, the molecular mechanisms underlying the pathogenesis of BPDCN have not yet been elucidated. Since translocation (6;8)(p21;q24), specific anomaly for BPDCN, involves regions adjacent to RUNX2 and MYC, we demonstrated that a pDC-specific super-enhancer of RUNX2 was associated with the MYC promoter due to t(6;8). The inhibition of BRD4 and genetic deletion of the mutant-allele super-enhancer of RUNX2 markedly impaired the proliferative capacity of BPDCN cells following the significant reduction in MYC expression, indicating that the translocation juxtaposed the pDC-specific RUNX2 super-enhancer with MYC and aberrantly activated MYC expression. We also demonstrated that the transduction of RUNX2 and MYC was sufficient to initiate the transformation of BPDCN in mice lacking Tet2 and Tp53, providing a new model that recapitulates the human disease. Our results also support a new rationale for combined applications of BRD4 inhibitors with agents targeting pDC-signature genes driven by RUNX2 in patients.

▲TOP

[J-3091] J12-4 [Japanese]
Cancer immunity

2018 / 9 / 29 (Sat) 14:55-16:10 Room 11/12F Conference Hall, Osaka International Convention Center Room 11

Hiroaki Ikeda / Dept. Oncology, Nagasaki Univ., Grad. Sch. Biomed.

J-3091

Valproic acid reduces the immunosuppressive activity of myeloid-derived suppressor cells

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Myeloid-derived suppressor cells (MDSCs), immature myeloid cells, are major immunosuppressive cells that accumulate in cancer patients and mouse tumor models. Since MDSCs promote tumor growth by inhibiting anti-tumor immunity, MDSCs are an attractive target for cancer immunotherapy. In our study, we focus on the anticonvulsant drug valproic acid (VPA), which has additional functions including anti-cancer activity and immune regulation due its inhibition of histone deacetylases. We show for the first time that VPA greatly reduces the immunosuppressive activity of *in vitro* MDSCs in a dose-dependent manner. Additionally, VPA-conditioned *in vitro* MDSCs exhibited impaired ability to stimulate *in vivo* tumor progression. We showed that several mechanisms could be involved in the regulation of the immunosuppressive function of MDSCs, including the IL-4Rα/arginase axis, PD-L1 and TLR4 signaling pathways. *Rb1* derepression which inhibits the differentiation from monocytic MDSCs to polymorphonuclear MDSCs was also observed by VPA treatment. This research highlights the potential of VPA as an immunotherapy targeting MDSCs.

▲TOP

J-3092

Spontaneous immune responses in breast cancer patients against TWIST1: potential as a highly immunogenic shared antigen

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Recently cancer immunotherapy has been focused since immune checkpoint inhibitors (ICIs) show antitumor effect in a clinical setting. Because the ICIs don't have specificity to tumors, other drugs are still needed for activating tumor-specific immune system. TWIST1 is overexpressed in several tumors including melanoma, breast cancer, lung cancer, and head and neck cancer, and promotes epithelial-to-mesenchymal transition and metastasis in tumors. Therefore, it is anticipated that metastatic tumors would be cured or prevented by targeting TWIST1-expressing tumor cells. We developed a cancer vaccine peptide for activating TWIST1-specific helper T (Th)-cells. It binds to several HLA-class II alleles including DRB1^{+}01:01 and 15:02, and DRB4^{+}01:01. Vaccinated into HLA-DR1^{+}mice, the TWIST1 peptide efficiently increased TWIST1-specific Th cells and delayed growth of implanted TWIST1^{+} tumors. Moreover, TWIST1-reacting Th cells were more frequently detected in breast cancer patients than healthy donors, indicating that TWIST1 is high immunogenic and spontaneously activates Th cells in patients. Therefore, vaccine therapy targeting TWIST1 is a rational therapy for tumor metastatic patients.

J-3093

Identification of a CTL cancer-specific antigen encoded by a long non-coding RNA

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Cytotoxic lymphocytes(CTL) survey peptides presented by HLA molecules(pHLA) on target cell surfaces.It has long been argued whether HLA-presented peptides originate from unconventional translation products.Here, we screened peptides that are naturally presented by HLA-A24 of a colon cancer line using large-scale HLA-ligandome analysis, which combines pHLA affinity purification followed by LC-MS/MS analysis.We analyzed the source of a vast number of detected HLA-A24 peptides, and found that some peptides arose from long non-coding RNAs(lncRNAs). A representative 10-mer peptide(F10) was encoded by a well-known lncRNA, which transcript was expressed in a series of colon cancer tissues but not in a panel of normal tissues.Most likely due to its cancer specificity, the F10 peptide stimulation induced specific CD8+ T-cells from a healthy donor.Those T-cell clones produced IFN-γ upon recognition of target cancer cells expressing the responsible lncRNA as well as the synthetic F10 peptide pulsed on T2-A24 cells.Thus, we consider that lncRNA-derived peptide products are presented by HLA class I, and the peptide repertoire contains cancer-specific antigens monitored by CTL.

J-3094

T cell receptor repertoire analysis of lung adenocarcinoma harboring EGFR mutations

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Recent clinical trials of non-small cell lung cancer with immune checkpoint inhibitors revealed that patients with EGFR mutations showed more unfavorable outcome compared with those with wild-type EGFR. However, the underlying mechanism for the link between EGFR mutation and immune resistance still remains unclear. We performed T cell receptor repertoire analysis of resected lung adenocarcinoma tissues with/without EGFR mutations to investigate the characteristics of T cell receptor repertoire. We collected a total of 39 paired (normal and tumor) lung tissue samples (20 had EGFR mutations and 19 had wild-type) and conducted T cell receptor repertoire analysis, whole-exome sequence (WES), and transcriptome analysis. T cell receptor diversity index in EGFR-mutant tumors was significantly higher than that in EGFR wild-type tumors. In WES, EGFR-mutant tumors showed lower numbers of nonsynonymous mutations and predicted neoantigens than EGFR wild-type tumors. The diversity index showed a negative correlation with the number of nonsynonymous mutations. This study revealed distinct characteristics of T cell receptor repertoire between EGFR-mutant and EGFR wild-type tumors.

▲TOP

J-3095

Characterization of immune-suppressive microenvironment in head & neck cancer

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Flow cytometric and immunohistochemical analysis were performed to characterize immune-suppressive microenvironment in head & neck cancer for further development of immunotherapy. Lymphocytes from peripheral blood (PBL) and tumor tissues (TIL) of patients with head & neck cancer were analyzed on eTreg positive rate and expression of immune-checkpoint molecules on eTregs and conventional T-cells (T-conv) by flowcytometry. eTreg positive rate in CD4+T-cells in the TIL was much higher than the PBL, and the expression of immune-checkpoint molecules on eTregs in the TIL was much higher than the T-conv. While, expression of PD-L1 on tumor cells and PD-1 on T-cells in tumor tissues and those topological analysis were performed by multi-fluorescent immunohistochemistry. Highly expression of PD-L1 was observed in most cases of squamous cell carcinomas, and PD-1 was expressed on the most of T-cells. Interaction of PD-L1+ tumor cells with tumor infiltrated PD-1+T-cells was observed in some cases. These findings suggest that not only immune-checkpoint system but also eTregs were important target of immunotherapy for H&N cancer based on improvement of immune-suppressive microenvironment.

J-3096

Phenotypic and genetic characteristics of tumor-reactive CD8+ T cells existing in human colorectal tumor tissue

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It has become increasingly important to have better knowledge about their recognizing epitopes, phenotypic and genetic characteristics of tumor-reactive T cells for establishing of effective personalized immunotherapies. We reported our data about the characteristics of tumor-reactive CD8+ TILs in colorectal tumor tissues at the 77th annual meeting of JCA. To further extend this study, we analyzed tumor samples in details from sixteen colorectal cancer patients. In line with our results, most of tumor-reactive CD8+ TILs usually reside in PD-1+ population. In addition, we observed that these CD8+ T cells most likely exist in the group of T cells expressing TCRs with high repertoire size. Indeed, we successfully identified tumor-reactive TCR in most cases. Correlation with tumor reactivity of other molecules such as 4-1BB, LAG3, TIM3 have been investigated, however, we think that PD-1 might be the most useful marker of tumor reactiveness at the present time. We are trying to determine whether these TCRs are effectively suppress tumor growth in autologous tumor inoculated NOG mice model. These data might be useful for development of future effective personalized immunotherapies.

▲TOP

[ML23] ML23 [Japanese]
Morning Lectures 23

2018 / 9 / 29 (Sat) 8:00-8:50 Room 12/12F 1202, Osaka International Convention Center Room 12

Hiroki Yamaue / 2nd Dept. Surg., Wakayama Med. Univ.

ML23

How to utilize chemistry-based fluorogenic probes for biological researches and clinical medicine

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Discussant：Kenji Tamura
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Fluorescence imaging with various probes and microscopes is a widely used technique in biological fields as one of the most powerful means currently available for continuous observation of dynamic intracellular processes in living cells. In this lecture, among various probes, chemistry-based fluorogenic probes will be showcased, and the concept of chemical biology, examples of live fluorescent molecular imaging, and the possible contribution of this technology for future biological and medical experiments will be overviewed. Also, it will be discussed what can be realized in the field of clinical medicine with chemistry-based live imaging techniques, especially for finding out novel biomarkers for various diseases.

▲TOP

[J-3037] J14-17 [Japanese]
Novel targets for lung cancer therapy

2018 / 9 / 29 (Sat) 9:00-10:15 Room 12/12F 1202, Osaka International Convention Center Room 12

Yasuhiko Nishioka / Dept. Respir. Med. & Rheumatol, Grad. Sch. Biomed. Sci, Tokushima Univ.

J-3037

The Subunit eIF2 β of Translation-Initiation Factor EIF2 Is a Potential Therapeutic Target for Non-Small Cell Lung Cancer

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To identify novel therapeutic targets for non-small cell lung cancer (NSCLC), we conducted an integrative study. A dropout viability screening with semi-genome wide shRNA library was performed in NCI-H358 cells, and its result was integrated with data from our previous screen in NCI-H460 cells. Among the 24 genes identified as potential targets in the both screens, we focused on eIF2β, which is a subunit of heterotrimeric G protein EIF2 and functions as a transcription initiation factor. The eIF2β protein is highly expressed in lung cancer cell lines compared with normal controls, and gene copy number analysis revealed that eIF2β is amplified in a subset of NSCLC cell lines. Furthermore, high eIF2β expression was correlated with poor survival in patients with lung adenocarcinoma, as shown in other cohorts using publicly available online tools. RNAi-mediated depletion of eIF2β suppresses growth of lung cancer cells in part through G1 cell cycle arrest. Our data suggest that eIF2β is a therapeutic target for lung cancer.

▲TOP

J-3038

Antitumor activity of YAP1 inhibitor in K-Ras mutant lung cancer cells

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Lung cancer is the leading cause of cancer death worldwide, and non-small cell lung cancer (NSCLC) accounts for more than 80% of all new lung cancers with poor prognosis. Recent advances in the understanding of the molecular pathogenesis of the disease have enabled the development of drug discovery. The Hippo pathway plays a critical role in cell proliferation, tissue repair, and regulation of organ size. Yes-associated protein (YAP) 1 is the major effector of the mammalian Hippo pathway and regulates the transcriptional enhancer activator domain (TEAD) transcription factors to activate the transcription of target genes. In this study, we identified that the treatment with verteporfin, a YAP1 inhibitor, reduced cell viability in K-Ras mutant lung cancer cells. To determine the role of YAP1 in these cascades, we carried out knockdown of YAP1 with siRNAs and assessed the effect on cell viability in K-Ras mutant lung cancer cells. We are now investigating the function of the YAP1 inhibitor in vivo. These analyses will provide additional insights into the pathogenesis of K-Ras mutant lung cancer cells.

J-3039

Inhibitors of pituitary differentiation reduce cell proliferation of small cell lung carcinomas

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Small cell lung carcinoma (SCLC) shows neuroendocrine differentiation and produces anterior pituitary hormones. We recently found that SCLCs use pituitary developmental pathway to differentiate neuroendocrine-like cells; however, whether the neuroendocrine differentiation changes drug sensitivity has remained elusive. Here we show that agents modulating pituitary differentiation reduce cell proliferation of SCLCs. By using 3D culture system, we established two SCLC cell lines, both of which showed high expression of endogenous pro-opiomelanocortin, precursor of pituitary hormone, compared with parental cells. Among 445 chemicals, the differentiated SCLC cells were sensitive to inhibitors of neuronal differentiation or dendritic spine formation, while the parental cells were sensitive to inhibitors of sonic hedgehog signaling, which is essential for early embryonic development of anterior pituitary gland. Collectively, neuroendocrine differentiation of SCLC altered drug sensitivity to inhibitors of pituitary differentiation.

J-3040

Prorenin receptor is involved in cell proliferation and migration of lung cancer cells through regulation of autophagy

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Prorenin receptor (PRR) is the specific receptor for renin and prorenin and functions as subunit of Vacuolar-type H^+-ATPase (V-ATPase). V-ATPase regulates autophagy through acidification in vesicles and is involved in the pathology of various cancers including lung cancer. However the expression of PRR and relationship between PRR and autophagy is unknown in lung cancer. The aim of this study is to explore the function of PRR in the autophagy and development of lung cancer. In vitro study, we used A549 derived from lung cancer. siRNA-mediated PRR knockdown decreased 40% to 60% of cell proliferation and inhibited migration of lung cancer cells. Moreover, western blot analysis showed that autophagy related protein (LC3-I, LC3-II and p62) were accumulated in lung cancer cells by PRR suppression. Immunohistochemistry indicated that PRR immunoreactivity was detected in lung cancer cells and co-localized with LC3. These results suggest that PRR may be involved in the regulation of autophagy and be related to the pathophysiology of lung cancer.

▲TOP

J-3041

TrkB/BDNF signaling pathway could be a therapeutic target for lung cancer

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Background：It has been reported that TrkB and BDNF signaling are associated with poor prognosis in some kinds of malignancies. In the present study, the biological significance of TrkB/BDNF signaling in several types of lung cancer including LCNEC, SCLC and SCC was investigated, and whether TrkB/BDNF signaling pathway could be a therapeutic target for lung cancer was evaluated. Materials and methods：LCNEC, SCLC and SCC cell lines were used as target cells. Mice xenograft experiments were performed using BALB/c nude female mice. Surgically resected human lung cancer specimens were used for immunofluorescent staining. Results：Exogenous BDNF addition enhanced the invasiveness. Inhibition of TrkB or BDNF suppressed matrix MMP-2 and -9 activities and the invasiveness. In vivo experiments, implanted LCNEC cells pretreated with TrkB-siRNA developed no subcutaneous tumor in all six nude mice, although those with control-siRNA formed tumors in four of six nude mice. Conclusions: These results suggest that TrkB/BDNF signaling pathway contributes to proliferation and invasiveness in lung cancer and that it could be a therapeutic target for lung cancer.

J-3042

Low DNA methylation epigenotype of squamous cell lung cancer with idiopathic pulmonary fibrosis

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Purpose: Patients with idiopathic pulmonary fibrosis (IPF) have higher risk of developing lung cancer, especially squamous cell carcinoma (SCC). DNA methylation alteration is associated with both lung carcinogenesis and pathogenesis of IPF. This study aimed to assess the impact of DNA methylation on lung SCC associated with IPF. Method: Genome-wide methylation analysis was performed using Infinium 450k beadarray for clinical 20 SCC tumor samples with/without IPF, 11 surrounding non-cancerous tissues, and 2 normal lung tissues. The methylation status of 7 genes was quantitatively validated in 78 SCC tumor samples. Results: Hierarchical clustering analysis classified SCC tumor samples into two methylation subtypes: low and high-methylation epigenotypes (LME and HME, respectively). While genes related with negative regulation of growth were significantly hypermethylated in HME SCCs, SCC with IPF significantly correlated with LME. Conclusions: DNA methylation analysis in SCC revealed two subtypes of states such as HME and LME. SCC with IPF was prominent in high frequency of LME, suggesting that they might require molecular aberrations other than DNA methylation for tumorigenesis.

▲TOP

[J-3043] J14-18 [Japanese]
Cutting edge of lung cancer research

2018 / 9 / 29 (Sat) 10:15-11:30 Room 12/12F 1202, Osaka International Convention Center Room 12

Yoshitaka Sekido / Aichi Cancer Ctr. Res. Inst., Div. Cancer Biol.

J-3043

In vitro evaluation of EGFR secondary mutations at front-line osimertinib progression in lung cancers

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Osimertinib is a third generation (G) EGFR-TKI and was designed to overcome T790M secondary EGFR mutation in NSCLC. Several “tertiary” EGFR mutations have been reported as resistant mechanisms to osimertinib. Based on a recent FLAURA trial, osimertinib will be used as a front-line TKI in the near future, and it is hypothesized that these “tertiary” EGFR mutations may cause acquired resistance to front-line osimertinib as “secondary” mutations. To evaluate the role of these mutations and to investigate adequate TKIs after osimertinib progression, we transfected EGFR exon 19 deletion plus one of these mutations (G724S, L792F, L792H, G796S, C797G, and C797S) in cis into Ba/F3 cells. The transfection of EGFR exon 19 deletion with G796S or C797G did not confer IL-3 independent growth. The magnitudes of osimertinib resistance were small in G724S, L792F/H transfected cells compared with C797S. Ba/F3 cells with L792F/H or C797S showed sensitivity to 2G-TKIs or 1G-TKIs, respectively. Ba/F3 cells with G724S were sensitive to both 1G and 2G-TKIs. These results suggest that the choice of second line TKI should be based on the type of secondary mutations of the EGFR gene.

▲TOP

J-3044

SEMA7A-ITGB1 axis plays pivotal roles for EGFR-TKI resistance in human EGFR mutant lung cancer

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Most lung cancer patients harboring EGFR mutations experience resistance for EGFR tyrosin kinase inhibitors (TKI). Therefore, it is necessary to identify not only the mechanisms underlying EGFR-TKI resistance. We found that the GPI-anchored protein semaphorin 7A (SEMA7A) is highly induced by the EGFR pathway via mTOR signaling, and that expression levels of SEMA7A in human lung adenocarcinoma specimens were correlated with mTOR activation. Investigations using cell culture and animal models demonstrated that loss or overexpression of SEMA7A made cells less or more resistant to EGFR-TKIs. The resistance was promoted by aberrant activation of ERK signaling. Furthermore, higher SEMA7A expression in clinical samples predicted the worse response to EGFR-TKI treatment in patients with EGFR mutant tumors. The combination of EGFR-TKI and MEK inhibitors showed the synergic effectiveness for SEMA7A expressing lung cancer cells. Collectively, we show that SEMA7A-ITGB1 axis plays pivotal roles for EGFR-TKI resistance by ERK activation, and our results reveal the significance of SEMA7A as a predictive biomarker and a novel therapeutic target in EGFR-mutant lung adenocarcinoma.

J-3045

Axl kinase drives immune checkpoint and chemokine signalling pathways in lung adenocarcinomas

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Axl receptor tyrosine kinase is involved in the growth and metastasis of many cancers. Although a mitogen-activated protein kinase (MAPK) pathway and an epithelial-to-mesenchymal transition (EMT) program are critical, molecular mechanisms underlying the Axl-driven cancer progression have not been fully elucidated. We aimed to identify downstream molecules of Axl kinase in lung adenocarcinomas. Through gene expression analyses of three independent cohorts, we found that AXL expression positively correlated with mRNA expressions of immune checkpoint molecules (PD-L1) and chemokine receptors (CXCR6) especially in lung adenocarcinomas with epidermal growth factor receptor (EGFR) mutation. Pharmacological inhibition of Axl kinase activity decreased mRNA expressions of PD-L1 and CXCR6 in EGFR mutation-positive cell lines. Our data indicates the novel role of Axl kinase as a driver of immune checkpoint molecules and chemokine signalling pathways in the progression of lung adenocarcinomas. This study also highlights the necessity of clinical trials in order to test the efficacy of Axl kinase inhibition in the Axl-highly expressing subset of lung adenocarcinomas.

J-3046

Anti-PD-1 antibody enhances antitumor efficacy of oncolytic HSV-1 G47Δ in a mouse lung cancer model

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Cancer immunotherapy blocking PD-1 immune checkpoint has demonstrated clinical efficacy in a range of malignancies. The use of third generation oncolytic herpes simplex virus type 1 G47Δ has been shown to be a promising therapeutic approach for a variety of cancer. G47Δ elicits antitumor immune responses while killing tumor cells via viral replication. In this study, we evaluated the efficacy of an anti-PD-1 antibody when used in combination with G47Δ. In a bilateral subcutaneous tumor model using KLN-205 lung cancer cells in syngeneic DBA/2 mice, unilateral intratumoral inoculation with G47Δ significantly inhibited the tumor growth of both inoculated and noninoculated contralateral tumors compared with mock. This tumor growth inhibition was significantly enhanced by systemic administration of anti-PD-1 antibody. In addition, CD8+/Treg ratio of tumor infiltrating lymphocytes of the combination treatment group was significantly greater in both sides than the G47Δ alone group. These findings suggest that the antitumor efficacy of G47Δ can be augmented when used in combination with immune checkpoint inhibitors in the treatment of lung cancer.

▲TOP

J-3047

Perspective of targeting cancer-associated fibroblasts in non-small-cell lung cancer

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The tumor microenvironment is composed of different types of stromal cells that represent a key component of tumor progression. We have reported that TGF-β signaling mediates epithelial mesenchymal transition (EMT) in non-small-cell lung cancer (NSCLC) cells, and that TGF-β induced changes are associated with insensitivity to treatment such as chemotherapy or radiation. In order to identify new targets for prevention of metastasis, it is important to understand the molecular mechanisms that drive EMT. Interaction between cancer-associated fibroblasts (CAFs) and tumor cells have shown increased tumor cell survival via several pathways including EMT. We showed that IL-6 from CAFs enhanced TGF-β induced EMT in NSCLC cells. Furthermore, an anti-fibrotic agent pirfenidone (PFD) significantly inhibited production of IL-6 or TGF-β1 of CAFs, resulting in inhibition of the EMT change in NSCLC cells. In in vivo examination, co-implantation of CAFs promoted tumor progression and PFD suppressed the tumor progression with inhibitory effect of stroma outgrowth. Targeting CAFs as a therapeutic strategy against cancer is an intriguing concept that would benefit from further study.

J-3048

The role of cancer associated fibroblasts in immune suppressive microenvironment of lung cancer

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More than half of patients with lung cancer showed a resistance to immune therapy, indicating that cancer tissue constructs immune suppressive microenvironment. The cancer tissue consists of 3 cell types: cancer cells, immune cells and stromal cells such as cancer-associated fibroblasts (CAFs), however, especially, the relationship between CAFs and immune suppressive cells such as Tregs and myeloid-derived suppressor cells (MDSCs) is unclear. Here, to characterize the immunological phenotypes of CAFs, we examined the expression of MDSC-related cytokines in 11 kinds of CAFs derived surgical specimens of non-small-cell lung cancer. The protein array and ELISA showed that cytokine expression profile is much heterogeneous, whereas 63% of CAFs secretes IL-6, which is associated with differentiation of MDSCs, 55% of CAFs secrete HGF, which stimulates proliferation and 45% of CAFs secrete CCL2, which is associated with migration. The results suggest that CAFs play an important role to exert the function of MDSCs. It may be possible to classify the CAFs into several types from the viewpoint of immunological characteristics, which leads to the development of individualized immune therapy.

▲TOP

[LS34] LS34 [Japanese]
Recent advances in the molecular pathogenesis of B-cell lymphomas

2018 / 9 / 29 (Sat) 11:50-12:40 Room 12/12F 1202, Osaka International Convention Center Room 12
：Medical Affairs Division, Janssen Pharmaceutical K.K.

Koichi Akashi / Department of Medicine and Biosystemic Science,

LS34
Recent advances in the molecular pathogenesis of B-cell lymphomas

Masao Nakagawa
Department of Hematology, Hokkaido University Faculty of Medicine

No Abstract

▲TOP

[J-3097] J14-20 [Japanese]
Prostate cancer

2018 / 9 / 29 (Sat) 13:40-14:55 Room 12/12F 1202, Osaka International Convention Center Room 12

Motohide Uemura / Dept. Urol., Osaka Univ. Grad. Sch. Med.

J-3097

Establishment of a dog primary prostate cancer organoid using the urine cancer stem cells

Tatsuya Usui
Tokyo Univ. of Agricul & Tech. Vet Med. Vet Pharmacol

Dogs spontaneously develop prostate cancer (PC) like humans. Stem cell-derived 3D organoid culture could recapitulate organ structure and physiology. A recent study showed that urine cells also possess the characteristics of stem cells. However, the urine cell-derived PC organoids have never been produced. We therefore generated PC organoids using dog urine samples. After dogs were diagnosed with prostate tumor, urine samples were collected by catheterization and used for the organoid culture. The organoids were assessed microscopically (haematoxilin and eosin staining) and in terms of marker expression by means of immunofluorescence staining, immunohistochemical staining and flow cytometry. Additionally, mouse xenograft assay and cell viability assay were carried out. Urine organoids from each PC dog were successfully generated. Each organoid showed cystic structures and resembled the epithelial morphology of the original tissue. Analysis of these organoids revealed that PC organoids using urine might become a useful tool to investigate the mechanisms of pathogenesis and treatment of canine PC.

▲TOP

J-3098

Development of a novel anti-cancer drug targeting for γ-glutamylcyclotransferase

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Background & Objective: γ-Glutamylcyclotransferase (GGCT) is expressed at higher levels in tumors than normal tissues. Previous studies showed that depletion of GGCT using RNA interference blocks proliferation of cancer cells. However, the detailed underlying mechanism is unclear. We have identified N-glutaryl-L-alanine (GA) as a GGCT inhibitor and developed its cell-permeable prodrug with methyl-acetoxymethyl ester named "pro-GA". In this study, we evaluated efficacy of the pro-GA for anti-cancer activity in vitro and in a xenograft model. In addition, we examined the combination effects of pro-GA with docetaxel on antiproliferative activity in PC3 cells.
Results & Discussion: The pro-GA inhibited proliferation of PC3 cancer cells in vitro and in a xenograft model in immunocompromised mice. The combined treatment with pro-GA and docetaxel exerted enhanced anti-cancer activity compared to pro-GA or docetaxel alone that induces cellular senescence and apoptosis respectively. These results indicate that the pro-GA may be promising as a novel therapeutic reagent against prostate cancer.

J-3099

Modulation of AKR1C2 by Curcumin Decreases Testosterone Production in Prostate Cancer

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Intratumoral androgen biosynthesis has been recognized as an essential factor of castration resistant prostate cancer. The present study investigated the effects of curcumin on the inhibition of intracrine androgen synthesis in prostate cancer. After treatment of curcumin, testosterone and dihydrotestosterone concentrations in prostate cancer cells were determined through LC-MS/MS assay. Curcumin inhibited cell proliferation and induced apoptosis of prostate cancer cells. Curcumin decreased the expression of steroidogenic acute regulatory proteins, CYP11A1 and HSD3B2 in prostate cancer cell lines, supporting the decrease of testosterone production. After one-month oral administration of curcumin, Aldo-Keto reductase 1C2 (AKR1C2) expression was elevated in prostate tissues from the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. Simultaneously, decreased testosterone levels in the prostate tissues were observed in the TRAMP mice. These results suggest that curcumin's natural bioactive compounds could have potent anticancer properties due to suppression of androgen production, and this could have therapeutic effects on prostate cancer.

J-3100

Novel therapeutic strategy for prostate cancer treatment by targeting extracellular vesicles

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Extracellular vesicles (EVs) play key roles in cancer progression and have a great potential in clinical application. In patients with prostate cancer (PCa), bone metastasis (BM) is a major concern that leads to skeletal-related events and consequently increases mortality. Here, we present a novel molecular mechanism of BM involving EVs from PCa cells. We found that EVs from PCa promoted the osteoclast (OC) fusion. In addition, RNA-seq confirmed the drastic change of gene expression essential for osteoclastogenesis. Moreover, we have found the molecule on the EV surface which is responsible for OC differentiation. This molecule can utilize not only for diagnosis of PCa metastasis but also the novel target molecule for antibody-based therapy against BM. Further, by microRNA-based screening approach, we have identified the regulator of EV secretion from PCa, which can also be the therapeutic target for BM of PCa. In this presentation, we would like to introduce our current work and discuss EV-targeting therapy in PCa toward clinical application.

▲TOP

J-3101

Dual inhibition of BRD4 and DOT1L as an epigenetic treatment strategy for prostate cancer

Hiroaki Sato
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While androgen deprivation therapy targets upstream of androgen receptor (AR) and is effective for prostate cancer (PCa) in first, most tumors acquire life-threatening resistant state called CRPC (castration resistant PCa). To get insight into development of therapeutic strategies targeting downstream of AR, presumably effective for both primary PCa and CRPC, we performed comprehensive analysis of transcriptome by RNA-seq and epigenome by ChIP-seq for H3K4me1, H3K4me3, H3K27ac, H3K79me2, AR, FOXA1, and BRD4. In primary PCa cell line LNCaP, we found 5,975 regions positive for AR, FOXA1, and BRD4, where 70.6% were enhancers with upregulated H3K27ac by DHT stimulation, located nearby important PCa stimulating genes. Using H3K27ac, we defined 1,073 regions as super enhancers and identified neighboring 967 genes. Of these, 78.6% were positive for H3K79me2, which were downregulated by DOT1L inhibitor. While inhibition of BET proteins including BRD4 suppressed cell growth, DOT1L inhibitor alone had only mild effects. DOT1L inhibitor, however, boosted BET inhibitor effects under dual treatment, through repressing both super enhancers and BET, suggesting a possibility of a novel strategy.

J-3102

Identification of critical AR-V7 target genes in castration resistant prostate cancer

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Aberrant expression of splicing variant of androgen receptor (AR), called AR-V7, is one of the mechanisms for castration resistant prostate cancer (CRPC). Regulatory function of AR-V7 and its difference from AR, however, are mostly unknown. Here we performed comprehensive analysis of transcriptome and epigenome by RNA-seq and ChIP-seq for H3K4me1, H3K4me3, H3K27ac, and AR/AR-V7 target regions, using a primary prostate cancer cell line LNCaP, and CRPC cells expressing AR-V7 in the absence of androgen. While AR bound and activated 2,955regions in LNCaP and 2,109 regions in CRPC cells, AR-V7 targeted 399 regions in CRPC cells. While most of AR-V7 target regions could be commonly activated by AR, 22 regions were identified as AR-V7 specific target regions. Knockdown of AR-V7 by its specific shRNA decreased cell proliferation of CRPC cells, with repression of genes nearby those common and specific AR-V7 target regions. Among these candidates of critical AR-V7 downstream genes, we identified two genes contributing to cell proliferation and significantly upregulated in clinical CRPC samples with high AR-V7 expression. These unveil molecular genesis of CRPC via aberrant AR-V7 expression.

▲TOP

[J-3103] J14-21 [Japanese]
Non-prostate genitourinary cancer

2018 / 9 / 29 (Sat) 14:55-16:10 Room 12/12F 1202, Osaka International Convention Center Room 12

Seiichi Mori / Japanese Foundation for Cancer Res., CPM Ctr.

J-3103

Establishment and analysis of renal cell carcinoma reactive tumor-infiltrating T cell

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Objective: Tumor-infiltrating lymphocyte (TIL) in tumor tissue is supposed to be the effectors. However, tumor-reactivity of TILs in renal cell carcinoma (RCC) are still elusive. In this study, we investigated the tumor-specific reactivity of TILs to renal cell carcinoma. Methods: 24 patients with RCC who received nephrectomy or partial nephrectomy were enrolled in this study. Bulk TILs were isolated from tumor tissues. CD8+ T cell clones were established from TILs by single cell sorting using a flow cytometer. Tumor cell reactivity were addressed by an interferon gamma ELISPOT assay. Results: The median age was 66 years old. Of the 24 patients, the median frequency of TIL and CD8+Tcell were 5.2% and 0.8% in each single cell, respectively. Bulk TILs were established in 5 patients; however, bulk TILs did not show specific activity to primary tumor. Therefore, 151 CD8+Tcell clones were established from TILs. ELISPOT assay revealed a TIL clone showed reactivity to tumor cells. Conclusion: Although tumor-infiltrating CD8+Tcells were few in RCC, TIL clone cells could recognize autologous cancer cells. Further analysis might reveal a molecular insight of immune checkpoint inhibitor.

▲TOP

J-3104

Accumulation of tumor associated macrophages by miR-27b regulated CSF1 in renal cell carcinoma

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Gene expression analyses revealed higher gene expression of macrophage markers (CD68, CD163) in renal cell carcinoma tissues compared to non-tumor tissues in two different cohorts (TCGA and NCCJ), and high CD68 expression was associated with lower overall survival (OS). We analyzed expression of miRNAs which were predicted to target CSF1 mRNA by in silico analysis. Among the identified mRNAs, miR-27b was found to have lower gene expression in renal cell carcinoma compared to normal tissues, and was associated with lower overall survival. In CSF1 expressing human renal carcinoma cell lines (A498, 786O), miR-27b and CSF1 were inversely expressed. Overexpression of miR-27b decreased the expression of CSF1 and CD274 in A498 and 786O, and miR-27b decreased the activity in human CSF1-3'UTR luciferase reporter assay using 293T cells. Furthermore, miR-27b in 786O cells was downregulated by treatment with hIL6 which is frequently over-produced by renal carcinoma cells. These results indicated that the miR-27b-CSF1 axis may be involved in the accumulation of tumor associated macrophages (TAM) and its poor prognosis in renal cell carcinoma.

J-3105

Genetic, epidemiologic and clinicopathologic studies of Japanese patients with Birt-Hogg-Dube syndrome, 2018 update

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Birt-Hogg-Dube syndrome (BHD) is a familial cancer syndrome characterized by skin fibrofolliculomas, pulmonary cysts / pneumothoraces, and renal cell carcinomas (RCCs). The affected individuals have germline mutations in the folliculin gene (FLCN). We investigated the genetic spectrum and clinicopathologic findings of 490 patients from 190 families. We detected a total of 45 FLCN germline variants. The most prevalent mutation pattern was 11c.1285delC (n=55), followed by 12c.1347_1353dupCCACCCT and 13c.1533_1536delGATG (n=30 each). Almost all the patient had pulmonary cysts (n=488, 99.6%). Up to 373 patients suffered from recurrent pneumothoraces. Skin papules were observed in 103 and RCCs were in 96 patients. Among 90 RCCs histologically investigated, the frequent subtypes were hybrid oncocytic/chromophobe tumors (n=38) and chromophobe RCCs (n=31). The majority of patients with RCCs have a favorable prognosis; however, 3 of 4 patients with papillary RCCs had distant metastases. The possibility of aggressive phenotype for BHD-associated papillary RCC should be taken into account.

J-3106

MUC1C Oncoprotein Contributes to Acquiring Cisplatin and Gemcitabine Resistance in Urothelial Carcinoma Cells

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Mucin 1 C-terminal peptide (MUC1C), a well -known oncoprotein in breast carcinoma, has been introduced as a key regulator for acquiring drug resistance recently. Since little is known about the biological mechanisms of acquiring drug resistance in urothelial carcinoma (UC), our aim is to investigate how MUC1C contributes to the mechanism of drug resistance in UC cells. From the two- human bladder cancer cell lines T24 and UMUC3, we established cisplatin resistant (CR) cells; T24CR and UMUC3CR, and gemcitabine resistant (GR) cells; T24GR and UMUC3GR. We confirmed the high expression of MUC1C in all drug resistant cell lines, and the knockdown of MUC1C by siRNA led to the recovery of drug sensitivities. To determine the mechanism of drug resistance, we found that MUC1C drives PI3K/AKT pathway through p-AKT overexpression and therefore upregulates multiple drug resistance (MDR) transporter expression. Moreover, we confirmed MUC1C stabilizes the CD44 variant 9- cystine transporter linkage which leads to enhance antioxidant defense by modulating intracellular glutathione synthesis. From these findings, MUC1C may become a new therapeutic target for overcoming chemo-resistance in UC.

▲TOP

J-3107

Bladder cancer patient-derived cells and xenografts enable to characterize cancer stem-like cell phenotype

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Advanced bladder cancer usually needs chemotherapy, although cancer stem-like cells (CSCs) may contribute to acquired chemo-resistance and tumor progression. Generation of CSC-enriched patient-derived cancer cells and xenografts (PDCs and PDXs) will be useful to define cancer stemness and molecular targets. We here generated PDCs from bladder cancer patients' specimens under a spheroid culture condition. Notably, the PDXs using these PDCs in immunocompromised mice had a similar pathological feature as those of the original patients' specimens. PDCs have a unique signature with abundant expression of stem cell markers and we focused on aldehyde dehydrogenase (ALDH) pathway. ALDH inhibitors and ALDH1A1 knockdown impaired PDC proliferation and spheroid formation. Tumor growth of the PDXs could be suppressed by ALDH inhibitor. Microarray analysis further identified another ALDH1A1-related gene, which could be a poor prognostic marker for bladder cancer patients based on public database. Overall, the spheroid-generated PDCs and PDXs exhibit cancer stemness and will be a powerful tool to identify alternative diagnostic and therapeutic targets for advanced bladder cancer.

J-3108

Comparison of Immunological Condition in Tumor Microenvironment between Bladder Cancer and Upper Urinary Tract Carcinoma

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Objective: In this study, we aimed to clarify the difference of immunity status and its clinical significance depending on the tumor site in urothelial carcinoma. Material and Methods: Tumor tissue infiltrating lymphocytes were extracted from 52 bladder cancer (BCa) and 18 upper urinary tract carcinoma (UTUC) patients. The immunological classification was established by unsupervised clustering analysis according to the expression ratio of 9 kinds of immunocheckpoint molecules.Results: The immunological condition was classified into two groups, (Group Ⅰ: CD4 T cells dominant group, Group Ⅱ: immunologically activated group). This immunological classification was significantly correlated with tumor grade not tumor location in multivariate analysis. In invasive BCa patients, cancer specific survival (CSS) of Group Ⅱ were significantly poorer than Group Ⅰ (p=0.020), while there was no significant difference in invasive UTUC patients. Conclusions: Although there was no difference in the immunological condition between BCa and UTUC, it was shown that its significance as a prognostic predictor varies depending on the tumor site.

▲TOP

[ML24] ML24 [Japanese]
Morning Lectures 24

2018 / 9 / 29 (Sat) 8:00-8:50 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13

Yasuhiro Kodera / Dept. Gastroenterol Surg., Nagoya Univ.

ML24

Kenjiro Kohri
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I've sent an e-mail to The 77th Annual Meeting of the Japanese Cancer Association Administration Office.

▲TOP

[S17-1] S17 [English]
Current status and prospects in translational research

2018 / 9 / 29 (Sat) 9:00-11:30 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13

Atsushi Ohtsu / Natl. Cancer Ctr. Hosp. East、Shoji Natsugoe / Dept. Digestive Surg., Breast & Thyroid Surg., Kagoshima Univ., Sch. Med.

Oncology agent developments are major targets in many pharmaceutical industries. Various targets/methodologies including immuno-oncology have recently been developed which provided remarkable impacts in clinical oncology areas. With regards to the origin of new agents, many compounds have been produced from academia-industry collaborations based on various cutting-edge technologies. Although the number of new active agents developed in Japan is still limited, remarkable progress in the academia seeds developments have recently been observed with an increasing number of collaborations with industries and regulatory authorities. This symposium is focusing on new promising oncology agents/therapies development from academia in Japan, particularly from preclinical to early exploratory clinical trials. The audience will learn recent progress on academia seeds developments and how to implicate new scientific findings into early clinical developments.

S17-1
Risk Management in Translational Research: Critical insights for effectively transferring the results

Shuichi Furuya
Neutron Therapy Res. Ctr., Okayama Univ.

The recent trend of open innovation is a desirable development for researchers. Collaborative research between universities and industry is now common in biomedical medical technology. Researchers in academia can apply for the public TR projects to the take initiative in such research. However, huge problems exist in clinical projects. That is, appropriate risk management must be introduced for TR projects. The biggest risk in TR is that successful clinical applications are extremely low. Most researchers in academia do not pay sufficient attention to this risk. This is the most important point to consider in discussion about this risk. However, it seems that the "mechanism to accept failure risk" is not cultivated well in Japan. Researchers in academia should understand that in order to minimize the cost of failure the research outcome must be transferred to a company as soon as possible when the POC is obtained. The degree of risk depends on the type of diseases, while the scientific risk can be minimized if the outcome of TR by academia is smoothly transferred to companies. This is the second and an important opportunity to transfer the social assets of academia.

▲TOP

S17-2

Development of next-generation oncolytic viro-immuno-therapy and investigator-initiated first-in-human clinical trial

Ken-ichiro Kosai
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Oncolytic virotherapy (OV) attracts the worldwide oncologists and anticancer market. We developed a novel platform technology of the next-generation OV, i.e., m-CRA (conditionally replicating adenovirus that can target cancers using multiple factors). We first developed survivin-responsive m-CRA (Surv.m-CRA-1), which induced more potent anticancer and tumor-specific effects than competitors and increased effectiveness against cancer stem cells. After GMP-manufacturing and nonclinical GLP studies, we performed the first-in-human investigator-initiated ICH-GCP clinical trial for refractory malignant bone and soft tissue tumors. Promising results are being confirmed in safety and therapeutic efficacy, including long-term drastic antitumor effects only by a single injection of 1/100 of the predicted maximal dose. After phase I study, we will start Phase I/IIa study (multiple injections) to get early approval. Moreover, we started the nonclinical development of Surv.m-CRA-2, which has an immunity gene to efficiently treat metastasis. Furthermore, we are systematically developing numbers of candidates for next-generation OV immunotherapies using our proprietary platform m-CRA technology.

S17-3

Clinical development of a humanized de-fucosylated anti-CD4 antibody as a cancer therapeutic

Kouji Matsushima
Inst. BioMed. Sci., Tokyo Univ. Sci.

Depletion of CD4+ cells by the depleting antibody in tumor-bearing mice shows strong antitumor effects with robust oligoclonal proliferation of tumor-specific CD8 T cells in the draining lymph node and increased infiltration of PD-1+CD137+CD8 T cells into the tumor. Gene expression analysis revealed the increase of IFN-gamma and TNF-alpha at the tumor site after CD4+ cell depletion. Combination treatment with the anti-CD4 antibody and immune checkpoint antibodies, particularly anti-PD-1/PD-L1 antibody, synergistically suppressed tumor growth and greatly prolonged survival in several types of tumors. In pre-clinical studies in nonhuman primates, no serious adverse effects were observed after several weeks of treatment with a de-fucosylated humanized anti-human CD4 antibody with potent ADCC activity, IT1208. Phase I physician-initiated clinical trial (first-in-human) of IT1208 is being conducted at National Cancer Research Center East Hospital.

S17-4

Therapeutic strategies targeting cancer stem cells

Hideyuki Saya
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Cancer stem cells (CSCs) are a subset of tumor cells that are responsible for initiating and maintaining the disease. In the clinical point of view, the most important characteristics of CSCs include their resistance to various therapeutic interventions. However, the underlying mechanisms of the resistance remain unclear.

We have recently found that expression of a CSC marker, CD44, in particular variant forms of CD44 (CD44v), contributes to the defense against reactive oxygen species (ROS) by promoting the synthesis of reduced gluathione (GSH), a primary intracellular antioxidant. CD44v interacts with and stabilizes xCT, a subunit of a glutamate-cystine transporter, and thereby promotes the uptake of cystine for GSH synthesis (Cancer Cell 2011). Therefore, ablation of CD44 reduced GSH levels and increased ROS levels, leading to suppression of tumor growth and metastasis in both transgenic and xenograft tumor models (Nat Commun 2012; Cancer Res 2013). Based on these preclinical findings, we conducted clinical trials using an xCT inhibitor for cancer patients (Gastric Cancer 2016; Cancer Sci 2017).

▲TOP

S17-5

Early clinical trials and development from academia seeds in National Cancer Center Hospital East (NCCHE)

Toshihiko Doi
Natl. Cancer Ctr. Hosp. East Dept. Exp. Therap.

The 'valley of death,' between academia and pharma is a place where ideas go to die. Academic scientists struggle to transform their research into compounds that pharma want to bring to market. But many drugs never reach patients due to lack the business mind. We established exploratory Oncology Research & Clinical Trial Center (EPOC) and joint development with NCCHE for academia innovative development. NCCHE took over management of clinical trials, allowing EPOC to focus on non-clinical studies aimed at proof of concept for academia drug to carry out exploratory translation research. Clinical trials under IND are supported by "Clinical Research Support Office(CRSO)" . Our bed-side collaborative system enables us to combine the best practices and industry to design innovative programs. We also promote the consultation for well maturated seeds, and support from regulatory science view, also project management. We have conducted many trials using academia seeds . Several products have succeeded to be delivered to companies. The global drug development is changing, we are more focusing to discover germination and promote seamless development.

S17-Special_Remarks

Special Remarks

Hideaki Shimada
Dept. Surg., Sch. Med., Toho Univ.

No Abstract

[LS35] LS35 [Japanese]
Current Status and Future Perspectives of Carbon Ion Radiotherapy

2018 / 9 / 29 (Sat) 11:50-12:40 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13
：Osaka Heavy Ion Therapy Center

Hidetoshi Eguchi / Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University

LS35

Current Status and Future Perspectives of Carbon Ion Radiotherapy

Kazuhiko Ogawa
Department of Radiation Oncology, Graduate School of Medicine, Osaka University

No Abstract

[S20-1] S20 [English]
Revolution in basic research and clinical practice by genome editing

2018 / 9 / 29 (Sat) 13:40-16:10 Room 13/3F Korin1, RIHGA Royal Hotel Osaka Room 13

Takashi Takahashi / Div. Mol. Carcinog., Nagoya Univ. Sch. Med.、Tomoji Mashimo / GERDC/IEXAS, Med, Osaka Univ.

The genome editing has made 'revolution' in bioscience and clinical research. Researchers are now developing new genome editing tools such as new CRISPR, epigenome editing, gene transcription regulation, cell screening and so on. Various mouse models for human diseases and humanized models has been progressed by the genome editing, and applied to translational research and regenerative medicine. Gene therapy, cell therapy, and drug discovery using genome editing technology have been advanced in universities, research institutes, pharmaceutical companies and venture companies. On the other hand, regulation, governance and risk management related to the genome editing are important issues.
In this symposium, we invite prominent scientists who are conducting cutting edge research in each field on genome editing. They present their medical application, latest development trends and future prospects. We would like to discuss the latest findings on the genomic editing technologies, future medical applications and future prospects with participants.

S20-1
An overview of recent genome editing technologies

Tomoji Mashimo
GERDC, Grad. Sch. Med., Osaka Univ., IEXAS, Grad. Sch. Med., Osaka Univ.

The genome editing has made 'revolution' in bioscience and medical research. Researchers are now developing new genome editing tools such as new CRISPR, epigenome editing, gene transcription regulation, cell screening and so on. Various mouse models for human diseases and humanized models has been progressed by the genome editing, and applied to translational research and regenerative medicine. Gene therapy, cell therapy, and drug discovery using genome editing technology have been advanced in universities, research institutes, pharmaceutical companies and venture companies. On the other hand, regulation, governance and risk management related to the genome editing are important issues. At this symposium, we invited prominent scientists who were conducting cutting edge research in each field on genome editing. They will present their medical application, latest development trends and future prospects. So I would like to briefly explain the basic principles of genome editing, technology use, basic research before their tall. I would also like to discuss the latest findings on the genomic editing technologies, future medical applications and future prospects with participants.

▲TOP

S20-2

Oncology drug candidates identified from genome-wide CRISPR screening of 204 cancer cell lines

Kosuke Yusa
Wellcome Sanger Inst., Ins. Front. Med. Sci., Kyoto Univ.

An increasing number of molecular target drugs have been developed and currently under clinical evaluation. These drugs targets vulnerabilities of cancer cells and thus are thought to be safer and more efficacious. However, a substantial number of cancer patients do not benefit from molecular targeted therapies due to unavailability. Systematic approach is needed to identify and prioritise new therapeutic targets. Here we performed genome-wide CRISPR-KO screens in 204 human cell lines across 12 cancer types and developed a data-driven computational framework to identify oncology drug candidates. Using newly generated fitness profiles, we determined new sets of core cellular fitness genes at pan-cancer level and individual cancer-type levels. In addition, we identified thousands of context-specific fitness deficiencies, such as in the presence of a specific oncogenic alteration. Our framework prioritizes potential anti-cancer targets by integrating multiple lines of evidence obtained from the CRISPR screens and target tractability for drug development. This work presents an analytical framework to prioritise oncology targets and its application to large-scale CRISPR screen datasets.

S20-3

Forward genetic screens of genes determining the efficacy of molecular target therapy in hepatocellular carcinoma

Takahiro Kodama
Dept. Gastro. & Hep. Osaka Univ. Sch. Med.

Co-author：Yuta Myojin、Hayato Hikita、Ryotaro Sakamori、Tetsuo Takehara
Dept. Gastro. & Hep. Osaka Univ. Sch. Med.

Several multi-kinase inhibitors have been recently developed for advanced hepatocellular carcinoma (HCC). However, due to the high inter-tumor and intra-tumor heterogeneity of HCC, its efficacy is limited and it eventually becomes refractory to these therapies. To improve poor prognosis of HCC, it is important to clarify molecular mechanisms of chemo-resistance and discover predictive biomarker of these drugs. Forward genetic screens (FGS) such as insertional mutagenesis and cDNA/siRNA library are versatile genetic tools to discover genes involving phenotype of interest. Recently, CRISPR/Cas has been also applied to FGS. In this symposium, we would like to introduce the results of our FGS-based gene discovery efforts involving HCC drug resistance as follows; 1) In vitro sleeping beauty transposon mutagenesis screen using murine liver tumor cell lines containing hundreds copies of mutagenic transposon, 2) In vitro pooled whole-genome CRISPR/Cas library screen using human HCC cell lines, and 3) In vivo pooled cDNA library screen using hydrodynamic delivery of oncogene plasmids into the mouse liver.

S20-4

Gene therapy using adeno-associated virus vectors

Shin-ichi Muramatsu
Neurology, Jichi Med. Univ., CGCT, IMS, Univ. Tokyo

Adeno-associated virus (AAV) vectors are derived from non-pathogenic parvoviruses. Genome of this vector does not integrate into the host chromosome and persists as an episome in the nucleus. Although small size of expression cassette can limit the use of AAV vector, most therapeutic genes fall within this range. The expression of transgenes persisted 15 years after gene transfer into the brain of a primate model of Parkinson disease without significant toxicity. Some AAV vectors can cross the blood or cerebrospinal fluid-brain barrier after systemic injection. To date, AAV vectors have been used in more than 183 clinical trials conducted for various diseases such as retinal degeneration, hemophilia, Parkinson disease, and spinal muscular atrophy. Some of them are recently progressed or anticipated to receive approval from the FDA in near future. Various advantages of AAV vector-based gene therapy over ordinary drugs indicate the requirement to develop large-scale production system for GMP-grade vectors. Since the effects of gene transfer last for a lifetime with a single dose, construction of new business model is also required.

▲TOP

S20-5

Genome-Editing Therapy of SCID in Mouse and Pig Models

Yutaka Hanazono
CDAMTec, Jichi Med. Univ.

X-linked severe combined immunodeficiency (X-SCID) is a congenital disorder of hematopoietic stem cells (HSCs) caused by genetic defect of the interleukin-2 receptor gamma-chain (IL2RG) gene on the X chromosome. Boys suffering from the disease are extremely vulnerable to infection. Although X-SCID can be cured by allogeneic HSC transplantation, more than half of the cases fail to find their donors. Gene therapy of HSCs has been attempted as an alternative treatment. It has utilized retroviral or lentiviral vector to randomly insert the normal IL2RG gene to patients' genome, having caused leukemia in some subjects. Therefore, it is desirable to repair per se the mutated genome of patients' HSCs with genome-editing technology. Gene-editing therapy of HSCs consists of 1) harvest of HSCs from patients, 2) induction of double-strand break into the mutated site of HSC genome with genome-editing tools such as CRISPR/Cas9, 3) knock-in of correct sequence, and 4) transplantation of the repaired HSCs back to patients. We have generated X-SCID mice and pigs with genetic mutations mimicking human X-SCID and are carefully testing each step. We will see some of the results in the symposium.

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[ML25] ML25 [Japanese]
Morning Lectures 25

2018 / 9 / 29 (Sat) 8:00-8:50 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Naoya Fujita / Cancer Chemother Ctr., JFCR

ML25

Dissecting cancer biology with reprogramming technology

Yasuhiro Yamada
Div. Stem Cell Path., Inst. of Med. Sci., Univ. Tokyo

Discussant：Yasuhiro Fujiwara
Natl. Cancer Ctr. Hosp.

Cancer arises through the accumulations of both genetic and epigenetic alterations. Although the causal role of genetic mutations on cancer development has been established in vivo, similar evidence for epigenetic alterations is still limited. Moreover, mutual interactions between genetic mutations and epigenetic alterations remain unclear. Cellular reprogramming technology can be used to actively modify the epigenome without affecting the genomic information. Here I introduce our recent studies that utilized this property for cancer research. The faithful shutdown of the somatic program occurs in the early stage of reprogramming. Here, we examined the effect of in vivo reprogramming on Kras-induced cancer development. We next demonstrate that Kras and p53 mutations are insufficient to induce ERK signaling in the pancreas. Notably, the transient expression of reprogramming factors in Kras mutant mice is sufficient to induce the robust and persistent activation of ERK signaling in acinar cells and rapid formation of pancreatic ductal adenocarcinoma. These results underscore a crucial role of dedifferentiation-associated epigenetic regulations in the initiation of pancreatic cancers.

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[IC7] IC7 [Japanese]
Introduction Course for Current Cancer Research 7

2018 / 9 / 29 (Sat) 9:00-9:35 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Tomotaka Sobue / Div. Environ Med, Osaka Univ. Sch. Med.

IC7
Introductory course for statistics to intuitively understand big data

Yukinori Okada
Lab. Stat. Genet., Osaka Univ. Grad. Sch. Med.

Recent development of genome sequencing technology have provided large amount of human genome data in the field of life science. Especially in the field of cancer research, genome big data analysis become a critical bottleneck process of both basic and clinical studies. Even for the society members who do not directly handle data analysis, it is important to know the scheme of big genome data for appropriate interpretation of cancer genome data. In this course, we overview the current status of genomic analysis in both basic and clinical cancer researchers. Further, we provide introductory and "non-mathematical" lecture on statistical genetics, which would be necessary to understand cancer genomics. We note that precise understanding of mathematical theory of statistics may not be required for most of the researchers, but understanding of the framework of how to analyze and how not to analyze big data is necessary. We would like also to introduce our recent institutional efforts to give basic lecture on statistical genetics, such as "Summer Schol of Statistical Genetics in Osaka University".

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[IC8] IC8 [Japanese]
Introduction Course for Current Cancer Research 8

2018 / 9 / 29 (Sat) 9:35-10:10 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Eiji Miyoshi / Dept. Mol. Biochem. & Clin. Invest. Osaka Univ. Grad. Sch. Med.

IC8
Medical Ethics in Genomic Medicine

Akiko Shibata
NCC, Ctre for Can. Cont. & Info. Services

Ethics is the study of morality-careful and systematic reflection on and analysis of moral decisions and behavior. In liberal societies, individuals have a great deal of freedom to decide for themselves what is ethical. In most situations, physicians have to decide for themselves what is the right way to act, but in making decisions, it is helpful to know what other physicians would do in similar situations. Policy statement such as guidelines reflect a general consensus about the way physicians should act. Laws set minimum standards of ethical behavior. Quite often ethics prescribes higher standards of behavior than does the law. Medical ethics is the branch of ethics that deals with moral issues in medical practice. It must respect all three of these principles: compassion, competence and autonomy. Advances in medical science and technology raise new ethical issues that cannot be answered by traditional medical ethics. Main ethical issues on genomic medicine are discrimination against based on one's genetics and privacy. In Japan, ethical guideline in genomic research and amendment privacy act have been implemented, however policy for protection for discrimination is immature.

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[IC9] IC9 [Japanese]
Introduction Course for Current Cancer Research 9

2018 / 9 / 29 (Sat) 10:10-10:45 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Hisashi Wada / Clin. Res. in Tumor Immunol.

IC9

Basics and current topics of cancer immunotherapy

Koji Tamada
Dept. Immunology., Yamaguchi Univ., Sch. Med.

Cancer immunotherapy has been explored to treat patients in whom conventional anti-cancer therapeutic modalities are ineffective. An early phase of cancer immunotherapy includes Coley's toxins, non-specific immune-stimulators derived from plants and bacteria, cytokine therapy, LAK therapy, dendritic cell vaccine, and peptide vaccine, but these approaches rarely demonstrated clinical efficacies superior to the standard therapies in large scale phase III clinical trials. However, recent development of immune-checkpoint blockade therapy including anti-PD-1 antibody has demonstrated potent clinical efficacy superior to the standard therapies, inducing paradigm shift in the field of oncology. In addition, further novel approaches such as gene-modified T cells expressing chimeric antigen receptor (CAR) have been developed. Based on these progress, it is now important to explore mechanisms and biomarkers related with these therapies. This seminar will introduce basic concept of cancer immunology, briefly explain mechanisms and clinical application of checkpoint blockade and CAR-T cell therapy, and discuss current situation of biomarker studies and combination immunotherapies.

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[IC10] IC10 [Japanese]
Introduction Course for Current Cancer Research 10

2018 / 9 / 29 (Sat) 10:45-11:20 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Hiroaki Kataoka / Dept. Path., Facul. Med., Univ. Miyazaki

IC10

Application of primary culture to cancer research

Masahiro Inoue
Dept. CL Bioresource R&D, Kyoto Univ. Sch. Med.

Co-author：Jumpei Kondo
Dept. CL Bioresource R&D, Kyoto Univ. Sch. Med.

The goal of culturing cancer cells is to faithfully reproduce characteristics of original cells in vitro as feasible as possible. Recently the technology of culturing primary cancer cells has greatly progressed to meet the necessity. Such primary culture can be applied to find new targets which are lost in established cancer cell lines. Moreover, given the primary culture represents the characteristics of individual original cancer cells, a panel of primary cultured cancer cells would be useful for assessing inter patient heterogeneity. Ultimately, it would be applicable to the functional precision medicine by determining the best drug of treatment for individual patient. Among the technologies, we developed a novel primary culture system of cancer cells, cancer tissue-originated spheroid (CTOS) method. The principle of the CTOS method is to maintain cell-cell contact throughout the preparation and the culture process. CTOS retains differentiation status of original patient tumors. CTOS can be applied to the drug sensitivity assay as well as the high-throughput screening. A CTOS panel would be useful for assessing inter patient heterogeneity.

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[LS36] LS36 [Japanese]

Immuno-oncology & Molecular Imaging~Molecular mechanisms of immune checkpoint molecules and chimeric antigen receptor, CAR~

2018 / 9 / 29 (Sat) 11:50-12:40 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14
：MSD K. K. / Taiho Pharmaceutical Co., Ltd.

Norio Nonomura / Department of Urology, Osaka University Graduate School of Medicine

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LS36

Immuno-oncology & Molecular Imaging~Molecular mechanisms of immune checkpoint molecules and chimeric antigen receptor, CAR~

Tadashi Yokosuka
School of Medicine Department of Immunology, Tokyo Medical University

No Abstract

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[IC11] IC11 [Japanese]
Introduction Course for Current Cancer Research 11

2018 / 9 / 29 (Sat) 13:40-14:15 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Hideaki Tahara / Advanced Clin. Res. Ctr., Inst. Med. Sci., the Univ. of Tokyo

IC11
Basic course of genome editing for cancer research

Masaki Ohmuraya
Dept. Genetics, Hyogo College of Med.

Cancer is caused by gene alterations and epigenetic changes, resulting in activation of oncogenes and/or inactivation of tumor suppressor genes. The clustered regulatory interspaced short palindromic repeat (CRISPR)-associated 9 (Cas9) system is a powerful tool for editing to the genome of many eukaryotic cells and organisms. Modifications to adaptive immune system in bacteria and archaea enable scientists to efficiently edit DNA or modulate gene expression in vitro and in vivo environments. Thus, lots of laboratories can perform critical experiments to accelerate cancer research by providing an efficient technology to approach the mechanisms of tumorigenesis, identify drug targets and cell based therapies. The CRISPR-Cas system shows promising potential for modeling, repairing and correcting genetic events in different types of cancer. The accuracy and versatility of CRISPR-Cas9 have been established in biological and medical research and bring new hope to cancer research. This course reviews the basic concept of CRISPR-Cas9 system, its application and advantages in cancer research.

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[IC12] IC12 [Japanese]
Introduction Course for Current Cancer Research 12

2018 / 9 / 29 (Sat) 14:15-14:50 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Eiichi Morii / Dept. Pathol, Osaka Univ. Grad. Sch. Med.

IC12
Basic knowledge for cancer investigations in pathology

Yoshinao Oda
Dept. Anatomic Path. Grad. Sch. Med. Sci. Kyushu Univ.

1. The detailed observation of morphology in cancer tissue is essential and very important for pathological cancer investigations. New disease entity or tumor classification often arise from this careful observation. Such examples in our research group, combined with molecular technique in pathological specimens, are introduced.
2. Tissue samples should be prepared from cancer tissue carrying no necrotic and hemorrhagic areas for appropriate genomic analysis. Snap-frozen samples should be collected at the latest within about 3 h of storage at 4^{o}C and they should be stored in liquid nitrogen until use. To secure high quality nucleic acid for genomic research, 10% neutral buffered formalin should be used for formalin-fixed paraffin embedded specimens. Insufficient fixation or overfixation in formalin cause serious degradation of nuclei acid.
3. Assessment of the proportion of cancer cells are important to interpret the results of genomic analysis, even if at-a-glance assessment. Micro dissection is effective to obtain genomic information in only cancer cells, preventing the contamination of stromal cells. ISH technique is useful to compare molecular alteration and morphology.

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[IC13] IC13 [Japanese]
Introduction Course for Current Cancer Research 13

2018 / 9 / 29 (Sat) 14:50-15:25 Room 14/3F Korin2, RIHGA Royal Hotel Osaka Room 14

Shoji Nakamori / Osaka Natl. Hosp.

IC13

Important issues to plan a clinical research

Narikazu Boku
Div. Gastrointestinal Med. Oncol., Natl. Cancer Ctr. Hosp.

First step of planning a clinical research is to have a clear clinical question to be investigated. Whichever the objective of the clinical research is either confirmative or explorative, the clinical research requires rationales for the expecting the outcomes, and we should estimate the impact of the clinical research on current unmet needs, unsolved problems, or future research. It should be kept in mind that research of so many biomarkers, so called fishing expedition, is easy to plan, but accidental good results will be obtained. The results obtained by the explorative research should be confirmed in future. In the second step, the optimal study design should be considered: 1) endpoint (main outcome), 2) sample size, 3) eligibility criteria, 4) treatment, 5) evaluation of the efficacy and toxicity, 6) clinical data to be collected and its handling, 7) samples and methods for translational research, 8) statistical analysis plan, 9) budget and study period. In this step, we have to comply with laws, guidelines and other requirements (ethical, scientific, regulatory). Quality of the research should be monitored and reported regularly and the all the processes should be audited

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[ML26] ML26 [Japanese]
Morning Lectures 26

2018 / 9 / 29 (Sat) 8:00-8:50 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15

Taroh Satoh / Dept. Frontier Sci. for Cancer & Chemother. Osaka Univ. Grad. Sch. of Med.

ML26

The Japan premiere of the cancer genome medicine

Katsuya Tsuchihara
Div. Translational Informatics, EPOC, Natl. Cancer Ctr.

Discussant：Minoru Tanabe
Dept. Hepatobiliary & Pancreatic Surg., Tokyo Med. & Dent. Univ.

Though the cancer genomics had remained in the field of basic science for decades since the discovery of oncogene, it has been drastically changed by the appearance of molecular targeted therapy and next generation sequencing. Mutations of targetable oncogenes and tumor suppressor genes and tumor mutational burden are now widely used for the choice of precise treatment arms including molecular targeted drugs and immunooncology drugs. Quality assured diagnostic systems have been introduced in US and other countries. In 2018, Japanese Ministry of Health, Labour and Welfare first designated 11 core hospitals and 100 affiliated hospitals for cancer genome medicine. They are demanded to satisfy the requirements for the systems of informed consent, sample preparation, sequencing, molecular tumor board, genetic counseling and data collection. These systems are well considered the protection of patients' right. On the other hand, to maximize their benefit, we have to expand the patients' access to the approved and investigating new drugs in daily clinical use.

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[S18-1] S18 [English]
Radiation oncology in cancer research and treatment

2018 / 9 / 29 (Sat) 9:00-11:30 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15

Hiroyuki Kuwano / Dept. General Surg. Sci. Gunma Univ., Grad. Sch. of Med.、Hiroshi Harada / Lab. of Cancer Cell Biol., Grad. Sch. Biostudies, Kyoto Univ.

Recent researches yielded development of innovative and effective radiation modalities against cancer cells, such as intensity modulated radiation therapy and heavy ion beam radiotherapy. However, further investigations are needed to establish the indication and limitation of the radiation therapies and would improve the prognosis of cancer patients. This symposium will highlight recent progress in radiation oncology fields from various perspectives and approaches. Deep understanding of sensitization and resistance against radiation may provide important information on mechanisms to eradicate the residual cancer cells by radiation. We look forward to sharing the latest and interesting information on the basic and clinical researches in radiation oncology.

S18-1

K63-ubiquitination signaling promotes end-processing of double-strand breaks for subsequent nonhomologous end joining

Shunichi Takeda
Dept. Radiation Genetics, Grad. Sch. Med., Kyoto Univ.

Co-author：Hiroyuki Sasanuma、Remi Akagawa
Dept. Radiation Genetics, Grad. Sch. Med., Kyoto Univ.

UBC13 catalyzes K63-linked polyubiquitination, and facilitates the recruitment of BRCA1 onto DNA double-strand break(DSB)sites via RAP80. BRCA1 and Mre11 then promote the DSB resection of homologous recombination at S/G_2phases. Etoposide(ETP)stabilizes Topoisomerase 2(Top2) covalently associating with 5'DSB ends. We here show that BRCA1, RAP80, UBC13, Mre11, and NHEJ factors are all required for repair of ETP-induced DSBs in G_1 phase. UBC13, RAP80, BRCA1, and endonuclease activity of Mre11, but not NHEJ, contribute to the removal of 5' Top2 adducts from DSB sites. Moreover, UBC13, RAP80, and BRCA1 promote the recruitment of Mre11 to DSB sites.Thus, K63 ubiquitin signaling involving UBC13, RAP80, and BRCA1 promotes Mre11-mediated removal of 5'adducts from DSBs for subsequent NHEJ. Inactivation of either K63 ubiquitin signaling or MRE11 endonuclease activity causes significant delay in repair of ionizing-radiation (IR)-induced DSBs but not in repair of restriction-enzyme-induced DSBs in G_1 phase. We propose that K63 ubiquitin signaling is required for efficient removal of blocking adducts and restore canonical 5'-phosphate and 3'-hydroxyl moieties at IR-induced DSBs.

▲TOP

S18-2

Roles of endosome proteins Samd9/L in radiation-induced MDS associated with monosomy 7

Toshiya Inaba
Dept. Mol. Oncol, RIRBM, Hiroshima Univ.

Co-author：Akiko Nagamachi[1]、Akinori Kanai[1]、Hirotaka Matsui[2]
[1]Dept. Mol. Oncol, RIRBM, Hiroshima Univ.、[2]Dept. Mol. Lab. Med., Univ. Kumamoto

Survey of atomic-bomb survivors revealed two types of radiation-induced leukemia and myelodysplastic syndromes (MDS). One is acute or chronic leukemia with short latency among the young, who's relative risk (RR) is extremely high (> 10/Gy), while the other is age- and latency-independent leukemia and MDS with relatively low risk (RR is around 3-4/Gy). The former is most likely due to chromosomal translocations caused by powerful radiation effects to create double-stranded DNA breaks, whereas we still don't know the exact mechanisms through which radiation causes the latter. Monosomy 7 is a prominent alteration in radiation-induced MDS. Haplo-insufficiency of multiple responsible genes is considered to promote the development of MDS. One such gene Samd9 and related Samd9L (Samd9/L) control endosome trafficking. Virtually all mice lacking one allele of Samd9L gene developed MDS. Intriguingly, gain-of-function mutations of Samd9/L were recently identified in human autosomal dominant diseases, which are characteristic of pancytopenia with the development of MDS in early infancy. In this talk, roles and molecular mechanisms of 7q- in radiation-induced MDS will be discussed.

S18-3

Investigating the molecular mechanism underlying PD-L1 expression after DNA damage for precision radioimmunotherapy

Atsushi Shibata
ERSC, Grad. Sch. Med., Gunma Univ.

Immune checkpoint therapy has recently emerged as a promising next-generation treatment for cancer. Recent studies suggest that, in cancer cells, exogenous cellular stress upregulates PD-L1 expression, which may contribute to the formation of an immunosuppressive tumor environment. DNA double-strand break (DSB) is the most critical type of genotoxic stress; however, the involvement of DSB repair in PD-L1 expression is yet to be investigated. Here, we demonstrate the upregulation of PD-L1 expression in cancer cells, which is mediated by ATM/ATR/Chk1 kinase activities, in response to DSB. By targeting DSB repair genes in an siRNA library, we noted enhanced PD-L1 upregulation by the depletion of either BRCA2 or Ku70/80 after X-ray. Moreover, DSBs activated the signaling of STAT1/3 and upregulated the expression of IRF1, both of which are regulators of PD-L1 expression. Thus, our findings reveal that DSB repair regulates PD-L1 expression, providing a mechanistic insight into DSB-induced upregulation of PD-L1. This presentation will also discuss our latest findings regarding PD-L1 upregulation following heavy-ion irradiation and oxidative DNA damage.

S18-4

Development of a novel radiosensitizer against hypoxia-induced radiation resistance

Takehiko Yokobori
Dept. Gen Surg Sci, Gunma Univ.,

Co-author：Gombodorj Navchaa[1]、Kei Hagiwara[1]、Takayuki Asao[2]、Hiroyuki Kuwano[1]、Ken Shirabe[1]、Dai Yamanouchi[3]
[1]Dept. Gen Surg Sci, Gunma Univ.,、[2]Big Data Ctr. for Integrative Analysis, Gunma Univ.、[3]Div. Vascular Surg. Univ. Wisconsin Sch. Med. Public Health

Radiation resistance has been known to be induced by hypoxic conditions via HIF1a accumulation. To overcome the hypoxia-induced resistance, we developed ultrafine oxygen nanobubble water in the single-nanometer range using a newly developed method. Smaller bubbles in water has better stability than bigger bubbles, therefore our nanobubble might have some advantages such as in vivo distribution of oxygen nanobubble to improve the intra-tumoral hypoxic condition. Cell viability and HIF1alevels were evaluated in cancer cells treated with or without the oxygen nanobubble and radiation under normoxic and hypoxic conditions in vitro. As a result, we could clarified that cancer cells grown in oxygen nanobubble media showed repression of hypoxia-induced HIF1acompared to the control. The oxygen nanobubble medium significantly suppressed the hypoxia-induced radiation resistance compared to normal medium under radiation treatment. This newly created single-nanometer range oxygen nanobubble water may be a promising agent to overcome the hypoxia-induced therapeutic resistance of cancers via HIF1a suppression.

▲TOP

S18-5

The development of radiotherapy and carbon-ion radiotherapy for lung cancer

Katsuyuki Shirai
Dept. Radiology, Saitama Ctr., Jichi Med. Univ.

Co-author：Keiko Akahane[1]、Masaru Wakatsuki[2]、Osamu Tanaka[1]
[1]Dept. Radiology, Saitama Ctr., Jichi Med. Univ.、[2]Dept. Radiology, Jichi Med. Univ.

Radiotherapy plays an important role of organ preservation for lung cancer patients. Recent advances of high precision photon therapy represented by stereotactic body radiotherapy (SBRT) have contributed to improve the treatment outcomes for stage Ⅰ lung cancer. SBRT achieved better local control than the conventional radiotherapy without adverse events. Chemoradiotherapy for unresectable stage Ⅲ lung cancer is a standard therapy, however, the new regimens improving the treatment outcomes have not been developed in the last decades. Adjuvant immunotherapy, durvalumab, after chemoradiotherapy was recently reported as a promising therapy. Particle radiotherapy, including carbon-ion radiotherapy (C-ion RT) and proton beam therapy, has been developing as an effective and less-invasive modality. Particle radiotherapy has the physical advantage that the dose can be focused on the tumor without exposure of the surrounding normal tissues. Furthermore, C-ion RT has a high biological effectiveness compared with photon therapy, which is expected to be effective and safe for lung cancer. In this session, the recent development of photon therapy and C-ion RT for lung cancer will be reviewed.

S18-6

Accurate and Precise Cancer Treatment and Radiotherapy: past, present, and future

Hiroki Shirato
Dept. Rad. Med., Hokkaido Univ. Fac. Med., Hokkaido Univ., GI-CoRE, GSQ

The physical preciseness and accuracy of external radiotherapy has been improved last 50 years.However, it does not necessarily translate to the preciseness and accuracy of the selection of cancer treatment for an individual patient.Clinical considerations and experiences alone are often insufficient for guiding the selection of a specific cancer treatment.Other considerations such as the biological impact of the treatment on the tumor and on normal tissues, the quality of life, and its impact on the society are required.To overcome the shortcomings of randomized clinical trials in the selection of new treatment technologies, model based approaches for predictions of treatment outcomes are expected to be a new paradigm.These theoretical biophysical models are expected to address physical, biological, clinical and social issues and useful in determining the most appropriate cancer treatment for individual disease types and sites.Good collaboration among biologists, clinicians, statisticians and informaticis is necessary.The evolving machine learning methods are important to convert the knowledge into clinical prediction using data science models.

S18-Special_Remarks

Special Remarks

Yasumasa Nishimura
Dept. Radiation Oncol., Kindai Univ. Faculty of Med.

No Abstract

[LS37] LS37 [Japanese]
Topics of cancer immunology: Lessons learned from immune checkpoint blockade

2018 / 9 / 29 (Sat) 11:50-12:40 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15
：Chugai Pharmaceutical co., ltd.

Hideaki Shimada / Department of Surgery, Toho University Graduate School of Medicine

LS37

Topics of cancer immunology: Lessons learned from immune checkpoint blockade

Yutaka Kawakami
Division of Cellular Signaling, Institute for Advanced Medical Research, Keio University School of Medicine

No Abstract

▲TOP

[YSA-1] YSA [English]
The Young Scientist Award Lecture

2018 / 9 / 29 (Sat) 14:10-16:40 Room 15/3F Korin3, RIHGA Royal Hotel Osaka Room 15

Yasufumi Kaneda / Gene Ther. Sci., Gr. Sch. Med., Osaka Univ.

YSA-1

Lack of IL-6 in tumor microenvironment augments type-1 anti-tumor immune responses

Yosuke Ohno
Dept. Gastroenterological Surg. I., Hokkaido Univ., Sch. Med.

Co-author：Hidemitsu Kitamura[1]、Yujiro Toyoshima[2]、Huihui Xiang[2]、Kentaro Sumida[3]、Shun Kaneumi[3]、Shigenori Homma[4]、Hideki Kawamura[4]、Norihiko Takahashi[4]、Akinobu Taketomi[5]
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(Background)Improving of immunosuppression is crucial for effective cancer immunotherapy. It is well-known that IL-6 is produced in tumor-bearing state. In this study, we investigated the precise effects of IL-6 on antitumor immunity.(Material and Method) CT26 cells were inoculated into wild type or IL-6-/- mice, and the tumor size was measured. Tumor tissues were obtained from mice, and the population of immune cells in tumor tissue was analyzed by flow cytometry. Cytokine profile of T cells was analyzed by intra cellular cytokine staining method.(Result)Tumor growth significantly decreased in IL-6-/- mice mice. T cells were highly accumulated in the tumor sites of IL-6-deficient condition. Higher numbers of IFN-γ-producing T cells were present in the tumor tissues of IL-6-/- mice. (Discussion and Conclusion) Lack of IL-6 enhanced induction of type-1 immunity in tumor microenvironment. The helper function of antigen-specific Th1 cells was essential for inducing fully activated killer T cells in tumor-bearing hosts.IL-6 signaling will be a promising target in the development of effective cancer immunotherapy purposing induction of type-1 immunity.

▲TOP

YSA-2

Intratumoral bidirectional transitions between epithelial and mesenchymal ce lls in triple-negative breast cancer

Mizuki Yamamoto
Div. Cell. & Mol. Biol., IMSUT

Co-author：Jun-ichiro Inoue
Div. Cell. & Mol. Biol., IMSUT

Epithelial-mesenchymal transition (EMT) and its reverse process, MET, are crucial in several stages of cancer metastasis. EMT allows cancer cells to move to proximal blood vessels for intravasation. However, because EMT and MET processes are dynamic, mesenchymal cancer cells are likely to undergo MET transiently and subsequently re-undergo EMT to restart the metastatic process.
To elucidate such regulation, we analyzed HCC38, a human TNBC cell line, because HCC38 cells are composed of EpCAM+, CD44low, E-cadherin+ epithelial and EpCAM-, CD44high, Vimentin+ mesenchymal populations at a fixed ratio. We demonstrated that the efficiency of EMT is about an order of magnitude higher than that of MET and that the two populations enhance the transition of cells from the other population to their own. In addition, our data suggest that several ligands including TGF β and EMT-related transcription factors such as ZEB1 and SLUG are involved in the EMT and MET.
Consequently, we propose HCC38 cell line as a suitable model to analyze EMT-MET dynamics that could affect development of triple-negative breast cancer.

YSA-3

Effects of SMYD2-mediated EML4-ALK methylation on the signaling pathway and growth in non-small-cell lung cancer cells

Rui Wang
Section of Hematology

Co-author：Xiaolan Deng[1]、Yuichiro Yoshioka[1]、Theodore Vougiouklakis[1]、Jae-Hyun Park[1]、Takehiro Suzuki[2]、Naoshi Dohmae[2]、Koji Ueda[3]、Ryuji Hamamoto[4]、Yusuke Nakamura[1]
[1]Section of Hematology

A specific subtype of non-small-cell lung cancer (NSCLC) characterized with an EML4-ALK fusion gene, shows good clinical response to ALK inhibitors. Through exploring biological significance of methylation on non-histone proteins in human carcinogenesis, we found that a lysine methyltransferase, SET and MYND domain-containing 2 (SMYD2), could methylate lysine residues 1451, 1455, and 1610 in ALK protein. The phosphorylation levels of the EML4-ALK protein significantly attenuated either by SMYD2 siRNA or SMYD2 inhibitor in two NSCLC cell lines. Substitutions of each of these lysine residues to an alanine (K-A) partially or almost completely diminished in vitro methylation of ALK. Besides, exogenous introduction of EML4-ALK K1610A protein reduced the phosphorylation levels of AKT in the EML4-ALK pathway, and suppressed cancer growth. Furthermore, the combination of SMYD2 inhibitor and ALK inhibitor additively suppressed cancer growth compared with single-agent. Our results suggest a novel mechanism that modulates the kinase activity of the ALK fused gene product and imply that SMYD2-mediated ALK methylation might be a promising target for treatment for tumors with the ALK fused gene.

YSA-4

p62 as an oncotarget mediates cisplatin resistance through RIP1-NF-KappaB pathway in human ovarian cancer cells

Xiao-Yu Yan
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Co-author：Yu Zhang、Juan-Juan Zhang、Li-Chao Zhang、Ya-Nan Liu、Yao Wu、Ya-Nan Xue、Sheng-Yao Lu、Jing Su、Lian-Kun Sun
Dept. Pathophysiol., College of Basic Med. Sci., Jilin Univ.

Platinum-based therapeutic strategies have been widely used in ovarian cancer treatment. However, drug resistance has greatly limited therapeutic efficacy. Recently, tolerance to cisplatin has been attributed to other factors unrelated to DNA. p62 (also known as SQSTM1) functions as a multifunctional hub participating in tumorigenesis and may be a therapeutic target. In this study, we demonstrate that the activity of the NF-KappaB signaling pathway and K63-linked ubiquitination of RIP1 was higher in cisplatin-resistant ovarian (SKOV3/DDP) cells compared with parental cells. In addition, cisplatin resistance could be reversed by inhibiting the expression of p62 using siRNA. Furthermore, deletion of the ZZ domain of p62 that interacts with RIP1 in SKOV3 cells markedly decreased K63-linked ubiquitination of RIP1 and inhibited the activation of the NF-KappaB signaling pathway. Collectively, we provide evidence that p62 is implicated in the activation of NF-KappaB signaling that is partly dependent on RIP1. p62 promotes cell proliferation and inhibits apoptosis thus mediating drug resistance in ovarian cancer cells.

▲TOP

[MV2] MV2 [Japanese]
The JCA-Mauvernay Award Lecture

2018 / 9 / 29 (Sat) 8:00-8:50 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16

Tomoki Naoe / Nagoya Med. Ctr.

MV2

Tumor regulation by cell competition

Tatsushi Igaki
Lab. of Genetics, Grad. Sch. of Biostudies, Kyoto Univ.

Normal epithelial cells often exert anti-tumor effects against nearby oncogenic cells. In Drosophila epithelia, clones of oncogenic cells mutant for apico-basal polarity gene scribble are actively eliminated by cell competition when surrounded by wild-type cells. We have shown that JNK signaling and downstream Slit-Robo-Ena/VASP signaling play a crucial role in this cell elimination. However, the initial event occurring at the interface between normal cells and polarity-deficient cells remained unknown. Through a genetic screen in Drosophila, we identified the ligand Sas and the receptor-type tyrosine phosphatase PTP10D as the cell-surface ligand-receptor system that drives cell competition. At the interface between wild-type winner and polarity-deficient loser clones, Sas-PTP10D signaling is trans-activated in oncogenic loser cells, which restrains EGFR signaling and thereby enables elevated JNK signaling to trigger cell elimination. These findings uncover the mechanism by which normal epithelial cells recognize and eliminate oncogenic neighbors by cell competition. I will also discuss our recent data on the systemic control of tumor-suppressive cell competition.

▲TOP

[SST5-1] SST5 [Japanese]
Recent progress of urologic oncology

2018 / 9 / 29 (Sat) 9:00-11:30 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16

Norio Nonomura / Dept. Urology, Osaka Univ., Grad. Sch. Med.、Yasuhisa Fujii / Dept. Urol, Tokyo Med. Dent. Univ.

We selected six speakers who were the most energetic researchers in the field for urothelial cancer, renal cell carcinoma and prostate cancer among urological cancers.
First, with regard to urothelial carcinoma, Prof. Kazutoshi Fujita from Osaka University will talk about the latest research results on urine biomarkers and Prof. Soichiro Yoshida from Tokyo Medical and Dental University will show the results of research on predictive factors of chemo-and radiosensitivity in invasive bladder cancers.
Next, with regard to renal cell carcinoma, Prof. Toshiya Baba from Kumamoto University will present the carcinogenesis mechanism in Xp11.2 translocation type renal cell carcinoma and Prof. Koji Ueda from Japanese Foundation for Cancer Research will introduce the development of a new therapeutic strategy targeting protein antigen in secretory granules called exosome.
Regarding prostate cancer, Prof. Takahiro Inoue from Kyoto University will talk about the importance of liquid biopsy which is more recent and highly regarded in this field and Prof. Masaki Shiota from Kyushu University will present the latest research results on the mechanism to acquire resistance against castration.
At the end, we could get overviews as special remarks by Prof. Mototsugu Oya from Keio University.
Every speakers are leading researchers in the field for urological cancers. We hope to be able to study the latest perceptions in this symposium.
We are convinced that this symposium will be a good chance for all participants, especially young researchers and will surely be useful for developing their knowledge.

SST5-1

Development of urinary tests for urothelial carcinoma through the analysis of gene mutations

Kazutoshi Fujita
Dept. Urology, Osaka Univ. Grad. Sch. Med.

Co-author：Yujiro Hayashi[1]、Motohide Uemura[1]、George Netto[2]、Norio Nonomura[1]
[1]Dept. Urology, Osaka Univ. Grad. Sch. Med.、[2]Dept. Path., Univ. of Alabama at Birmingham

Urothelial carcinoma (UC) of the upper and lower urinary tracts differs in etiology, but they have many common features, such as the somatic alterations that drive their growth. High rates of activating mutations in the upstream promoter of the TERT gene are found in most of urothelial carcinoma of both upper and lower tracts. Other important mutations include those in FGFR3, RAS, PIK3CA TP53, CDKN2A, MLL and ERBB2 genes. As urothelial cells are in direct contact with urine, genetic analyses of exfoliated urinary cells or urinary cell free DNA could be used to detect UC. We developed a massively parallel sequencing-based assay, termed UroSEEK, for the detection of bladder cancer and upper tract urothelial carcinoma (UTUC) through the genetic analysis of urinary cell DNA as an international collaborative research. UroSEEK has three components: detection of intragenic mutations in regions of ten genes frequently mutated in UC; detection of mutations in the TERT promoter; and detection of aneuploidy. We also developed the gene panel detecting hot-spot mutations by digital PCR and assessed its performance for the detection of UTUC with urinary cell free DNA.

▲TOP

SST5-2

High cell proliferation as a predictive factor for favorable response to chemoradiotherapy against bladder cancer

Soichiro Yoshida
Dept. Urology, Tokyo Med. & Dent. Univ.

Co-author：Yasuhisa Fujii
Dept. Urology, Tokyo Med. & Dent. Univ.

Radical cystectomy is the standard of care for patients with muscle-invasive bladder cancer (MIBC). Reportedly, high Ki-67 status indicates poor prognosis after RC. Recent guidelines recommend chemoradiotherapy (CRT) based bladder-sparing treatment as an alternative. In CRT-based protocols, CRT response is of primary importance in terms of bladder preservation and survival outcomes. Molecular profiling, which characterizes their distinct clinical behaviors, may help to select optimal treatment options. We have applied a tetra-modality protocol incorporating with maximal TUR, low-dose CRT followed by partial or radical cystectomy with curative intent for MIBC patients. In MIBC patients treated with our tetra-modality protocol, high Ki-67 expression, high restriction status of water molecule diffusion on diffusion-weighted MRI, and genomically unstable and squamous cell cancer-like subtypes in the Lund University model, all of which are the features of highly proliferating tumor, are associated with favorable CRT response. Assessment of cell proliferative activity might provide valuable information to identify patients who might benefit from CRT-based multimodal approaches.

SST5-3

Studies to uncover the molecular mechanism for cancer development in Xp11.2 translocation renal cell carcinoma

Masaya Baba
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Co-author：Wenjaun Ma[1]、Takanobu Motoshima[2]、Yorifumi Satou[1]、Hisashi Hasumi[3]、Tsuyoshi Kadomatsu[4]、Mitsuko Furuya[3]、Yoji Nagashima[5]、Masahiro Yao[3]、Masatoshi Eto[6]、W. Marston Linehan[7]、Yuichi Oike[8]、Tomomi Kamba[2]
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Xp11.2 translocation renal cell carcinoma (tRCC) is a newly defined subset of RCC involving chromosomal translocations of transcription factor TFE3 at Xp11.2. All translocations seen in tRCC produce chimeric TFE3 proteins including SFPQ-TFE3, PRCC-TFE3 and others that retain the basic helix-loop-helix leucine zipper structure for DNA binding, suggesting that these chimeric TFE3 proteins function as oncogenic transcription factors. To prove chimeric TFE3 oncogenicity, we generated a genetically engineered mouse model, in which chimeric PRCC-TFE3 was expressed in kidney epithelial cells. These mice developed RCC by 6 months. To characterize the transcriptional function of chimeric TFE3s, we performed comprehensive gene expression analysis to identify genes whose expressions were significantly changed by induction of chimeric or wild type TFE3 expression in inducible cell lines. We mapped binding of chimeric and wild type TFE3s to the genome by chromatin immunoprecipitation sequencing. Cells expressing chimeric TFE3s demonstrated distinct gene expression alterations and characteristic genome binding patterns. These findings shed light on the molecular mechanism of tRCC development.

SST5-4

Diagnostic and therapeutic potential of exosomal proteins in kidney cancer

Koji Ueda
Can. Proteomics. Gr, CPM Ctr, JFCR

The incidence and mortality rate of kidney cancer increased 2.5– and 2.0–fold in the latest 15 years, respectively. To manage and improve them, innovative diagnostic and therapeutic technologies are urgently required. Recently, exosome has come to be known as an attractive resource of diagnostic and therapeutic molecular targets since it delivers molecular cargoes from original cancer cells into body fluids. We here present a result of global proteome profiling for exosomes extracted from viable renal cell carcinoma (RCC) tissues or adjacent normal kidney tissues. Comprehensive LC/MS analysis identified 3,871 exosomal proteins, in which azurocidin (AZU1) and TME19 exhibited RCC-specific expression patterns. Importantly, these exosomal surface antigens were confirmed to be upregulated in RCC patients ´ sera also. We further found that these proteins induced gene-independent manipulations of tumor microenvironment, such as promotion of hematogenous metastasis or protection from hypoxic condition. These findings provide novel insights regarding relationship between behaviors of exosomes and cancer biology, leading to development of novel diagnostic and therapeutic strategies.

▲TOP

SST5-5

Genomics and lipidomics analysis of blood and urine toward prostate cancer precision medicine

Takahiro Inoue
Dept. Urol, Kyoto Univ. Grad. Med.

Co-author：Xin Li[1]、Takayuki Sumiyoshi[1]、Kenji Nakayama[2]、Kei Mizuno[1]、Kosuke Okasho[1]、Takayuki Goto[1]、Shusuke Akamatsu[1]、Osamu Ogawa[1]
[1]Dept. Urol, Kyoto Univ. Grad. Med.、[2]Shimazu Techno-Reserach

Recent advancements of drug development for castration-resistant prostate cancer (PCa) have led the cost for the treatment to be increase dramatically. Thus, early diagnosis and appropriate application of the drugs is mandatory especially for the economical view.We have been focusing on genomic and lipidomics analysis using human biomaterials. We have established method for detecting cfDNA of CRPC patients and analyzed it from tumors focusing androgen receptor (AR) amplification and mutations. AR amplification together with AR mutation might be an effective molecular marker for selecting appropriate PCa patients for delivering abiraterone acetate. We also have performed urinary lipidomics by MALDI-TOF/MS in the urine after digital rectal examination (DRE) and identified phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine and phosphatidylglycerol species by MALDI-TOF/MS^2. The compositions of PC and LPC in the urine samples were significant different between the PCa and BPH groups and the relatively quantitative analyses of PC and LPC concentrations in the DRE urine samples might be feasible and be a simple and non-invasive biomarker for the PCa.

SST5-6

Up-to-date on the mechanism of castration resistance in prostate cancer

Masaki Shiota
Dept. Urol., Kyushu Univ., Grad. Sch. Med. Sci.

Co-author：Masatoshi Eto
Dept. Urol., Kyushu Univ., Grad. Sch. Med. Sci.

Numerous researches on the mechanism of castration-resistant prostate cancer (CRPC) have revealed significant findings, leading to the development of novel anticancer agents. The reactivation of androgen receptor (AR) signaling even in castrate condition has been recognized to be main contributors. Recently, neuroendocrine prostate cancer has emerged as another major cause of CRPC, since the development of resistance to novel AR-targeting agents evoked non-AR driven CRPC. On neuroendocrine CRPC development, numerous researches have been performed intensively. Accordingly, genetic alterations in neuroendocrine CRPC as well as curious molecular mechanisms regulating suppressing AR signaling and promoting neuroendocrine signaling have recently been revealed. Thus, great advances on the mechanism of CRPC development have been achieved. In this session, we would summarize and introduce up-to-date findings on the mechanism of CRPC development.

SST5-Special_Remarks

Special Remarks

Mototsugu Oya
Dept. Urology, Keio Univ. Sch. Med.

No Abstract

▲TOP

[LS38] LS38 [Japanese]

RamDA-seq: Single-cell full-length total RNA sequencing method to uncover expression and full-length structure of total RNA

2018 / 9 / 29 (Sat) 11:50-12:40 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16
：TOYOBO CO., LTD.

Akira Watanabe / Center for iPS Cell Research and Application, Kyoto University

LS38

RamDA-seq: Single-cell full-length total RNA sequencing method to uncover expression and full-length structure of total RNA

Itoshi Nikaido
Laboratory for Bioinformatics Research, RIKEN Center for Biosystems Dynamics Research

No Abstract

▲TOP

[SST6-1] SST6 [Japanese]
Advances in treatments for lung cancer

2018 / 9 / 29 (Sat) 13:40-16:10 Room 16/2F Katsura, RIHGA Royal Hotel Osaka Room 16

Tetsuya Mitsudomi / Thorac. Surg., Kindai Univ. Fac. Med.、Yuichi Ishikawa / Pathol. Div., JFCR Cancer Inst.

The recent progress of drug therapy for lung cancer has been remarkable. As for molecular targeted therapy against lung cancers harboring driver gene mutations, approval of gefitinib in Japan in 2002, and discovery of EGFR gene mutation as a predictive biomarker in 2004 marked the start for this type of therapy. Following this, targeted therapies against lung cancer with mutations in the ALK, ROS1, or BRAF gene were shown to provide similar significant clinical response, and has become standard of care. In the near future, it is expected that targeted therapies against lung cancer with rarer mutations (HER2, MET, NTRK, and RET, etc.) will be available. Dr. Kohno will summarize current status molecular genomic research of lung cancer and Dr. Okamoto will talk on current targeted therapies of lung cancer from the clinical point of view. Dr. Goto will mainly touch on novel molecular targets through his experience in the SCRUM-Japan project. Dr. Kobayashi will discuss the recent knowledge on acquired resistance against these targeted therapies which is inevitable. Nivolumab, the first immune-checkpoint inhibitor for lung cancer, was approved in Japan in 2015. Subsequently, pembrolizumab, atezolizumab, and durvalumab have been also approved to date. First, these agents became standard of care for the second-line treatment of lung cancer and then pembrolizumab became a standard of care for the first-line treatment of lung cancer with high PD-L1 expression. Furthermore, positive clinical trials of immune-checkpoint inhibitors in combination with chemotherapy or anti-angiogenic agent, and maintenance immunotherapy after chemoradiation for locally-advanced lung cancer have recently been reported. Dr. Hayashi will summarize recent clinical trials and future direction. However, patients who benefit from these therapies are still limited and thus biomarker study that identify these patients is important. This point will be touched on by Dr. Togashi. Finally, Dr. Ohe will give special remarks on drug therapy in general from his abundant clinical experience.

SST6-1
Genome profile and mutational signature of lung cancer

Takashi Kohno
Div. Genome Biol., Natl. Cancer Ctr. Res. Inst., Div. Translational Genomics, EPOC, Natl. Cancer Ctr.

Lung carcinogenesis is diverse as demonstrated by differential driver oncogene aberration fractions according to histological types, smoking habit and UIP (usual interstitial pneumonia) association. Genome profiling studies have identified critical driver oncogenes in the majority of LADCs (lung adenocarcinomas), but their alterations are infrequent in LADCs of smokers and those associated with UIP. Other histological types of lung cancers are also often negative for driver oncogene alterations. Instead, inactivating mutations in chromatin-regulating genes, such as SMARCA4/BRG1 and CREBBP, and pulmonary surfactant system genes, such as NKX2-1/TTF1 and SFTPs, might contribute to the development and/or progression of lung cancer. Development of EGFR-mutated LADC is influenced by HLA variations, therefore, both exogenous and endogenous risk factors underlie the diversity of lung carcinogenesis. Mutational signature studies have revealed the mutational events underlying lung cancer development/progression associated with risk factors. Genome profile and mutational signatures of lung cancer will be summarized and their significance in precision lung cancer medicine will be discussed.

▲TOP

SST6-2

Molecularly targeted therapies for oncogene-driven advanced non-small-cell lung cancer

Isamu Okamoto
Dept. Resp. Med., Kyushu Univ., Sch. Med.

Over the past decade, driver gene alterations that play major roles in oncogenesis and tumor progression have been identified in non small cell lung cancer (NSCLC). The success of molecularly targeted tyrosine kinase inhibitors (TKIs) for EGFR mutated and ALK rearranged NSCLC patients (pts) has dramatically changed overall survival for those pts and also given treatment opportunity even for poor PS pts. Despite of initial remarkable clinical benefit, such individuals inevitably progress due to the development of acquired resistance; however 2nd or 3rd generation TKIs have been currently incorporated in this setting, leading to further prolonged survival. More recently, effective targeted therapies for ROS1 rearranged NSCLC pts and activating BRAF mutated pts have been approved and more comprehensive genomic screening such as next-generation sequencing will be employed in daily clinical setting. Recent broad advances in molecular-targeted therapies and diagnostics will be reviewed.

SST6-3

Development of Nationwide Genomic Screening Platform (LC-SCRUM-Japan) to Establish Precision Medicine in Lung Cancer

Koichi Goto
Dept. Thoracic Oncology, Natl. Cancer Ctr. Hosp. East

Background: Recently many druggable driver oncogenes such as EGFR, ALK, RET, ROS1, BRAF and MET have been identified in non-small cell lung cancer (NSCLC). However, most of these driver oncogenes are encountered rather rarely, that is, in only about 1-2% of lung adenocarcinoma.
Methods: From February 2013, LC-SCRUM-Japan was initiated. From March 2015, it has been amended to an academic-industrial collaboration project (SCRUM-Japan), and the samples are subjected to NGS system.
Results: From February 2013 to May 2018, 6440 patients were enrolled into LC-SCRUM-Japan. In non-Sq NSCLC, various rare targetable genomic alterations including ALK/RET/ROS1/FGFR fusions, ERBB2/BRAF mutations and MET/ERBB2/FGFR amplifications were detected. Through this genomic screening, many patients with rare driver oncogenes were successfully enrolled into the various clinical trials. Based on our project, crizotinib and dabrafenib/trametinib were approved for ROS1 fusions and BRAF mutation, respectively in Japan.
Conclusion: LC-SCRUM-Japan enabled efficiently detecting various rare targetable genomic alterations in NSCLC, contributing to promote cancer precision medicine.

SST6-4

Mechanisms and strategies to overcome resistance to tyrosine kinase inhibitors in lung cancer

Susumu Kobayashi
Div. Translational Genomics, EPOC, NCC, Div. Hem-Onc, Beth Israel Deaconess Med. Ctr.

The discovery of somatic mutations in oncogenic kinases such as EGFR or ALK and development of tyrosine kinase inhibitors (TKIs) have revolutionized treatment for lung cancer. These oncogenic kinase mutations trigger both pro-survival and anti-apoptotic signals, and inhibition of these aberrant signals causes massive apoptosis of tumor cells and tumor regression. Despite dramatic initial responses, acquired resistance to TKIs emerges in almost all patients. Recently, newer generation inhibitors have been developed to overcome the resistance. For example, osimertinib is third-generation EGFR TKI designed to target the gatekeeper EGFR-T790M mutation and has shown excellent responses even to classic EGFR mutations, leading to approval for its use as a first-line treatment for metastatic non-small cell lung cancer with EGFR mutations. However, these new inhibitors eventually become ineffective during the course of treatment. The mechanisms of resistance include: 1) emergence of resistance mutations; 2) kinase-bypass; and 3) histological transformation. I will dicuss these resistant mechanisms and efforts to establish strategies to win these whack-a-mole battles.

▲TOP

SST6-5

Future directions in immune-checkpoint inhibitors in NSCLC

Hidetoshi Hayashi
Dept. Med. Oncol., Kindai Univ.

Treatment with immune-checkpoint inhibitors (ICIs) has dramatically improved outcomes for advanced non-small cell lung cancer (NSCLC) and a monoclonal antibody to the immune-checkpoint protein PD-1 (programmed death-1) or PD-L1 (programmed death ligand 1), has been approved for the treatment of NSCLC. However, single agent PD-1 blockade has limited activity with one-year progression free survival (PFS) rate of 15-20%, therefore, more effective treatment has been needed.
Cytotoxic chemotherapies can enhance antitumor immune responses through a variety of mechanisms. On this basis, several clinical trials to evaluate the efficacy of the combination of cytotoxic agents and ICIs were conducting and, more recently, demonstrated their positive impact in terms of overall survival and PFS. Additionally, the combination of PD-1/PD-L1 blockade and anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) antibody demonstrated the improved PFS in phase 3 trials in advanced NSCLC with high tumor mutation burden. Here, I will review the clinical findings about these ICIs combination therapy for NSCLC and how we can translate these understanding into future treatment strategy in such patients.

SST6-6

Translational Research for Predictive Biomarkers in Cancer Immunotherapy

Yosuke Togashi
Div. Cancer Immunol., Natl. Cancer Ctr.

Recent success of cancer immunotherapy, particularly PD-1 blockade, makes it a key strategy for cancer treatment including lung cancer. The efficacy, however, is not satisfied yet. In our laboratory, variable immune cells both in tumors and in the periphery are being investigated using several techniques such as flow-cytometry, CyTOF, and single cell RNA sequencing. Our analyses revealed that immune responses exhibited marked changes in tumor microenvironment during treatment courses of certain anti-cancer reagents, leading to investigator-initiated trials. Tumor microenvironment of patients treated with PD-1 blockade were also analyzed, resulting in identification of predictive biomarkers and resistant mechanisms. We also analyzed the association between gut microbiome and response to PD-1 blockade in solid cancers, showing a predictive biomarker candidate which is also related to immune status in tumor microenvironment. Based on these findings, we aim at establishing the next precision medicine including both genome alterations and immune responses.

SST6-Special_Remarks

Special Remarks

Yuichiro Ohe
Dept. Thoracic Oncol., Natl. Cancer Ctr. Hosp.

No Abstract

▲TOP

[P-3001] P3-1 [English]
Virus, bacteria infection, inflammation and cancer (1) [English]

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Naoko Kamiya / Dept. Microbiol., Grad. Sch. Med., Univ. Tokyo

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P-3001

Radiotherapy-induced cell death activates HMGB1-TLR2/4 signaling and regulates stemness of resident cancer stem cell

Haitao Zhu
Affiliated Hosp. of Jiangsu Univ.

Radiotherapy is one of the major tools of pancreatic cancer treatment. Dying cells and their released inflammatory mediators are major components of the niche that pancreatic cancer stem cells relied on following radiotherapy and their interactions may profoundly affect pancreatic cancer progression. Inflammatory mediators have been considered as a critical factor for CSCs maintenance. In this study, we found high level of HMGB1 in the cancer cells culture media following radiation and pancreatic carcinoma following radiotherapy. Mechanistically, dying cells released HMGB1 can activate its receptor TLR2 expressed by pancreatic cancer cells to enhance their stemness and tumourigenicity, while TLR4 abrogate this effect. HMGB1-TLR2 axis regulated self-renewal and core pluripotency genes via Wnt/&beta-catenin signaling. Our study indicated that HMGB1 was up-regulated following radiotherapy and associated with the carcinogenesis and CSCs self-renewal activation in pancreatic carcinoma. Dying cells derived HMGB1 and TLR2 on the cancer cells can be potential therapy target for preventing pancreatic cancer stem cell induced recurrence.

▲TOP

P-3002

Lon-ROS axis induces mtDNA release that activate cGAS-STING-IFN signaling and pack in exosome in tumor microenvironment

An Ning Cheng
Natl. Inst. of Cancer Res., NHRI

Co-author：Li-Chun Cheng、Alan Yueh-Luen Lee
Natl. Inst. of Cancer Res., NHRI

Mitochondrial Lon functions in protein quality control and stress response pathways in mammals. Lon also has been shown to be overexpressed in various types of cancer including colon cancer, prostate cancer and oral cancer. Recently, we observed that Lon overexpression promotes ROS-dependent NF- κ B and interferon (IFN) signaling in oral cancer, suggesting that Lon may induce a NF- κ B-dependent inflammatory response. Herein, we used microarray expression analysis to demonstrate that Lon overexpression induces the interferon-stimulated genes, such as ISG15, IRF7 and STAT1 in a ROS-dependent manner in OSCC. We further found that mitochondrial DNA is released into cytosol in the Lon-overexpressing oral cancer cells and Lon-ROS axis induced the cGAS-STING pathway, then the mtDNA transfers to extracellular space by exosomes. In addition, we observed that Lon overexpression induces the ROS-dependent expression of indoleamine 2,3 dioxygenase (IDO) in OSCC, which is involved in mtDNA-STING activation tumor tolerogenic response. Taken together, these results suggest that inflammation induced by Lon overexpression may have an immunosuppression effect on tumor microenvironment.

P-3003

SLPI is a chronic inflammation induced-factor and exerts tumorigenicity in cholangiocarcinoma

Suchada Phimsen
Faculty of Med. Sci., Naresuan Univ., Phisanulok, Thailand

Co-author：Chaiwat Chouiphuk[1]、Sarawut Kumphune[2]、Sopit Wongkham[3]
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Cholangiocarcinoma (CCA) is a chronic inflammation induced-cancer with poor prognosis. Previous report has revealed that secretory leukocyte protease inhibitor (SLPI) was positively correlated with CCA progression. However, the association of the overexpression of SLPI and the progression of CCA carcinogenesis was still unclear. Therefore, we investigated the expression of SLPI in human CCA tissues. The results showed that SLPI was abundant in the CCA tissues compared with adjacent tissues, indicating that it may be a potential factor for the malignant transformation. Subsequently, we investigated the effects of the pro-inflammatory cytokine interleukin 6 (IL-6) treatment on the expression of SLPI, nuclear factor-kB (NF-kB) pathway and its tumorigenicity of CCA cells.The finding revealed that IL-6 promoted NF-kB suggesting the chronic inflammation. Furthermore, SLPI was significantly enhanced after IL-6 treatment. Moreover, colony formation rate of IL-6 treated CCA cells was higher than untreated cells. In addition, overexpression of SLPI was enhancing tumorigenesis. These data demonstrated that SLPI is an important factor for CCA development.

P-3004

Biological properties of Epstein-Barr virus positive oral squamous cell carcinoma cell lines

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We have previously detected oncogenic Epstein-Barr virus (EBV) genomes in 32.5% of the tumor lesions of oral squamous cell carcinoma. However, there is no in vitro model for studying contribution of EBV in the development of oral squamous cell carcinoma. Latently EBV-infected cells were established from HSC1 (well-differentiated) and SCC25 (poorly-differentiated) cell lines. Viral copy numbers in EBV-positive HSC1 cell and EBV-positive SCC25 cell are 2 and 5, respectively. Although EBV copy number was two, spontaneous EBV replication was observed in EBV+ HSC1 cell. On the other hand, lytic activation was not observed in EBV+ SCC25 cells having five EBV copies. Proliferation and migration of cells were activated more strongly in EBV+ HSC1 cells than uninfected cells. TPA treatment of EBV+ HSC1 cells induced expression of viral lytic BZLF1 gene. However, these observations could not be observed in EBV+ SCC25 cells. Suggesting, EBV can persistently infect to oral squamous cells, but promotion of cell proliferation or lytic EBV replication may change between stages of cell differentiation. The possible role of oral squamous cell for secretion and transmission of EBV will be discussed.

▲TOP

P-3005

Viral marker genes potentially useful for understanding geographical distribution of various EBV strains

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We recently applied a CRISPR/Cas9 genome editing technology, which enabled efficient transgene insertion to the targeted region of the EBV genome, to clone and characterize EBV genomes (SNU-719 and YCCEL1 strains, derived from Korea). In order to apply the system to EBV strains maintained by Japanese individuals, EBV-infected naturally-arising B-lymphoblastoid cell lines were obtained from tonsillar tissues of eleven Japanese patients undergoing routine tonsillectomies. Based on the nucleotide sequence polymorphisms that were found within the targeted region (spanning viral early genes), the Japanese EBV strains were categorized into two groups, either SNU719-type or YCCEL1-type. Interestingly, while Japanese and Korean EBVs share the same sequences of the targeted region, they are distinct from M81-type EBVs, which are commonly found in China and Southeast Asian countries. Blast search indicated that the sequence of a gene encoding a small capsid protein can also be used to distinguish SNU719 and YCCEL1-type EBVs from M81-type EBVs. These results imply that there are nucleotide sequence polymorphisms which can be used to rapidly categorize EBV strains worldwide.

▲TOP

[P-3012] P3-3 [English]
Virus, bacteria infection, inflammation and cancer (3) [English]

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Dai Iwakiri / Nat.Cent.Child Health and Dev.

P-3012

Disrupting the acetylation of ISX-BRD4 by PCAF Suppresses Tumor Metastasis

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The epithelial-mesenchymal transition is an important process in the cancer progression, but its occurrence and the regulatory mechanism are not fully understood. Intestine specific homeobox acted both as an proto-oncogene and upstream regulator of EMT markers, by which modulates tumorigenic initiation and progression in lung cancer cells. ISX acetylated by PCAF recruits chromatin reader, bromodomain-containing protein 4, to initiate chromatin remodeling, and up-regulated EMT downstream regulators in tumors cells. Ectopic expression of ISX were shown to enhance TWIST1, Snail1, vascular endothelial growth factor expression by recruiting BRD4 to enhance Pol II dependent transcription, leading to remodeling of the tumor microenvironment. Neutralizing VEGF by avastin effectively abrogates metastasis induced by the ISX-BRD4 complex. In NSCLC carcinoma, increased ISX expression was noted, correlating with distinct clinical metastatic features and poor prognosis. These results suggest that the ISX-BRD4 axis mediates EMT signaling and exerts significant regulatory effects on tumor initiation and metastasis.

▲TOP

P-3013

Intestine-specific homeobox (ISX) upregulates E2F1 expression and related oncogenic activities in HCC

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ISX, a proto-oncogene, is involved in cell proliferation and progression of HCC. However, the mechanisms linking gene expression and tumor formation remain unclear. We found that ISX activated E2F1 and associated oncogenic activity by directly binding to its promoter. Forced expression of ISX increased the expression of and phosphorylated the serine residue at position 332 of E2F1, which may be translocated into the nucleus to form the E2F1-DP-1 complex, suggesting that the promotion of oncogenic activities of the ISX-E2F1 axis plays a critical role in hepatoma cells. Coexpression of ISX and E2F1 promoted p53 and RB-mediated cell proliferation and anti-apoptosis, and repressed apoptosis and autophagy. In contrast, shRNAi-mediated attenuation of ISX and E2F1 decreased cell proliferation and malignant transformation in hepatoma cells. The mRNA expression of E2F1 and ISX in 238 HCC patients, and the adjacent, normal tissues exhibited a tumor-specific expression pattern which was highly correlated with disease pathogenesis, patient survival time, progression stage, and poor prognosis. Our results indicate that E2F1 is an important downstream gene of ISX in hepatoma progression.

P-3014

TIP60-dependent acetylation of the SPZ1-TWIST complex promotes EMT and metastasis in liver cancer

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Metastasis is the main cause of cancer mortality. However, the triggering mechanisms and regulation of EMT factors in the commitment of metastasis have not been well characterized. Spermatogenic Zip 1 acts as a proto-oncogene and an upstream regulator of EMT during tumorigenesis. Here, we report that the HIV-1 Tat-interacting protein 60 kDa acetyltransferase mediates acetylation at lysine residues of SPZ1 at positions 369 and 374 and of TWIST1 at positions 73 and 76, which are required for SPZ1-TWIST1 complex formation and cancer cell migration in vitro and in vivo. Ectopic SPZ1 and TWIST1 expression, but not that of TWIST1 alone, enhanced vascular endothelial growth factor expression via the recruitment of bromodomain-containing protein 4, thus enhancing RNA-Pol II-dependent transcription and inducing metastasis. Neutralization of VEGF using humanized monoclonal antibodies, such as Avastin, effectively abrogated the EMT and oncogenesis induced by the acetylated SPZ1-TWIST1 complex. Our findings highlight the importance of acetylation signaling in the SPZ1-TWIST1-BRD4 axis in the mediation of EMT and its regulation during tumor initiation and metastasis.

P-3015

Intestine-Specific Homeobox Gene ISX Integrates IL6 Signaling, Tryptophan Catabolism, and Immune Suppression

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ISX, a proto-oncogene, is thought to promote HCC, but the mechanisms remain unclear. We proposed an ISX-mediated positive feedback loop integrating inflammation, tryptophan metabolism and immune suppression. ISX was shown to mediate IL-6-induced expression of IDO1 and TDO2 encoding tryptophan-metabolizing enzymes, resulting in ISX-dependent increase of a tryptophan metabolite, kynurenine, and its receptor, AHR. The resultant kynurenine-AHR axis, in a positive feedback mechanism, targeted ISX expression and enhanced proliferation and tumorigenic potential, while either ISX or AHR knockdown reversed such effects. Overexpression of ISX induced, in an IDO-dependent manner, the expression of the genes encoding two critical immune modulators, CD86 and PD-L1, through which ISX conferred a significant suppressive effect on the CD8+ T-cell response. In patients, expression of IDOs, kynurenine, AHR, and PD-L1 correlated negatively with survival. These results suggested a self-perpetuating loop of regulation involving ISX-mediated kynurenine-AHR signaling and PD-L1-associated immune suppression, providing evidence supporting its potential utility as a therapeutic target in HCC.

▲TOP

P-3016

Ubiquitin Specific Protease 4 Regulates Lung Cancer Progression by Control of Inflammation and Stemness

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Ubiquitination and deubiquitination are important posttranslational modifications involved in various cellular processes. To investigate the role of deubiquitinase (DUBs) in lung cancer progression, we analyzed correlation of DUB expression with survival data in a database linking TCGA survival data to gene expression. We found that low expression of ubiquitin specific protease 4 (USP4) was associated with low survival rate of lung cancer patients. Moreover, USP4 was downregulated in stemness enriched cancers. Thus, the effect of USP4 downregulation on tumor propagation in lung cancers were evaluated. Knockdown of USP4 in lung cancers enhanced NF-&kappaB activation and inflammatory responses. In addition, the capability of sphere formation and expression of stemness associated genes were elevated in USP4 knockdown cells. Further, knockdown of USP4 enhanced proliferation and expression molecule for resistant to immune therapy in lung cancers as well as enhanced resistance to chemotherapy drugs. Animal studies showed that reduction of USP4 expression in lung cancers enhanced tumor growth and increased inflammatory and stemness properties in tumors.

P-3017

DUB3 regulated tumor associated inflammation and stemness in lung cancer cells

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Inflammatory stimuli such as TLR ligands, IL-1&beta, and TNF-&alpha in tumor microenvironment are capable of activating the NF- &kappaB controlled inflammatory responses in cancer cells. The activation of cellular responses by these stimuli are modulated by various molecules including those control ubiquitination and deubiquitination in the NF-&kappa B signaling pathway. The DUB-3 has been identified as a deubiquitinating enzyme that belongs to a family of inducible DUBs. In this study, we found that expression of DUB-3 is increased in lung cancers and associated with poor prognosis, macrophage and inflammatory marker expressions, and cigarette smoking. DUB-3 regulated deubiquitination process in the inflammatory signaling pathways, and controlled inflammation and stemness in cancer cells. In animal studies, overexpression of DUB3 in cancer cell promoter tumor growth. Taken together, there results suggest that DUB3 regulate inflammation and stemness in cancer cells and promoter lung cancer growth.

▲TOP

[P-3025] P3-5 [Japanese]
Virus, bacteria infection, inflammation and cancer (5)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Futoshi Okada / Div. Pathol. Biochem., Tottori Univ. Facul. Med.

P-3025

Analysis of biological differences of human papillomavirus 58 E7 variants

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HPV58 is frequently detected in patients with cervical neoplasia and invasive cervical cancer in eastern Asia including Japan. HPV58 consists of multiple lineages of genetic variants harboring less than 10% differences between its complete genome sequences. Among variant lineages/sublineages of HPV58, sublineage A3 has been suggested to pose a higher risk for cervical cancer development, and our recent data also showed a trend of a higher prevalence of A3 in cervical cancer in Japan; however, the underlying mechanisms for higher carcinogenicity remain unclear. Since the A3 genome codes for a characteristic variant of E7 T20I/G63S, we explored biological differences of HPV58 E7 across variant lineages/sublineages. When HPV58 E7s from A1, A2, A3, and C were exogenously expressed in human cervical keratinocytes immortalized with telomerase reverse transcriptase, protein levels of E7 from A3 and C were significantly lower than those from A1 and A2, which may reflect their higher capability to degrade cellular target proteins, including Rb, PTPN14, and UBR4. These results suggest that the E7 variants may differently affect the cellular pathways.

▲TOP

P-3026

Dynamic change in frequency of abnormal findings in cervical cytology depending on birth year in Japan

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[Background] In Japan, HPV vaccination rate fell dramatically after the suspension of the governmental recommendation in 2013.
[Purpose] We aimed to analyze the change in frequency of abnormal findings in cervical cytology of 20-year-old females during 2010– 2015 (birth years: 1990–1995).
[Method] We analyzed the data for each birth year, for the cumulative HPV vaccination rates achieved as of age 16, and for the corresponding results of cervical cancer screening at age 20. The data were obtained from seven local governments in Japan.
[Results] HPV vaccination rates of the targets of 20–year–old cervical cancer screening in and before 2013 were 0% because it was not yet publicly introduced. HPV vaccination rates of the targets of 20–year–old cervical cancer screening in 2014 and 2015 were 63.9% and 74.7%. The rate of abnormal findings (LSIL) in cervical cytology was 2.11% in 2010–2013, but significantly dropped to 0.58% in 2014–2015 ($p<0.001$).
[Conclusion] Our results indicate that the yearly rate of abnormal cervical cytology suspected for CIN was statistically significantly reduced in possible correlation with the widespread administration of the HPV vaccine.

P-3027

Roles of NF κB-dependent degradation of Human papillomavirus E1 in the viral persistence

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Persistent infection of high risk human papillomaviruses (HPVs) causes several cancers such as cervical and oropharyngeal cancers. The viral persistence is mainly attributed to the ability of HPVs to stably maintain viral genomes as low copy-number episomes with minimal expression of the viral genes in basal cells of infected epithelium. Intervention in viral persistence can prevent cancers, thereby it is important to understand its molecular mechanisms. Previously, we reported that NF κB activation induced by expression of E1, the viral DNA helicase, limits the viral genome replication by promoting proteasomal degradation of E1 itself. In the present study, we found that HPV16 E1 encodes a motif known as a phospho-degron recognized by F-box protein, β-TrCP and this motif is well conserved among most of, if not all, papillomaviruses. Alanine substitution at putative phosphorylation sites in the E1 abrogated NF κB-dependent degradation. Interestingly, the E1 mutant incapable of activating NF κB was resistant to degradation induced by overexpression of β-TrCP. These results suggest that NF κB induces $SCF^{\beta\text{-}TrCP}$ mediated degradation of E1 by promoting phosphorylation.

P-3028

Delta-like 3 is silenced by HBx via histone acetylation in HBV-associated HCCs

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Background Delta-like3 (DLL3) is a member of Delta/Serrate/Lag2 ligands for Notch receptor and suppressed Notch signaling. We show that i) DLL3 expression in normal function livers, ii) the relationship between clinicopathological features and expression of DLL3 in HCCs, iii) the relationship between silencing of DLL3 and the hepatitis B virus (HBV) X protein (HBx), and iv) epigenetic change of DLL3 in HCC cell lines. Materials and Methods HepG2 and HepG2.2.15 cells were used to investigate the relationship between silencing of DLL3 and HBx. Results i) DLL3 expression was confirmed in 9 of 10 normal function livers. ii) DLL3 expression was strongly positive in non-cancerous cirrhosis livers. Silencing of DLL3 was closely related to HBV infection. iii) HepG2.2.15 cells showed lower DLL3 expression than the parent cell line, HepG2 cells.Treatment with HBx small interfering RNA significantly upregulated DLL3 expression in HepG2.2.15 cells. iv) Treatment of cells with a histone deacetylase inhibitor induced DLL3 expression in HepG2.2.15 cells. Conclution DLL3 expression is silenced during hepatocarcinogenesis in association with HBV infection via an epigenetic mechanism.

▲TOP

[P-3034] P4-8 [English/Japanese]
Translational research in colorectal cancer

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Sonshin Takao / Tanegashima Med. Ctr.

P-3034

Biological and clinic-pathological significance of Uc.63+ in colorectal cancer

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The transcribed ultraconserved regions (T-UCRs) are a novel class of long non-coding RNAs that are absolutely conserved across species and are involved in cancer developement in several types of cancer. We have reported the oncogenic role of Uc.63 in prostate cancer. But there are no reports mentioning its expression and biological function in colorectal cancer (CRC). In this study, we examined Uc.63+ expression in CRC cell lines by qRT-PCR, and found that knockdown of Uc.63+ repressed CRC cell growth. We also evaluated Uc.63+ expression by qRT-PCR using 40 CRC samples and assessed the correlation between Uc.63+ expression and clinico-pathological factors. Higher levels of Uc.63+ expression were significantly correlated with more advanced clinicopathological parameters in CRC and CRC patients with higher Uc.63+ expression showed significantly poorer clinical outcome. Our results suggest that Uc.63+ is more likely to be involved in CRC progression.

▲TOP

P-3035

The function and regulation of Golgi-Associated PDZ And Coiled-Coil Motif Containing (GOPC) in Colorectal Cancer

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We extracted 2463 genes correlated with the prognosis of colorectal cancer using the public database, Gene Expression Omnibus. We focused on GOPC from the gene group.First, we assessed the correlation GOPC expression and colorectal cancer prognosis using our clinical samples. It was identified the GOPC as poor prognostic factors.Next, we analyzed the function of GOPC using colon cancer cell lines DLD-1, HT-29, RKO, KM12SM. As a result, it was shown that the proliferation and invasion of cancer cells are significantly increased in GOPC knockdown. The conflicting results were obtained in GOPC over expression.These results showed that GOPC played a role as a tumor suppressor gene. In addition, the EMT-related molecules such as N-cadherin and ZEB-2 were related with the GOPC expression.In colorectal cancer, the methylation in the promoter site for GOPC was occured more frequently than in the normal mucosa. And the methylation in the promoter site and GOPC gene expression showed inverse correlation.

P-3036

Downregulation of ARID1A in colorectal cancer

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ARID1A is a subunit of the SWI/SNF chromatin remodeling complex, which possesses DNA binding activity. Mutation or dysfunction of ARID1A is believed to various carcinogenesis. The present comprehensive gene expression analyses, isolated a molecule, designated CA8, which was increasingly expressed by dysfunction of ARID1A. Methods: ARID1A and CA8 expression were examined by immunohistochemical staining in patients with colorectal cancer resected in our hospital. We compared the groups to clinicopathological features, OS and RFS. We employed a colon cancer cell line, LS174T which expressed CA8 at cell surface to evaluate the anti-tumor effect of anti-CA8 monoclonal antibody. We transplanted LS174T into the subcutaneous of Scid Beige mice and injected anti-CA8 antibody into intraperitoneal. Results: About ARID1A, differentiated type were significantly less in the partial positive group. OS and RFS were significantly worse in the partial positive group. About CA8, there was no significance in clinicopathological features, OS and RFS between two groups. Anti-CA8 antibody significantly decreased LS174T cell growth in vitro. Anti-CA8 antibody delayed the tumor progression in vivo.

P-3037

Biological significance of ArfGAP with GTPase domain, ankyrin repeat and PH domain 3 (AGAP3) in colorectal cancer (CRC)

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Background & Aim: Specific chromosomal arms including 7q were frequently amplified in CRC, and novel driver genes may be located on these arms. We aimed to identify a novel driver gene on 7q and to clarify its biological function.Methods & Results: We focused on AGAP3 as a candidate driver gene which fulfilled the following criteria in CRC data from The Cancer Genome Atlas (TCGA). 1) The concordant association between DNA copy number and mRNA expression. 2) Overexpressed in CRC tissues compared to normal tissues. In TCGA and GEO dataset, AGAP3 expression was significantly higher in CRC and colon adenoma tissues compared with normal tissues, while there was no difference between CRC tissues in each pathological stage. CRC cells were transfected with expression vector bearing AGAP3 isoform-a or isoform-b (CRMP5-associated GTPase (CARG)) which are major splicing isoforms of AGAP3. Cell proliferation and colony formation were increased in CRAG transfected cells, but not in isoform-a transfected cells. Forced expression of CRAG elevated c-Fos and c-Jun protein expression.Conclusion: CRAG, splicing isoform of AGAP3, may contribute to development of CRC via activator protein 1 activation.

▲TOP

P-3038

Crosstalk among oncogene products revealed by CRISPR/ Cas9-based knock out

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Colorectal cancer is one of major lethal human malignancy, therefore, we have carried out comprehensive analysis of novel monoclonal antibody (mAb)-defined cell-surface molecules in various human colorectal cancer cell lines. In this context, we have recently found that expression of HER3 and MET proteins are closely correlated and co-stimulation of HER3 and MET by neuregulin-1 (NRG) and hepatocyte growth factor (HGF), a ligand of HER3 and MET, respectively, potentiated the growth of SW1116 human colorectal cancer cells, as compared with each treatment alone. Co-inhibition of HER3 by anti-HER3 mAb and that of MET by PHA665752 (MET inhibitor) caused a reduction of cell growth in vitro. CRISPR/Cas9-based knockout (KO) of HER3 in SW1116 cells, has result in the decrease of MET expression and abolishment of MET phosphorylation with or without stimulation by NRG. Furthermore, expression of HER4 proteins was remarkably downregulated in HER3-KO SW1116 cells. These findings suggest that HER3, MET, and possibly HER4 might form heteromeric molecular complexes and these oncogene products are concomitantly involved in the growth of colorectal cancers. Collaborators: Kazue Masuko, Kinji Yoshida

P-3039

Identification of pathways associated with tumor invasion in mouse model for colon cancer with Pten haploinsufficiency

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Background and Aim: We previously showed that Pten (phosphatase and tensin homologue) haploinsufficiency promoted tumor invasion in mouse colon epithelium with Apc (Adenomatous polyposis coli) deficiency. We aimed to identify pathways associated with advanced phenotype of colon tumors in the mouse model with Pten haploinsufficiency by using gene expression profiling analyses. Method and Result: Two mouse models, CDX2P9.5-NLSCre:$Apc^{flox/+}$;$Pten^{flox/+}$and CDX2P9.5-NLSCre:$Apc^{flox/+}$(;$Pten^{+/+}$), were generated and tumors were harvested at 12 weeks of age. Genome-wide gene expression profiling by microarray were performed to analyze alterations in gene expression in tumors between two mouse models (n=3/group). The microarray data were further investigated using Gene Set Enrichment Analysis (GSEA) for pathway identification. GSEA identified 8 up-regulated pathways (e.g. Oxidative phosphorylation, PPAR, lysosome and regulation of autophagy) and 28 down-regulated pathways (e.g. Nod-like receptor, apoptosis, MAPK and TGF- β) in tumors from Pten haploinsufficiency. Conclusion: Specific signaling pathway alterations were identified in tumor invasion phenotype induced by Pten haploinsufficiency.

▲TOP

[P-3045] P4-10 [English/Japanese]
Cancer related genes / metabolome

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Hiroshi Fukamachi / Dept. Mol. Oncol., Tokyo Med. Dent. Univ.

P-3045

Cytoplasmic maspin increases EMT-associated gene expression and promotes breast cancer cell invasion

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[Aim] Maspin is known as a tumor suppressor gene. However, we revealed that cytoplasmic maspin expression was positively correlated with a poor prognosis in breast cancer patients. Therefore, we investigate the function of cytoplasmic maspin in breast cancer. [Methods] Maspin subcellular localization were examined by immunofluorescence. Cell invasion was investigated using transwell invasion assay. Gene expression profiles were evaluated by cDNA microarray and qPCR. [Results] Expression of maspin in MDA-MB-231 mainly exhibited a cytoplasmic localization, whereas that in MCF10A exhibited a pancellular localization. Maspin overexpression promoted cell invasion in MDA-MB-231, while downregulation of maspin induced the opposite effect. Furthermore, to investigate how cytoplasmic maspin regulates cancer cell invasion, we focused on the EMT. In MDA-MB-231 overexpressing maspin, E-cadherin expression was decreased, while N-cadherin expression was increased. In addition, maspin overexpression increased EMT-related transcription factors, namely Slug, Twist, ZEB1, and ZEB2. [Conclusion] Cytoplasmic maspin may be contributes to regulate cancer cell invasion via a process consistent with EMT.

▲TOP

P-3046

PRDM14 directly interacts with heat shock proteins HSP90α and GRP78 in breast cancer cells

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【Aim】 PRDM14 is specifically expressed in embryonic stem cells and primordial germ cells. There is no expression of PRDM14 in the non-cancerous tissues, however PRDM14 is expressed in 50% of triple-negative breast cancer (TNBC), approximately. To develop compounds against TNBC, we focused on the exploration of the binding partners of PRDM14 in cancers.
【Results】 We selected candidates interacting to PRDM14 via pulldown assay followed by mass spectrometry in HCC1937 cells, one of TNBC cells. We confirmed the candidates by immunoprecipitation assay using two TNBC cell lines, and consequently, found the interactions PRDM14 with heat shock protein 90α (Hsp90α), and glucose-regulated protein 78 (GRP78). In surface plasmon resonance (SPR) analysis, both proteins directly interact with PRDM14, however, the interactions disappeared in PRDM14 C-terminus deletant lacked most of zinc finger domains. To screen inhibitors of the interactions as a next step, we constructed NanoLuc luciferase-based bioluminescence resonance energy transfer (NanoBRET) assay for these interactions in living cells.
【Conclusion】 PRDM14 directly binds to HSP90α and GRP78 in breast cancer cells.

P-3047

CBP/p300 acts as a tumor suppressor in epidermal keratinocytes in mice

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Germline loss-of-function mutations in CREBBP or EP300 increase the susceptibility of humans to tumors. Moreover, a cancer genome database shows that acetyltransferase-inactivating mutations of CBP and p300 are common in human cutaneous squamous cell carcinomas, suggesting that such mutations might be cancer drivers; however, it has been unknown how dysfunction of CBP/p300 contributes to tumorigenesis of epidermal keratinocytes.
Using epidermal keratinocyte-specific genetically modified mice, we examined the role of CBP/p300 in epidermal development and homeostasis. We found that a single copy of either Crebbp or Ep300 was necessary and sufficient for maintaining normal epidermal development in mice; however, reduced CBP/p300 expression strengthened the Ras-Erk signaling-induced hyperplastic phenotype of epidermal keratinocytes by upregulating ligand-induced EGFR signaling. Reduction of CBP/p300, in combination with increased Ras-Erk signaling, accelerated epidermal tumor formation. Our findings suggest that CBP/p300 acts as a tumor suppressor in epidermal keratinocytes by counteracting EGFR-Ras-Erk signaling.

P-3048

Targeting FoxM1 in ATL cells: application of FoxM1 inhibitor, thiostrepton

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FoxM1 is a pro-proliferating transcription factor targeting genes in cell-cycle progression, thus in tumor development and progression. Adult T cell leukemia (ATL) is an aggressive malignancy caused by human T cell leukemia virus type-I (HTLV-1) infection, without conclusive cures due to its mostly unclear course of leukemogenesis. We have been demonstrating evidences that overexpression of FoxM1 provides basis for proliferative/invasive phenotypes of ATL cells via overwhelmed transactivation of its target genes. Here, we propose a new therapeutic approach for ATL cells by targeting FoxM1 using its inhibitor, thiostrepton (TS). Downregulation of FoxM1 target genes by specific knockdown of FoxM1 was reproduced by TS treatments in HTLV-1 infected cell-lines. We found that TS effectively induced cell-death only in malignant, but not in uninfected healthy cells in CD4+ T cells from ATL patients. Finally, we confirmed significant suppression of tumor-burdens in ATL-xenograft mice when treated with TS. Taken together, targeting FoxM1 by TS is a promising approach to combat ATL cells, worth to make further efforts for development and optimization of ATL cell-specific TS-delivery system.

▲TOP

P-3049

Regulation of amino acid aminotransferase gene by hamartin

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The products of tuberous sclerosis complex (TSC) tumor suppressor genes, hamartn (TSC1) and tuberin (TSC2) form a complex and suppress mTORC1. Because of insufficient efficacy and adverse effects of of rapamycin-related drugs in the treatment of TSC, new options of therapeutic method are expected. To explore new therapeutic target pathways, we have identified amino acid, retinol, and other metabolism-related genes down-regulated by hamartin in Tsc1-deficient cells form animal model. Rapamycin suppressed their expressions suggesting mTORC1-dependent controls for those genes. Among those genes, we further examined the branched chain amino acid (BCAA) aminotransferase gene (Bcat1) because its related pathways were implicated in human cancers and epilepsy-related diseases. Interestingly, Bcat1 shRNAs or siRNAs promoted the growth of Tsc1-deficient cells. This suggests an unexpected role of Bcat1 to counteract tumorigenesis caused by Tsc1-deficiency. We are further exploring the function of Bcat1 and BCAA metabolism in TSC-related pathogenesis.

▲TOP

[P-3056] P9-2 [English]
Histone modification (1) [English]

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Satoshi Yamashita / Divison of Epigenomics, Natl. Cancer Ctr. Res. Insitute

P-3056

EZH2-based biopathological classifier stratifying oral cancer patients for outcome prediction and treatment selection

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Radiotherapy is an important post-operative modality for managing oral cancer patients, however, no reliable biomarkers are available for selecting potential responders to radiotherapy. We investigated the role of EZH2 on oral cancer patients treated with surgery and radiotherapy. We found that EZH2 level was escalated along the axis from dysplasia to invasive cancers, while decreased in cancer progression, disclosing two facets of EZH2 function in both oncogenic and tumor-suppressing roles. Positive correlation between EZH2 and H3K27me3 was also observed. Remarkably, higher EZH2 expression represented a biomarker of clinical radiosensitivity, in terms of loco-regional control, disease-free survival, and disease-specific survival. In multivariate analysis, we can stratify patients into low-, intermediate, and high-risk groups according to five independently adverse factors (low EZH2, close margin, nodal metastasis, poor differentiation, and advance stage) that are ifentified. Our study reveals EZH2 as a potential biomarker for predicting post-irradiation outcomes, and also emphasizes potentially adverse effect of EZH2 inhibitors.

▲TOP

P-3057

Cyproheptadine as a novel epigenetic modifier in the expression of NK cell receptor ligand, ULBP2 in bladder cancer

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Bladder cancer is one of the most common cancer in the world. Our previous study showed that cyproheptadine (CPH), an antihistamine and serotonin antagonist, inhibited tumor growth in human bladder cancer cells both in vitro and in and animal model. To determine the molecular mechanism of the anti-tumor activity, we performed RNA-Seq in BFTC905 bladder cancer cells treated with CPH. The results showed that UL16 Binding Protein 2 (ULBP2), a MHC class I-related molecule and ligand for NK cell receptor, was among the genes showing upregulation after treatment with CPH. ULBP2 re-expression could be confirmed by qRT-PCR in UMUC3 and BFTC905 bladder cancer cell lines treated with CPH as well as using HDAC inhibitor. Importantly, the re-expression was in concomitant to the enrichment of H3K27Ac and H3K4me3 in the promoter region of ULBP2 in CPH treated BFTC905 cells. In conclusion, CPH could be a novel epigenetic modifier in modifying the histone codes in re-expression of ULBP2 in bladder cancer cells. The role of CPH in restoring innate anti-tumor immune response in bladder cancer deserves further investigation.

P-3058

KDM4C Promotes Prostate Cancer Metastasis via Modulation of c-Myc and Metabolic Enzymes

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Prostate cancer (PCa) is the 5th most common cancer overall in the world. Recent studies suggest that metabolic reprograming in cancer is connected to changes at the epigenetic level. Our study focusing on investigating the role of histone lysine demethylases 4C (KDM4C) in PCa. The mRNA and protein expression of KDM4C is higher in human PCa tissues as compared to normal. Cox Survival Analysis revealed that PCa patients with higher KDM4C expression correlates to poor outcome. Knockdown of KDM4C by siRNA in PCa cells reduced cell proliferation, soft agar colony formation, migration, and invasion. Micro-Western Array (MWA) analysis indicated that reduction of KDM4C protein caused down-regulation of proteins involved in cell cycle regulators, c-Myc and epithelial-mesenchymal transition (EMT). Chromatin-IP experiment result suggested that KDM4C directly binds to c-Myc promoter region and regulates c-Myc genes expression. KDM4C regulates metabolic reprograming of cancer through regulation of c-Myc. Our finding suggested that knockdown of KDM4C shifts glycolytic metabolism to mitochondrial oxidation in prostate cancer cells, which may contribute to the suppression of cancer metastasis.

P-3059

Novel prognostic marker EHMT2 involves cell proliferation via HSPD1 regulation in breast cancer

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Molecular classifications of breast cancer, such as HER2, luminal A and luminal B, have been developed to reduce needless treatment by dividing breast cancer patients into low- and high-risk progression groups. However, these methods do not cover all the pathological features of breast cancer, and investigations into novel prognostic/therapeutic markers are thus constantly required. In this study, we identified overexpression of the histone methyltransferase EHMT2 in breast cancer samples, normal samples derived from the TCGA portal, and a breast cancer tissue microarray, and EHMT2 overexpression was clearly associated with poor prognosis in multiple breast cancer patient cohorts. Furthermore, knocking down EHMT2 expression affected cell apoptosis via the down-regulation and re-localization of HSPD1. Finally, a statistically significant positive correlation between EHMT2 and HSPD1 was revealed in the clinical cohorts. Thus, our results may assist the development of novel therapeutic strategies and provide a prognostic marker (EHMT2) for breast cancer patients.

▲TOP

P-3060

EHMT2 is a metastasis regulator in breast cancer

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Various modes of epigenetic regulation of breast cancer proliferation and metastasis have been investigated, but epigenetic mechanisms involved in breast cancer metastasis remain elusive. Thus, in this study, EHMT2 (a histone methyltransferase) was determined to be significantly overexpressed in breast cancer tissues and in Oncomine data. In addition, knockdown of EHMT2 reduced cell migration/invasion and regulated the expression of EMT-related markers (E-cadherin, Claudin 1, and Vimentin). Furthermore, treatment with BIX-01294, a specific inhibitor of EHMT2, affected migration/invasion in MDA-MB-231 cells. Therefore, our findings demonstrate functions of EHMT2 in breast cancer metastasis and suggest that targeting EHMT2 may be an effective therapeutic strategy for preventing breast cancer metastasis.

P-3061

Significance of histone methyltransferase SETDB1 expression in colon adenocarcinoma

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This study investigated the clinical implications of SETDB1 (also known as KMT1E) in human colon adenocarcinoma. Expression levels of SETDB1 proteins were analyzed by IHC, and TMA were used to examine expression profiles in human patients. Our results revealed that SETDB1 protein expression was significantly higher in various tumor tissues. Moreover, an analysis with SurvExpress software suggested that SETDB1 was highly expressed in colon adenocarcinoma and associated with poor prognosis. High SETDB1 expression was also found to be significantly correlated with histological grade, TNM stage, T class/primary tumor, and N class/regional lymph nodes; and Kaplan Meier survival curves indicated that SETDB1 protein expression was significantly associated with poor survival. Finally, univariate analysis demonstrated that SETDB1 protein expression was related to TNM stage and SETDB1 score, whereas multivariate analysis showed that the influence of SETDB1 on overall colon adenocarcinoma survival was independent from other risk factors. Taken together, our results suggest that the SETDB1 protein could serve as a clinical prognostic indicator for colon adenocarcinoma.

P-3062

The role of G9a in relation to colorectal cancer and SASP associated with cellular senescence

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Cellular senescence has been thought to arrest cell proliferation irreversibly when cell damage. Recently, it has been reported that senescent cells survive long-term, and it secrete inflammatory cytokines, which may conversely promote carcinogenesis. This phenomenon associated with cellular senescence is called senescence-associated secretory phenotype (SASP). The relationship between SASP and carcinogenesis is being studied (Nature, 2013). Epigenetics attracted attention, and involvement of G9a (histone methyltransferase) was reported (Mol Cell, 2012). G9a suppresses transcription of SASP factors such as IL-6 and IL-8 by dimethylation of H3K9. Open database analysis of correlation with G9a expression and prognosis of colorectal cancer (CRC) revealed that high G9a expression correlated with better survival ($p<0.05$). Immunohistochemical staining performed on 235 CRC specimens in our department showed the same result. Western blot showed higher G9a expression in microsatellite instability (MSI-H) CRC cell line than microsatellite stable (MSS). MSI-H CRC is known as with hereditary juvenile CRC. These findings suggest that G9a is involved in the biological properties of CRC.

▲TOP

[P-3069] P9-4 [Japanese]
DNA methylation (2)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3069

SWI/SNF defects induce CpG island methylator phenotype in gastric cancers

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The CpG island methylator phenotype (CIMP) is associated with prognosis and drug sensitivity in cancer patients. However, the mechanism of CIMP induction is still unclear. In this study, we aimed to clarify the molecular mechanism of CIMP induction. Among 50 gastric cancers (GCs), all of the CIMP-high GCs and 38% of the CIMP-moderate GCs had mutations of SWI/SNF components. Especially, *ARID1A* mutation was most strongly associated with the CIMP. To clarify whether or not CIMP is induced by SWI/SNF defect, methylation induction in knockout cells of SWI/SNF subunits was analyzed. In *ARID1A* knockout cells, methylation levels of 4,445 regions were increased. And in double knockout cells of *SMARCA2* and *SMARCA4*, that of 4,220 regions were increased. Mechanistically, *DNMT*s mRNA levels were up-regulated in double knockout cells of *SMARCA2* and *SMARCA4* (*DNMT1*：11.7-fold, *DNMT3A*：4.1-fold, *DNMT3B*：2.4-fold). These results showed that the CIMP in gastric cancers was induced by SWI/SNF defects.

▲TOP

P-3070

Excess androgen exposure induces aberrant DNA methylation in the prostate

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Aberrant DNA methylation is frequently observed in prostate cancer cells. However, it is unclear whether or not aberrant DNA methylation can be induced in apparently normal prostate tissues by androgen exposure. Here we show that DNA methylation levels in the genes Amn1 and Mmp23 were increased by testosterone treatment in a rat testosterone-inducible cancer model. Expression levels of DNA methyltransferases; Dnmt1, Dnmt3a, and Dnmt3b; and Tet (tet methylcytosine dioxygenase) genes, Tet1, Tet2, and Tet3, were not changed by treatment with high levels of testosterone. These observations suggest that excess androgen exposure can induce aberrant DNA methylation in the prostate by a mechanism other than increasing the expression of DNA methylating enzymes. Aberrant methylation may be due to enhanced enzyme activity or altered localization of DNMTs and Tets on chromatin.

P-3071

The Tumor Suppressor microRNA-34a Suppresses Organoids Derived from Human Cholangiocarcinoma

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Cholangiocarcinoma is one of the most aggressive malignancies, characterized by a poor prognosis. We have reported the successful establishment and long-term in vitro culture of organoids derived from human cholangiocarcinoma. In the present study, we investigated global DNA methylation levels in cholangiocarcinoma organoids and normal bile duct-derived organoids. We found that expression of the tumor suppressor microRNA-34a (miR-34a) was suppressed in cholangiocarcinoma organoids relative to normal bile duct-derived organoids via aberrant DNA methylation of its CpG island promoter. Enforced expression of miR-34a markedly suppressed the growth of cholangiocarcinoma organoids with down-regulation of its target oncogenes. These findings indicate that miR-34a is a potentially important therapeutic target against refractory cancers.

P-3072

OR21, a newly oral DNA methyltransferase inhibitor for MDS and AML

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DNA methyltransferase (DNMT) inhibitors have provided significant clinical benefits for patients with a high-risk myelodysplastic syndrome (MDS). OR21 is a novel oral decitabine prodrug with a potential oral absorbability. In this study, we investigated the efficacy of OR21 in MDS-L and AML cell lines. OR21 induces cell growth inhibition and cell apoptosis in a dose dependent manner. OR21 and decitabine strongly reduced DNMT protein level and LINE-1 methylation levels in MDS-L and HL60, using western blotting and pyrosequencing, respectively. In addition, increased CEBPE mRNA level and CD11b expression level were observed in MDS-L and HL60, indicating OR21 can induce cell differentiation. OR21 significantly prolonged survival (p=0.008) without inferiority to decitabine (p=0.08) in xenograft mouse model injected HL60 cells. Decreased LINE-1 methylation levels were observed in mouse bone marrow cells (median: vehicle, 85.7%; DAC, 57.8%; OR21, 71.1%). Above these findings, OR21 with a potential oral absorbability have a DNMT inhibition effect and an anti-tumor effect similar to decitabine.

▲TOP

P-3073

Clinical significance of promoter DNA methylation of HOPX gene in colorectal carcinogenesis

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Background; Homeobox only protein homeobox (HOPX) is a critical tumor suppressor gene, and stem cell marker. However its molecular mechanism is largely unknown in human carcinogenesis.Methods; we investigated the molecular HOPX status during colorectal carcinogenesis, and revealed its clinicopathological relevance. We then assessed functional relevance of HOPX in cancer cells through molecular mapping using the deletion mutants.Results; (1) HOPX promoter DNA methylation steadily increased from normal mucosa to adenoma and carcinoma ($p<0.0001$). HOPX protein reduced in adenoma/carcinoma with HOPX methylation. (2) HOPX transfection subsequently with flowcytometry elucidated they got much larger and more complex structures, and complete loss of cell division capacity. (3) Molecular mapping by deletion mutant critically identifies region essential for cell division. Conclusion; HOPX is proved to be involved in cell division without DNA suppression through the specific region which can bind with other proteins at least to inhibit Wnt pathway. This molecular function may be consistent with epigenetic change of HOPX during adenoma-carcinoma sequence and/or normal mucosal development.

P-3074

Characteristics of DNA methylome by viral infection status of hepatocellular carcinoma: a machine learning approach

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[Background/Aim]: Integrating DNA methylome information across multiple studies (acquired in Array express), we investigate its differences due to virus infection status in hepatocellular carcinoma.[Method]: Finding the characteristics of DNA methylome in each infected state through construction of mathematical model from about 27,000 probe information.[Results]: Five studies on hepatocellular carcinoma (HCC) was selected (HBV 62, HCV 60, and NBNC 188 cases). Many genes showed hypermethylation specific to HBV, and showed profiles that were significantly different from the other two infection states. Hypermethylation of the Wnt signaling pathway and genes related to apoptosis was remarkable as a common methylation abnormality in all the states.With the multinomial logistic regression model by LASSO, the discrimination between cancer and noncancer was extremely high diagnostic rate of 97.7%, and even seven patterns of combination of cancer / non-cancer and infection status were clearly separated with high accuracy of 90%. The goal is to approach NBNC's carcinogenesis mechanism through commonalities and differences with virus oncogenesis.

▲TOP

[P-3082] P9-6 [Japanese]
Histone modification (2)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3082

Histone methyltransferase SMYD2 might be a candidate therapeutic target in high-grade serous ovarian carcinoma (HGSC)

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Dysregulation of histone methylation could be involved in human carcinogenesis. It is reported that histone methyltransferases are overexpressed in various types of cancers. The aim of our research is to elucidate the dysregulation of histone methyltransferases in high-grade serous ovarian carcinoma (HGSC).We analyzed the expression of 9 genes by quantitative real-time PCR (q-PCR) with 35 HGSC clinical tissues. Six out of nine histone methyltransferase mRNA expression levels were significantly upregulated in HGSC clinical tissues ($P<0.01$), compared to 6 normal ovarian tissues. The role of histone lysine methyltransferase SMYD2 in HGSC has not been explored. We performed functional analysis of SMYD2 by SMYD2 siRNA knockdown and treatment with SMYD2 inhibitor (LLY-507) in HGSC cells. Suppression and inhibition of SMYD2 showed growth suppression by increasing apoptotic cells in MTT assays, FACS analysis, immunoblotting. Immunohistochemistry of HGSC clinical tissue specimens showed high expression of SMYD2. From the present findings, histone lysine methyltransferase SMYD2 might be a novel candidate therapeutic target for HGSC.

▲TOP

P-3083

Identification of a histone reader involved in cancer stem cell properties

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Reader proteins of histone modifications are required to translate the information encoded by histone marks into cellular phenotypes, including pluripotency and malignancy. We identified a potential histone reader, Cdyl2 (chromodomain protein Y-like2), and showed that it is important for normal differentiation of mouse embryonic stem cells [JCA, 2016]. This year, we explored potential involvement of human CDYL2 in cancer stem cells (CSCs). CDYL2 overexpression was observed in human breast (7/18) and colorectal cancer (6/18) cell lines. CDYL2 was higher in the CSC population, defined as ALDH positive population, than in the non-CSC population of breast cancer cell lines. Expression of exogenous CDYL2 increased the CSC population in two breast and three colorectal cancer cell lines, and upregulated genes involved in cancer invasion and cancer metastasis, including ALDH1A1 and ITGBL1. These indicated that CDYL2 is important for normal and cancer stem cells.

P-3084

Effect of inhibition of histone demethylase KDM6A on breast cancer development

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KDM6A is a histone demethylase that specifically targets di- and tri-methyl histone H3 lysine 27 (H3K27me2/3) and regulates transcriptional gene expression. In breast cancer cell lines, high expression of KDM6A enhances hormonally responsive breast cancer carcinogenesis (Xie et al. Oncogene, 2017), whereas, the loss of KDM6A expression induces epithelial mesenchymal transition (EMT) (Choi et al. EMBO Rep, 2015). H3K27me3 levels have been reported to be elevated in the cancer stem cell (Van Vlerken et al. Stem Cells Translational Medicine, 2013). However, the relationship between KDM6A and breast cancer progression is still largely unknown.To study the effect of Kdm6a loss on breast cancer, we generated mammary gland specific-deletion of Kdm6a in mouse mammary tumor virus-polyoma middle T antigen (MMTV-PyMT) mouse model, and found that Kdm6a deficiency accelerated tumor development and lung metastasis. The expression of KDM6A is low in basal-like breast cancer, which is more aggressive and drug-resistant cancer. Thus, our data suggest that Kdm6a deletion may promote human breast cancer progression, implicating clinical relevance of KDM6A in breast cancer treatment and/or diagnosis.

P-3085

Deregulation of the histone demethylase LSD1 is involved in hepatocellular carcinoma

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We previously reported that dysregulation of the histone demethylase LSD1 is involved in some types of cancer such as small cell lung carcinoma, but it is still unclear whether LSD1 affects hepatocellular carcinoma (HCC) patients' outcome. Therefore, we aimed to clarify the significance of LSD1 dysfunction in HCC using clinical samples and cell lines. The expression profiles of LSD1 examined by immunohistochemical analysis in 303 HCC clinical tissue samples showed that HCC patients with positive LSD1 expression had significantly lower both overall survival (OS) and disease-free survival (DFS) rates than patients with negative expression. Moreover, LSD1 expression was an independent prognostic factor for both OS and DFS in patients with HCC in multivariate analysis. Furthermore, knock-out of LSD1 by CRISPR/Cas9 system significantly suppressed the growth of HCC cell lines according to the results of colony formation assays and cell proliferation assays. In summary, we demonstrated that LSD1 overexpression promoted poor prognosis in HCC, and the inhibition of LSD1 resulted in the growth suppression of HCC cells. These results imply that LSD1 may be an ideal target to treat HCC.

▲TOP

P-3086

JARID1 family inhibitor reduces generation of drug resistant EGFR mutation-positive lung cancer cell lines

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H3K4 trimethylation (H3K4me3) is epigenetic marks which exist surrounding transcription start site. JARID1 family is demethylase of H3K4me2/me3. Especially, JARID1a and JARID1b have been suggested to have oncogenic properties and be associated with anti-cancer drug resistance. We previously showed that JARID1 family inhibitor (2-4(4-methylphenyl)-1,2-benzisothiazol-3(2H)-one; PBIT)) recovers sensitivity of drug- tolerant lung cancer cells. Recent report revealed that secretomes induced by targeted therapies paradoxically stimulate the outgrowth of drug tolerant subpopulation, which are driven by downregulation of transcription factor FRA1. Here we have examined whether PBIT affects the generation of drug resistant cells. We showed that PBIT reduced EGFR-TKI-induced secretomes by preventing decrease of active marker H3K4me3 at FRA1 promoter regions, which led to prevent downregulation of FRA1. Furthermore, we detected IGF-1 is one of the major secretomes in EGFR-TKI treatment and PBIT reduced generation of gefitinib resistant cells by inhibiting IGF-1-AKT pathway. These results indicate that JARID1 inhibitor may have potential to overcome anti-cancer drug resistance in lung cancer.

P-3087

Influence of incorporation of histone H3 variants on breast cancer cell

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Histone H3 variants act as transcriptional activators or repressors of cancer-related genes. However, the role of differential expression of the histone H3 variant genes in tumors remains unknown. The aim of this study was to clarify whether or not the expressions of histone H3 variants may be regulated by signal transduction activated in cancer cells as well as histone modification and cooperatively to form the gene profiles of breast cancer subtypes and their cancer cell phenotypes. Incorporation of histone H3 variant did not lead to complete change of the gene expression profiles which distinguished between hormone receptor positive and triple negative breast cancer subtypes, however, some important genes related to cancer cell phenotype were commonly up-regulated in both breast cancer subtypes. Promoter analysis revealed that HIST2H3C and H3F3A were upregulated by the same promoter sequence and HIST1H3A and H3F3B were not among breast cancer subtypes. The growth rate of cancer cells varied due to differences in histone variants that were highly expressed, and the expression of genes controlling cell proliferation was altered, which was supported by the results by ChIP-sequence.

▲TOP

[P-3092] P9-8 [Japanese]
Epigenetics and others

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yasuhito Nannya / Dept. Pathol. & Tumor Biol., Kyoto Univ.

P-3092

Analysis of epigenetic heterogeneity in triple-negative breast cancer

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Patients with triple-negative breast cancer (TNBC) have a higher rate of distant recurrence and a poorer prognosis than women with other breast cancer subtypes, since targeted therapies are not available for them. TNBC is a highly heterogeneous and the underlying biology is thought to be diverse. We have previously found that common core epigenetic programs in luminal tumors are defined by FOXA1 super-enhancers, however the basal-like (TNBC) phenotype is not defined by a specific epigenetic state and displays a high degree of heterogeneity (Cell Reports, 2015). To further elucidate the epigenetic heterogeneity in TNBC, we have performed ATAC-seq analysis on a panel of breast cancer cell lines. Comparison of open chromatin landscapes across different cell lines revealed that ER-negative cells showed more diverse epigenetic patterns than ER-positive. We also performed motif analysis of ATAC-seq data and identified several transcription factors that might be important for a subset of TNBCs. We identified a transcription factor X whose function is currently unknown, is upregulated in a subset of TNBCs and the expression level of the gene is associated with poor prognosis.

▲TOP

P-3093

Clinical significance of m6A reader YTHDF1 expression in colorectal cancer

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N6-methyladenosine (m6A) was first reported and is most common modification among various RNA modifications. The YT521-B homology (YTH) domain family are representative m6A-binding proteins, but how the YTH domain family is involved in cancer remains to be clearly understood. Clinical sequence data in colorectal cancer (CRC) indicate that overexpression of YTHDF1 is outstanding among other family members. We clarify the clinicopathological significance and the role of YTHDF1 in CRC. Immunostaining of Ythdf1 showed that its expression was associated with various malignant tumor behaviors, such as depth, lymph node metastasis, and poorer cancer stages. In vitro study showed that the knockdown of YTHDF1 resulted in the suppression of cancer proliferation and sensitization to the exposure of anticancer drugs such as fluorouracil and oxaliplatin. The study upstream of the YTHDF1 gene indicated that an oncogenic transcription factor c-Myc was associated with YTHDF1 in both expression and chromatin immunoprecipitation data. YTHDF1 plays an important role in CRC progression. YTHDF1 could be a useful prognostic indicator and a therapeutic target in CRC.

P-3094

Genistein regulates long non-coding RNA and epithelial-to-mesenchyme transition in renal cancer cells

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Mitsuho Imai-Sumida, Rajvir Dahiya and Soichiro Yamamura Renal cell carcinoma (RCC) is one of the most common malignancies. Despite the development of therapeutic regimens, the prognosis of patients with RCC is poorly understood. Genistein, a naturally occurring isoflavone, has been reported to have anti-cancer effects on a variety of cancer types. In malignancies, long non-coding RNAs (lncRNAs) are differentially expressed in various tissues. HOX transcript antisense RNA (HOTAIR) is necessary to target the polycomb repressive complex 2 (PRC2). HOTAIR is highly expressed in various cancers. In our study, we investigated the molecular mechanisms of genistein action through a novel pathway that represses HOTAIR. We found that HOTAIR expression is higher in renal cancer cell lines. Genistein treatment was found to significantly decrease HOTAIR expression in renal carcinoma cell lines. Genistein treatment also reduced expression of epithelial-to-mesenchyme transition (EMT)-related proteins. In this study, we revealed detailed mechanism of antitumor action of genistein. These insights indicate that genistein is a potent therapeutic agent for renal cancer.

P-3095

Tumorigenic role of non-coding RNA, TUG1 in pancreatic cancer

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Pancreatic cancer is a lethal disease with 5-year survival rate of less than 5%. Dysregulation of the long non-coding RNA (lncRNA) is known to drive important cancer phenotypes via interactions with other molecules including DNA, protein, and RNA. Here, we investigated the roles of lncRNA, TUG1 in pancreatic cancers. Pancreas cancer cell line, BxPC3, was subcutaneously inoculated into nude mice. After inoculation, antisense oligonucleotides targeting TUG1 coupled with drug delivery system (TUG1-DDS) was administered intravenously every three days for 30 days. TUG1-DDS significantly reduced tumor growth. Furthermore, we found that miRNA-X (miR-X) was profoundly increased upon inhibition of TUG1, together with downregulation of its candidate targets, mindbomb E3 ubiquitin protein ligase 1 (MIB1). MIB1 activates Notch signaling, which is known to play critical roles in tumorigenesis in different types of cancers including pancreas cancer. Our data indicate that oncogenic roles of TUG1 in pancreas cancer cell line and provide a new paradigm whereby targeting TUG1 might be an effective novel strategy for pancreas cancer treatment.

▲TOP

P-3096

Epitranscriptomic regulation of cell cycle in cancers

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BACKGROUND: Epigenetic modifications on genetic DNA and histone protein play critical roles to regulate gene expression. RNA also has more than 100 types of chemical modifications and recent studies have revealed that RNA modifications are crucial for biological and physiological processes. The purpose of this study is to examine the role of RNA modification in relation to cancers.METHOD: RNA modification related genes were knocked down in several types of cancer cells. The differences of gene expression and protein expression were examined between control and knock-down cell. Furthermore, the expression of RNA modifications in oral cancer tissues and normal oral tissues were measured by mass spectrometry. RESULT: RNA modifications tend to correlate with cell proliferation. The expression of RNA modifications tends to correlate with clinical T-stage in oral cancer patients.CONCLUSION: This study suggests that RNA modifications have critical roles in relation to cancer growth.

[P-3104] P11-6 [English/Japanese]
Cancer stem cell (2)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yukinari Kato / Dept. Antibody Drug Development, Tohoku Univ. Grad. Sch. Med.

P-3104

Analysis of gastric cancer stem cells and regulatory mechanism

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Atrophic gastritis by Helicobacter pylori infection is known as one of risk factors for the initiation of Gastric Cancer (GC), but the detailed molecular mechanism leads to the tumorigenesis is still largely unknown. Recently, it has been shown that the resistance for chemotherapy in cancer stem cells is cause of recurrence and drug-resistance.Our group focus on a Leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5) gene which is thought to be a cancer stem cell marker in various tumors. To evaluate the contribution of Lgr5 expression to the GC initiation, we crossed an inflammation-driven GC mouse model (Gan) with mouse for visualization of Lgr5 expression and depletion of Lgr5 positive cells by Diphtheria toxin (DT) treatment (Lgr5-DTR-EGFP). Histologically, 8 months old mice showed polyps in corpus of stomach. From the results of immunostaining, we found that some gastric mucosal epithelium cells in tumors were strongly GFP positive. Furthermore, when those Lgr5 positive cells were depleted by the DT treatment, polyps were significantly attenuated. These results indicate that Lgr5 expression could be important for the development of inflammation-driven GC.

▲TOP

P-3105

Involvement of CD44v+ cells in drug resistance in gastric cancer patient-derived cells and the underlying mechanism

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Intratumor heterogeneity of cancer cell populations may allow the minimal residual disease (MRD) to remain after chemotherapy. However, nature of the drug-tolerant persister cells, which constitute MRD and cause relapse, is elusive. Here, by using gastric cancer patient-derived cells (PDCs) under approval by JFCR Institutional Review Board, we identified ABCG2, a member of ABC transporter family, as a driver of drug resistance in the persister cells. Upon treatment with SN-38, an active metabolite of irinotecan, gastric cancer PDCs, such as SC15-3, gave persister cells, in which a cancer stem cell marker CD44 variant-positive (CD44v+) cells were enriched. Our cell sorting-coupled transcriptome analyses revealed that the CD44v+ cells express ABCG2, a resistant factor against multiple anticancer drugs, higher than the CD44v-negative cells. SN-38 increased the ratio of CD44v/ABCG2 double-positive cells in SC15-3. ABCG2 inhibitors enhanced the sensitivity of SC15-3 to SN-38. These observations suggest that ABCG2 mediates drug resistance in the CD44v+ persister cells and its inhibition may provide an opportunity to target MRD of gastric cancer. Collaborator: Naomi Kawata[1,5]

P-3106

The significance of autophagy of gastric cancer stem cells

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Background: Cancer stem cells (CSCs) have been thought to be responsible for tumor initiation, distant metastasis, and chemoresistance. Side population (SP) cells are thought to be enriched for CSCs. Autophagy is an intracellular degradation system that is induced under stress, such as starvation. The significance of autophagy in CSCs has still remained to be unknown. The aim of this study is to clarify the role of autophagy of CSCs.Materials and Methods: Two gastric cancer cell lines, OCUM-2MD3 and OCUM-12 were used. SP cell lines were sorted by FACS. The effects of starvation on the CSCs were examined. The expression level of autophagy associated proteins, LC3 was evaluated by RT-PCR and western blotting. The effects of autophagy on the proliferation were examined by MTT assay. Chloroquine was used as an autophagy inhibitor.Results: The expression level of LC3 was high in both SP cells, in comparison to that in parent cells. Starvation increased the autophagy status of SP cells. Chloroquine significantly decreased the proliferation activity of SP cells.Conclusion: CSCs might sustain stemness by the induction of autophagy. Chloroquine might suppress the proliferation of CSCs.

P-3107

CD168 expression marks a highly-concentrated human colorectal cancer stem cell population

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Cancer stem cells (CSCs) possess the capacity for self-renewal and the potential to differentiate into their progenies. Colorectal CSCs can be prospectively isolated based on specific surface marker such as CD44, CD133, LGR5, and CD166. However, the employment of these CSCs markers is still insufficient to prospectively isolate the colorectal CSCs. To overcome the cellular heterogeneity of previously reported CSCs fraction, we performed single cell transcriptome analysis of 72 cells within organoid. By conducting single cell transcriptome analysis, we identified stem-like cells expressing MKI67 and Cyclin families within these cells. Remarkably, high expression of HMMR, which encodes CD168, was observed in the stem-like cells. Prospective isolation of CD44+CD168+ cells revealed higher organoid- and tumor-forming abilities than those in CD44+CD168- cells. Furthermore, comprehensive gene expression analysis also demonstrated the stem-like gene expression within CD44+CD168+ cells. These results clearly show that CD168 is a useful surface marker to concentrate human colorectal CSCs within the conventional CD44+ population.

▲TOP

P-3108

Cancer stem cell marker CD133 attenuates colon cancer cell death induced by serum deprivation

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CD133 has been considered to be a representative molecular marker for cancer stem cell (CSC); however, its roles in tumor progression remain largely unclear. Our previous studies demonstrated that CD133 significantly promotes xenograft tumor growth of human colon cancer cells. It has been also shown that a resistance to various stresses is an additional theoretical property of CSC. We therefore sought to ask the functional significance of CD133 in colon cancer cell death caused by serum deprivation. For this purpose, we transduced shRNA or cDNA for CD133 into colon cancer cells and checked their viability exposed to a lower serum. Under our experimental conditions, CD133 depletion resulted in a decreased cell viability relative to non-depleted control cells. Consistent with these observations, forced expression of CD133 increased cell survival under the lower serum condition. Intriguingly, the expression level of CD133 was up-regulated in response to serum deprivation. Thus, it is highly likely that CD133 contributes to the acquisition/maintenance of the resistance to the stress arising from nutrient deficiency of avascular tumor tissues.

P-3109

Biological significance of full-length LGR5 in colorectal cancer

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[Background] We analyzed the biological significance of full length Lgr5, produced anti-LGR5 antibody originally, and reported its effect. [Objective] To clarify the physiological significance of full length LGR5 positive cells. [Methods and Results] mRNA was extracted from clinical specimens of human small intestine, colon and rectum, and the variant form of Lgr5 was analyzed by RT-PCR. Western blot confirmed that these variants are also expressed at the protein level. In situ hybridization demonstrated that full-length Lgr5 was expressed only in crypt base columnar cells of normal intestinal tract, whereas splice variant was also expressed in transit amplifying cells differentiated from intestinal stem cells. The response of the WNT signal by stimulation of R-spondin 1 was evaluated using a cell line overexpressing each isoform of Lgr5. As a result, a difference in WNT activity was observed. In sphere culture using colon cancer cell line, addition of anti-full length Lgr5 antibody increased both number and size of spheres. [Summary] It was suggested that full length Lgr5 could maintain dormancy in stem cells.

P-3110

Withdrawn

No Abstract

▲TOP

[P-3118] P11-8 [English/Japanese]
Cancer stem cell (4)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3118

Effect of Type I interferon in cancer stem cell maintenance and tumorigenesis

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Type I interferon (IFN-α/β : IFN) are a family of secreted signaling proteins that modulate resistance against viral and pathogenic infections, enhance immune responses. IFN also had alternative functions that activate dormant haematopoietic stem cells, and IFN-inducible protein plays critical microenvironmental factor for cancer cells. However, the precise role of IFN is still unclear. We found that, in several human breast cancer cell lines, IFN were produced during sphere formation and enhanced the number and size of sphere-forming cells. To determine the effect of IFN in cancer stem cells, we analysed mRNA expressions in breast cancer cells stimulated by IFN. Interleukin-6, Glut-3, Sox2 and Snail expression were upregulated in response to IFN. Next, we generated MCF-7, expressing mouse IFN receptor (IFNAR1) or IFNAR1 knockout by CRISPR-Cas9, and analyzed xenograft assay in nude mouse. Interestingly, in mouse transplanted with mouse IFNAR1 expressing cells, tumor growth was enhanced, whereas in mouse transplanted with IFNAR1 knockout cells, tumor growth was suppressed. Overall, IFN is involved in tumor growth and malignant progression through cancer stem cell maintenance.

▲TOP

P-3119

Cytotoxicity of hesperidin on MCF-7 breast cancer cell monolayer and mammosphere

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Cancer stem cells (CSCs), a small population of cells within tumors, contribute to drug resistance, tumor recurrence, and metastasis, and therefore, CSCs targeted therapy are needed for the management of this disease. Previously, hesperidin exhibited cytotoxicity on several cell lines (e.g. MCF-7, T47D, NALM-6, and HepG2) and increased the cytotoxicity of classical chemotherapy doxorubicin on doxorubicin-resistant MCF-7 cells. This study aimed to investigate the potential of hesperidin to target breast CSCs (BCSCs) in MCF-7 cells cultured under 2D and 3D models. Spheroid formation assay was used to enrich BCSCs. MTT and colony counting were used for measuring cytotoxicity and mammosphere forming potential, respectively. Flowcytometry and real-time PCR were used to determine the different expression levels of stem cell marker at protein and mRNA level. Our results showed that hesperidin exhibits cytotoxicity in both monolayer cells and mammosphere by cell cycle and reduction of stemness properties. Hesperidin is potential for eradicating cancer stem cells and overcoming chemoresistance

P-3120

Mesothelial cells facilitate cancer stem-like properties in spheroids of ovarian cancer cells

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Ovarian cancer is characterized by widespread peritoneal dissemination with ascites. Spheroids observed in the ascites of ovarian cancer patients are a mixture of cancer cells and mesothelial cells. In this study, we evaluated whether mesothelial cells facilitate tumor spheroid formation and give rise to cancer stem-like properties in ovarian cancer cells. Spheroids from the CAOV3 and A2780 ovarian cancer cell lines grew much larger in co-culture with mesothelial cells than in monoculture. The spheroids in co-culture displayed high Ki-67 expression in the peripheral zone and low expression in the central zone area. The expression of CD133 emerged in the inner portion of spheroids at later time-points, indicating that cancer cells expanded to the inner spheroid and acquired stem cell-properties. The mRNA levels of cancer stem cell markers significantly increased in co-cultured CAOV3 and mesothelial cells compared to CAOV3 cells alone. Furthermore, the mesothelial cells promoted the growth of the CAOV3 cells in a mouse xenograft model compared to cancer cells alone. In conclusion, mesothelial cells promoted spheroid formation and facilitated cancer stem-like properties.

P-3121

Exploration of useful markers to sort out endometrial cancer stem cells selectively

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Cancer stem cells (CSCs) possess the ability for self-renewal, differentiation and tumorigenesis, and play an important role in cancer recurrence and metastasis. It is well-known that CD44, CD133, and CXCR4 are widely expressed in CSCs and used as a surface marker of CSCs. However, these markers were not available marker as the separation of CSCs from endometrial cancer cells (ECCs). The present study aimed at sorting out CSCs, selectively, from ECCs using several markers. CSCs were sorted out by FCM and we tried to represent the colony formation assay. Cell sorting with two surface markers, such as CD44/CD133, CD44/CXCR4, and CD133/CXCR4, could not exclude contaminated non-CSCs. However, cell sorting with one of these surface markers significantly increased the number of colony formation as compared with negative control. It is a very important to find out another useful marker to collect CSCs efficiently and selectively from ECCs. We are going to search new molecules to sort out CSCs from ECCs, efficiently, in unconcern with Grade of ECCs. We hope that the selectively CSCs marker will lead to develop the anti-cancer drug.

▲TOP

P-3122

Basic and Translational Research in Carbon-Ion Radiobiology: Focused on Cancer Stem Cells

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Here we report our recent novel findings about the high radiocurability produced by carbon ion beams alone or in combination with DNA damaging drugs or with micro RNA 200c mimic focused on cancer stem cells (CSCs). The relative biological effectiveness values for the carbon ion beams relative to X-rays at the D10 levels for CSCs were 2.1-2.4. The colony and spheroid formation capability of CSCs was significantly inhibited by carbon ion beam combined with DNA damaging drugs (gemcitabine or cisplatin) or with micro RNA 200c mimic. Carbon ion beam combined with DNA damaging drugs significantly induced irreparable cluster DNA damage, apoptosis- and autophagy-related gene expression compared to carbon ion alone in several cancer cells. Xenograft tumors from pancreatic, breast cancer and mesothelioma cells were effectively disrupted by 25 Gy of carbon ion beam combined with gemcitabine for pancreatic xenograft tumors or 15 Gy of carbon ion beam combined with cisplatin for breast and mesothelioma xenograft tumors. In conclusion, carbon ion beam has superior potential to kill pancreatic, breast and mesothelioma CSCs when combined with DNA damaging drugs or with microRNA 200 mimic.

P-3123

CSCs in mouse skin carcinogenesis and inhibitory effects of EGCG on expression of stemness markers in human CSCs

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Last year, we published two new review articles entitled Human cancer stem cells are a target for cancer prevention using EGCG, and Cancer prevention with green tea and its principal constituent, EGCG: from early investigations to current focus on human cancer stem cells. In 1987, we first found that EGCG is a cancer preventive from experiments in two stage mouse skin carcinogenesis: Repeated topical applications of EGCG completely prevented tumor promotion of okadaic acid from 73.3% to 0. Since we assumed that EGCG inhibits growth of cancer initiated cells, we have studied development of stem cell theory in mouse skin carcinogenesis and the effects of EGCG on human CSCs, based on the results by other investigators. This paper deals with: 1) CSCs in mouse skin carcinogenesis by way of introduction, 2) expression of stemness markers of human CSCs compared with their parental cells, and 3) EGCG induced, decreases or increases in the expression of stemness related mRNAs and proteins, such as Oct, Nanog, and Sog2 in human CSCs. EGCG inhibits growth of human CSCs through the inhibition of self-renewal and expression of pluripotency maintaining transcription factors.

P-3124

Targeting RSPO3 Reduces Stem Cell Function in RSPO3-Fusion-positive Colon cancer and RSPO3 high Lung cancer

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Recent studies have revealed that the R-spondins (RSPOs) can mediate with Lgr4 and Lgr5 proteins/Frizzled/LRP receptor complexes as an independent (noncanonical) control of the Wnt pathway. Several lines of evidence supported that RSPOs play a positive role in the regulation of Wnt/beta-catenin signalling. We have identified an anti-RSPO3 antibody (DBPR117; hB1) using a rational design approach. DBPR117 was identified as an ideal candidate to be developed as a therapeutic antibody. DBPR117 was well characterized in a variety of assays including the binding assays, in vitro bioassays, in vivo PDX (patient-derived xenograft), lung cancer or colon cancer CDX (cell line-derived xenograft) models. DBPR117 is capable of binding specifically to the human RSPO3 with novel amino acid sequences in the complementary determining regions (CDRs). DBPR117 showed efficacy in human colon and lung cancers with RSPO3 fusion/overexpression. We are currently evaluating whether antagonizing RSPO3 by DBPR117 would synergize with anti-PD-L1 antibody to combat cancers using syngeneic murine models.

▲TOP

[P-3130] P11-10 [English/Japanese]
Cell culture (3)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Tohru Kiyono / Div. Carcinogenesis & Cancer Prevention, Natl Cancer Ctr. Res. Inst.

P-3130

Engineering of hydrogels for rapid induction of cancer stem cells

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To completely cure cancer patients, eradication of cancer stem cells (CSCs) is required. CSCs are resistant to chemo- and radiotherapies, and are a source of recurrence. However, detection of CSCs is extremely difficult. Here, we present the novel potential of a double-network (DN) hydrogel to rapidly generate CSCs. By placing six human cancer cells onto DN hydrogels, sphere formations were observed within 24 hours that expressed stemness markers including Sox2, Oct3/4, and Nanog. These DN hydrogel-induced CSCs were highly tumourigenic in SCID mice. The DN hydrogels rapidly modulated cellular gene expression and facilitated reprogramming of differentiated cancer cells towards CSCs. In this process, osteopontin played an essential role in the induction of cancer stemness. The DN hydrogels could reveal CSC-specific expression of the tyrosine kinase (TK) receptor, therefore suggesting possible eradication of CSCs by a TK inhibitor. Thus, DN hydrogels are a powerful tool for detecting CSCs when analysing the molecular mechanism of cancer cell reprogramming, and may contribute to discovery of therapeutic reagents to eradicate CSCs.

▲TOP

P-3131

Pancreatic cancer cells forming spheres differentiate in serum containing culture media

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Cancer stem cells (CSCs) contribute to the initiation, metastasis, and drug resistance of cancers. Sphere formation assay is used to identify CSCs. Previously, spheres of PANC-1 pancreatic cancer cells cultured with serum-free RPMI 1640 medium containing EGF and FGF-2 showed round to oval shapes and expressed high CSC markers. Low-vacuum scanning electron microscopy (LV-SEM) showed most sphere-forming PANC-1 cells had a smooth surface. In this study, we formed PANC-1 spheres in a serum containing RPMI 1640 medium. PANC-1 cells cultured in this media formed irregular oblong spheres. LV-SEM revealed the spheres to have a grape-like appearance, harboring cancer cells with smooth or rough surfaces. Transmission electron microscopy showed four types of cancer cells within the spheres: smooth cell surface, irregular large protrusions, protrusions and a small number of microvilli, and many microvilli throughout the cell surface. These spheres still expressed higher levels of CSC markers compared with PANC-1 cells cultured under adherent conditions. These findings among pancreatic cancer cells may indicate the differentiation process, from CSCs to non-CSCs, in the serum containing media.

P-3132

An efficient and low-cost method for propagating patient-derived colorectal cancer spheroids

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Recent advances allowed culturing and examination of patient-derived colorectal cancer (PD-CRC) cells as organoids or spheroids. To be applied to practical personalized medicine, however, current methods still need to be strengthened for higher efficiency. Here we report an improved method to propagate PD-CRC cells in spheroid culture. We established > 100 cancer spheroid lines derived from independent colorectal cancer patients employing a serum-containing medium with additional inhibitors, Y27632 and SB431542. Because colorectal cancer spheroids showed wide-range growth rates depending on the patient tumors, we searched for supplementary factors that accelerated proliferation of slow-growing CRC spheroids. We found that epidermal growth factor (EGF) and/or basic fibroblast growth factor (bFGF) were critical for steady propagation of a subset of CRC spheroids carrying the wild-type RAS and RAF genes. We also identified 5'-(N-ethyl-carboxamido)-adenosine (NECA), an adenosine receptor agonist, as an essential supplement for another subset of spheroids. Our method provides a versatile tool that can be applied to personalized chemotherapy evaluation in prospective clinical studies.

P-3133

Analysis of glioblastoma stemness-inducing master regulated molecules on double-network hydrogel

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Cancer tissue is composed of heterogeneous cells, in which cancer stem cells (CSCs) are the therapeutic target because of their drug- and radiotherapy-resistance. However, the detection of CSCs is difficult owing to the small numbers of them in cancer tissues. We developed the novel induction method for CSCs using a double-network (DN) hydrogel composed of poly-2-acrylamido-2-methylpropanesulfonic acid (PAMPS) and poly-N, N ´-dimethylacrylamide (PDMAAm). DN gel had a potential to induct CSCs within 24 hours from heterogeneous cancer cells via several signal transductions, however, the master regulated molecule related to cancer stemness was not clarified. To clarify the master regulated molecule, gene expression profiles by DNA microarray and cascade analysis, an integrated promoter-pathway analysis were performed in glioblastoma (GBM) cell line KMG4 cultured on DN hydrogel and polystyrene (PS) dish as normal culture condition. These analyses revealed that suggestive 105 candidate molecules involved in cancer stemness in KMG4 cells on DN hydrogel, and we finally focused on 5 molecules. These molecules might be the novel targets to abrogate GBM stem cells.

▲TOP

[P-3140] P11-12 [English/Japanese]
Cell-to-cell interaction (1)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3140

Production of monoclonal antibodies against three-dimensional culture cancer cells

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Multicellular tumor spheroids, one type of three-dimensional (3D) culture, are well-studied in vitro tumor tissue model, because the preservation of the 3D structure is important for cell-to-cell and cell-to-matrix interactions. 3D cell culture systems mimic tissue-like structures more effectively than traditional two-dimensional (2D) monolayer cell culture systems. In the present study, we performed Monoclonal antibody (MAb) shotgun approach. The membrane extracts from 3D culture DLD-1 cells were immunized as antigen and MAbs were generated using rat medial iliac lymph node method to produce a functional antibody. The hybridoma supernatants were screened by the observation of the morphological change of 2D or 3D culture DLD-1 cells after treating MAbs and the immunocytochemistry of 2D culture DLD-1 cells. We plan to examine the synergy with cytokines or anticancer drugs in 3D culture DLD-1 cells. Moreover, we will analyze the characteristic feature of MAbs and perform MS analysis to determine of the antigen recognized by MAbs.

▲TOP

P-3141

Analysis of the mechanism of kinase inhibitors resistance by pancreatic tumor-stromal cell interactions

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Pancreatic ductal adenocarcinoma (PDAC) is the most common pancreatic cancer and one of the most lethal malignancies. Current standard therapies are ineffective. Interactions between PDAC cells and stromal cells, including macrophages and fibroblasts, are important for tumor growth and drug resistance. We revealed that tumor growth is regulated by tumor-stromal cell interactions in prostate and stomach cancer, and identified novel compounds that suppress tumor growth in tumor cells and stromal cell cocultures. To examine the drug sensitivity in pancreatic tumor-stromal cell cocultures, we screened chemical libraries of the Platform of Advanced Animal Model Support. Screening revealed that the cocultured pancreatic tumor and stromal cells were more resistant to kinase inhibitors than monocultured pancreatic tumor cells. Pancreatic tumor cells incubated in conditioned medium from stromal cells also exhibited resistance to kinase inhibitors. We are currently searching for kinase inhibitor resistance targets in conditioned medium from pancreatic stromal cells.

P-3142

Fibroblasts disturb the expression of cancer-related genes in non-transformed human prostatic epithelial cell line BPH-1

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Deregulation of epithelial-stromal interactions is considered to play a critical role in the initiation and promotion of prostate cancer (PCa). Fibroblasts secret a number of growth factors, cytokines, ECM proteins, and miRNAs that stimulate cellular proliferation of prostatic epithelial cells. In this study, we investigated the effects of fibroblasts on non-transformed human prostatic epithelial cell line BPH-1 and human PCa cell line LNCaP focusing on the expression of cancer-related genes. As for tumor suppressor genes, mRNA expression of GSTP1 in BPH-1 cells was decreased by co-culturing with fibroblasts, whereas that in LNCaP cells was not detected. In contrast, mRNA expression of NKX3-1 in LNCaP cells was decreased by co-culturing with fibroblasts, whereas that in BPH-1 cells was not affected. As for oncogenes, mRNA expressions of NFKB1 and SRC in LNCaP cells was increased by co-culturing with fibroblasts, whereas that in BPH-1 cells was not affected. Our data showed that mRNA expressions of tumor suppressor gene in BPH-1 cells were highly disturbed by co-culturing with fibroblasts compared with those in LNCaP cells.

P-3143

Histone deacetylase mediates tumor-promoting phenotypes in breast carcinoma-associated fibroblasts via TGF-β signaling

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Carcinoma-associated fibroblasts (CAFs) stably acquire TGF-β autocrine signaling to maintain myofibroblstic traits and tumor-promoting ability during tumor progression. Since genetic alterations were rarely detected in primary cultured human CAFs, epigenetic alterations might mediate their tumor-promoting ability. However, such epigenetic alterations underlying the tumor-promoting trait remain poorly elucidated in CAFs. We show here significant enrichment of the histone deacetylase (HDAC)-regulated gene expression profile and the decreased histone H3 and H4 acetylation in CAFs relative to those in control human mammary fibroblasts. Of note, the increased TGF-β-regulated gene expression profile and myofibroblastic state were substantially inhibited in CAFs treated with trichostatin A (TSA), a broad HDAC inhibitor. Furthermore, inhibition of HDAC1 expression by the corresponding shRNA in CAFs, significantly suppressed their tumor-promoting traits in vivo compared to the effect of the control GFP-shRNA. Taken together, these findings reveal promising roles of epigenetic alterations modulating histone acetylation to mediate myofibroblstic traits and tumor-promoting ability in CAFs.

▲TOP

P-3144

Intratumoral bidirectional transitions between epithelial and mesenchymal cells in triple-negative breast cancer

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Epithelial-mesenchymal transition (EMT) and its reverse process, MET, are crucial in several stages of cancer metastasis. EMT allows cancer cells to move to proximal blood vessels for intravasation. However, because EMT and MET processes are dynamic, mesenchymal cancer cells are likely to undergo MET transiently and subsequently re-undergo EMT to restart the metastatic process. Therefore, spatiotemporally-coordinated mutual regulation between EMT and MET could occur during metastasis. To elucidate such regulation, we chose HCC38, a human TNBC cell line, because HCC38 is composed of epithelial and mesenchymal populations at a fixed ratio.
Using HCC38, we demonstrate that the efficiency of EMT is about an order of magnitude higher than that of MET and that the two populations enhance the transition of cells from the other population to their own. In addition, we found that several signaling pathways and EMT-related transcription factors are involved in efficiency of EMT and MET.
Consequently, we propose HCC38 as a suitable model to analyze EMT-MET dynamics that could affect development of triple-negative breast cancer.

P-3145

Macrophages in ascites from cancer patients are primed to transdifferentiate into fibroblasts

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Fibroblasts localized in tumor, called cancer-associated fibroblasts (CAFs), play an important role in cancer progression. The origin of CAF, however, remains unclear. Malignant tumor with ascites often develops peritoneal fibrosis. We here show that macrophages in malignant ascites are primed to transdifferentiate into CAF-like cells. Ascites were collected from 122 cases of gastrointestinal cancer patients. Macrophages within ascites were purified by FACS, and were cultured in vitro. In most cases, purified CD45+CD14+ macrophages within ascites transdifferentiated into CD45-CD90+ fibroblast-like cells that exhibited spindle shape after 2-3 week of culture. cDNA microarray analysis revealed that macrophage-derived CAFs (MDCAFs) had fibroblast-specific gene expression signature, and produced growth factors for epithelial proliferation. When DLD-1 cells xenotransplanted into immunodeficient mice together with MDCAFs, they formed significantly larger tumors as compared to DLD-1 cells alone. Thus, macrophages in ascites might be an important source for CAFs. Targeting the transdifferentiation machinery could be a therapeutic option for solid tumors associated with fibrosis.

P-3146

It takes two to tango: HLEC and 5-8F cells interaction in lymphatic metastasis of nasopharyngeal carcinoma

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Nasopharyngeal carcinoma (NPC) is a prevailing malignant tumor of head and neck in Southeast Asia. Lymph node metastases in the neck are presented in 85% of NPC cases, which are very important prognostic factors. However, the molecular mechanism of the metastatic process is poorly understood. In this study, we performed a co-coculture of NPC cell line 5-8F and HLEC. Through Live Cell Station, we found the migration capacity of 5-8F was significantly enhanced after co-culturing with HLEC. In xenograft model, 5-8F cells co-cultured with HLEC facilitated lymph node metastasis in nude mice. To further study its possible mechanism, we prepared condition medium (CM) using supernatant of 5-8F culture and HLEC culture, respectively. We found that HLEC growth was promoted when cultured in the CM from 5-8F, meanwhile, migration capacity of 5-8F was enhanced when cultivated in the CM from HLEC. Using RNA-seq, Protein chip for antibody screening, RT-PCR and WB, we found that TGF-β pathway was activated in 5-8F cells after co-cultured with HLEC, and mir-17-92 clusters, which are closely related to angiogenesis and lymphangiogenesisin, was upregulated in HLEC when cultured in the CM from 5-8F.

▲TOP

[P-3151] P11-14 [English/Japanese]
Glycosylation and glycosyltransferase

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3151

Expression mechanisms of cancer-related glycosyltransferase genes using signal transduction inhibitors

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GD3 is a glycosphingolipid containing sialic acid, highly expressed in human melanomas. There have been few studies on ganglioside expression and their function in human normal melanocytes. Analysis of gene expression of glycosyltransferase genes during evolution of melanomas from melanocytes is important to understand roles of ganglioside in melanomas. We reported that TNFα secreted from UVB-irradiated keratinocytes induced expression of ganglioside GD3 synthase gene, and recently found that elimination of cyclic AMP (cAMP) from the medium also resulted in GD3 synthase gene expression in melanocytes. Then, we added a PKA inhibitor (H89) to melanocytes. As a result, the expression level of GD3 synthase gene was significantly enhanced. On the other hand, an IKK inhibitor (Wedelolactone) treatment significantly reduced expression levels of GD3 synthase gene in melanocytes and melanoma cells. Consequently, it was suggested that signals via TNFα or cAMP oppositely regulate GD3 synthase gene expression in melanocytes. We are investigating differences in the regulatory mechanisms for GD3 synthase gene expression between melanocytes and melanoma cells by various signal inhibitors.

▲TOP

P-3152

Elevated O-GlcNAcylation stabilizes FOXM1 protein via suppression of its proteasomal degradation

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O-GlcNAcylation is a potent post-translational modification which adds an O-linked N-acetylglucosamine (O-GlcNAc), to cytonuclear proteins. The modification is involved in a wide range of cellular signaling in cancer progression. In many types of cancer cells, O-GlcNAcylation is elevated and contributes to the transformed phenotypes, but the molecular mechanisms is not fully understood. In this study, we examined O-GlcNAcylation-mediated cancer cell proliferation focusing on FOXM1, one of a key oncogenic transcription factor to regulate cell cycle. FOXM1 was not O-GlcNAcylated, while it was polyubiquitinated and the ubiquitination (Ub) was reduced by elevated O-GlcNAcylation. We found O-GlcNAcylation of two molecules involved in proteasomal degradation of FOXM1. One is GSK-3 β Ser/Thr protein kinase mediating the phosphorylation of FOXM1 to induce the polyUb. Elevated O-GlcNAcylation of GSK-3 β increased FOXM1 protein level. The other is an ubiquitin E3 ligase, FBXL2, mediating polyUb of FOXM1. Elevated O-GlcNAcylation reduced FBXL2 protein level via elevation of its polyUb. These data suggest that O-GlcNAcylation-mediated FOXM1 stabilization cloud promote cancer progression.

P-3153

Compositions and secretion mechanisms of extracellular vesicles from glyco-remodeling cancer cells

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Extracellular vesicles secreted from cancer cells are considered to play critical roles in the cancer microenvironments and regulation of tropism of cancer metastasis. We have reported roles of cancer-associated glycolipids in lipid rafts. In this study, we have analyzed compositions of extracellular vesicles derived from ganglioside GD3-positive (+) and GD3-negative (-) melanoma cells with focus on the membrane molecules such as integrins and growth factor receptors. Furthermore, we analyzed involvement of lipid rafts in the secretion of exosomes. Consequently, integrin alpha2, alpha5, beta1 and beta2 were markedly increased in exosomes from GD3+ cells compared with those from GD3- cells, while expression levels of them were almost equivalent in GD3+/- cell lines. EGF receptor 1 also showed increase in exosomes from GD3+ cells compared with GD3- cells. Then, we examined changes in exosomes after the treatment of cells by methyl-beta-cyclodextrin to investigate roles of lipid rafts in exosome secretion. Consequently, destruction of lipid rafts resulted in the reduction of an exosome marker, TSG101 in the exosomes, suggesting the involvement of lipid rafts in their secretion.

P-3154

change of anti-cancer effect by alteration of glycolipid compositions in ovarian carcinoma-derived cells

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The amounts of Gb3Cer, in paclitaxel-resistant KF28TX cells were higher than those in the original KF28 cell, and the ABC-transporter MDR1 gene, which was absent in KF28 cells, was characteristically expressed in KF28TX cells, suggesting that coexpression of MDR1 and glycolipids is involved in extrusion of hydrophobic paclitaxel in the raft of plasma membrane of KF28TX cells. In fact, cultivation of KF28TX cells with D-PDMP, an inhibitor of GlcCer synthase, for 48 hr resulted in the reduced amounts of Gb3Cer and the acquirement of sensitive property to paclitaxel, in comparison to those in KF28TX cells. Whereas, gangliosides, particularly GM3, were absent from cisplatin-resistant KFr13 cells, suggesting that loss of ganglioside-derived negative charge prevents the binding and incorporation of basic cisplatin in the plasma membrane of KFr13 cells. Actually, cultivation of KFr13 cell with GM3 for 48 hr resulted in the increased amounts of GM3 and the acquirement of sensitive property to cisplatin, in comparison to those in KFr13 cells. These findings indicate that glycolipids are involved in anticancer drug resistance in different ways to paclitaxel and cisplatin.

▲TOP

P-3155

C-mannosylation of R-spondin2 as a potential cancer biomarker

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R-spondin2 (Rspo2) is a secreted protein that has agonistic activity for Wnt/β-catenin signaling. Although human Rspo2 has two putative C-mannosylation sites, it has been unclear whether these sites are C-mannosylated or not. We first demonstrated that Rspo2 is C-mannosylated at W^{150} and W^{153} by mass spectrometry. Using wild-type (wt) and C-mannosylation-defective mutant Rspo2-overexpressing cell lines, we investigated the effect of C-mannosylation on Rspo2 functions. As a result, we showed that C-mannosylation of Rspo2 is required for its secretion and transport from endoplasmic reticulum to Golgi apparatus. Further, the agonistic activity of mutant Rspo2 for Wnt/β-catenin signaling is weakened than that of wt Rspo2. It has been reported that Rspo2 is involved in cancer metastasis. We finally conducted wound-healing assay and transwell migration assay to reveal whether C-mannosylated Rspo2 enhances cell migration. Indeed, the expression of wt Rspo2 significantly increased cell migration ability whereas that of mutant Rspo2 increased less. Collectively, we propose the C-mannosylation of Rspo2 might be a potential biomarker for cancer.

▲TOP

[P-3163] P12-8 [English/Japanese]
Innate immunity (2)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3163

IL-10 producing regulatory B cells are involved in immune evasion in gastric cancer patients

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Backgroud: The recent studies identified regulatory B cells (Bregs) in both human and mouse. However, the clinical significance of Bregs remains unclear in gastric cancer.**Methods**: The prevalence of Bregs was evaluated by multicolor flow cytometry. Immunohistochemical analysis with double staining method was also performed to evaluate cells showing both CD19 and IL-10 expressions in gastric cancer tissues.**Results**:1.Circulating Bregs were significantly more numerous in gastric cancer patients than in controls ($P = 0.0023$) and significantly more numerous before surgery than after surgery ($P < 0.0001$). 2.There were significantly more Bregs in $CD19^{+}CD24^{high}CD27^{+}$ population than in other population ($P < 0.0001$). 3.Bregs were significantly more in the tissue of gastric cancer than in peripheral blood ($P < 0.0001$). 4. The 5-year survival rate of patients with $Bregs^{high}$ ($\geq 12.5\%$) and $Bregs^{low}$ ($< 12.5\%$) were 34.6% and 67.7%, respectively ($P = 0.0013$). 5.Multivariate analysis indicated that the number of Bregs in the tissue of gastric cancer was an independent prognostic indicator. **Conclusions**: Bregs may be one of the key mechanisms responsible for immune evasion by tumors in gastric cancer.

▲TOP

P-3164

Study of the effect of indoleamine 2,3-dioxygenase on murine skin allograft rejection

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Background: Acute rejection of allografts is mediated by the coordinated infiltration and effector functions of alloantigen-specific T cells. Objective: The aim of this study is to investigate whether indoleamine 2,3-dioxygenase (IDO) might play an important role in the skin allograft rejection.Methods: Tail skin grafts from adult BALB/c, B6.C-H2bm1 and B6.C-H2bm12 mice were transplanted onto the left flank of adult recipient C57BL/6 mice. Skin graft rejection was defined as necrosis of more than 80% of skin graft. Engrafted mice were administered 10mg /mouse of 1-MT daily, or as a control, PBS by intraperitoneal injection.Results: The day of rejection was indistinguishable between PBS injected group and 1-MT injected group in the full MHC + minor H disparate model and the class II MHC disparate model. However, the mean day of rejection in the MHC class I disparate model was slightly but significantly decreased in the 1-MT injected group compared to the PBS injected group.Conclusion: These results suggested that only slight significant difference in the day of rejection and only in the MHC class I disparate model.

P-3165

Indoleamine 2,3-dioxygenase Activity During Letrozol Therapy for Elderly Breast Cancer Patient

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Background: Indoleamine 2,3-dioxygenase (IDO) is an enzyme that catabolizes tryptophan, which can result in the death of T lymphocytes.Objective: The aim of this study is to investigate the clinical significance of indoleamine 2,3-dioxygenase (IDO) during Letrozol therapy for elderly local advanced breast cancer patient.Methods: We took serum from elderly woman with local advanced breast cancer during Letrozol therapy. Then we measured Trp/Kyn ratio in these samples. IDO activity can be measured by Tryptophan (Trp) / Kynurenine (Kyn) ratio. Trp and Kyn were measured by HPLC. The correlations about Trp/Kyn ratio between intra-cystic papilloma group and the intra-cystic cancer group were studied.Results: The serum Trp/Kyn level of the patient with local advanced breast cancer was lower than the patient after endocrine therapy. IDO activity decreased after endocrine treatment. The IDO activity correlated with the number of metastatic lymph nodes lesions during Letrozol therapy.Conclusion: These results suggested that to measure Trp/Kyn ratio is useful to evaluate immunological metastatic status during endocrine therapy for elderly local advanced breast cancer patient.

P-3166

Cytokine Expression and Macrophage Localization in Xenograft and Allograft Tumor Models Stimulated with LPS

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Human cancer cells transplanted xenografts tumor model have been utilized to evaluated antitumor efficacy of drug treatment. T cell deficient mouse such as nude mice are often applied to generate tumor xenograft model for the development of anticancer agents. However, the functionality of the other immune cells including macrophages, dendritic cells (DCs), and myeloid derived suppressor cells (MDSCs) in nude mice are largely unknown. The better understanding of the immune system in xenograft tumor model will help drug development and discovery. The tumor microenvironment in xenograft model, comprising human donor cancer cells and mouse host cells, exhibits more complex bidirectional signaling and function than the tumor microenvironment of allograft tumor model. Here, we evaluated the differences of macrophages, DCs, and MDSCs between xenograft and allograft tumor model using nude mice transplanted with human or mouse colorectal cancer cells.

▲TOP

P-3167

Differences of tumor-recruiting myeloid cells and sensitivity to the TLR7 agonist between two murine SCC models

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Recruitment of $CD11b^+$ myeloid cells in tumor microenvironment (TME) greatly affects the outcomes of immunotherapy. We previously reported that a low-dose administration of resiquimod (RQ), a TLR7 agonist, efficiently reduced tumor growth with increased effector $CD8^+$ T cells and decreased $CD11b^+$ cells in TME using a SCCVII model. In this study, we compared the status of $CD11b^+$ cells and the efficacy of RQ treatment between two squamous cell carcinoma (SCCVII and NR-S1) models. Recruitment of $CD11b^+$ cells was correlated with tumor growth in both models. Based on the levels of Ly6G and Ly6C, $CD11b^+$ cells were divided into three fractions (G-MDSC, M-MDSC, and TAM). NR-S1 contained preferentially higher (60%>) $SCC^{lo}Ly6G^{hi}Ly6C^{med}$cells (G-MDSC), whereas SCCVII contained much higher (90%>) $SCC^{hi}Ly6G^{lo}Ly6C^{lo}$ cells (TAM). NR-S1-derived G-MDSC did not substantially express MHC class II, CD86, and Arginase 1. In contrast, SCCVII-derived TAM consisted of heterogenous cells with high and medium MHC class II, and some Arginase 1^+ cells, suggesting combined fractions with M1- and M2-like macrophages. We will further introduce differential effects of RQ treatment in both models.

▲TOP

[P-3173] P12-10 [English/Japanese]
Other immunotherapies (1)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Takashi Masuko / Cell Biol. Lab., Sch. Pharm ., Kindai Univ.

P-3173

Anti-tumor immunity via the superoxide-eosinophil axis induced by lipophilic component of Mycobacterium lipomannan

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Mycobacterium bovis Bacille Calmett Guerin (BCG) has been shown to possess potent antitumor activity particularly in various animal models, while, the cellular and molecular mechanisms underlying its activity are not well understood. We found that lipomannan (BCG LM), a lipophilic component of the mycobacterial cell envelope, specifically inhibits tumor growth and induces the infiltration of eosinophils at local tumor invasion sites. BCG LM enhances cytotoxic activity of eosinophils via the increased production of superoxide. Global transcriptomic analyses of BCG LM pulsed DCs identified CC motif ligand (CCL)5 as a crucial chemokine for the anti tumor immunity induced by BCG LM, indicating that CCL5 plays an important role for the accumulation of eosinophils in the tumor microenvironment. Furthermore, BCG LM and memory Th2 cells exerted a synergetic effect on tumor progression by cooperatively enhancing the eosinophil function. Thus, this study revealed un identified BCG LM mediated anti tumor mechanism via superoxide produced by infiltrated eosinophils in the tumor microenvironment.

▲TOP

P-3174

Identification of small molecule inhibitors of Foxp3

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Regulatory T cells (Tregs) suppress the anti-tumor immune response. Development and function of Treg are dominantly controlled by the transcription factor Foxp3. Inhibition of Foxp3 is the attractive approach for cancer immunotherapy. We have developed a novel cell-based assay system in which the NF-κB luciferase reporter signal is suppressed by the co-expressed Foxp3 protein. Using this assay, we screened approximately 10,000 compounds and discovered compound A by its ability to restore the Foxp3-inhibited NF-κB activity in a concentration-dependent manner without influencing cell viability. We examined the effect of compound A on the Treg activity in vitro by using splenocytes from BALB/c mice. Compound A decreased the proportion of Treg ($CD4^+$ Foxp 3^+) in $CD4^+$ cells and suppressed expression of the markers (GITR, CTLA-4) in Tregs. We next administered compound A to mice bearing CT26 colon carcinoma. Compound A (5 mg/kg iv qd x 5 days) decreased the proportion of tumor-infiltrating Tregs and inhibited the tumor growth. These data show the potential that functional inhibition of Foxp3 in Tregs by compound A will constitute a strategy for cancer immunotherapy.

P-3175

Anti-cancer drugs induce senescence in cancer cells and increase their sensitivity to CAR-T cells

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Chemotherapeutic drugs trigger DNA damage and induce apoptosis in cancer cells. However, survived cancer cells turn to be senescent and promote tumor recurrence with senescence-associated secretary phenotype (SASP). In this study, we determined whether senescence could be induced in human lung and breast cancer cells after chemotherapy treatment and tested the susceptibility of senescent cancer cells to anti-EGFR CAR-T cells. Two human lung cancer cell lines (PC9 and A549) and two human breast cancer cell lines (MDA-MB-231 and MCF-7) were used. These cell lines were examined for their sensitivity to pemetrexed (PEM) and doxorubicin (DXR), respectively. PEM suppressed proliferation of PC9 and A549 cells, and induced the expression of SA β-Gal, a marker of senescence. Similar results were observed when MDA-MB-231 and MCF-7 were treated with DXR. Importantly, these pretreated cancer cell lines increased their sensitivity to CAR-T cells. These results may provide a rationale of combination of chemotherapy and immunotherapy and suggest that chemotherapeutic drug-induced senescent cancer cells is a good target of T cell-based anti-cancer immunotherapy.

P-3176

Withdrawn

No Abstract

▲TOP

P-3177

DNA alkylating pyrrole-imidazole (PI) polyamide inhibits the expression of immunity checkpoint molecules

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Immune checkpoint blockade (ICB) is an attractive cancer therapeutic strategy by activating the patient's own immune system. However, ICB occasionally shows the immune-related adverse effects and is extremely expensive. Those may cause the immunotherapy discontinuation. Thus we synthesized a novel DNA alkylating PI polyamide (mPPC2-CBI) that designed to bind to the consensus sequences of mouse Pd-1, Pd-l1 and Ctla-4 to simultaneously suppress their expressions. The growth inhibition effect of mPPC2-CBI on B16F1 and LLC cancer cells was observed in WST assay. qPCR and western blot analysis demonstrated that mPPC2-CBI inhibited Pd-l1 expressions at mRNA and protein levels. Furthermore, mPPC2-CBI didn't show inhibitory effect of cell proliferation and suppression of Pd-1 and Ctla-4 expressions in normal mouse T cells. These date suggest that mPPC2-CBI suppresses the expression of immune check point genes and cancer cell proliferation without affecting T cell viability, representing the mPPC2-CBI is an appropriate test chemical to confirm alternative ICB strategy in immune competent mouse models.

P-3178

Structure-activity correlation analysis by using 1st-generation CARs with modified of hinge/transmembrane domain

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For ensuring the efficacy and safety of CAR-T cell therapy, it is important to understand the relationship between CAR structure and CAR-T cell function. In this study, we analyzed the functions of 1st-generation CAR variants whose hinge domain (HD) or/and transmembrane domain (TMD) were replaced to the components derived from CD3 ζ , CD4, CD8 α , or CD28. The expression intensity and stability of CAR on murine T cells were greatly affected by the modification of TMD rather than HD, and antigen-specific functions, such as proliferation, cytokine secretion, and cytotoxicity, of most CAR-T cells were dependent on their CAR expression levels. However, some kinds of CAR-T cells were found which showed significant differences in function despite equal expression levels of their CAR, suggesting that HD/TMD modification might influence not only the CAR expression but also signal transmission intensity with the antigen recognition. We are now investigating a possibility of the interaction or the complex formation of the CAR variants in themselves or with other molecules on the T-cell membrane.Non-member co-author: Keisuke Imaeda (Grad. Sch. Pharm. Sci., Osaka Univ.)

▲TOP

[P-3186] P12-12 [English/Japanese]
Antitumor effector cells and their induction (1)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Shin Kaneko / Ctr. for iPS Cell Res. & Application (CiRA), Kyoto Univ.

P-3186

The expression pattern of immune checkpoint molecules and the subset of T lymphocytes in locoregional esophageal cancer

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[Background] Previously we reported that tumor Infiltrating Lymphocyte (TIL) was a good indicator for the prognosis of esophageal cancer patients. [Purpose] To reveal about what type of lymphocyte subset is permeating into esophageal cancer lesion [Materials and Methods] Forty-seven esophagectomy cases were enrolled in this study. Immunohistochemical (IHC) was performed to investigate the expression of CD3,CD8,FOXP3,CD45RO,PDL1,HLA. Clustering analysis was performed following IHC staining pattern. [Results] Each T cell markers were expressed significantly stronger in perimeter of tumor. Each T cell markers' expressions was significantly associated with each other. Patients were divided into 5 groups by clustering analysis. The feature of the each group was as follows. Group 1 and 2: Accumulation of lymphocyte was significantly poor. Group 3: CD8/CD45RO expressing lymphocytes were significantly higher. Group 4 and 5: CD8/CD45RO/Foxp3 expressing lymphocytes were relatively higher. The expression of PD-L1 was significantly higher in group 4 and the expression HLA was not significantly different between the groups.

▲TOP

P-3187

The relationships of peripheral Tr1 and tumoral PDL-1 expressions reflect tumor immunity in pancreatic cancer patients

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Background. We have reported that the peripheral regulatory T cells (Tregs) reflected the aggressiveness of pancreatic neoplasms, however, the mechanism was still unclear. Recently we focused on the interaction among Foxp3+ Tregs, type 1 regulatory T cells (Tr1) and PDL-1 expression. Here we show that the strong correlations between Tr1 and PDL-1 for the tumor immunity. Methods. Peripheral blood was collected from 12 resectable pancreatic cancer patients and subjected to FACS analysis. Foxp3 and PDL-1 expressions in resected specimens were assessed immunohistochemically and compared with clinicopathological factors. Results. Combined pre-/post-operative ratios of Foxp3+Treg and Tr1 predicted early recurrence with 83.4% sensitivity, 91.3% specificity. OS and DFS were better in patients with high PDL-1 expression in resected specimens (P=0.01 and P＜0.05, respectively). The low expression of tumoral PDL-1 completely matched increased peripheral Tr1 population. Conclusions. These results indicate that patients selection can be made by peripheral Tr1 for the benefits of the immune-check point inhibitor though cross-talk of Tr1 and Foxp3+Tregs.

P-3188

Plasma cell-free DNA integrity analyses for ovarian and non-small cell lung cancer patients with peptide vaccination

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Finding useful biomarkers early in the cancer immunotherapies is very important because not all patients receive the benefits. As a promising candidate, we focused plasma cell-free DNA (cfDNA) and examined the DNA integrity, the ratio of the necrotic cell-derived longer cfDNA fragments to the total cfDNA. In this study, we used plasma samples collected before and after one cycle of peptide vaccination from 39 ovarian and 131 non-small cell lung cancer (NSCLC) patients enrolled in the phase II study. We assessed the DNA integrity by real-time PCR of Alu DNA repeats. We found, when the DNA integrity was higher at pre-vaccination, it tended to decrease more after vaccination in both cancer patients. In the ovarian cancer patients, the vaccinated peptide-specific IgG and CTL responses significantly related to the decreased DNA integrity after vaccination (p=0.0445 or 0.0283, respectively). In the NSCLC patients, the group with high DNA integrity at pre-vaccination showed the better overall survival than one with low DNA integrity (p=0.0087, 534 days vs 275 days MST). These results suggested the plasma DNA integrity as a useful biomarker in peptide vaccination.

P-3189

Improve Effector T-cells against Hepatocellular Carcinoma by Activated with Dendritic Cells Pulsed with Pools of Antigen

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T-cells activated with dendritic cells (DCs) pulsed with tumor associated antigens show the significantly benefit for HCC elimination. This strategy is worthy to develop for overcome the low survival rate and high recurrent rate in hepatocellular carcinoma (HCC) patients. In this study, therefore, we aim to elucidate the efficacy of DCs when loaded with total cell lysate or total RNA from three different HCC cells lines for their competency to activate and improve T-cells function. The result show that both total cell lysate and total RNA pulsed did not impel the phenotypic markers expression level of mature DCs. Total RNA pulsed DCs activated T-cells were remarkable superior than pulsed with total cell lysate which revealed with higher number of IFN γ producing CD4+ and CD8+ cells as well as with higher killing activity. Function of T-cells were significantly improved when activated with DCs pulsed with pools of antigens prepared from more than one cell lines. Two folds of improvement in killing activity of effector cells were observed in pooled antigen from three cell lines. In conclusion, our result indicated that pooled antigens are worthy for using in DC-based immunotherapy.

▲TOP

P-3190

Current status and future perspectives of immunotherapy for gastrointestinal cancer

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Although immune-checkpoint inhibitors (ICI) have revealed that patients already carry the neoantigens specific immunity (Neo-CTL), the response rate is still remain within 0-30% for gastrointestinal (GI) cancers. What we should do next are, detecting immunosuppressive biomarker and overcoming the immunosuppression by novel immune-modifiers, and enhance Neo-CTL. One matter is the suppressive tumor microenvironments including cytokines, immune cells, and immune checkpoint. Another matter is the low tumor mutation burden. We found that the exhaustion markers (PD-1, TIM-3) are the critical matters for the efficacy, and that the combination adjuvants of Poly-ICLC and LAG-3Ig activates cancer specific immunity. From a phase I study of novel immunotherapy composed by the adjuvants and novel tumor antigens against various GI cancers, we observed the CTL induction in 16 of 17cases, the reduction of tumor markers in 10 of 17. Moreover, to enhance Neo-CTL, we have constructed a unique binding prediction system to HLA. We can narrow down to 20 mutations as candidate neoantigens by the prediction system. These strategy is a promising alternative to previous immunotherapy against GI cancers.

P-3191

Evaluation of T cell response of immunological synapse-like superficial molecules aggregations after stimulation

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Background: T cells immune responses are initiated by communication with antigen presenting cells (APCs). It became elucidated that an immunological synapse (IS) is formed at the T cell-APC interface containing the T cell receptors (TCR) or CD28 microclusters (MCs). Based on this finding, we hypnotized that ex vivo T cells could be examined IS-like molecular aggregations. Method: To simplify IS formation of T cells, we used human monoclonal T cells by α CD3 and/or α CD28 antibody and replaced to evaluate the aggregation of MCs by detecting the CD3 or CD28 on the surface of T cells. This phenomenon was defined as IS-like formation(ISLF). Next, ISLF rates of T cells derived from PBMCs of healthy donors or esophago-gastric cancer patients were measured. Result: We have succeeded in observing that these molecules on a T cell aggregated in one area after stimulation as ISLF by imaging cytometry. Accordingly, we could measure ISLF rate of T cell clones and obtained T cells from PBMC in healthy donors or cancer patients. Conclusion: ISLF of T cells succeeded to observe after antibody stimulation. We established the method to quantify and evaluate ISLF degree by imaging cytometry.

▲TOP

[P-3199] P12-14 [English/Japanese]
Cancer vaccine (1)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Tsutomu Takeda / OCICC

P-3199

Immunomonitoring of rare cancer patients treated with WT1 Trio peptide-based cancer immunotherapy

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We have developed a WT1 peptide-based cancer immunotherapy and demonstrated its clinical potential. In the present study, patients with advanced rare cancer who met the eligibility criteria, which included having HLA-A*24:02 or 02:01 and WT1 positive tumors, were selected. WT1 Trio vaccine (containing two CTL peptides: WT1-126 and modified WT1-235, and HTL peptide: WT1-332, Montanide ISA51 adjuvanted) was administered seven times at an interval of 2 weeks. Two surrogate markers, the production of IgG antibodies and DTH skin test against WT1 peptides, were analyzed and compared to patients treated with WT1-235 CTL peptide vaccine alone (12 doses administered weekly). Of the 25 patients recruited in the study, 15 patients completed the protocol treatment. Increase in serum levels of WT1-235, WT1-126, and WT1-332 IgG Ab at 3 months in the 15 patients treated with WT1 Trio were all significantly higher than that in patients treated with WT1-235 vaccine. WT1-235 DTH positive rate at three month of WT1 Trio vaccine was higher than that observed in WT1-235 vaccine administered patients. Compared to WT1-235 vaccine, WT1 Trio vaccine may induce a more robust WT1 specific immune response.

▲TOP

P-3200

Predictive biomarkers for the efficacy of vaccine treatment against advanced pancreatic cancer

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The purpose of the present study was to explore predictive biomarkers for the efficacy of peptide vaccine treatment. From a phase Ⅱ trial using three human leukocyte antigen (HLA)-A *2402-restricted peptides for advanced pancreatic cancer (PC), we obtained peripheral blood samples from 36 patients of an HLA-A*2402-matched group and 27 patients of an HLA-A*2402-unmatched group. Multivariate analysis (p=0.0231) and log-rank test (p=0.0036) showed that a high expression level of programmed death-1 (PD-1) on CD4+ T cells was a negative predictive biomarker of overall survival in the HLA-A*2402-matched group. After treatment, we found that the upregulation of PD-1 and T cell immunoglobulin mucin-3 (Tim-3) expression on CD4+ and CD8+ T cells was significantly associated with a poor clinical outcome in the HLA-A*2402-matched group (p=0.0330, 0.0282, 0.0046, and 0.0068, respectively). Our results indicate that the upregulation of PD-1 and Tim-3 expression on CD4+ and CD8+ T cells may restrict T cell responses in advanced PC patients; therefore, combination immunotherapy with blockade of PD-1 and Tim-3 may be a potential therapeutic approach.

P-3201

The presence of Proline preceding HLA class I epitope sequences inhibits antigen presentation

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CTL recognize peptide-HLA class I complexes presented on target cell surfaces; however, the precise mechanisms of antigen processing by which T-cell epitopes are generated still remains unclear. Here, by means of HLA-class I ligandome analysis using LC-MS/MS, we collected a vast number of peptide sequences that were naturally presented by a cancer cells. The analysis revealed the frequency of each amino acid at given positions, ranging from -15 to -1 AA outside of HLA ligands. We found that the frequency of Proline residue (Pro) constantly declined while the other amino acids did not, implying that the peptide sequences following Pro were not efficiently presented by HLA class I. To further investigate this hypothesis, we prepared 293T cells expressing the constructs of a known CTL 9-mer epitope coupled with a series of N-terminal extensions containing Pro, and ultimately found that the presence of Pro at -3 to -1 positions prior to the epitope significantly decreased CTL responses to the cells. Thus, we consider that the presence of preceding Proline outside of epitope sequences hinders efficient HLA-class I antigen presentation, thereby influencing following CTL responses.

P-3202

cancer peptide vaccine therapy focused on dendritic cell subset

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Tumor-derived peptides can induce anti-tumor cytotoxic T lymphocyte(CTL) responses but the effects are limited. Dendritic cells (DCs) consist of various subsets with subset-specific functions. A chemokine receptor, XCR1, is selectively expressed on a DC subset with high ability to induce CTL. We thought selective targeting of a cancer Ag peptide to the DC subset should facilitate the peptide to provoke anti-cancer immunity. To achieve this, we have designed a fusion protein, mXCL1-OT-I, consisting of an OVA-derived peptide presented with MHC Class I, and an XCR1 ligand, XCL1. In mice injected with mXCL1-OT-I fusion protein plus poly(I:C) which is used as immune adjuvant, CTL responses were more potently induced than OT-I peptide plus poly(I:C). And mXCL1-OT-I plus poly (I:C) inhibited the growth of a OVA-expressing melanoma more efficiently than OT-I peptide plus poly(I:C). Furthermore, administration of mXCL1-OT-I plus poly(I:C) with anti-PD-1 Ab could suppress tumor growth longer. Thus, we concluded chemokine receptor directed antigen delivering to a certain DC subset could provoke an effective anti-tumor immunity and bring about synergistic effects with anti-PD-1 Ab.

▲TOP

P-3203

A practical strategy to pancreatic cancer immunotherapy using resected tumor lysate vaccines expressing α-gal epitopes

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α-gal epitope is a major carbohydrate antigen, expressed by non-primate mammals. One useful application of α-gal epitope is the enhancement of the immunogenicity of tumor-associated antigens (TAA) that promote effective uptake by antigen-presenting cells. This study presents a novel immunotherapy for pancreatic cancer (PC) expressing α-gal epitopes using tumor lysates obtained from PC patients. Resected tumors were processed to enzymatically synthesize α-gal epitopes on the carbohydrate chains of membrane glycoproteins. Processed membranes were analyzed for the expression of α-gal epitopes, and vaccine efficacy was assessed in vitro and in vivo. Effective synthesis of α-gal epitopes was demonstrated and tumor lysates readily bound an anti-Gal monoclonal antibody. These tumor lysate vaccines elicited strong antibody production against multiple TAAs and activated multiple tumor-specific T cells. The lysate vaccines stimulated a robust immune response in animal models, resulting in tumor suppression and a significant improvement in survival. We conclude that these tumor lysate vaccination may be a practical and effective new immunotherapeutic approach for treating PC.

P-3204

Antigen specific antitumor effect induced by antigen-electroporated, NKT cell ligand-loaded dendritic cells

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Background: With transfection of antigen coding mRNA, α-GalCer loaded CD1d expressing cells act as a vector to enhance the antigen specific immune responses. Electroporation (EP) has been reported as a way to induce substances easily into cells. In this study, we aimed to show a feasibility of EP as a method to deliver the antigen into α-GalCer loaded DCs which induce antigen specific antitumor responses. Methods: We compared the antitumor responses of α-GalCer loaded DCs with or without electroporated OVA in murine subcutaneous tumor model using EG7, which is OVA induced EL4, thymoma. Results: With electroporated OVA, tumors didn't grow in mice administered α-GalCer loaded DCs whereas tumors in those without electroporated OVA aggressively progressed. Survival rate was significantly better for mice administered α-GalCer loaded DCs with electroporated OVA. Conclusion: α-GalCer loaded DCs with electroporated OVA showed significantly higher rate of tumor rejection and longer survival in the mice model inoculated OVA-induced tumor. EP was feasible as a way to deliver the antigen into the vector, which induced antigen specific antitumor responses.

▲TOP

[P-3211] P13-1 [English/Japanese]
Factors regulation growth and differentiation

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Hugh Colvin / Kagawa Prefectural Central Hospital

P-3211

HGF accelerate RANKL expression in bone marrow stromal cells and osteoblasts

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<Purpose> Osteolytic lesions are rapidly progressive during the terminal stages of myeloma. In relation to the etiology of this bone destruction, it has been reported recently that HGF, produced in large amounts in myeloma patients, although the details of this process remain obscure. In the present study, we examined whether HGF induces RANKL expression in bone marrow stromal cells and osteoblasts, and investigated the mechanism by which osteoclasts are activated by HGF .<Methods> Expression of RANKL was examined by RT-PCR and western blotting. <Results> RANKL expression increased in both ST2 cells (bone marrow stromal cells) and MC3T3-E1 cells (osteoblasts) by the addition of HGF. Treatment with HGF promotes the NF-κB activation. Moreover, NF-κB inhibitor inhibited the RANKL expression in ST2 cells and MC3T3-E1 cells. <Discussion> These findings of this investigation suggested that activation of the NF-κB pathways were involved in RANKL expression induced by HGF in bone-marrow stromal cells and osteoblasts. This finding may be useful in the development of an osteoclastic inhibitor that targets intracellular signaling factors.

▲TOP

P-3212

Clathrin adaptor complex-dependent sorting of EGFR at endosomes

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Mechanisms for EGFR signaling and its downregulation by endocytosis have been extensively documented. However, we have only poorly understood how cells maintain steady-state protein expression of EGFR. Previously, we have demonstrated that one of clathrin adaptors, Golgi-localized, γ-adaptin ear-containing, ARF-binding protein 2 (GGA2), functions for the stabilization of EGFR presumably by enhancing its recycling from endosomes. Here, we report an involvement another type of clathrin adaptor, AP-1, in the EGFR trafficking. In RNAi experiments, AP-1-depletion greatly reduced the steady-state expression levels of EGFR. The reduction was due to enhanced lysosomal degradation of EGFR. Interestingly, TGN/endosome-localization of AP-1 was not dependent on the expression GGA2. A proximity ligation assay suggested that interaction between EGFR and AP-1 occurs at endosomes. Moreover, AP-1-depleted cells showed reduced EGF signaling and thus lower proliferation rates. Together, these results indicate that AP-1 acts to support cell growth by inhibiting EGFR degradation, which could be an effective target of drug discovery for cancer.

P-3213

The role of beta-adrenoceptor and catecholamine stimuli in renal cell carcinoma

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【Objective】Several reports suggested that activation of beta-adrenoceptor (ADRB) stimulates the secretion of proinflammatory cytokines in several cancers but not in renal cell carcinoma (RCC). We examined the expression of ADRB and the act of catecholamine stimuli in RCC.
【Methods】We examined expression of β1-adrenoceptor (ADRB1), β2-adrenoceptor (ADRB2), and β3-adrenoceptor (ADRB3) in human RCC cell lines by RTqPCR and Western blotting methods. To evaluate the role of CA, we added Isoproterenol (ISO), Noradrenaline (NA) and Adrenaline (AD) to RCC cells (A704 and 786O). In addition, we measured the quantity of Interleukin (IL)-6 in the supernatant of A704 after adding ISO, NA, and AD.
【Results】ADRB1 and ADRB2 were expressed in all cell lines except for ADRB1 in A704. A tendency of proliferation was observed in A704 and 786O when ISO was added. ISO and NA stimuli increased IL-6 secretion in A704.
【Conclusion】CA may promote secretion of IL-6 via adrenoceptors in RCC.

P-3214

Negative feedback regulation of ErbB4 by non-canonical phosphorylation at threonine-674 and serine-1026

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ErbB4 is a member of ErbB family of receptor tyrosine kinases, which has four different isoforms that classified by variants at extracellular juxtamembrane domain (JM-a and JM-b) and an intracellular domain (CYT-1 and CYT-2). We used ErbB4 JM-b CYT-1 to investigate the roles of Ser and Thr residues in MEK-ERK pathway-dependent feedback control of ErbB4 activation. TPA (an activator of ERK pathway) exhibited strong induction of phosphorylation of Thr-674 and downregulation of tyrosine phosphorylation in HEK 293 cells. Substitution of Thr-674 in the juxtamembrane domain to Ala and Ser-1026 in the C-terminal domain to Ala impaired the feedback inhibition of ErbB4. The double mutant TSAA (Thr-674/Ser-1026 to Ala) significantly upregulated tyrosine phosphorylation than each single mutant. This result suggests that phosphorylation of Thr-674 and Ser-1026 are crucial for negative feedback regulation of ErbB4. Given the fact that ErbB4 mutation is the most commonly altered protein tyrosine kinase gene in melanoma cells, now is being done study of TSAA combined with oncogenic mutation in melanoma to discover the potential contribution of the feedback phosphorylation in cancer progression.

▲TOP

P-3215

Broad-complex, Tramtrack and Bric-abrac (BTB) proteins are related to tumor invasion, metastasis in colorectal cancer

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Background. Colorectal cancer (CRC) still cause the cancer-related deaths of CRC patients despite intensive treatments. Recently, the ubiquitination has been revealed that it plays a crucial role in managing cancer progress. We have discovered Broad-complex, Tramtrack and Bric-abrac (BTB) protein X which is an adaptor protein of RING E3 ubiquitin ligase complex as a kind of CRC modulator. Therefore, we evaluated the effect of BTB protein X. Subjects and Methods. 1.The expression of BTB X in colorectal cancer cell lines was evaluated. 2. We assessed the effect of BTB X for proliferation. 3. The expression of BTB protein X in CRC was analyzed by performing immunostaining of clinical specimens. Results. 1. The expression of BTB X was detected in All CRC cell lines. 2. We discovered that the knocking down of BTB X suppressed the tumor proliferation and invasion. 3. The expression of BTB protein X was getting higher in invasive lesion and related to CRC progression. Conclusion. This study showed BTB protein X plays crucial roles in the proliferation and invasion of CRC. Further studies that clarify the mechanisms how BTB protein X is involved in CRC progression is needed.

P-3216

PDGFRA signal is a potential therapeutic target in neuroblastoma

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Neuroblastoma (NB) is the most common extracranial malignancy in pediatric cancers. Although previous studies have uncovered several genomic alterations critical for the tumor onset and progression in high-risk NBs (MYCN amplification, TERT rearrangement, and ATRX inactivation), further genomic aberrations remain to be identified.Our target DNA sequence captured a nonsynonymous mutation of PDGFRA, which encodes a receptor tyrosine kinase, in high-risk NBs. To validate the biological effects of PDGFRA wild-type (WT) and the mutant, we exogenously expressed either of them in NB cell lines. Intriguingly, the mutant PDGFRA enhanced phosphorylation of the receptor and downstream molecules, and then accelerated NB cell growth. Exogenous PDGFRA WT also increased intracellular signal and cell proliferation albeit marginal effects compared to the mutant. Inversely, the knockdown of endogenous PDGFRA suppressed NB cell proliferation. In several cohorts studies PDGFRA was highly expressed in high-risk and MYCN-amplified groups of NB.Therefore, we conclude that the nonsynonymous mutation and high expression of PDGFRA are to be a potential therapeutic target in a part of high-risk NBs.

P-3217

Ligand-independent EGFR activity reduces anti-cancer effect of cetuximab via ErbB3 signaling in non-stem cancer cells

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We previously showed that the growth inhibitory ability of cetuximab was markedly lower than that of EGFR TKI in non-stem oral squamous cell carcinoma (NSOSCC) cells. When treated with EGFR TKI, not only phosphorylation of EGFR but also phosphorylation of ErbB3 almost disappeared, and at the same time AKT phosphorylation and cyclinD1 level decreased. In contrast, treatment with cetuximab reduced EGFR and AKT phosphorylation levels, but had no effect on ErbB3 phosphorylation. In addition, EGFR phosphorylation level increased, but ErbB3 phosphorylation level remained unchanged by addition of EGFR ligands. Furthermore, NSOSCC did not grow and phosphorylation levels of EGFR, ErbB3, and FAK decreased under suspension culture conditions. Phosphorylation of EGFR/ErbB3 was inhibited by Src inhibitor instead of FAK inhibitor under adherent culture. In addition, the Src inhibitor exerted an additive effect on the suppression of NSOSCC proliferation by cetuximab. These results suggested that combination with an inhibitor targeting cell-substratum adhesion dependent EGFR-ErbB3 pathway may be an important key for enhancing cetuximab's anticancer effect.

▲TOP

[P-3225] P13-3 [English/Japanese]
TGF-β / Smad, others

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3225

Cell cycle arrest in oral squamous carcinoma cells undergoing TGF-β-induced migration

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Transforming growth factor-β (TGF-β) has been implicated in progression of multiple types of epithelial cancer, While TGF-β elicits multiple effects on epithelial cancer cells, it remains to be elucidated whether there is a link between increased migration and cell cycle arrest induced by TGF-β. Here, we utilized SAS oral squamous cell carcinoma (OSCC) cells carrying Fluorescent ubiquitination-based cell cycle indicator (Fucci) system in order to study this correlation at a single cell level. We found that TGF-β induced cell cycle arrest and increased motility in SAS-Fucci cells. Interestingly, the SAS-Fucci cells residing in G1 phase were more motile than cells residing in S/G2/M phase suggesting a correlation between TGF-β-dependent cell cycle progression and migration. These results were confirmed by HSC4 OSCC cells carrying Fucci system. Furthermore, cDNA microarray analyses revealed that this elevated migration is not caused by TGF-β-induced EMT. These findings suggest that OSCC cells under cell cycle arrest are prone to migrate, and can be a novel target of cancer metastasis.

▲TOP

P-3226

RUNX3 expression mediates TGF-β and SDF-1 autocrine signaling in human breast CAF myofibroblasts

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Runt-related transcription factor 3 (RUNX3) is known as a tumor suppressor in various human cancers. However, the roles of RUNX3 in tumor-associated stroma have not yet been fully elucidated. We show here that RUNX3 expression is progressively upregulated in human breast CAFs compared to their control fibroblasts during the course of tumor progression. Of note, inhibition of either TGF-β-Smad2/3 or SDF-1-CXCR4 signaling by the cognate shRNA significantly suppressed RUNX3 mRNA expression in CAFs. Conversely, RUNX3 mRNA was boosted in human normal mammary fibroblasts, in which each of constructs encoding a constitutively active form of TGF-β;1, SDF-1 and CXCR4 cDNA had been introduced. Furthermore, inhibition of RUNX3 expression attenuated TGF-β and SDF-1 autocrine signaling and the myofibroblastic state in CAFs. Taken together, these findings indicate that RUNX3 expression is induced by TGF-β and SDF-1 signaling during tumor progression and its expression in turn requires such autocrine signaling to be maintained in CAF myofibroblasts.

P-3227

Inhibitory action in intestinal tumor of TMEPAI knockout mice

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TMEPAI (Transmembrane prostate androgen-induced RNA) is a negative regulator of TGF-β signaling due to its competition of AR-Smad (activing/TGF-β receptor-regulated Smad) binding for SARA (Smad anchor for receptor activation). Thus, AR-Smads cannot be phosphorylated by the TGF-β type I receptor. However, it is not well understood how TMEPAI is involved in tumorigenicity in vivo. For this purpose, we analyzed the essential role of TMEPAI in tumorigenicity using the genetic or chemical-induced tumor model for TMEPAI knockout (TMEPAI-KO) mice. Interestingly, the number of tumors decreased in intestines from the TMEPAI-KO mice in both in vivo tumor models compared to tumors from the control mice. Consistently, survival time was drastically prolonged in TMEPAI-KO mice. When the gene(s) which is specifically increased or decreased in tumors from Apc$^{\Delta 716}$/TMEPAI-KO mice was investigated using DNA microarray, we found several unique genes which might be involved in control of tumor development. To investigate the possibility that these genes are implicated in suppression of tumorigenicity in TMEPAI-KO mice, we are going to analyze its mechanism using organoid culture systems at present.

P-3228

FOXA1 confers resistance to TGF-β-induced apoptosis in ER-positive breast cancer cells

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TGF-β is a multifunctional cytokine that promotes apoptosis in normal epithelial cells. Mammary epithelial cells are susceptible to TGF-β-induced apoptosis, and TGF-β3 expression is strongly induced after weaning and causes involution of mammary glands in rodents. However, breast cancer cells have resistance to TGF-β-induced apoptosis, and this resistance is involved in breast cancer development. In this study, we investigated the molecular mechanisms underlying resistance to TGF-β-induced apoptosis in breast cancer cells. We analyzed gene expression profiles during mouse mammary gland development and found that expression of forkhead box protein A1 (FOXA1), which is the transcription factor involved in mammary development, was strongly repressed at the involution stage. FOXA1 is highly expressed and promotes proliferation in estrogen receptor (ER)-positive breast cancer cells. Knockdown of FOXA1 enhanced TGF-β-induced apoptosis in the ER-positive human breast cancer MCF7 cells, suggesting that FOXA1 has suppressive role on TGF-β-induced apoptosis in these cells. Analysis of the molecular mechanisms of FOXA1-mediated repression of TGF-β signaling is currently underway.

▲TOP

P-3229

HIF-1 maintains a functional relationship between pancreatic cancer cells and stromal fibroblasts by upregulating Shh

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Hypoxic and stroma-rich microenvironment, a characteristic feature of pancreatic cancers, has been strongly associated with poor prognosis of patients. However, mechanisms underlying the hypoxia-mediated increase in stromal compartments remain largely unknown. Here we show that hypoxia-inducible factor-1 α (HIF-1 α), a transcription factor responsible for cellular adaptive responses to hypoxia, induces sonic hedgehog (SHH) secretion from pancreatic cancer cells, resulting in the accelerated growth of stromal fibroblasts through the activation of hedgehog signaling pathway. We found that pancreatic cancer cell lines expressed more SHH when exposed to hypoxic conditions, and secreted it in a HIF-1-dependent manner. Recombinant SHH, which was confirmed to activate the hedgehog signaling pathway, accelerates the growth of fibroblasts in a dose-dependent manner. These results indicate that HIF-1-mediated secretion of SHH under hypoxic conditions is responsible for the formation of the detrimental microenvironment in pancreatic cancers; and therefore, provide rational basis to target it for cancer therapy.

P-3230

Insulin-like growth factor-1 signaling is responsible for cathepsin G-induced aggregation of breast cancer MCF-7 cells

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Neutrophils frequently invade in tumor mass, however, the pathology of these cells in tumor cell proliferation and metastasis is still unclear. We have revealed that cathepsin G (CG), which is a neutrophil protease, induces an increase in cell motility, cell-cell adhesion via E-cadherin and formation of multicellular cell aggregates in human breast cancer MCF-7 cells. Multicellular aggregates cause tumor emboli, followed by metastasis. However, the molecular mechanism of tumor cell aggregation induced by CG have not been elucidated. In this study, we identified the intracellular signaling pathway that is activated by CG. As the results of screening assay, the CG-induced cell aggregation was strongly inhibited by multi-kinase inhibitors and receptor tyrosine kinase inhibitors. Antibody array indicated that CG activated insulin-like growth factor-1 (IGF-1) receptor (IGF-1R). Transfection of IGF-1R siRNA into MCF-7 cells decreased the CG-induced cell aggregation. CG evoked the release of IGF-1 in the conditioned medium. From these results, we concluded the CG induces release of IGF-1 followed by IGF-1R activation to induce the cell aggregation.

P-3231

Exploratory research of factors regulating the expression of AR splice variants

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In recent years, androgen receptor variants (AR-Vs) have been identified as potential important players in prostate cancer progression and therapeutic resistance. AR-Vs lack the ligand-binding domain, and is constitutively active as transcription factors in the absence of androgens. We established a subline of human prostate cancer 22Rv1 cells (defined as 22Rv1-500 cells) that showed different expression patterns of AR. Although 22Rv1 expressed full-length AR and AR-Vs, western blot analysis showed a higher level of full-length AR protein expression and a significant reduction in AR-Vs protein in 22Rv1-500 compared with the parent cell lines. Moreover, we obtained the similar results in the mRNA expression by Real-Time PCR analysis. In addition, we found that 22Rv1-500 showed significant higher drug sensitivity to AR-antagonist enzalutamide. To identify the factors that affect AR variants expression, we performed global gene expression analysis and found a few genes showed a significant difference between 22Rv1-500 and the parent cell lines. The relationship between these candidate genes and AR variants expression is now under investigation. (collaborator: Ohba Shun-ichi)

▲TOP

[P-3006] P3-2 [English]
Virus, bacteria infection, inflammation and cancer (2) [English]

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3006

HBx induces hepatocarcinogenesis via activation of cancerous signaling pathways and alteration of metabolism

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Different HBV genotypes are associated with varying levels of pathogenecity, while the genetic mechanisms behind this remain unclear. This study attempts to elucidate the mechanisms contributing to tumour development of HBx genotype B (HBx-B) *in vivo*. To investigate the potential tumorigenic effects of HBx-B on HCC, the *Sleeping Beauty* transposon system was used to deliver HBx-B gene variants into the livers of *fumarylaccetoacetate hydrolyse*-deficient mice by hydrodynamic tail vein injection. Short hairpin RNA directed against *transformation-related protein* was co-injected to accelerate the tumorigenic effect. A trend towards higher tumor burden in the mutant HBx-B variants. HBx-B induced high-grade inflammation, necrosis and fibrosis in our *in vivo* model. Proteomic and RNA-Seq analyses indicated the activation of phosphorylated thymoma viral proto-oncogene 1 and reconfirmed the activation of non-phosphorylated catenin (cadherin associated protein) beta 1 in tumors induced by HBx-B. Additionally, RNA-seq analyses on HBx-B injected animals revealed the involvement of metabolic pathways in HBx-induced tumorigenesis.

▲TOP

P-3007

RSV ameliorates LLC bearing mice partially through decreasing G-MDSCs accumulation, impairing its suppressive ability

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Myeloid-derived suppressor cells (MDSCs) are heterogeneous population of immature myeloid cells which consist of two subsets: granulocytic MDSCs (G-MDSCs) and monocytic MDSCs (M-MDSCs). MDSCs expand in tumor-bearing hosts and contribute to immunotherapeutic resistance by remarkably blocking effector T-cell activation via different mechanisms. Resveratrol (RSV) is a polyphenol and it has been widely used for its various health benefits. However, the underlying mechanism of its anti-tumor properties remains incompletely clear. In this study, a transplantable mouse model was used to investigate the effects of RSV on MDSCs. The results showed that RSV ameliorated tumor development by decreasing G-MDSCs accumulation, impairing its suppressive ability on CD8+T cells and promoting M-MDSCs differentiation into dendritic cells (DCs) and macrophage. Our results indicated that RSV should be emphasized as a modulator of MDSCs suppressive function and RSV can be used as a novel booster for tumor immunotherapy.

P-3008

Losartan, an antagonist of AT1R, inhibits colon cancer development, AT1R, a survival predictor of colon cancer

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Angiotensin Ⅱ type 1 receptors (AT1R) are involved in all aspects of human diseases and regulate different pathological and biological processes. Recent studies indicated that AT1R also involved in tumor development and metastasis. Losartan, an antagonist of AT1R, has drawn attention for its potential inhibition of tumor progression and anti-fibrotic activity. Therefore, the present work was to address whether losartan can attenuate the proliferation, epithelial-mesenchymal transition (EMT), and metastasis of colon cancer as well as to analyze the relationship between AT1R expression and colon cancer survival. The results showed that losartan up-regulated the expression of AT1R, inhibited the proliferation and decreased the ability of tumor metastasis. Losartan also significantly decreased TGF-beta1 expression and delayed the progress of EMT. Our results also showed that AT1R expression was closely associated with the survival rate of colon cancer. These results suggested that target the angiotensin Ⅱ-AT1R signaling pathway may be an effective strategy for the treatment of colon cancer.

P-3009

ZNF423 expression in relation to oxidative stress-induced CCA progression

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Cholangiocarcinoma (CCA) is a malignancy of the bile duct epithelial cells. The incidence of this cancer is highest in northeast Thailand in association with the infection of Opisthrochris viverrini (Ov) as the major risk factor. Chronic inflammation induced by Ov-infection generates persistent oxidative stress which causes CCA genesis and progression. Zinc finger protein 423 (ZNF423) is a transcriptional factor that involved in many stem cell differentiation and cancer progression. Our results demonstrated that ZNF423 is overexpressed in CCA cell lines and CCA tissues. High ZNF423 expression in CCA tissues tended to correlate with poor prognosis. Moreover, the expression of ZNF423 in CCA tissues was positively correlated with an oxidative stress marker (8-oxodG) formation. In addition, ZNF423 expression could be induced in immortal cholangiocyte cell line (MMNK1) by hydrogen peroxide treatment. Thus, we hypothesized that ZNF423 overexpression induced by oxidative stress may play essential roles in adaptation of cholangiocytes to oxidative stress and involved in CCA progression.

▲TOP

P-3010

Oxidative stress down-regulates Early B cell factor 1 resulting in cholangiocarcinoma genesis

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Early B cell factor 1 (EBF1) is a transcription factor involved in various stem cells differentiation and it is a negative regulator of estrogen receptors. However, the functional roles of EBF1 in carcinogenesis are unclear. Cholangiocarcinoma (CCA) is an oxidative stress- and estrogen-driven cancer of cholangiocyte cells. Our results demonstrated that EBF1 expression was suppressed in oxidative stress-resistant immortal cholangiocyte cell line compared with the parental cell line. In addition, EBF1 suppression was found in liver fluke-induced CCA model, human CCA tissues, and CCA cell lines. These suggesting that oxidative stress suppressed EBF1 expression and the reduced EBF1 level may induce CCA genesis. Moreover, EBF1 knockdown-immortal cholangiocyte cell line induced cell migration, stem cell markers expression and oxidative stress resistant properties. Also, cell migration was significantly enhanced after estrogen treatment. Therefore, EBF1 plays suppressive roles in CCA genesis via suppressions of stem-like cell, oxidative stress-resistant and estrogen response properties.

P-3011

Fatty liver with choline-deficient-model influences metastatic resistance

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Background: In the developed and developing countries, fatty liver disease has become common disease. The differences of the liver metastasis between fatty and normal liver remain unclear. We established the EL4-RFP lymphoma model in mice with fatty liver induced by a choline-deficient-diet (CDD) and researched the potential and tumor microenvironment of metastatic liver. Material and method: C57BL/6-GFP transgenic mice were fed with a CDD in order to establish a fatty liver model. EL4-RFP cells were injected in the spleen of normal-liver mice and fatty-liver mice. Metastases were imaged with the Olympus SZX7 microscope and the Olympus FV1000 confocal microscope. Result: Metastases of EL4-RFP were observed in the liver, ascites and bone marrow. The fewest metastases were observed in the fatty liver. In addition, the fewest cancer-associated fibroblasts were observed in the fatty liver. Conclusion: The relative metastatic resistance of the fatty liver may be due to the reduced number of CAFs. The fatty liver may not be a suitable environment for CAFs which may be due to re-programmed methionine metabolism which may have occurred during the time the animals were on the CDD.

▲TOP

[P-3018] P3-4 [Japanese]
Virus, bacteria infection, inflammation and cancer (4)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Kiichiro Tsuchiya / Dept. Gastroenterol., Tokyo Med. Dent. Univ.

P-3018

Genetic features of hepatocellular carcinoma developed in non-cirrhotic liver infected with hepatitis B virus

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Hepatocellular carcinoma (HCC) develops not only in a cirrhotic liver but in a non-cirrhotic liver tissue, especially in the cases with hepatitis B virus (HBV). To identify molecular features of HBV-related HCC in non-cirrhotic liver, we performed target deep sequencing of representative cancer-related genes and copy number analysis for 9 HCCs of non-cirrhotic HBV carriers and 10 HCCs of HBV-positive cirrhotic patients. The average age of the patients without cirrhosis was lower than those with cirrhosis, which represents the difference of the duration of hepatitis.The target deep sequencing and copy number analysis of HCC samples revealed that the landscape of gene abnormalities was different between non-cirrhosis and cirrhosis. For instance, the frequency of promoter mutations and/or copy number gain of TERT gene was lower in non-cirrhotic cases than cirrhotic patients. On the other hand, somatic mutations and/or copy number loss of TP53 gene was identified in a high frequency in non-cirrhotic cases compared to cirrhotic patients. These results indicate that molecular mechanisms of hepatocarcinogensis is different between non-cirrhosis and cirrhosis in the patients with HBV.

▲TOP

P-3019

Virus integration into the genomes of hepatocellular carcinoma patients with occult Hepatitis B virus infection

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The presence of hepatitis B virus (HBV) DNA in patients with hepatitis B surface antigen (HBsAg) negative is termed occult HBV infection (OBI). HBV DNA is frequently detected in the genome of hepatocellular carcinoma (HCC) in patients with chronic HBV infection, while it has not yet become clear in patients with OBI. To identify the integration of HBV to the HCC genome of OBI patients, we performed targeted virus capture sequencing for HCC samples obtained from 243 patients; 81 patients with chronic HBV infection, 73 HBsAg negative patients without hepatitis C virus (HCV) infection, 56 HBsAg negative patients with HCV infection, and 33 non-B non-C patients as negative control. We identified 4,014 non-clonal and 338 clonal HBV integration sites that distributed almost randomly through the genome. HBV integration was detected in 11 OBI patients but not detected in OBI patients with HCV infection nor 33 non-B non-C patients. Recurrent HBV integration at TERT and KMT2B loci, which was considered cancer driver alteration, were observed in OBI patients, suggesting a potential risk of HCC development even after the disappearance of serum HBsAg.

P-3020

Functional importance of JAK-STAT pathways in HTLV-1 infected cells

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HTLV-1 disturbs the order of host gene expression, contributing to cellular immortalization and lymphomagenesis. Here we show that JAK-STAT pathways are strongly activated in Tax-positive HTLV-1 infected cells, but not in Tax-negative ATL cells.
In order to identify the functions of JAK-STATs on the developmental course of HTLV-1-associated diseases, we performed gene expression profiling of JAKs inhibitor ruxolitinib- or shSTATs-treated HTLV-1-infected cells. We found that STATs promoted expression of many genes associated with cell cycle regulation (CDKs, CCNA2, CHEK1, E2F1, E2F8 etc), inflammatory response (IL6, IL10, etc), and lineage specification (TBX21, IFNG etc). Treatment of STAT shRNA and JAK inhibitors (ruxolitinib and tofacitinib) significantly decreased cell growth. It is interesting that target genes were significantly different between NF-κB and JAK-STAT pathways. Dysregulation of several transcription factors and signaling pathways depending on disease stage may cooperatively shape the ATL-specific transcription landscape.

P-3021

Helicobacter pylori infection down-regulates Sox2 expression through the methylation of its KLF4 binding site

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Aberrant DNA methylation by DNA methyltransferases (DNMTs) is involved in carcinogenesis. We previously reported that down-regulation of Sox2 induced gastric carcinogenesis upon H.pylori infection. Hence, we aimed to investigate whether Sox2 expression is regulated by DNA methylation. Quantitative image analysis revealed that the expression of DNMT1, 3a and 3b is increased in human gastric cancer tissues suggesting that Sox2 suppression in gastric cancers is due to DNA methylation. To investigate this in vitro, we employed two gastric cancer cell lines that express and that does not express Sox2, MKN45 and GCIY, respectively. Bisulfite sequencing analysis revealed that the CpG island (CpGi) (-594 to -496) of human Sox2 promoter was significantly more methylated in GCIY (47.5%) than in MKN45 (4.0%). Addition of 5-aza-dc demethylated the CpGi to 41.6% and enhanced Sox2 expression in GCIY. On the other hand, H.pylori infection methylated the CpGi to 21.2% in MKN45. This CpGi belonged to a KLF4 binding site and ChIP assay confirmed KLF4 binding to this site. In conclusions, H.pylori infection suppressed Sox2 expression through DNA methylation of KLF4 binding site in Sox2 promoter.

▲TOP

P-3022

Tenascin-C produced by intestinal myofibroblasts contribute to carcinogenesis of Colitis-Associated Cancer

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Background: The number of IBD patients is markedly increased and the frequency of Colitis-Associated Cancer (CAC) is expected to be increased. Intestinal myofibroblast (IMF) is reported influence the development of cancer, whereas the role of IMF in CAC has not been well studied. Thus, we explored the role of IMF involved in the CAC and sought to identify the candidate genes that could be targeted for preventing CAC. Methods: AOM/DSS model was used as CAC model. Flow cytometry was performed to obtain IMF, and their gene expression profile was analyzed by RNA-seq. Protein expression of candidate gene was analyzed by IHC. Then, inhibitor was administrated to assess the inhibitory effect of CAC. Results: RNA-seq revealed that 1045 genes were significantly changed in the comparison of CAC and normal IMF. Among them we focused Tenascin-C(TNC) which is known affect with various regulatory function in several cancers. TNC protein was expressed predominantly in the stroma around the tumor lesions. Administration of ATN-161, an inhibitor of TNC function, suppressed the tumorigenesis in CAC model. Conclusion: Inhibition of TNC function may have a possibility of preventing CAC carcinogenesis.

P-3023

Helicobacter pylori mutant strains after eradication therapy analyzed by quantitative pyrosequencing using gastric wash

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The eradication of Helicobacter pylori reduces the risk of gastric cancer. A clear understanding of the factors underlying mixed infection with multiple clarithromycin-susceptible and clarithromycin-resistant H. pylori strains is necessary to design more effective therapies against H. pylori. We aimed to assess how the abundance and prevalence of H. pylori strains vary after clarithromycin-based eradication therapy. We sequentially analyzed the abundance and prevalence of H. pylori DNA by pyrosequencing using gastric wash samples before and after eradication therapy. The abundance of H. pylori DNA decreased significantly until the 2-year follow-up, but it switched to an increase at the 3-year follow-up. Importantly, the ratio of the prevalence of mutant strains to the prevalence of wild-type strains had already increased at the first-year follow-up, suggesting the selection and growth of clarithromycin-resistant strains during the follow-up periods. Being sensitive and representative, our assay will be useful in effectively addressing gastric cancer development by enhancing the long-term success of intervention strategies and consecutive surveillance for H. pylori eradication.

P-3024

Expression of IRF7 correlates with expression of EBV LMP1 and neck metastasis in nasopharyngeal cancer

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Interferon regulatory factor 7 (IRF7) has oncogenic properties in several malignancies such as Epstein Barr virus (EBV) associated lymphoma. However, there is no evidence whether IRF7 is associated with the oncogenesis of nasopharyngeal cancer (NPC), the pathogenesis of which is closely associated with EBV. Herein, we report that expression of IRF7 was increased in normal nasopharyngeal cells that expressed the EBV principal oncoprotein, latent membrane protein 1 (LMP1). In addition, IRF7 was mainly expressed in the nucleus in both normal nasopharyngeal cells and nasopharyngeal cancer cells that expresses LMP1. On immunohistochemical analysis, IRF7 was predominantly localized in nucleus in biopsy samples of NPC tissues. Totally, IRF7 expression was detected with 36 of 49 specimens of these tissues. Furthermore, the expression score of IRF7 correlated with the expression score of LMP1. Moreover, the expression score of IRF7 is associated with cervical lymph-node metastasis, which reflects of the highly metastatic nature of this cancer. Taken together, our results suggest that expression of IRF7 is one of the metastatic effectors of LMP1 signalling in EBV-associated NPC.

▲TOP

[P-3029] P3-6 [Japanese]
Virus, bacteria infection, inflammation and cancer (6)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3029

Quantification of MCPyV DNA loads in the tumor tissues and nonlesional skins of patients with Merkel cell carcinoma

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Background: Merkel cell polyomavirus (MCPyV) is an etiological agent of Merkel cell carcinoma (MCC), an aggressive skin cancer that occurs on sun-exposed skin of the elderly. This oncogenic virus is also detectable in the normal skin of healthy individuals. The mechanism for transformation of MCPyV to an oncogenic form is unknown. We investigated the levels of MCPyV DNA and viral sequences both in the tumors and nonlesional skins of patients with MCC. Methods: Sun-exposed and -unexposed skin swabs were obtained from 6 patients with MCC, 30 healthy control donors and 19 patients with non-MCC skin cancers. DNAs from the swabs and MCC tumors were analyzed for MCPyV loads using quantitative real-time PCR. Results: MCPyV DNA levels were significantly higher in swabs from the nonlesional skins of patients with MCC compared with those from age-matched healthy donors and patients with other skin cancers. MCPyV strains obtained from the normal skin of patients with MCC had wild-type sequences without structural alterations, whereas the tumor-derived strains in the same patients showed tumor-specific mutation. Conclusion: MCC might develop in persons harboring higher MCPyV load in the skin.

▲TOP

P-3030

Analysis of cytotoxic factor contained in tumor supernatants

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We have shown that supernatants of human uterine carcinoma cell line (SiSo) contained a cytotoxic factor against several cell lines and human peripheral blood lymphocytes. Edman degradation sequence analysis suggested that this factor was expected to be an arginine deiminase (ADI) produced by mycoplasma. Indeed, cytotoxicity of SiSo supernatants was disappeared by mycoplasma remover treatment. To ascertain whether ADI have cytotoxicity, we obtained full length recombinant ADI and measured cytotoxicity. Also it is reported that a part of amino acid sequences (120 a.a~140 a.a) of ADI are different in mycoplasma subtype. We used some synthetic peptides containing 120 a.a~140 a.a and measured cytotoxicity. The results showed that cytotoxicity of recombinant ADI and each synthetic peptide were not observed. These results suggest that structure of purified ADI is characteristic and the unique site of amino acid sequence might have cytotoxicity. We also attempted to establish mouse monoclonal antibody (mAb) and gained some clones which react specifically to purified ADI. We will investigate the potential for various applications in basic and clinical research by using these mAbs.

P-3031

The mechanism of obesity-associated liver cancer through Toll-like Receptor Signaling and DNA sensing machinery

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Obesity has become more prevalent in most developed countries and is increasingly recognized as a major risk factor for several common types of cancer, including hepatocellular carcinomas(HCCs). However, the precise molecular mechanism through which obesity promotes HCC development remains unclear. Our previous studies have shown senescence-associated secretory phenotype(SASP) play an important role in promoting obesity-associated HCC development in mice. Dietary or genetic obesity induces alterations of gut microbiota, thereby increasing the levels of deoxycholic acid(DCA), a gut bacterial metabolite known to cause DNA damage. The enterohepatic circulation of DCA cause DNA damage, which in turn, cellular senescence and SASP phenotype were induced in hepatic stellate cells, thus facilitating HCC development in obese mice(Yoshimoto et al. Nature, 499, 97-101, 2013). Although our results have shown that the SASP plays an important role in the development of HCC, the molecular mechanism of how SASP is induced still unclear. Here, we show that the intracellular nucleic acid receptor, STING, and TLR2 play a crucial role in the induction of SASP and obesity-associated HCC development.

P-3032

Stromal cell activated by inflammation enhances gastric cancer progression

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BACKGROUND: Previous reports have reported that inflammatory cytokines such as IL-1 α , IL-1 β and TNF- α are involved in the proliferation, infiltration and poor prognosis of various cancer cells, however, the precise mechanism underlying gastric cancer (GC) progression related to inflammation is not clear.
METHOD: Growth ability, invasion ability, morphological change and activation of NF κ B were examined in the presence and absence of inflammatory cytokines using GC cell lines and non-cancer fibroblasts (NFs). We also performed proliferation evaluation in GC cells after co-culture with conditioned medium (CM) of cytokine stimulated NFs.
RESULTS: Neither GC cells nor NFs showed elevation in growth ability under cytokine stimulation. Whereas, NFs exhibited remarkable morphological change and high invasive ability. RNA-sequencing data revealed that NFs showed higher reactivity of NFkB target genes including secreted proteins that enhance the proliferation of GC cells.
SUMMARY: These finding suggest that specific secreted proteins derived from activated NFs in chronic inflammation significantly enhance the proliferation of GC cells.

▲TOP

P-3033

Cecal tumorigenesis in aryl hydrocarbon receptor-deficient mice and the effects of gut microbiota

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Aryl hydrocarbon receptor (AhR) known as a dioxin receptor is a transcription factor. Recently, AhR-deficient (AhR-/-) mice was revealed to develop cecal tumors with inflammation. In addition, germ-free AhR-/- mice or AhR-/- ASC-/- mice showed considerably reduced tumor development. Here, we investigated tumor histopathology, mechanisms of tumor development, and relationship between gut microbiota and tumor development. Seven of 19 AhR-/- mice developed tumors until 42 weeks, and tumor incidence was much lower than that previously reported. Most of tumors exhibited high-grade adenoma with hyperplasia, and remaining tumors were high-grade adenoma with invasive adenocarcinoma and hyperplasia alone. F4/80-positive macrophages and MPO-positive granulocytes infiltrated into or adjacent area of tumors. IL-1β, IL-6, CCL2, and CXCL5 were upregulated, and both p-ERK and p-Src were detected at tumors of AhR-/- mice. However, neither up-regulation of c-myc nor nuclear translocation of beta-catenin were noted. These results suggest that tumorigenesis of AhR-/- mice was dependent on different pathways from previously reported results.

▲TOP

[P-3040] P4-9 [English/Japanese]
Signaling of tumor-suppressor genes / novel diagnostic modality

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3040

p53 is sequestered by mitochondrial chaperone Lon in the matrix to restrain apoptosis under oxidative stress

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Accumulating evidences indicate that various stress signals induce transportation of p53 to mitochondria, leading to induction of apoptosis in a transcription-independent manner. However, the function of mitochondrial matrix translocation of p53 in apoptotic regulation remains unclear. Lon is a mitochondrial matrix protein with functions including chaperone activity. Lon overexpression promotes cell proliferation, apoptotic resistance to stresses. However, little literature undertakes detailed investigations on how Lon regulates apoptosis. Here, we found that Lon interactes with p53 in mitochondrial and restrains the apoptosis induced oxidative stress. Indeed, the mRNA expression of p53 target genes was significantly reduced when Lon was overexpressed. The ATPase mutant of Lon decreases the interaction with p53 and fails to inhibit apoptosis. These results indicate that the Lon is important for the control of p53 protein level and apoptotic function by sequestering p53 in mitochondrial under oxidative stress.

▲TOP

P-3041

AMOTL1 promotes gastric cancer by antagonising Hippo pathway

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AMOTL1 (Angiomotin-like protein 1) is associated with the tight junction between cells. Yet, its crosstalk with Hippo, a tumor-suppressive pathway, remains controversial. This study reveals the role of AMOTL1 in gastric cancer (GC). Mass spectrometry demonstrated AMOTL1 as a key binding-partner of YAP1. In clinical GC samples, AMOTL1 is overexpressed (P = 0.007), whose enrichment implied poor prognosis (P = 0.007, n = 394, TCGA cohort; P < 0.001, n = 512, multiple GEO cohorts). Besides, overexpression of AMOTL1 was related to worse clinical outcomes of patients in advanced stage (P = 0.025, n = 205, TCGA cohort), which was validated by gene set enrichment analysis (GSEA) (P = 0.025, n = 300, NCBI/GEO/GSE62254). Cox Regression analyses indicated that high AMOTL1 predicted poor survival (univariate analysis, P = 0.016; multivariate analysis, P = 0.022). Functional assay explicated that siAMOTL1 led to reduced cell growth (P < 0.001) and invasion (P < 0.001), suggesting its oncogenic role in GC. Our data propose that AMOTL1 is involved in Hippo pathway and it could be a potential therapeutic target for GC.

P-3042

Role of intestinal epithelial Src family kinases in the control of intestinal inflammation

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Colorectal cancer is linked to colonic inflammatory conditions, and its progression is associated with the increased activity of c-Src, a member of Src family kinases (SFKs), which is negatively regulated by carboxy-terminal Src kinase (Csk). We previously showed that intestinal epithelial cell (IEC)-specific ablation of Csk in mice (Csk CKO mice) resulted in the enhanced proliferation and turnover of IECs with SFK activity elevated. Here we show that Csk CKO mice develop more severe colonic inflammation than control mice in the dextran sodium sulfate (DSS)-induced colitis model. The mutant mice under the steady-state condition also showed the increased expression of mRNA for TNF-α, an inflammatory cytokine, in the distal colon. Moreover, the amounts of Occludin and Claudin-2, tight junction proteins, were reduced in the colon of the mutant mice. Following DSS treatment, the mutant mice displayed a marked increase in intestinal permeability, which is known to be involved in the development of inflammation. These results thus suggest that epithelial SFKs control inflammation in the colon. Disruption of such control by SFKs may contribute to the progression of colorectal cancer.

P-3043

Compare the focus points of the experts and novices on whole slice image of colorectal carcinoma tissues

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Interpretation of pathology teaching has progressed to the high-resolution image using whole slice scan mode. This study compared the eye movement trace and focus point between pathology experts and novices to use the eye tracker on normal tissue, adenomas, adenocarcinoma slice images of colorectal tumor sections. The total reading time of the experts on an image were shorter than the novices. The focus points of experts were often on deep area of colon wall, blood vessels, the junction of cancerous and normal tissue, and where a large number of blue particles accumulated. However, the focus points of the novices seemed to be on cancerous tissues and have no commonality between different novices. The trace was also no similarity either the experts or novices. These results could provide the information to design the teaching hand-held device or the learning of artificial intelligent for accelerated learning speed and interpretation results.

▲TOP

P-3044

A Versatile Nanowire Platform for Highly Efficient Isolation and Detection of Human Papillomavirus DNA from Urine

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As human papillomavirus (HPV) is mainly responsible for the development of cervicalCancer(CC), significant efforts have been devoted to develop novel strategies for detecting and identifying HPV DNA in urine. The analysis of target DNA sequences in urine offers a potential alternative methods as a non-invasive diagnostic assessment tool for the detection of HPV. However, the lack of efficient approaches to isolate and directly detect HPV DNA in urine has restricted its potential clinical use. In this study, we demonstrated a novel approach of using polyethylenimine-conjugated magnetic polypyrrole nanowires (PEI-mPpy NWs) for the extraction, identification, and detection of high-risk strains of HPV DNA sequences, particularly HPV-16 and 18, in urine specimens of CC patients. PEI/mPpy NWs-based isolation and detection strategy that we fabricated and characterized appears to be a cost-effective and practical technology with greater sensitivity and accuracy than other urine-based methods. The clinical performance of PEI-mPpy NWs was evaluated, demonstrating a type-specific concordance rate of 100% between urine and cervical swabs, even when using a small volume of urine (300 μ L).

▲TOP

[P-3050] P9-1 [English]
DNA methylation (1) [English]

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Takashi Sakatani / Dept. Diagnostic Path., Nippon Med. Sch. Hosp.

P-3050

Methylomics analysis identifies SPG20 as a sensitive non-invasive biomarker for early detection of gastric cancer

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Gastric cancer is one of the second leading cause of cancer worldwide. In this study, we performed Illumina methylation microarray in AGS gastric cancer cells and cells depleted with STAT3. Integrative computational analysis identifies SPG20 as one of the STAT3 targets. Bisulphite pyrosequencing found that promoter region of SPG20 is hypermethylated in gastric cancer cell lines including AGS cells but not in cells depleted with STAT3 and immortalized gastric epithelial GES cells. Accordingly, SPG20 is highly expressed in GES cells but not the gastric cancer cell lines while its expression can be restored by the treatment of DNMT inhibitor. Clinically, promoter methylation of SPG20 is significantly higher in cancer tissues (n=53) than that of gastritis (n=12) and adjacent normal. Importantly, methylation of SPG20 can also be observed in cfDNA isolated from serum samples of gastric cancer (n=53), IM (n=3) and gastritis (n=9), showing a progressive increase in tumor progression. Taken together, SPG20, a potential STAT3 target, is frequently methylated in gastric cancer. Methylation of SPG20 may be a novel non-invasive biomarker for early detection of gastric cancer.

▲TOP

P-3051

Global epigenomic analysis reveals the role of STAT3 in controlling enhancer methylation in gastric cancer

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Gastric cancer is one of the common causes of cancer death worldwide. Although activation of JAK/STAT signaling is frequently observed in gastric cancer, the role of this aberrant signaling remains unclear. In this study, by using Methylation EPIC array, we compared parental AGS gastric cancer cells, cells depleted with STAT3 as well as gastric cancer patients with different STAT3 activation status. Unexpectedly, the differentially methylated region (DMR) was highly enriched in the CpG island shore. Computational analysis by shuffling 500bp block with CpGs found that DMRs were significantly enriched with the enhancer histone modification H3K4me1. Interestingly, among those STAT3 targets, we found that the promoter/enhancer region of SMARCAL1 containing YY1 binding site and enriched with H3K4me1 was hypomethylated in cells depleted with STAT3. As SMARCAL1 is involved in alternative lengthening of telomeres, survival analysis found that gastric cancer patients with higher expression of SMARCAL1 is associated with poor survival. Binding of YY1, in a methylation-sensitive manner, may be crucial in controlling the expression of SMARCAL1 in promoting tumor growth in gastric cancer.

P-3052

Aberrant promoter methylation profile in low- and high-grade gastric lymphoma

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Aim: Epigenetic silencing of tumor-related genes owing to CpG island methylation has recently been reported in B-cell lymphomas, but its role in gastric lymphoma is unclear.Materials and Methods: We analyzed the methylation status of genes involved in cell cycle control (p16), apoptosis regulation (death-associated protein kinase, DAPK), and DNA mismatch repair (MGMT, hMLH1, and hMSH3) in 46 cases of low- and high-grade gastric lymphoma.Results: We found that p16, DAPK, and MGMT were methylated more frequently in high-grade lymphomas than in low-grade lymphomas (80, 80, and 93% vs. 71, 74, and 84%, respectively). The methylation of hMLH1 and hMSH3 was rare or absent. Comparing the lymphomas with matched normal gastric mucosa, five of the 46 demonstrated some microsatellite instability (MSI-low phenotype); two of these were low-grade lymphomas, and three were high-grade lymphomas.Conclusion: The methylation of p16, DAPK, and MGMT represents a major pathogenic event in gastric lymphomas that may contribute to early tumorigenesis. This may have clinical applications in the management and follow-up of low- and high-grade gastric lymphomas.

P-3053

The anti-histamine, cyprohepatdine, reverses epigenetic silencing of the tumor suppressor IRF6 in urothelial carcinoma

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Urothelial carcinoma (UC) is the most common malignancy of the urinary system. We have previously demonstrated that cyproheptadine (CPH), an anti-histamine, inhibited UC tumor growth in vitro and in vivo. In this study, we performed RNA-Seq in BFTC905 UC cells treated with CPH, showing several genes including IRF6 (interferon regulator factor 6) that are differentially upregulated. Real-time RT-PCR confirmed the restoration of IRF6 expression in a panel of UC cell lines treated with CPH. Similar to 5azaDC, bisulphite pyrosequencing found that CPH could decrease promoter methylation of IRF6 in UC cell lines. Interestingly, CPH could also increase total mono-methylation of H3K4 and acetylation of H3K27. Functional studies found that ectopic expression of IRF6 suppressed UC tumor growth in vitro and in vivo. Clinical studies also found that high-grade UC patient tissue samples has significantly higher IRF6 methylation than low-grade tissue samples. Taken together, our results suggested that CPH may be a novel epigenetic modifier exhibiting both DNMTi and HDACi activities in UC. The therapeutic potential of CPH in the treatment of UC deserves further investigation.

▲TOP

P-3054

Dual roles of ANGPTL4 in tumor tissue and its microenvironment in urothelial carcinoma

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ABSTRACTUrothelial carcinoma carcinogenesis has been occurred through epigenetic repression of tumor suppressor. We found that ANGPTL4, was expressed at low levels in all UC cell lines. Using MSP and bisulfite pyro-sequencing shown that ANGPTL4 promoter had hypermethylation in UC cell lines and primary tumor samples, as compared to adjacent noncancerous bladder urothelium. ANGPTL4 potently suppressed UC cell proliferation, invasion, migration in vitro and xenograft formation in vivo. As ANGPTL4 is a secreted factor, we therefore examined the cir-ANGPTL4 level in UC patients. Cir-ANGPTL4 was significantly higher in plasma samples from UC patients than normal control, suggesting it might be secreted from other cell types. Exogenous c-terminal fragment of ANGPTL4 could promote cell proliferation and cell migration via activation of signaling through the Erk/FAK axis. Finally, IHC shown that ANGPTL4 was downregulated in tumor cells but expressed in adjacent stromal tissues including muscle and macrophages. In conclusion, our data supports dual roles for ANGPTL4 in UC progression, either as a tumor suppressor or oncogene, in response to microenvironmental context.

P-3055

The Role of EZH2 in the Epigenetic Silencing of the TGF-beta Target, LTBP2 in Ovarian Cancer

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TGF-beta functions as a tumor suppressor in normal ovarian surface epithelium (OSE) cells but promotes EMT in ovarian cancer. We hypothesized that EZH2 may act as an epigenetic switch to facilitate the TGF-beta mediated EMT in ovarian cancer. NGS based experiments identified several TGF-beta/SMAD4 targets, including LTBP2 that are overexpressed in immortalized OSE cells but epigenetically silenced by DNA methylation and EZH2-mediated H3K27me3 in ovarian cancer. Combination treatment of 5azaDC and EZH2 inhibitor, GSK343 resulted in a dramatic increase of LTBP2 expression in MCP3 and CP70 cells. Interestingly, knockdown of SMAD4 in CP70 cells resulted in a further increase of promoter methylation in LTBP2. Finally, overexpression of LTBP2 inhibited invasion in CP70 ovarian cancer cells. Taken together, our result suggested that EZH2 might be involved in the epigenetic silencing of TGF-beta regulated tumor suppressors in ovarian cancer, turning the function of TGF-beta from a tumor suppressor into an EMT regulator. The therapeutic potential of targeting EZH2 in the inhibition of EMT in ovarian cancer deserves further investigation.

▲TOP

[P-3063] P9-3 [English]
Chromatin structure, others [English]

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3063

Nuclear non-coding RNAs Eleanors, define the active ESR1 chromatin domain in recurrent breast cancer cells

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Genomic DNAs are intricately folded into chromatin. Nuclear long non-coding RNAs (lncRNAs) are key regulators for the chromatin and epigenetic regulations in cancers. Estrogen receptor alpha (ER)-positive breast cancer that undergoes endocrine therapy often acquires therapy-resistance, resulting in recurrence. To elucidate the underlying mechanism, we performed transcriptome analyses, and found that the gene for ER alpha (ESR1) was up-regulated in the endocrine therapy resistant cell model, with overexpression of Eleanor lncRNAs, from 0.7 Mb chromatin region including ESR1. This “Eleanor chromatin domain” corresponded to topologically associating domain that harbors a region with gene amplifications in cancer. This domain is active A-compartment in breast cancer, while it is repressive B-compartment in non-mammary cells, suggesting that Eleanors create active chromatin domain in cancer. Depletion of Eleanors resulted in repression of ESR1 mRNA and LTED cell proliferation. Our results revealed a novel type of ncRNA-mediated chromatin domain formation, which could be a good diagnostic and therapeutic target for recurrent breast cancer.

▲TOP

P-3064

MUC1 regulates enhancer activation by mediating SWI/SNF complex function in triple-negative breast cancer

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Next generation sequencing (NGS) has revolutionized the study of genomics, and integrated analyses for NGS can provide further valuable insights.Mucin1 (MUC1) is well known as a useful cell surface marker in human mammary epithelial cell for identifying luminal cells from basal cells that do not have its expression. Although MUC1 is shown to play an important role as an oncogenic molecule, there are few reports about the intranuclear function of MUC1 C-terminal subunit (MUC1-C) in cancer cells and a luminal and basal difference for its function has not been investigated in detail.The present work demonstrates that MUC1-C is located in nucleus in triple-negative breast cancer (TNBC) cells, while it is almost not in luminal cells in spite of their higher expression of MUC1. RIME shows intranuclear MUC1-C makes complexes with SWI/SNF family, and MUC1-C peak locations overlap greatly those of SWI/SNF family. Integrated analyses of ChIP-seq, RNA-seq and Hi-C data reveal that MUC1-C promotes enhancer activation with SWI/SNF and regulates gene expression transcriptionally for cell identity. Our findings indicate that MUC1-C may serve as a novel therapeutic target in TNBC.

P-3065

Polycomb molecule L3MBTL2 suppresses apoptotic cell death cooperating with BMI1 in neuroblastoma

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Polycomb group protein (PcG) expression was increased in tumor cells and they seem to work as oncogenic. Previously, we reported that ATM was activated and gamma-H2A.X was induced by PcG BMI1 inhibitor PTC-209 treatment in neuroblastoma (NB) cells. Recently, it was reported that PcG member L3MBTL2 was mutated in rare cases of medulloblastoma and its C. elegans homolog LIN-61 protects the genome by promoting homologous recombination for the repair of DNA double-strand breaks. Here, we performed pathway analysis of microarray data and found that cell cycle and mitotic pathways were significantly suppressed in L3MBTL2 knockdown/PTC-209 treated NB cells. L3MBTL2 knockdown strongly induced G1 arrest. Moreover, L3MBTL2 knockdown up-regulated PTC-209-induced gamma-H2A.X and apoptotic cell death even in the BMI1 inhibitor-resistant NB cells. Importantly, we confirmed that L3MBTL2 and BMI1 formed different PcG complexes. High expression of L3MBTL2 related to unfavorable NB patient prognosis in several cohorts. BMI1 and L3MBTL2 appear to be promising targets of PcG inhibition in tumors.

P-3066

BET Inhibitors Suppress ALDH Activity by Targeting ALDH1A1 Super-enhancer in Ovarian Cancer

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The emergence of tumor cells with certain stem-like characteristics, such as high aldehyde dehydrogenase (ALDH) activity due to ALDH1A1 expression, contributes to chemotherapy resistance and tumor relapse. However, clinically applicable inhibitors of ALDH activity have not been reported. It has been suggested that epigenetic regulation of stem-related genes contributes to chemotherapy efficacy. In this study, we show that bromodomain and extraterminal (BET) inhibitors suppress ALDH activity by abrogating BRD4-mediated ALDH1A1 expression through a super-enhancer element and its associated enhancer RNA. The clinically applicable small-molecule BET inhibitor JQ1 suppressed the growth of cisplatin-treated ovarian cancer cells both in vitro and in vivo. Combination of JQ1 and cisplatin improved the survival of ovarian cancer-bearing mice. These phenotypes correlate with inhibition of ALDH1A1 expression through a super-enhancer element and other stem-related genes in promoter regions bound by BRD4. Thus, targeting the BRD4 using clinically applicable small-molecule inhibitors, such as JQ1, is a promising strategy for targeting ALDH activity in epithelial ovarian cancer.

▲TOP

P-3067

Analysis of histone dynamics using permeabilized cells and reconstituted histone complexes

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In eukaryotes, the genomic DNA is packaged into chromatin. It is known that chromatin regulates gene expressions through its dynamic structural changes. In cancer cells, such a chromatin-based gene regulation is compromised. The chromatin structure strongly correlates with the state of its components including histones H2A, H2B, H3 and H4. Each histone has non-allelic variants and their incorporation affects chromatin structure and functions. However, it is unclear how these histone variants are incorporated into chromatin. To address this question, we have developed a new method to analyze histone dynamics using permeabilized cells and in vitro reconstituted histone complex, which can discriminate newly deposited histones from previously incorporated ones. We found that canonical H2A and its variant, H2A.X, are incorporated into both transcriptionally active and inactive chromatin regions in replication-dependent manner, while, another H2A variant, H2A.Z, are rarely deposited on transcriptionally inactive chromatin. These results indicate that mechanisms of how these histone H2As target at chromatin regions are different, and this method is suitable for analyzing the mechanism.

P-3068

Significance and application of epitranscriptome in cancer

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Cancer is a genetic disease, while the significance of epitranscriptome (ETR) modification of RNA in cancer has been emerged with unexpected level. Recent studies have indicated abundant ETR modifications occur and that ETR plays a role in RNA function such as translation of proteins; nevertheless, the significance in cancer remains to be understood perfectly, i.e., how ETR is involved in cancer development, which alterations in ETR pathway associate with cancer malignancy, and whether ETR information may be useful for cancer profiling and detection. Here we studied pancreatic cancer model of transgenic animals with RNA methyltransferase, and found that it functioned as a writer in collaboration with other erasers and readers, demonstrating its critical role in cancer development and metastasis. We then developed the technology of MS based measurement for ETR methylation, and confirmed the results. In addition, we studied the RNA methylation in patients with gastrointestinal cancer and found methylation of small RNAs was reduced after surgical resections of tumors. The present study indicates that information of ETR may be useful for precise detection and diagnosis of cancer.

▲TOP

[P-3075] P9-5 [Japanese]
DNA methylation (3)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3075

5-Azacytidine Targets Chromatin Regulation through piRNA Pathway

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Background: The mechanism underlying the resistance to 5-azacytidine (AZA) is not fully understood. The role of piRNA pathway in chromatin regulation in AZA activity and AZA resistance is also still unknown. Therefore, we investigated the effcts of AZA on PIWIL4 (piwi like RNA mediated gene silencing 4) and MAEL (maestrom), the key players in piRNA pathway, in AZA-sensitive and AZA-resistant leukemia cells.Methods: We examined the effects of AZA treatment on PIWIL4 and MAEL proteins in U937 and HL-60 cells, and their AZA resistant derivertives named R-U937 and R-HL-60 cells, respectively.Results: PIWIL4 was detected in all cell lines without AZA treatment. AZA treatment decreased PIWIL4 in U937 and HL-60 cells, but not in R-U937 and R-HL-60 cells. MAEL was not detectable in U937 and HL-60 cells without AZA treatment, while AZA treatment induced MAEL expression in U937 and HL-60 cells. Although MAEL was detectable even before AZA treatment in R-U937 and R-HL-60 cells, AZA treatment failed to change the amount of MAEL in R-U937 and R-HL-60 cells.Conclusions: Our results indicate involvement of chromatin regulation via piRNA pathway in AZA-treated leukemia cells.

▲TOP

P-3076

UHRF1 depletion and HDAC inhibition synergistically reactivate epigenetically silenced genes in colorectal cancer cells

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UHRF1 is a major regulator of epigenetic mechanisms and is overexpressed in human malignancies. In this study, we examined the role of UHRF1 in colorectal cancer (CRC) epigenome. Transient UHRF1 knockdown rapidly induced genome-wide DNA demethylation in CRC cells. Infinium BeadChip assays and bisulfite pyrosequencing analyses revealed significant demethylation across entire genomic regions, including CpG islands, gene bodies, intergenic regions and repetitive elements. Nonetheless, UHRF1 depletion only minimally reversed CpG island hypermethylation-associated gene silencing. However, the combination of UHRF depletion and histone deacetylase (HDAC) inhibition synergistically reactivated the silenced genes and strongly suppressed CRC cell proliferation. UHRF1 depletion plus HDAC inhibition induced marked changes in gene expression profiles in CRC cells. Our results suggest that (i) maintenance of DNA methylation in CRC cells is highly dependent on UHRF1; (ii) UHRF1 depletion rapidly induces DNA demethylation, though it is insufficient to fully reactivate the silenced genes; and (iii) dual targeting of UHRF1 and HDAC may be an effective new therapeutic strategy.

P-3077

Epigenetic drugs suppress cholangiocarcinoma and pancreatic cancer organoids by inducing an anti-tumor immune response

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DNA methyltransferase inhibitors (DNMTis) such as 5-aza-2'-deoxycytidine (5-Aza-CdR) were initially thought to reactivate silenced tumor suppressor genes in cancer cells. However, recent studies demonstrated that DNMTis activated endogenous retroviruses (ERVs) and induced Interferon (IFN)-responsive genes.A newly developed 3D culture system allows cancer stem cells to form budding cyst-like structure (organoids) that resemble the properties of original tumors. The aim of this study is to investigate the anti-tumor effect of epigenetic drugs including DNMTis and histone deacetylase inhibitors on organoids derived from human cholangiocarcinoma and pancreatic cancer.5-Aza-CdR treatment significantly reduced cell proliferation of cancer organoids and activated expression levels of ERVs and IFN-responsive genes. In addition, suberoylanilide hydroxamic acid (SAHA) treatment also reduced cell proliferation of cancer organoids and activated expression levels of ERVs and IFN-responsive genes. These results indicate that epigenetic drugs could exert the anti-tumor effect on cholangiocarcinoma and pancreatic cancer organoids by activating ERVs and IFN-responsive genes.

P-3078

Epigenetic inactivation of G protein coupled receptors in differentiated thyroid cancer

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Differentiated thyroid cancer has a relatively favorable prognosis but sometimes it is difficult to control. Epigenetic analysis for differentiated thyroid cancer is under investigation to distinguish such cases. We have reported that methylation of the promoter region of G protein coupled receptors (GPCR) in head and neck squamous cell carcinoma (HNSCC) is related to prognosis and recurrence rate. Based on these results, the association between GPCR methylation status and prognosis in differentiated thyroid cancer was analyzed using The Cancer Genome Atlas (TCGA) database. GALR1-3, Tachykinin receptor (TACR) 1-3, Somatostatin receptor (SSTR) 1-5 genes were examined. Unlike HNSCC, in thyroid differentiated carcinoma, GALR3, TACR2, SSTR1 and SSTR2 were more frequently methylated than normal thyroid tissues. Furthermore, the frequency of TACR2 and SSTR1 is related to the overall survival rate of thyroid differentiated cancer, and the survival rate in unmethylated case of TACR2 is significantly lower than in methylated cases. Further investigations were necessary, but it was suggested that methylation of GPCR would be a potential biomarker in differentiated thyroid cancer.

▲TOP

P-3079

Genome-wide DNA methylation profile of young-onset endometrial cancer

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The aim of this study was to clarify the significance of DNA methylation alterations in young-onset endometrial cancer. EPIC Infinium assay and targeted sequencing of 50 tumor-related genes were performed in 105 tissue samples. Principal component analysis revealed distinct DNA methylation profiles of endometrial cancers. Genes showing significant differences of DNA methylation levels between young-onset endometrial cancer (YE) (aged 40 years or less) and old-onset endometrial cancer (OE) were overrepresented by “transcriptional factors”. Mutations of the CTNNB1 gene or DNA methylation alterations of genes participating in Wnt signaling were frequent in YE patients. Unsupervised hierarchical clustering analysis clustered the YE samples into Clusters YA (n=22) and YB (n=12). Clinicopathologically less aggressive tumors tended to be accumulated in Cluster YB. We identified eleven marker CpG sites discriminating YB samples from YA samples with 100% sensitivity and specificity. Genetically and epigenetically different pathways may participate in the development of YEs and OEs and DNA methylation profile may be biomarkers for predicting patients amenable to fertility preservation.

P-3080

Epigenomic alterations during hepatocarcinogenesis without viral, alcoholic and fatty liver injury

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The aim of this study was to clarify the significance of DNA methylation alterations during hepatocarcinogenesis without viral, alcoholic and fatty liver injury. Genome-wide DNA methylation analysis using Infinium HumanMethylation450 BeadChip was performed in 378 liver tissue samples. Principal component analysis revealed that samples of non-cancerous liver tissue showing no remarkable histological findings from patients without viral, alcoholic and fatty liver injury (U-N) and the corresponding hepatocellular carcinomas (HCCs) with unknown etiology (U-T) showed distinct DNA methylation profiles differing from those of non-cancerous liver tissues showing findings compatible with viral, alcoholic and fatty liver injury and the corresponding HCCs. Unsupervised hierarchical clustering clustered all non-cancerous liver tissue samples into 4 clusters. U-N samples were accumulated in a subclass consisting of elder patients and generating HCCs with larger diameter. These data suggested that genome-wide DNA methylation alterations may participate in hepatocarcinogenesis with unknown etiology in the histologically normal liver of the elder patients and may generate more aggressive HCCs.

P-3081

Analyses of the cytosine methylation status among IDH mutated gliomas and the mechanism of gliomagenesis

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We previously reported malignant transformatjon of low grade glioma (WHO gradeII) to secondary glioblastoma (WHO gradeIV). Isocitrate dehydrogenase (IDH) 1 or 2 mutations are widely recognized as the primary initiation event to sufficiently cause these tumors. Mutated IDH mediates the inhibition of Ten-Eleven Translocations (TETs), the enzymes involved in DNA demethylation, and causes global change of 5-Methylcytosine (5mC) and 5-Hydroxymethylcytosine (5hmC) distribution status. This status change may initiate gliomagenesis, but the detailed mechanism is unknown. We examine the distribution change of 5mC and 5hmC before and after IDH mutation with EPIC 850k bead array system. Recently, several methods have been developed to distinguish the 5mC and 5hmC as different methylated status. In OxBS method, where DNA oxidation step was added before bisulfite conversion in the bead array assay, 5mC can be differentiated from 5hmC in 850000 EPIC probe areas. Several glioma cell lines and surgically resected glioma samples with and without IDH mutation were analyzed. We will discuss the relationship between 5mC and 5hmC status and gliomagenesis.

▲TOP

[P-3088] P9-7 [Japanese]
Chromatin structure

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yoshimitsu Akiyama / Dept. Mol. Oncology, Tokyo Med. & Dentl. Univ.

P-3088

Eleanor RNAs affect the chromatin interaction involved in breast cancer fragility

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Estrogen receptor positive breast cancer cells depend on estrogen for cell proliferation, but often acquire estrogen-independent growth after long-term estrogen deprivation (LTED). This recapitulates endocrine therapy resistance. We have shown that *Eleanor* non-coding RNAs are induced from chromatin domain and activate *ESR1* and neighboring genes in LTED cells, and inhibited with resveratrol. However, it remains to be elucidated how *Eleanor* RNAs affect the chromatin structure. Our 4C-Seq analysis showed that *Eleanors* demarcated a topologically associating domain (TAD) which was interacted with other TADs with active transcriptions; A-compartments. We found that a TAD, 42.9 Mb away from *ESR1* interacts more often in LTED cells, and contains *FOXO3* gene involved in apoptosis. Inhibition of *Eleanors* suppressed the chromatin interaction and *ESR1* (proliferation), while *FOXO3* (death) expression was maintained, which caused apoptosis. These results indicate that the *FOXO3* is coordinately activated with *ESR1* by *Eleanors* , and easily induces apoptosis in LTED cells. This study shows a novel intra-TAD interaction mediated by non-coding RNAs which can be a therapeutic target.

▲TOP

P-3089

Effect of BET protein inhibitor JQ1 on colorectal cancer cells

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Bromodomain and extra-terminal domain (BET) family proteins consist of BRD2, BRD3, BRD4 and BRDT are epigenetic reader. BET proteins recognize acetylated lysine residues and transfer the cellular signals. Recently, cellular effects of the BET protein inhibitors have been extensively studied in many cancer cell types. We previously reported that BET inhibitors such as JQ1 suppressed the stem cell properties and stem-related gene expression in epithelial ovarian cancer cells. In this study, we investigated the effect of JQ1 on colorectal cancer (CRC) cells. By using CRC cell lines (HCT116, HT29, DLD1), we confirmed that JQ1 suppressed the expression of LIF (Leukemia Inhibitory Factor) and MYC, both of are known as a target gene of JQ1. Cell proliferation assay using WST assay showed that JQ1 markedly inhibited growth of CRC cells in a dose-dependent manner. We also found that JQ1 suppressed the expression of three genes (BMI-1, LGR5 and LRIG1) that are implicated in cancer stem cells. Taken together, it is suggested that BET protein may also regulate the stem cell property in CRC and that JQ1 could be expected as a hopeful treatment for cancer stem cells of CRC.

P-3090

A capture Hi-C analysis revealed the interactions between p53 binding sites and the target genes

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Gene activities are controlled by a combination of proximal and distal regulatory elements that interact with each other. The keys among these are enhancers, which associate with promoters to activate cell type- or disease-specific gene expression. A large number of enhancer regions have been annotated in the human genome by their hyper- sensitivity to DNaseI digestion and binding of chromatin modifier. To investigate globally how active enhancers dynamically interact with their target genes, we used Hi-C and Capture Hi-C assay in p53-positive HCT116 cells treated with a thymidylate synthase inhibitor 5-Fluorouracil (5-FU). We applied Bayes theorem (reported as 'Mango') for calculating the probabilities. Using even "SureSelect All Exon + Regulatory" (containing 220,000 bait regions), more than 181,000 significant interactions were found ($p < 10^{-4}$). Over 96% of those were inside of a topologically associated domain (TAD). Active enhancers have a tendency to interact with active promoters. For Capture Hi-C, our pipeline and viewer are able to find feasible genomic interactions using complicated Capture baits.

P-3091

Single stranded DNA in interphase is a key chromatin structure for mitotic chromosome organization

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DNA replication, transcription and chromosome construction are central to many biological processes such as cellular proliferation and cancer etiology, and these cellar events are enabled by the formation of higher order structures of chromatin. Recent studies based on Hi-C and CRISPR/Cas9 imaging technologies spotlight chromatin dynamics throughout the cell cycle; however, how chromatin structure in interphase converts into mitotic condensed chromosomes remains mostly unknown.
To address this outstanding question, we have focused on condensin complex which has a crucial role in mitotic chromosome condensation. We found that condensin II, which localizes in the nucleus, binds to single stranded DNA (ssDNA) more preferentially than double stranded DNA in S phase. Its ability to bind to ssDNA was remarkably reduced in mitosis by the phosphorylation of its subunits, which potentiates condensin II to induce chromosome condensation. Furthermore, we investigated whether the ssDNA mediated by DNA replication or transcription process is involved in the DNA localization of condensin II. These results significantly suggest the closely related chromatin dynamics between interphase and mitosis.

▲TOP

[P-3097] P11-5 [English/Japanese]
Cancer stem cell (1)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3097

Bisdemethoxycurcumin Suppresses Wilms' Tumor 1 and CD34 Protein Expressions in KG-1a Leukemic Stem Cells

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Remaining leukemic cells is the main problem in patients which are called leukemic stem cells. Many studies revealed that overexpression of Wilms' tumor 1 (WT1) protein relates to leukemogenesis. Curcuminoids were focused in this study. This study aims to investigate the cytotoxic effect of in-house curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) in human leukemic stem cell line, KG-1a compared to leukemic cell lines (KG-1 and K562). Curcumin exhibited the strongest cytotoxic activity in KG-1a cells with IC_{50} values of 13.6±1.9 μM. KG-1a cells had the highest LSC population by fluorescence microscope. To determine the effect of curcuminoids on WT1 and CD34 protein expressions, KG-1a cells were treated with non-cytotoxic concentration (IC_{20} value). Bisdemethoxycurcumin showed the strongest suppression of WT1 and CD34 protein expressions by 73.3±1.4 and 82.9±2.0%, respectively. In summary, curcuminoids could inhibit WT1 and CD34 protein expressions, especially bisdemethoxycurcumin.

▲TOP

P-3098

Reinforcing the Practicality of Chick Embryo Model to In Vivo Evaluate Engraftment of Human Leukemic Stem Cells

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Introduction: Several immunodeficient mice models were developed for leukemias with main limitations due to their high cost, demanding management and elongated assessment intervals. We aimed here at evaluating the engraftment of primary human AML $CD34^+$ cells in naturally immunodeficient chick embryo model.

Methods & Results: Mononuclear cells or magnetic sorted $CD34^+$ cells were injected into chick embryo chorioallantoic membrane veins. After 7 days, human CD34 transcript was detected by RT-PCR in blood, bone marrow (BM), spleen and liver from embryos injected with leukemic cells. Interestingly, an amplicon of the same length has been detected in BM and spleen from PBS injected embryos, although analysis via bioinformatics tools revealed no matches in chicken. Importantly, splenomegaly and hepatic lesions were observed in some $CD34^+$ cells injected embryos.

Conclusion: Collectively, our data confirm the engraftment of primary human $CD34^+$ leukemic cells in chick embryo liver, but other experiments are required to verify engraftment in BM and spleen, and to confirm the identity of a putative CD34 orthologous transcript in these 2 organs.

P-3099

A monocyte-recruiting phenotype defines functional heterogeneity of glioma cells with stemness and chemoresistance

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Intratumoral heterogeneity, especially the presence of functionally distinct subpopulations of cancer stem cells (CSCs) plays a critical role in therapy resistance and cancer recurrence. Previously, we reported that side population (SP)-defined glioma CSCs (GSCs) have a self-expanding strategy that exploit host cells to construct their own microenvironment. To investigate whether such an adaptive subpopulation could be a therapeutic target, by focusing on the monocyte chemoattractant CCL2 as a gene highly upregulated in SP and correlated with poor prognosis of glioma patients, we established reporter cell lines in which copGFP gene is cotranscribed with endogenous CCL2 gene (L2/cGFP). In all the established clones, L2/cGFP(high) cells were enriched in 1) SP of conventional cultures, 2) floating clusters of sphere cultures, and 3) cells treated with alkylating agents temozolomide and nimustine, suggesting a dominant contribution of monocyte-recruiting cells to stemness and chemoresistance. Our study identified a phenotypic marker, i.e. CCL2 that defines functional heterogeneity, which could provide the basis for new strategies to overcome therapy resistance and cancer recurrence.

P-3100

Development of SIRT2 inhibitor target to cancer stem cells

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Glioblastoma is the most malignant form of glioma. Despite great efforts, the median survival of glioblastoma patients has remained at around 1 year for the past decade. It has been reported that glioblastoma stem cells (GSCs), subsets of glioblastoma cells that possess the capability of self-renewal and exhibit extensive tumorigenicity, are resistant to both chemotherapy and radiotherapy, and thus are responsible for the poor prognosis of glioblastoma. Recent studies indicated that Sirtuins2 (SIRT2) is involved in the pathogenesis and development of several cancers. We found that SIRT2–mediated inactivation of p73 is critical for the proliferation and tumorigenicity of glioblastoma stem cells. In contrast, knockdown of SIRT2 or treatment with SIRT2 inhibitors did not affect the proliferation of normal cells, such as neural stem cells. These results suggest that SIRT2 may be a promising molecular target for the therapy of glioblastoma. In this session, we will introduce the effect of newly developed SIRT2 inhibitor to the proliferation of cancer cells, including GSCs.

▲TOP

P-3101

DJ-1 regulates stem cell function in glioblastoma

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DJ-1 was reported as an oncogene stimulating cell proliferation, cell invasion, and metastasis. Glioblastoma (GBM) classified as the most aggressive form of glioma (WHO grade Ⅳ), which have been correlated with the rate of cancer stem cell population. In this study, we clarified the upregulation of DJ-1 in the GBM stem cells (GSCs) and their significant role in maintenance of GSCs.By culturing in an ultra-low attachment plate satisfied with a non-serum media, GBM cell lines (U251-MG and U87-MG cells) proliferate to form spheres with cancer stem cell property. These spheres increased DJ-1 expression in a time-dependent manner. Sphere-forming efficiency in DJ-1-knockdown U87-MG (DJ-1 KD) cells was significantly lower than that in non-targeting siRNA-treated cells. The overall survival rates of tumor-bearing mice that were implanted with DJ-1 KD cells were not changed in primary transplantation, but significantly prolonged in secondary transplantation by DJ-1 depletion. Using PrognoScan database, these results were validated as a positive correlation between DJ-1 expression and overall survivals in GBM patients. Our findings propose that DJ-1 is a novel therapeutic target for GSCs.

P-3102

Relationships between cancer stem cell markers (ALDH1 & CD133) and disease-free intervals in human lung adenocarcinoma

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[Background and Purpose] Cancer stem cells (CSC) may have abilities of self-renewal and multi-potent differentiation. Aldehyde dehydrogenase 1 (ALDH1) and CD133 have been identified as CSC marker in patients with lung adenocarcinoma (ad patients). We investigated the relationship CSC markers (ALDH1 and CD133 expression) and disease-free intervals (DFI). [Materials and Methods] We examined 183 of 249 (73.5%) consecutive ad patients, using a standard immunoperoxidase technique. Semi-quantitative analysis (ALDH1-score) was performed (Shenton method). We defined as positive cases (ALDH1 positive cases) when more than 100 of the ALDH1-score were calculated. We also defined as CD133 positive cases when more than 10% of tumor was stained. [Results] The ALDH1 and CD133 positive cases were observed in 31 (16.9%) and 53 (29.0%) from 183 cases. The CD133 positive rate of ALDH1-score positive cases (41.9%) tended to be higher than that of ALDH1-score negative cases (26.3%). A uniivariate DFI analysis demonstrated that the ALDH1-score and CD133 positive cases were associated with an increasing risk of poor DFI.

P-3103

Stemness control by iron chelator is a novel strategy for cancer treatment

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Iron is an essential element for human health. However, iron overload can also cause some types of cancer. Cancer stem cells (CSCs) have been proposed to be responsible for tumor initiation, drug resistance, and recurrence. CSCs are considered to be involved in the earlier phase of tumor progression. In this study, we examined the importance of iron in maintenance of stemness, and revealed that DFX, an oral iron chelator, but not 5-FU and CDDP, suppressed the stemness marker expression in several cell lines, including miPS, miPS-LLCcm (CSCs model cells), NT-2 (Embryonal cell carcinoma), HSC-2 (Oral squamous cancer) and OE33 (Esophageal adenocarcinoma). DFX also suppressed sphere formation ability, indicating that DFX suppressed not only the phenotype but also function of CSCs. A combination therapy by DFX and CDDP synergistically suppressed the cancer cell proliferation in vitro and tumor growth in vivo. IHC staining of Nanog in 134 human esophageal cancer tissue revealed that high expression of Nanog was correlated with low overall survival and disease-free survival. These findings suggests that stemness control by iron chelator can be a novel strategy for cancer treatment.

▲TOP

[P-3111] P11-7 [English/Japanese]
Cancer stem cell (3)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3111

Miltefosine abrogates survival of cancer stem cells through mitotic catastrophe in colorectal cancer

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Lipid Raft, a signaling platform, is a highly ordered cholesterol-rich membrane domain modulating signal transduction as well as maintain structural and morphological features of the cell. Its dynamic features of the cancer cell surface may modulate the malignant phenotype of cancer. Miltefosine, a widely applied anti-leishmaniasis drug is engaging attentions as potential anti-tumor drug by its properties of altering lipid raft, though its mechanism of action is not yet cleared. Here we report that lipid raft is further enriched in CSCs compared to tumor burden, and miltefosine efficiently targets CSCs via interrupting this scaffolding structure. Enriched sub-population of actively repairing cells against DNA damage is a distinctive feature of CSCs which defends themselves against anticancer drugs. Mechanistically, miltefosine, an inhibitor of checkpoint kinase 1(CHK1), curtails these occulting population by leading them to enter mitotic catastrophe. Purging of CSCs by targeting CHK1 to stimulate mitotic catastrophe may thus provide the fundamental evidence of repositioning miltefosine into oncological application especially in colorectal cancer.

▲TOP

P-3112

Characteristics of carbonic anhydrase 9 expressing cells in human intestinal crypt base

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Though stem cells of many tissues are reported to be harbored in hypoxic microenvironment, little is known about the relationship between hypoxia and intestinal crypt base, where intestinal stem cells are supposed to exist. In this study, we focused on carbonic anhydrase IX (CA9), a hypoxia-inducible membrane-tethered protein, in normal intestinal crypt base, adenoma and early colorectal cancer (CRC). Using surgically resected human CRC specimen, we searched for the expression pattern and functional association of CA9 in human adult normal intestinal epithelia, adenoma and early CRC by IHC staining, flow cytometry, and qRT-PCR. We demonstrated that almost all crypt base slender cells in ileum and crypt base cells with eosinophilic structure in their basal cytoplasm in right and left colon were $CA9^+$ with the ratio of 25 to 40%, and that adenoma and T1 CRC showed broad expression of CA9. Flow cytometrically sorted $CA9^+$ population showed increased mRNA level of a Wnt signaling factor AXIN2. In conclusion, these observations indicate that CA9 expression in normal crypt base cells has association with intestinal epithelial stemness and CA9 may be involved in the carcinogenesis of CRC.

P-3113

PTPRC is a novel target for attenuating colorectal cancer stemness and radioresistance

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Radiotherapy is an essential for colorectal cancer (CRC) that is used either before surgery or after primary treatment. However, subpopulations survive and succeed at colonizing distant organs, preventing a permanent cure. To identify a novel therapeutic target for such radioresistance, we analyzed gene expression data for CRC patient tumors from primary and metastatic lesion, then identified radioresponsive genes whose transcription varies after radiation using public databases, CRC cells, and patient-derived tumor xenografts. We found that protein tyrosine phosphatase receptor C (PTPRC) was transcriptionally activated following radiotherapy and drove resistant phenotypes, such as increased clonogenic survival and reduced apoptosis. Interestingly, PTPRC is expressed preferentially in cancer stem cells (CSCs) than in bulk tumor cells and promotes certain CSC properties, including tumor initiation, repopulation, metastatic growth, and therapy resistance by promoting Wnt/β-catenin signaling. Correspondingly, PTPRC deletion combined with radiation treatment synergistically reduces metastasis and primary tumor burden, revealing PTPRC as an important mediator of radioresistance in CSCs.

P-3114

Effects of Carbon Ion Beam Alone or in Combination with 5-FU on Colorectal Cancer Stem Cells In Vitro

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In this study we investigated how effective on killing colorectal cancer (CRC) cells by carbon ion beam alone or in combination with 5-FU in vitro. Human CRC cells HCT116 and HT29 were treated with carbon ion beam or in combination with 5-FU, and then cell viability assay, colony and spheroid formation ability assay, apoptotic assay, and real time PCR analysis of apoptosis- and autophagy-related gene expression were performed. Carbon ion beam dose-independently suppressed CRC cell viability and in combination with 5-FU significantly enhanced its action. We found that the percentage of apoptotic cells was significantly increased by carbon ion beam in combination with 5-FU, and also the expressions of some of apoptosis- and autophagy-related genes such as BAX, Bcl2, Beclin1, ATG7 were significantly induced by carbon ion beam alone and it was further enhanced by carbon ion beam in combination with 5-FU in HCT116 cells. Spheroid formation ability of CD133+ cells was significantly inhibited by carbon ion beam combined with 5-FU. In conclusion, carbon ion beam combined with 5-FU has superior potential to kill CRC cells including cancer stem cells with enhanced apoptosis and autophagy.

▲TOP

P-3115

Mycn induces development of poorly differentiated liver cancer in vivo

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The transcription factor MYCN is a member of MYC family. Amplification of MYCN is often observed in poorly differentiated cancer. However, the detailed molecular basis of MYCN-mediated undifferentiated cancer development is largely unknown. In this study, we first generated a novel mouse model in which Mycn can be induced under the control of doxycycline. Mycn overexpression at this mice induced liver tumors. Histological analyses revealed that Mycn-driven liver tumors consist of undifferentiated dysplastic cells. Notably, Mycn-driven liver tumors showed significant upregulation of pluripotency-related proteins.We next showed that MYCN was highly expressed in SALL4-expressing HCCs. We also found that this HCCs exhibit partial activation of pluripotency-related genes, suggesting that the activation of pluripotency-related transcriptional network is associated with this HCCs.Collectively, these results indicate that Mycn overexpression is sufficient for development of poorly differentiated Sall4-expressing HCCs. Our results also suggest that Mycn-mediated activation of embryonic network may drive these cancers.

P-3116

Metastatic ability and the epithelial-mesenchymal transition in induced cancer stem-like hepatoma cells

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Background:Previously, we successfully induced sphere cancer stem-like cells (CSLCs) and observed the property of chemoresistance. In the present study, we examined the metastatic potential of these induced CSLCs.Methods: CSLCs were induced from a human hepatoma cell line (SK-HEP-1) in a unique medium containing neural survival factor-1. Splenic injection of cells into immunedeficient NRG mice was used to assess liver metastasis. Comprehensive mRNA expression analysis were performed by next generation sequencer; NextSeq 500.Findings:Splenic injection to NRG mice showed increased liver metastasis potential in CSLCs compared to parental cells ($P < 0.05$). GSEA with hallmark gene sets showed EMT were enriched in CSLCs. Quantification of CD44 isoforms showed CD44 standard isoform was down-regulated in CSLCs compared to parental cells (0.6-fold). Conversely, CD44v8-v10 was up-regulated (2.9-fold). Interestingly, CD44 isoform short-tail isoform was more up-regulated (7.4-fold) compared to the CD44v8-v10.Conclusion:Induced CSLCs possess an increased metastatic potential in which promotion of the EMT and upregulation of CD44v8-v10, especially short-tail, were observed.

P-3117

Characterization of the microRNA in PDAC tending to increase c-Met expression with preoperative chemo-radiation therapy

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Background: C-Met was identified as a dominant pancreatic Cancer Stem Cell (CSC) marker. We hypothesized that remnant PDAC tissue after CRT might harbor cells with high c-Met expression in some patients, and these cells may exacerbate patients' prognosis. Methods: The expressions of c-Met in resected specimens of PDAC were evaluated with immunohistochemistry, and we compared their prognosis and backgrounds including comprehensive microRNA expressions in patients' pretreatment serum.Results: The c-Met high group showed significantly shorter survival time in both overall survival and recurrence free survival. The c-Met high group included significantly more patients undergoing preoperative CRT before surgery. Microarray analysis for the pretreatment serum from patients who had received preoperative CRT revealed twelve microRNA statistically.Conclusions: A part of patients may be increased c-Met expression in PDAC by preoperative CRT. To identify the genes which are related to c-Met expression, we have performed microarray analysis. This study would be a help to individualize the multimodal treatment strategy for PDAC patients.

▲TOP

[P-3125] P11-9 [English/Japanese]
Cancer stem cell (5)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Tsukasa Okuda / Dept. Biochem. Molec. Biol., Kyoto Pref. Univ. Med.

P-3125

Non-mutagenic Chemical Compounds enhance the Conversion of iPSCs into CSCs

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n the previous study, a model of CSC was evaluated from iPSCs with the effect of cancer cells derived conditioned medium. Making use of the procedure of developing CSCs, we tried to assess the effect of various non-mutagenic chemical compounds that are inhibiting various signaling pathways on the conversion of iPSCs to CSCs. As the result, we found that some inhibitors such as GSK&3beta and MEK were enhancing the development of CSCs. The exposure of these inhibitors in the presence of conditioned medium exhibited some significant changes in phenotypes that are the maintenance of stemness, sphere forming ability and the malignant tumorigenesis in vivo. The gene expression profiling by microarray analysis implied that the inhibitors affected the pathways related with cancer development and poor prognosis. We report in this study that the inhibitors have tuning effect on the development of CSCs under the condition provided by the conditioned medium from cancer derived cells.

▲TOP

P-3126

Tumor initiating cell in immunocompetent animal defined by immunological features

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The tumor-initiating cell also called cancer stem cells, the theory provides one explanation for the phenotypic and functional heterogeneity among cancer cells in some tumors. According to this tumor-initiating cell concept, only particular tumor cells have a capacity to initiate the tumor, so targeting of tumor-initiating cells is promising therapeutic strategy of cancer therapy. Many kinds of tumor-initiating cells defined by surface markers or selective gene expression have identified. However, unfortunately, the challenge of the clinical trial that targeting tumor-initiating cells has not been succeeded yet. Recent study indicating that host immune system is quite important in tumor control, but historically, tumor-initiating cells identification had done mainly performed by the immunodeficient animal. Different from immunocompromised mice, the human body has the immune system, commonly. So, to develop effective tumor therapy, we should discover the tumor-initiating cells and key factor(s) for tumorigenicity in the immunocompetent animal. In this paper, we report that the immuno-related factor(s) which correlated with tumorigenicity of tumor cells in immunocompetent animals.

P-3127

Functional analysis of CD44 variants and xCT in canine cancer cells

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Cancer stem-like cells (CSCs) are thought to be responsible for therapy resistance, recurrence and metastasis. Recently, it was reported that the CSC marker CD44 has various splicing variants and one of them, CD44v8-10 isoform, promotes the synthesis of the antioxidant glutathione (GSH) by associating with xCT transporter in human CSCs. Thus, CD44v8-10 and xCT are considered important factors that enhance resistance to reactive oxygen species (ROS). In this study, we investigated the expression and function of CD44v8-10 and xCT in canine cancer cells. Consequently, the expression of canine CD44v8-10 and xCT mRNA was upregulated in canine cancer tissues as compared to normal tissues. When we transfected to canine tumor cells with CD44v8-10 cDNA, GSH levels and resistance to ROS in CD44v8-10 transfectants were significantly increased as compared to control. Furthermore, resistance to ROS in canine tumor cells was decreased by treatment with xCT inhibitor sulfasalazine (SSZ). Taken together, these results suggested that the interaction between canine CD44v8-10 and xCT plays important roles in CSC defense mechanism against ROS as well as human case.

P-3128

Can killing cancer stem cells using chemotherapy result in a complete cure?

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Cancer stem cells (CSCs) are regarded as being chemoresistant, but in vivo experiments on the chemosensitivity of side population (SP) cells derived from a mouse ascites tumor (FAT) suggest otherwise. A single intraperitoneal (i.p.) dose of bleomycin (BLM; 37.0 mg/kg), cyclophosphamide (CPA; 108 mg/kg), or 5-fluorouracil (FU; 39.0 mg/kg) did not extend the survival time of FAT-bearing mice. Nevertheless, an i.p. dose of each drug inhibited a single SP-cell injected i.p. into mice from developing into an FAT and killing the host, and also lowered the SP-cell fraction percentage in FAT-bearing mice. Thus, BLM, CPA, and FU chemotherapy resistance in FAT might not be solely attributable to the chemoresistance of CSCs. Flowcytometric analyses of the cell cycle and the numbers of i.p. FAT cells after an i.p. dose of each drug into FAT-bearing mice revealed that a decrease in the i.p. FAT cell number may recall some G0 phase of cells into the division cycle, followed by regaining of the proliferative ability of the surviving FAT cells. Cancer chemotherapy requires “Total cell kill” including CSCs for the complete cure of cancer.

▲TOP

P-3129

Promoter-dependent visualization of cancer cells

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[Background & Aim] Cancer stem cells (CSCs) and undifferentiated (potentially pluripotent) tumor cells may be relatively rare among the tumor cells. We currently attempted to establish the visualization system for Lgr5-, Bmi1- expressing CSCs and for Sox2-expressing undifferentiated tumor cells.[Methods] Lentiviral construct containing the cassette of the specific promoter of each gene encoding GFP/DsRed fluorescence sequence at its downstream was generated, infected to colon and pancreatic cancer cell lines. Cells were analyzed by FACS, microscope, western blot, and RT-PCR. [Results] Fluorescence signals for Bmi1 were evident in HCT116 and RKO and only minimal in Panc1 and Miapaca2. Each signal was concordant to the Bmi1 protein expression. As for Lgr5, similar results were obtained in Panc1 and Miapaca2, but an intense fluorescence was not always correlated to the protein expression in HCT116. Sox2-positive cells were rare in 2.5% for HCT116 and 0.4% for HT29 and the positive cells displayed high mRNA expression of Nanog and Oct3/4. [Conclusion] Our visualization system may be useful to identify CSCs and undifferentiated tumor cells.

▲TOP

[P-3134] P11-11 [English/Japanese]
Cell culture (4)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yoshiyuki Rikitake / Lab. Med. Pharma., Kobe Pharm. Univ.

P-3134

Establishment and Analysis of 5-FU resistant organoids from gastric cancer

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5-FU is one of the key drugs in the treatment of Gastric cancer(GC). However, GC often acquires drug resistance. Although several evidences have demonstrated that cancer stem cells (CSCs) play a key role in the acquisition of drug resistance, the details of mechanisms how CSCs endure the environment with anti-cancer drugs. Organoid is a novel 3D cell culture system through using the specific niche factors in a dish. Since organoid is believed to harbor abundant stem cells, cancer organoid could possibly be useful for scrutinizing CSC biology. In this study, we established GC organoids from patient derived specimens, and gradually treated them with higher and higher concentration of 5-FU in order to establish 5-FU resistant organoids. We have successfully harvested a couple of 5-FU resistant GC organoids that can stand higher dose of 5-FU than IC50 of 5-FU in parental GC organoids. The key enzymes and metabolites that are involved in metabolic pathway of 5-FU were significantly upregulated in 5-FU resistant organoid. We are carrying out more extensive studies, such as expression levels of stem cell and drug resistance related markers, morphological change and so on.

▲TOP

P-3135

Organoids with Cancer Stem Cell-like Properties Secrete EpCAM-Exosomes and HSP90 in a 3D NanoEnvironment

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Ability to form cellular aggregates is a morphological marker of cancer stem cells (CSC). We examined morphologies of 67 cell lines cultured on three dimensional (3D) morphology-enhancing NanoCulture Plates (NCP) and classified the types of cellular aggregates. Among these, 49 cell lines formed spheroids whereas 8 cell lines formed grape-like aggregation (GLA). Seven GLA-forming cell lines were derived from adenocarcinoma among the 8 lines. A neuroendocrine adenocarcinoma cell line PC-3 formed GLA with ductal structures on the NCPs and rapidly growing asymmetric tumors that metastasized to lymph nodes. Culture in the 3D nanoenvironment enabled the neuroendocrine adenocarcinoma cells to form slowly growing organoids that expressed multiple stem cell markers, neuroendocrine markers, adhesion molecules, and oncogenes. The more commonly used 2D environment reduced intercellular adhesion and induced mesenchymal transition and promoted rapid growth of the cells. Of note, the 3D stemness nanoenvironment promoted secretion of HSP90 and EpCAM-exosomes, a marker of CSC phenotype, from the neuroendocrine organoids.

P-3136

Organoid culture of pancreatic acinar cell carcinoma

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Pancreatic acinar cell carcinoma (PACC) is a rare type of cancer, accounting for only 1% of pancreatic tumors. Its etiology remains poorly understood, partly due to the lack of established cell lines for detailed analyses. Matrigel-based 3D culture is an emerging technique that enables long-term propagation of both normal and tumor cells in a physiological condition, which has been applied to many research fields. We here report that the organoid culture of patient-derived PACC from a particular patient was technically feasible. Organoids were propagated from the biopsy, bile, and surgically resected tumor, which were taken at different time points from the patient. Upon inoculation in subcutis of nude mice, the organoids developed tumors, confirming its malignant features. Moreover, they retained similar histological properties as the primary tumor from the patient. Thus, we concluded that organoids indeed derived from PACC. To the best of our knowledge, this is the first report for 3D organoid culture of patient-derived PACC. These newly established cell lines will likely provide clues for analyzing not only PACC but also other diseases like diabetes mellitus or pancreatitis.

P-3137

Patient derived sarcoma models: investigation of proteome profiling and applied to FDA-approved drugs screening

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Sarcomas are malignant mesenchymal tumors, arising from almost everywhere in human body. Sarcomas are also rare malignancies and complex disease; accounting for less than 1% of all malignancies, and they comprise of more than 50 histological subtypes which exhibit different biological and clinical features. Thus, in terms of its diversity, rarity, and complexity, sarcomas are very challenging malignancies. A lack of in vitro models profoundly hindered sarcoma research. We launched a project to develop the patient-derived sarcoma models in 2014. Challenging with surgical specimens from 240 sarcoma patients, 40 patient-derived xenografts and 30 cell lines were established. These cancer models were included in sarcomas whose models had not been established before. Using the models, we examined the effects of anti-cancer drugs which were approved in the use of treatments of other malignancies, but not previously tried in sarcomas. Additionally, the transient proteome profiling of the cancer models were revealed during the establishment. Further establishment of cancer model will be continued, and much better uses of the model will be achieved in our challenging.

▲TOP

P-3138

Establishment and characterization of cell line (UROC-1) originating from a human renal cell carcinoma

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A new human renal cell carcinoma cell line, designated UROC-1, was established from the right renal tumor of a 54-year-old man. UROC-1 was successively subcultured in 212 months. The monolayer cultured cells appeared to be epithelial and had tendency to pile up without contact inhibition. The cytology revealed anaplastic and pleomorphic features. The chromosomal number shows aneuploidy and the modal chromosomal number are 32 and 63. The population doubling time was 60 hours, the saturation density was 7.6×10^4 cells/cm^2, the plating efficiency was 2.5% and the mitotic index was 9.8%. The UROC-1 cells were transplanted subcutaneously to SCID mice and produced tumors that resembled the original tumor. Ten thousand UROC-1 cells did not produce tumor markers (CA 125, CA 19-9, CEA, HCG, SCC, AFP, TPA) during 7 days in culture media. UROC-1 may be useful in investigating renal cell carcinoma.

P-3139

Characterization of human prostate cancer LNCaP sublines differing in androgen-sensitivity

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The reduced androgen-sensitivity of prostate cancer (PCa) cells is associated with the cell' progression to castration-resistant prostate cancer (CRPC). Tumor stroma is enriched in fibroblasts secreting androgen receptor (AR)-activating factors. In this study, we used an androgen-sensitive human PCa cell line (LNCaP) and its sublines (androgen-low-sensitive E9 and F10 cells, and androgen-insensitive AIDL cells) to investigate the responsiveness to fibroblasts. Cell growth of E9 and AIDL cells was significantly increased by co-culturing with fibroblasts, while that of F10 cells was not affected. In the condition of co-cultures with fibroblasts, PSA production was directly increased in only E9 cells. Cell growth of E9 and AIDL cells was directly increased by treatment with several growth factors and cytokines. Tumor growth in E9 cells + fibroblasts became diminished post castration but that in F10 or AIDL cells + fibroblasts was gradually increased even post castration. Serum PSA was gradually increased even in F10 cells inoculated alone because of AR-V7 expression. The use of LNCaP sublines may allow us to investigate the characteristics of PCa cells progressing to CRPC.

▲TOP

[P-3147] P11-13 [English/Japanese]
Cell-to-cell interaction (2)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

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P-3147

Purification and structural analysis of a fusion factor derived from a cell line infected with murine leukemia viruses

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Cell fusion occurs in wide varieties of essential biological phenomena such as endocytosis, phagocytosis, etc. Furthermore, cell fusion is very useful in making hybrids and genetic analyses. We reported that syncytium formation of RFL cells was induced by infection of murine leukemia viruses in 1997. Although cell fusion induced by paramyxoviruses, herpes virus and retroviruses, which are viruses with envelopes composed of lipids and proteins same as cell membranes, has been widely studied, the mechanism and actual factors that induce cell fusion have not been unveiled.
In this study, we purified a substance that induces cell fusion in culture media of RFL cells infected with murine leukemia viruses by column chromatography after obtaining concentrated media and further determined the structural formula of this factor. This purified factor induced cell fusion as well as viruses or cells infected with viruses. It was also confirmed that cell fusion was induced by this factor in other cell lines as well.
We expect this fusion factor could have a potential for development of novel antitumor agents and also for application as one of gene transfer tools.

▲TOP

P-3148

Development of a treatment strategy for liver metastasis of CRCs by targeting a factor secreted from liver stromal cells

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Metastasis is the major cause of cancer mortality. Hence, development of effective treatment for cancer metastasis is needed. In 1989, Paget's "seed and soil" theory explained the importance of interaction between cancer cells (seed) and organ microenvironment (soil) when cancer cells metastasize. However, it is unclear whether which types of cancer cells preferentially interact to certain organs. To answer this, we implanted several different organ-derived human cancer cells into some organs of nude mice, which are preferred sites for metastasis. The results showed that colorectal cancer cells (CRCs) grow faster than other cancer cells in the liver, compared to the growth in the subcutaneous site. We then established the liver stromal cells from mouse liver tissue and found that the culture supernatant of liver stromal cells accelerates growth of CRCs in vitro. Because, the same phenomena were observed using human liver stromal cells, we explored the secreted factors and identified protein X, which enhances the growth of CRCs by itself. These data suggest that X might be involved in the preferential interaction and targeting X may be effective against CRC liver metastasis.

P-3149

Adhesion molecule CADM1 is an osteoblastic differentiation marker for EMT in carcinomas

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Epithelial carcinoma occasionally has the sarcomatous component and manifests as sarcomatoid tumors. These lesions are believed to emerge as a result of epithelial-mesenchymal transition (EMT) of carcinoma cells. Cell adhesion molecule 1 (CADM1) is expressed in not only epithelial cells, but also mesenchymal osteoblasts . We performed immunohistochemistry for CADM1, osteoblastic markers (Osterix, CD151 and alkaline phosphatase) and EMT markers (vimentin and N-cadherin) using sarcomatoid carcinomas (n=8) and the sarcomatous component in various carcinomas (n=11). Almost all of these sarcomatous lesions were EMT marker-positive, suggesting EMT may be involved in these sarcomatous changes. Immunohistochemical stains were scored for each of CADM1 and three osteoblastic markers, and were summed to speculate the degree of osteoblastic differentiation; then we analyzed correlation between the total scores and each score of the markers. Notably, CADM1 expression was significantly associated with larger total scores. These results suggest that osteoblastic differentiation occurs during EMT in carcinomas and that CADM1 is a useful marker for identifying this phenomenon.

P-3150

Three clones derived from a spontaneous mouse sarcoma representing distinct immunogenicity and vasculogenic activities

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We established 3 cell clones from a spontaneous, subcutaneous sarcoma developed in an old female C57BL/6-EGFP mouse. Fibroblast-like cells grew from pieces of the tumor tissue in vitro and the bulk cells developed tumors in wild type C57BL/6 (WT) mice. We then got 3 clones, currently named B12, D2 and D11 respectively, by limiting dilution. All clones represented similar growth rate in vitro, however, tumorigenicities of 3 clones were different. In immuno-competent syngeneic WT mice, both B12 and D11 tumors were rejected within 30 days. On the other hand, B12 and D11 tumors stayed dormant and pale for more than 50 days, then grew progressively in immuno-deficient nude, or CD8(+) lymphocyte-depleted WT mice. D2 tumors grew faster in nude mice and color of the tumors was reddish at an early stage of tumor development. Immunofluorescence studies represented higher expression of CD31, a marker of vascular endothelial cells, in D2 tumor as compared to B12 and D11 tumors. These single tumor-derived 3 cell clones that have different immunogenicities and vasculogenic activities will be useful in the study of tumor immunology and angiogenesis.

▲TOP

[P-3156] P12-7 [English/Japanese]
Innate immunity (1)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Shoichi Hazama / TR & Develop. Therap. against Cancer, Yamaguchi Univ.

P-3156

Granulocytes-mediated phagocytosis in insect

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Mammals have evolved humoral and cellular immunity by developing non-adaptive and adaptive antigen-antibody responses. However, most insects have only innate immune response that does not have adaptive antigen-antibody responses. Therefore, the immune response in insect have well developing by rapid, immediate, maximal immune responses to kill pathogens. In particular, the cellular immune response by the hemocytes includes various functions such as phagocytose, encapsulated, and nodulate photogenic microorganism. Insects usually have four to seven hemocyte types and among these types, the granulocytes are considered as main hemocyte in cell-mediated immunity. In this study, we used various microscopes, molecular probes, and flow cytometric analyses to characterize the hemocytes in several insects. Then, we identified the professional phagocytes, granulocytes, which mediate encapsulation and phagocytosis of pathogens. In addition, we showed that the phagocytosis by granulocytes is associated with cell death mechanisms such as autophagy or apoptosis that could be an efficient way to eliminate pathogens in this system.

▲TOP

P-3157

Dendritic Cell-Derived Exosomes Increase the Efficacy of anti-PD-L1 Antibody in Melanoma Model

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Immunotherapy is a promising treatment for metastatic melanoma. However, treatment of melanoma with an immune checkpoint inhibitor, such as anti-PD-L1 antibody, has lower response rate in patients. Dendritic cells play a critical role as central orchestrators of the immune response. Dendritic cell-derived exosomes might inherit some special functions from dendritic cells and exert these functions in vivo. We find that microRNAs and proteins are present in dendritic cell-derived exosomes and could affect other immature dendritic cells. Then, we induced melanomas in C57BL/6 mice and then intravenously injected dendritic cell-derived exosomes into mice, observing that the combination of dendritic cell-derived exosomes enhanced the efficacy of anti-PD-L1 antibody, but treatment with either dendritic cell-derived exosomes or anti-PD-L1 antibody alone, did not. These were suggested that combination of dendritic cell-derived exosomes and anti-PD-L1 antibody will significantly improve overall survival time.

P-3158

Targeting M-MDSCs to relieve aggressive liver fibrosis-associated hepatocellular carcinoma development

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Liver fibrosis is a major pathological precursor of hepatocellular carcinoma (HCC), which exhibits low response rate to immunotherapy. Here, we identify monocytic myeloid derived suppressor cells (M-MDSCs) as pivotal immunosuppressive cells that contribute to aggressive HCC in fibrotic liver. Mechanistically, activated hepatic stellate cells induce M-MDSC generation by activating myeloid-intrinsic p38 MAPK, which represents a nexus of fibrotic liver-elicited signaling inputs to trigger enhancer remodeling for M-MDSC development and function. In a preclinical fibrotic HCC mouse model, treatment of p38 MAPK inhibitor significantly decreased liver-infiltrating M-MDSCs and prevented HCC tumorigenicity. Clinically, the proportion of M-MDSCs was significantly higher in the tumor-surrounding fibrous livers than the non-fibrous counterparts of HCC patients. As we also showed profound suppression of HCC patient-derived M-MDSCs by a clinically-trialed p38 MAPK inhibitor, our findings may facilitate the development of combination strategies to reinstate immunosurveillance in immune-refractory HCCs. Acknowledgement: This work is supported by the RGC CRF (C4017-14G) and HMRF (03141376).

P-3159

Administration of IL-18 sustains recruitment of effector-like NK cells into the tumor microenvironment in animal models

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In the present study, a more detailed mechanism of IL-18 action was investigated.WT or IL-18KO mice (BALB/c) were inoculated (ip) with CT-26 colon tumor cells, and C57BL/6 mice were injected (iv) with B16 melanoma cells. At appropriate days, mice were treated by anti-PD-1 Ab with or without IL-18 for several weeks, and examined for survival, tumor growth, and cellular component in the tumor microenvironment. The results indicated that IL-18 significantly reduced the number of nodules in the peritoneum or lung, and prolonged overall survival of mice. By the treatment of combination of anti-PD-Ab and IL-18, the number of DX5+/B220+ NK cells with effector phenotypes was increased in the spleen, lung, and ascites, and this was maintained while IL-18 was administered. However, after termination of IL-18 injection, NK cells with features of exhaustion began to increase and finally became dominant. These results indicated that IL-18 has an important role in NK-cell-based cancer immunotherapy, and suggested that combination of IL-18 and immune-checkpoint inhibitors is beneficial for cancer immunotherapy.

▲TOP

P-3160

The significance of lymph node macrophages in anti-tumor immune response

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It is well known that many macrophages are distributed in lympho-reticular organs including spleen and lymph node (LN). CD169 is specifically expressed on macrophages, including lymph node sinus macrophages. Animal studies suggested that CD169-positive lymph node sinus macrophages (LySM) have tumor preventing properties. We investigated the significance of CD169-positive LySM in malignant tumors including malignant melanoma, colorectal cancer, endometrial cancer, breast cancer, bladder cancer, and esophageal cancer. The high expression of CD169 on LySM was found to be significantly associated with a longer overall survival in some cancers. Positive correlations were noted between the expression of CD169 in LySM and the density of CD8-positive cytotoxic T cells in tumor tissues. In vitro studies using human macrophages demonstrated that CD169 expression was up-regulated by interferons (IFNs), and this indicates high expression of CD169 is linked to high production of IFNs in lymph node. CD169 in lymph node may be a useful marker for assessing the clinical prognosis and monitoring antitumor immunity in patients with malignant tumors.

P-3161

Development of a new innovative multifunctional immune checkpoint inhibitor

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Blockade of PD-1, PD-L1 or CTLA-4 is an attractive strategy for Immuno-Oncology (I-O) therapy. Here, we developed a novel alkylating Pyrrole-Imidazole polyamide capable of disrupting expressions of human PD-1, PD-L1 and CTLA-4 genes (CCC07-01) by binding to a common motif. CCC07-01 administration significantly suppressed PD-1, PD-L1 and CTLA-4 mRNA and protein expressions at low nanomolar doses in several cell lines and should share a characteristics of enhanced permeability and retention (EPR) effect. Additionally, elevated doses of CCC07-01 induced substantial cytotoxicity. Preliminary results from human RKO colon carcinoma xenograft NGS mice, following the engraftment of human HLA-matched peripheral blood mononuclear cells (PBMC), showed significant tumor reduction 14 days after the CCC07-01 injection and displayed detectable amounts of predominant effector memory CD8 T cells in the spleen 14 days post-administration. The induction of tumor- and tumor-environment-specific combinatorial I-O therapy and preferable cancer cell cytotoxicity by only a single agent of CCC07-01 may be a promising anti-cancer therapeutic strategy minimizing adverse effects.

P-3162

VISTA expressed in tumor cells regulates T cell function

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V-domain Ig suppressor of T cell activation (VISTA) is a novel inhibitory immune checkpoint protein. Its expression in tumor cells and its role in antitumor immunity have not been fully characterized. In this study, we investigated VISTA expression and function in tumor cells and evaluate its mechanism and activity.A series of in vitro assays were used to determine the function of tumor-expressed VISTA. In vivo efficacy was evaluated in syngeneic models.VISTA was highly expressed in human ovarian and endometrial cancer specimens. In particular, upregulation of VISTA in endometrial cancers was related to the methylation status of the VISTA promoter region. VISTA in tumor cells suppressed T-cell proliferation and cytokine production in vitro and decreased the abundance of tumor-infiltrating CD8+ T cells in vivo. Treatment with anti-VISTA antibody prolonged survival of tumor-bearing mice.VISTA is widely expressed in human ovarian and endometrial cancer cells and regulates T-cell distribution and function in tumor sites. Therefore, it represents a novel therapeutic target for gynecologic cancers and other solid tumors.

▲TOP

[P-3168] P12-9 [English/Japanese]
Dendritic cells / antigen-presenting cells

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Shinichi Kageyama / Immuno-Gene Ther. Mie Univ. Grad. Sch. Med.

P-3168

Cancer Vaccine Therapy Using CEA expressing Dendritic Cells generated from Induced Pluripotent Stem Cells

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The clinical application of dendritic cells (DCs) vaccine therapy is hindered by the need for a large quantity of DCs generated from the peripheral blood monocytes of patients. We investigated whether genetically modified human iPS cells-derived dendritic cells (hiPSDCs) expressing CEA could induce CEA-specific cytotoxic T cells in a human model, and whether genetically modified mouse iPSDCs (miPSDCs) expressing CEA would show an actual antitumor effect using a CEA transgenic mouse model. We differentiated hiPSDCs from iPSCs of three healthy donors and transduced the CEA cDNA into hiPSDCs. The cytotoxic T cells induced by hiPSDCs-CEA exhibited CEA-specific cytotoxic activity against the target cells expressing CEA. Furthermore, in the CEA transgenic mouse model, the cytotoxic T cells generated in mice immunized with miPSDCs-CEA showed CEA-specific cytotoxic activity against MC38-CEA. In the subcutaneous tumor model, vaccination with miPSDCs-CEA achieved a significant growth inhibitory effect on MC38-CEA. Genetically modified iPSDCs expressing CEA is a promising tool for clinical application on vaccine therapy against gastrointestinal cancer patients (Sci Rep 2018).

▲TOP

P-3169

Immune sensitivity of tumor is governed by the mechanism for differentiation of tumor-associated macrophages (TAMs)

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Currently, novel therapeutic strategies for the treatment of low immunogenic tumors are required in the field of cancer immunotherapy. Our previous work showed that an immune resistant murine CMS5a tumor does not possess immunogenic antigens and that tumor-associated macrophages (TAMs) in resistant CMS5a tumor stay in inactive state with limited antigen-presenting capacity as compared with those from immune sensitive tumors. TAM-targeted delivery of nanoparticles loaded with a tumor antigen peptide in combination with a TLR agonist followed by the transfer of tumor-specific CD8+ T cells resulted in augmentation of antigen-presenting capacity of TAMs, leading to the eradication of resistant CMS5a tumor. Thus, the importance of antigen presentation activity of TAMs in immune sensitivity of tumor was revealed; however, the differentiation mechanism(s) enabling TAMs to exert potent antigen-presenting activity was still unclear. In this study, we tried to identify TAM differentiation factors governing immune sensitivity of tumor. By whole gene expression analysis, we found that interferon-gamma signaling pathway contributed to the acquisition of antigen-presenting activity by TAMs.

P-3170

The basic research for a cancer vaccine therapy using iPS-derived dendritic cells

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We have employed a study of a cancer vaccine therapy using genetically modified DCs expressing tumor-associated antigen (TAA) gene. Clinically DCs are generated from peripheral blood monocytes of the patients. Thus the number of monocytes and the potential of them to differentiate into DCs are limited. Therefore, we have induced iPSDCs from murine iPS cells and clarified that genetically modified iPSDCs have an equal efficacy and therapeutic antitumor immunity to naive DCs. And we also have clarified that genetically modified iPSDCs expressing CEA gene have a TAA-specific therapeutic antitumor immunity. Now, we are engaging a new pre-clinical study using iPSDCs with mRNA instead of one TAA. We are examining whether a vaccine therapy using iPSDCs with mRNA including neoantigens can induce stronger therapeutic antitumor immunity. We transduced mRNA derived from B16 and CT26 to iPSDCs. We are examining the cytotoxic activity of CD8(+) CTLs by genetically modified iPSDCs and the efficacy of the vaccination in tumor models. We will report that the vaccination using iPSDCs with mRNA may be useful clinical applications as a cancer vaccine therapy.

P-3171

Development of dendritic cell-based immunotherapy using tumor endothelial cells as vaccine antigens

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Tumor blood vessels, constructed by tumor endothelial cells (TECs) play an important role in tumor growth. In comparison, angiogenesis also occurs under normal physiological conditions such as wound healing and in the formation of the corpus luteum with the implication that the clinical application of antiangiogenic agents in cancer patients may result in damage to normal blood vessels. Therefore, the selective inhibition of tumor angiogenesis is expected to result in the development of cancer therapy with fewer side effects. In this study, we have attempted to develop a dendritic cell (DC) vaccine therapy using TECs as antigens. DC vaccination using TECs as antigens significantly suppressed tumor progression and lung metastasis. Additionally, in TEC/DC vaccine-treated mice, the formation of new blood vessels allowing blood flow to tumor tissues during tumor growth was suppressed. On the other hand, vaccination with DCs pulsed with TECs did not inhibit physiological angiogenesis as evidenced by a wound-healing assay. Thus, these results show that DC vaccine therapy targeting TECs is an effective therapy against tumor angiogenesis, but does not affect normal blood vessel growth.

▲TOP

P-3172

Dendritic cell derived-exosomes activate immune systems by transferring exosome-involved factors to T cells

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Exosomes released from dendritic cells (DCs) are responsible for the persistence of antigen presentation. So, we considered that whether DCs-derived exosomes could induce suppress cancer cells and more effective response of an immune system and what factors in exosomes-involved DCs can activate T cells. Luciferase gene transferred-3LL cells (murine lung cancer cell line derived C57BL/6) were injected into C57BL/6J mice by intraperitoneal administration. And then, DCs, DCs-exosomes or 3LL-exosomes were weekly administrated to lung cancer-bearing mice. The exosomes derived from DCs decreased lung cancer cell growth in compared with DCs, DCs-exosomes and non-treated. DNA microarray analysis data showed that 44 genes increased as ratio LPS-treated DC mRNA vs non-treatment DC one. Western blot analysis showed one of the genes contained higher in exosomes derived from LPS-treatment DCs than that derived from non-treatments. This gene induces T cell proliferation and signals for T cell maturation. We concluded that DCs derived-exosomes activate anticancer immune systems by transferring exosome-involved the factor to T cells.

▲TOP

[P-3179] P12-11 [English/Japanese]
Other immunotherapies (2)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Mamoru Harada / Dept. Immunol., Shimane Univ. Facult. Med.

P-3179

Inflammatory soluble factors as potential biomarkers in non-small cell lung cancer treated with anti-PD-1 inhibitors

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No biomarkers are currently available for predicting clinical benefit from anti-PD-1 treatment in non-small cell lung cancer (NSCLC). Here we attempted to identify potential biomarkers in peripheral blood of patients with advanced NSCLC treated with anti-PD-1 therapy. The levels of 88 different soluble factors in plasma were assessed before and after anti-PD-1 treatment, and their relationships with clinical outcomes were statistically analyzed in two independent cohorts (training, n = 27; validation, n = 50). The levels of chitinase 3-like-1 and GM-CSF before treatment as well as the changes in CXCL2, VEGF, IFNA2, and MMP2 levels after treatment were identified as potential prognostic factors in the training cohort. In contrast, only changes in the levels of CXCL2 and MMP2 were validated in the validation cohort. A decreasing CXCL2 level and an increasing MMP2 level were significantly associated with better PFS, and were retained in long responders during the course of anti-PD-1 therapy. Since these inflammatory soluble factors identified in plasma can be easily measured by minimally invasive blood sampling, they could be clinically applicable as prognostic biomarkers.

▲TOP

P-3180

NK cell therapy in combination with IgG1 antibody in patients with gastric/colorectal cancer: A phase I clinical trial

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Background; We have reported our original expanded Natural killer (NK) cells were safe for monotherapy (J Transl Med (2015) 13:277). This phase I clinical trial evaluated the safety, toxicity, and immunological responses of NK therapy in combination with IgG1 antibody(Ab) focused on ab-dependent cellular cytotoxicity (ADCC).Patients and Methods; Patients with unresectable advanced gastric or colorectal cancer who have administered or planned to administer IgG1 ab with or without anti-cancer cytotoxic drugs. NK cells were injected 3 days after IgG1 ab administration in a dose-escalating manner (dose 0.5, 1.0, 2.0 $\times$ 10^9 cells/injection, three patients/one cohort), every 3 weeks for 3 cycles. We evaluated the safety, efficacy and the immunological responses.Results; 9 eligible patients were enrolled. This therapy was well tolerated. Among 6 evaluable patients, 4 presented SD, 2 presented PD. About immunological responses, whole blood IFNr production level and several serum cytokines was fluctuated and the proportion of Treg (Treg/CD4) was decreased.Conclusions; Our data provide evidence of good tolerability and anti-tumor activity and Th1-type immune response and reduced Tregs.

P-3181

STING is dispensable for low susceptibility for HF10 in pancreatic cell lines

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Therapy with oncolytic viruses (OVs) is one of the most promising anti-cancer treatments. We have reported that the efficacy and safety for patients with unresectable pancreatic cancer in phase I clinical trials of HF10, which is a spontaneously occurring herpes simplex virus type 1 mutant. Although therapy by HF10 has demonstrated superior results to the standard therapy, the response rate was not high enough. Most recently, it has been implicated that low expression level of cGMP-AMP synthase (cGAS) or stimulator of interferon genes (STING) in cancer cells could be associated with bad prognosis but also associated with low susceptibility to viral infection. However, it is still unclear if cGAS or STING actually contributes to susceptibility in cancer cells. Here, we show that there is little correlation between cGAS or STING pathway and susceptibility to virus in human pancreatic cancer cell lines. Knockout of cGAS or STING in cells with high susceptibility resulted in no difference in susceptibility to virus and vice versa. Now we are examining in detail if STING pathway is properly activated during response against virus infection.

P-3182

Relationship between regulatory T cells and Helicobacter pylori infection in gastric cancer

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<Background and Aim> Regulatory T cells (Tregs) with highly suppressive function in tumor immunity are characterized in a subpopulation of $Foxp3^+$ $CD4^+$ cells. We have reported that the expression of ICOS is a useful marker for functional Tregs in gastric cancer. In this study, we investigate the pathway for the induction of $ICOS^+$ Tregs in association with Helicobacter pylori (HP) infection. <Materials and Methods> Immune related cells infiltrated in gastric cancer tissues were purified and expression of ICOS and ICOS-L in $Foxp3^+$ cells and plasmacytoid dendritic cells (pDCs), respectively, were analyzed by flow cytometry and multicolor immunohistochemistry. HP antibody in patients' sera was detected by ELISA. <Result> Significant positive relations were observed among ICOS in $Foxp3^+$ cells, ICOS-L in pDCs and HP antibody positivity each other. Furthermore, $ICOS^+$ Tregs were significantly lower in HP eradicated patients. <Conclusion> HP infection, mediated by $ICOS^+$ Tregs, might have negative effect on tumor immunity in established gastric cancer. The HP eradicating therapy could be a potential immune therapy for gastric cancer.

▲TOP

P-3183

Peripheral T cell activity is a potential predictor for T cell function in the tumor microenvironment

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Purpose: Immune checkpoint inhibitors exerts beneficial effects in cancer patients. Companion diagnostics are needed in order to identify patients for whom these therapies are effective. We evaluated T cell function in tumor tissues and analyzed their relationship with peripheral blood T cells for the development of blood-based companion diagnostics. Methods: In order to evaluate the cytotoxic activity of T cells in fresh lung tumor tissue, we developed an assay system using bispecific T-cell engager (BiTE). We analyzed the immune profiles, T cell cytotoxicity, and TCR repertoire of peripheral blood and lung tumor tissue from non-small cell lung cancer (NSCLC) patients. Results: Among various factors in peripheral blood, we found that the cytotoxicity of peripheral T cells closely correlated with that of tumor-infiltrated T cells. We also found that the cytotoxicity of peripheral T cells has potential as a predictor of the effects of nivolumab in the tumor microenvironment. Conclusions: We demonstrated that the cytotoxicity of tumor-infiltrated T cells closely correlated with that of peripheral T cells. These results imply further applications to blood-based companion diagnostics.

P-3184

Combination of a STING ligand cGAMP and the COX-2 inhibitor celecoxib induces antitumor effects

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We have previously reported that intratumoral administration of a ligand for stimulator of IFN genes (STING) results in antitumor effects mediated by accumulation of $CD11b^{mid}Ly6C^{+}$ cells in the tumors. Myeloid cells (i.e., TAMs and MDSCs) often infiltrate intensively in the tumors and play a role in promoting tumor growth and suppressing antitumor immunity. Recent studies have demonstrated that TAMs frequently up-regulate COX-2 expression, thereby producing high amounts of PGE2, which is one of the key factors in development and accumulation of MDSCs. Inhibition of PGE2 biosynthesis delays tumor progression and reverts immunosuppressive functions of MDSCs. We therefore investigated whether a combination of a STING ligand cGAMP and the COX-2 inhibitor celecoxib augments antitumor effects. In vivo studies using murine tumor models showed that the combination therapy significantly inhibited tumor growth compared to monotherapy with cGAMP or celecoxib. The antitumor effect of the combination therapy was not abrogated by treatment with anti-CD8 mAb or clodronate liposomes. Further investigations are needed to determine the mechanisms underlying the effects of the combination therapy.

P-3185

mySORT: A web framework by using Deconvolution Approach to Estimating Immune Cell Composition from Complex Tissues

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MySort is a web implement for resolving relative proportions of twenty-one immune cell subclasses from a human tissue profiled transcriptome by microarray technology. We use statistical methods to select signature genes as features and then adopt ν-Support Vector Regression to construct a deconvolution model. The resolved proportion from uthe ser-uploaded dataset is in a column-wise layout as well as visualized of a bar chart with hierarchical clustering among submitted data. The diversity of immune cell components is estimated in alpha diversity (diversity of composition in a sample) and beta diversity (diversity of composition among samples). The performance of our system was finally evaluated using blood biopsies from 20 adults, in which 9 immune cell types were identified using flow cytometry. The present computations performed better than current state-of-the-art deconvolution methods. MySort is available at http://symbiosis.iis.sinica.edu.tw/mySORT/

▲TOP

[P-3192] P12-13 [English/Japanese]
Antitumor effector cells and their induction (2)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Kazunori Aoki / Dept. Immune Med. Natl. Cancer. Ctr. Res. Inst.

P-3192

CAR-T Cell Screening in Tumor Spheroids

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CAR-T cells, which are engineered to recognize target cell surface antigens expressed on tumor cells, have shown promise to affect complete remission in patients with B-cell malignancies. However, applying this approach to target solid tumors has resulted in adverse effects in clinical studies due to a toxicity of the normal tissues. Methods for testing different models of CAR-T cells *in vitro* can provide further insight into viable antigen targets before these models reach the clinical stage. Historically, 2D cell culture models have been used in drug discovery for the development of cancer therapeutics due to their ease of use and established compatibility with high throughput screening. Recently, more elaborate, 3D cell culture models have been developed, which better mimic the *in vivo* tumor microenvironment. Corning spheroid microplates are multiple well microplate with Ultra-Low Attachment surface coating. In coordination with DiscoverX KILR Cytotoxicity assay and ProMab Biotechnologies EGFR scFv-CD28-CD3ζ CAR-T cells, a novel assay system was investigated if it would be used as a high throughput screenable CAR-T cell assay that targets tumor spheroids.

▲TOP

P-3193

Structure-activity correlation analysis by using 2nd generation CARs with replacements of signal transduction domain

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Although CAR-T cell therapy has attracted attention as an innovative cancer treatment, it is unclear which CAR component contributes to which CAR-T cell function. To establish the theory for ideal CAR design and fabrication, we are promoting the correlation analysis between CAR structure and CAR-T cell function by using CAR variants. Herein, we compared the expression and functions among 2nd-generation (2G) CARs that possess an intracellular component in which CD3 ζ -derived signal transduction domain (STD) is tandemly linked after the STD derived from various T-cell costimulatory molecules. The expression intensity of the 2G CARs in murine T cells tended to be slightly lower than the first-generation CAR containing CD3 ζ -STD alone. The expected effect of the added 2nd STD was not always reflected in the function of 2G CAR-T cells; for example, some 2G CARs induced a reduction of cytotoxic activity or a proliferation activity independent on the antigen stimulation. These results indicated that the adding multiple STDs to CAR aimed at enhancing CAR-T cell function would require further ingenuity and methodology to keep intracellular components of CAR in proper conformation.

P-3194

Investigation of affecting factors for abscopal effect with radiotherapy

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BACKGROUND: Radiotherapy (RT) is one of the major therapeutics for the cancer treatment. Abscopal effect is known as tumor regression at a site distant from the irradiated lesions, but it rarely occur in the clinical cases. Recently, combination of RT and immuno-check point inhibitor is considered to enhance the abscopal effect. However, little is known about the factors on abscopal effect. METHODS: MC38 cells were subcutaneously injected into C57BL6 mice (n=6or8) at two sites on their back. The tumor in one side was irradiated and the other was not irradiated, and the both tumor sizes were measured periodically. The combination effect of anti-PD-1 antibody to RT on abscopal effect were also examined (n=6or8). We investigated the factors that influence abscopal effect. RESULTS: Abscopal effect was observed in some mice. It was enhanced by radiation dose- and/or tumor volume-dependently. Moreover, combination of RT with anti-PD-1 antibody significantly enhanced abscopal effect. CONCLUSION: RT dose, tumor volume and anti-PD-1 antibody were the affecting factor on abscopal effect. Further study is needed to elucidate the mechanism of abscopal effect.

P-3195

Immune profile of thymoma and thymic carcinoma

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Purpose: Cancer immunotherapy including immune checkpoint inhibitors exerts beneficial effects in cancer patients. However, the rationale of immunotherapy for thymoma and thymic carcinoma has not been well evaluated. Therefore, we analyzed immune profile of thymoma and thymic carcinoma as the basis for understanding immune responses in the tumor microenvironment. Methods: Fresh tumor samples from 17 patients were analyzed including 15 patients with thymoma and 2 patients with thymic carcinoma. The immune profiling of these tumors was evaluated by using multicolor flow cytometry. Results: The ratios of Tim-3+ and CD103+ in CD8 single positive T cells was higher in B3 thymoma and thymic cancer than AB, B1 and B2 thymoma ($p = 0.001$ and $p = 0.0003$, respectively). The ratio of regulatory T cells in CD4 single positive T cells was also higher in B3 thymoma and thymic cancer than AB, B1 and B2 thymoma ($p<0.0001$). Conclusions: We demonstrated the significant correlations between histology and immune profile. These results suggest the potential of immunotherapy for higher grade thymic tumors.

▲TOP

P-3196

Galectin 9, a ligand of an immune checkpoint TIM-3, is promising antigen that induces highly active CTLs against RCC

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We previously identified an RCC-antigen, galectin 9, probed by sera of RCC patients who responded cytokine therapy. Galectin 9 is expressed at higher level among all the clear cell carcinoma specimens examined (18/18). Galectin 9 is a ligand of an immune checkpoint TIM-3 (T cell immunoglobulin and mucin domain 3), of which activation causes tumor immune escape, and reported to be expressed in hematological malignancies. In this report, we show that HLA-A*2402-restricted, HLA-A*0201-restricted, and HLA-A*33-restricted cytotoxic lymphocytes (CTLs) against RCC were induced by galectin 9-derived peptides for each HLA-A type to exhibit specific and highly cytotoxic activities towards RCC cells. Specific CTLs were induced abundantly, as shown by flow cytometry analysis of the CTLs labelled with fluorescein isothiocyanate anti-CD107a and APC anti-CD8. The clonal expansion of the CTLs was shown by the clonality of T-cell receptor V β repertoires. In summary, induction of the CTL targeting galectin 9 seems promising therapy of RCC, the rationale being not only the high cytotoxicity of specific CTLs against cancer cells but also the inhibition of immune checkpoint TIM-3.

P-3197

The reasonability of the density of immune cells in H&E sections of colorectal cancer as the the immunological biomarker

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[Background] “Immuno Score” based on the density of tumor-infilrtating lymphocytes (TILs), in particular CD8+ and total T cells, have been reported to be associated with the clinical outcome in colorectal cancer. We have reported that the density of tumor-infiltrating immune cells (which was defined as TILs) in H&E sections were significantly associated with the clinical outcomes in colorectal cancer. However, the relationship between the density of TILs in H&E sections and that of TILs subsets still remains unclear.[Methods] 308 patients who underwent curative resection for stage II/III colorectal cancer were enrolled. We assessed the density of TILs in H&E sections and TILs subsets by IHC. [Results] The density of TILs in H&E sections were significantly associated with the density of tumor-infiltrating CD4+, CD8+ and total T cells (CD4+CD8).[Conclusions] The density of tumor-infiltrating immune cells in H&E sections was associated with the density of tumor-infiltrating CD8+ and total T cells which were key factors in “Immuno Score”. Therefore, the density of tumor-infiltrating immune cells in H&E sections may be a reasonable immunological biomarker.

P-3198

Anti-metastatic effect of thalidomide through the regulation of NK cell homeostasis

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Thalidomide have been used for the treatment of multiple myeloma, however the mechanism by which thalidomide show anti-tumor effect through natural killer (NK) cells remains unclear. In this study, we examined the role of NK cells in the anti-metastatic effect of thalidomide and its mechanism of action by focusing on in vivo NK cell homeostasis.We demonstrated the systemic thalidomide treatment strongly suppressed B16F10 melanoma lung metastases and the critical requirement of NK cells for the anti-metastatic effect of thalidomide. In align with the NK cell-dependent anti-metastatic effect of thalidomide, the proportion of terminally differentiated CD27lo NK cell subset were increased in the peripheral tissues of thalidomide-treated mice. In addition to the increase of CD27lo mature NK cells, we also observed the alteration in the expression of transcription factors that are known to regulate NK cell differentiation and maturation upon thalidomide treatment in vivo. Collectively, our results indicate that thalidomide may mediate its anti-metastatic effect through modulating in vivo NK cell homeostasis.

▲TOP

[P-3205] P12-15 [English/Japanese]
Cancer vaccine (2)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Yoshihiro Hayakawa / Inst. Nat. Med., Univ. Toyama

P-3205

Glypican-3 vaccine as an adjuvant therapy for hepatocellular carcinoma patients can prolong their overall survival

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[Introduction]Glypican-3(GPC3) is a specific marker for hepatocellular carcinoma(HCC) and GPC3 positive patients showed poor prognosis.[Method] A phase II trial of GPC3 vaccine as adjuvant therapy after HCC surgery was conducted from 2009 to 2012. We compared the prognosis between those 35 patients(A group) and 33 patients who underwent surgery alone(vaccine non-administered; B group) at the same time.[Result]GPC3 positive patients were 25(A) and 21(B). In B group, overall survival(OS) of GPC3 negative patients was better than GPC3 positive(HR3.1, 95%CI 1.1-8.6, P=0.029). In GPC3 positive patients,1-year recurrence rate was 24.0%(A) versus 52.4%(B). Early recurrence rate of A group was significantly lower than B group(p=0.047). On the other hand,5-year OS rate was 64.0%(A) versus 47.6%(B). OS was significantly increased in A group(HR 0.41, 95%CI 0.18-0.93, P=0.034). [Discussion] In GPC3 positive patients, A group showed better OS than B group,one of because re-exacerbation period of A group was longer than B group(8.5 vs 30.8 months, p=0.019).[Conclusion] It was suggested that adjuvant vaccine therapy for GPC3 positive patients can prolong their overall survival.

▲TOP

P-3206

Phase I clinical trial of peptide vaccine derived from HSP105 and analysis of immune response in vaccinated patients

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HSP105 is a polymeric heat shock protein belonging to the HSP105/110 family. We clarified that HSP105 is highly expressed in various cancer types and reported the utility as a tumor antigen. We identified two HLA-A2- and A24-restricted HSP105 peptides and conducted a Phase I clinical trial of HSP105 peptide vaccine for 30 patients with advanced esophageal or colorectal cancer. In the clinical trial, we succeeded in establishing two kinds of HSP105 peptide specific CTL clones from two patients, in whose PBMCs after vaccination, the frequency of HSP105 peptide specific CTL was increased.The one was established from PBMCs of a patient with colon cancer. It produced cytotoxic cytokines against HSP105 peptide pulsed cells and SW620 (HSP105+ colon cancer cell line). The other was obtained from resected lymph node of a patient with esophageal cancer, and which showed similar response against those cells. We demonstrated that these established CTL clones specifically recognized HSP105 peptide and exerted cytotoxicity against cells presenting HSP105 not only exogenously but also endogenously. In the future, we are considering TCR-T therapy using TCR repertoire obtained from the CTL clones.

P-3207

TCR repertoire analysis of peptide-specific T cells using immunospot array assay on a chip (T-ISAAC) technology

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Peptide vaccination has come into the limelight as a combination therapy of immune check point inhibitors. Confirmation of T cell reactivity to the peptide by ELISPOT assay is pre-requisite for the therapy. However, the assay has a major problem that T cell receptor (TCR) specificity of T cells detected in ELISPOT is not confirmed. To solve the problem, we developed a novel system, T-ISAAC (single cell manipulation system using microarray chip), that enables us to efficiently detect peptide specific T cells and verify the TCR antigen-specificity. In this study, we applied the T-ISAAC system to detect Wilms tumor antigen (WT-1)-specific T cells. We first stimulated HLA-A24$^+$ healthy donor's PBL with mutant (mut)WT-1 peptide and expanded mutWT-1-specific CD8$^+$ T cells. Then, we detected mutWT-1 peptide specific-T cells on a microarray chip and verified their TCR specificity to the peptide. We also confirmed that T-ISAAC system could be applied for analyzing class-II restricted CD4$^+$ T cells. These data suggest that T-ISAAC system could be a powerful tool for analyzing peptide-specific T cells of cancer patients who received peptide vaccination.

P-3208

NY-ESO-1 expression and antibody related to poor outcome in MAGE-A4-vaccinated esophageal and head/neck cancer patients

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MAGE-A4 antigen is one of the cancer-testis antigens frequently expressed in tumor tissues. Cholesteryl pullulan (CHP) is a novel antigen delivery system for cancer vaccines. This study evaluated the safety, immune responses and clinical outcomes of patients who received CHP-MAGE-A4-protein-complexed vaccine. Twenty-two patients with advanced/metastatic cancer were enrolled, and they were subcutaneously vaccinated with either 100 or 300 μg of CHP-MAGE-A4. No serious adverse events were observed. Two patients out of 7 (29%) who received 100 μg dose and 6 patients out of 15 (40%) who received 300 μg dose exhibited the responses. No survival differences were seen between 100 and 300 μg-dosed patients, or between immune-responders and non-responders. In 16 patients with esophageal or head/neck squamous cell carcinoma, the survival time was significantly shorter in patients who had NY-ESO-1-coexpressing tumors or preexisting antibody responses to NY-ESO-1. Limited immune spreading to NY-ESO-1 during CHP-MAGE-A4 vaccination shown in three patients did not improve survival. Therefore CHP-MAGE-A4 vaccination is recommended for MAGE-A4-expressing and NY-ESO-1-negative cancer patients.

▲TOP

P-3209

Immunohistological analysis of the unresectable pancreatic carcinoma after survivin 2B peptide vaccination

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Immune checkpoint inhibitor (ICI) is a great revolution in cancer treatment providing us the alternative options in addition to existing type of therapies. On the other hand, immune therapy with tumor-specific peptide vaccination is a still attractive way because of its expected specifity for malignant cell and less harmful side effect. We identified a 9 mer peptide sequence, that is survivin 2B, derived from survivin, an inhibitor of apoptosis protein family, as a candidate of tumor specific immunotherapy for the various cancer including pancreatic carcinoma. Consequently, survivin 2B vaccination-based phase II randomized clinical trial targeting unresectable and refractory pancreatic carcinoma have performed. It was unfortunate, however, that we could not have statistically significant prolonged progression free survival time. We performed autopsy analyses to investigate whether there was immunological effect in the local tumor microenvironment. Here, we show immunohistochemical analysis using formalin fixed paraffin embedded specimen derived from 13 cases of autopsies of pancreatic carcinoma including 7 cases with the vaccination.

P-3210

Evaluation of serum immune biomarkers for breast cancer patients who treated by personalized peptide vaccination

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Purpose: To evaluate the association between the immunologic factors including cytokines, haptoglobin (HP), B-cell activating factor (BAFF) etc. and the clinical outcome of patients(pt) with metastatic breast cancer (mBC) who treated by personalized peptide vaccines (PPV). Methods: Blood samples from 57 pts and 52 pts obtained before and after PPV treatment, respectively. Serum Hp and IL-6 were analyzed by ELISA. The bead-based multiplex assays were performed for GM-CSF,IFN- γ , BAFF, IL-8 etc. The association between each factor and clinical outcome was statistically evaluated. Results: Pt's group with low BAFF and high Hp level (>544 pg/ml), high IFN- γ and high Hp level or low IL-8 and high Hp level before PPV therapy showed a significantly shorter median survival time (MST; $p<0.05$). There was no difference in high BAFF, low IFN- γ or high IL-8 level group. In contrast, pt's group with low serum level of IL-10, GM-CSF, IL-5, IL-4 and low Hp level after PPV therapy associated with significantly shorter MST ($p<0.005$). Conclusions: The combination of serum levels of Hp and cytokines might be useful prognostic biomarkers for mBC pts who treated by personalized peptide vaccine.

▲TOP

[P-3218] P13-2 [English/Japanese]
Cytokines

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(A)/3F Event Hall, Osaka International Convention Center Room P(A)

Masao Saitoh / Ctr. for Med. Sci., Grad. Sch. Med., Univ. of Yamanashi

P-3218

Tumor necrosis factor-α induces prostate cancer cell migration in lymphatic metastasis via CCR7 upregulation

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Prostate cancer (PCa) is one of the most frequently diagnosed malignancies in men worldwide and the detection of lymph node metastasis indicates a poor prognosis for patients. Understanding the mechanism of lymph node metastasis and the further dissemination of the disease is important to develop novel treatment strategies. Recent studies have reported that C-C chemokine receptor 7 (CCR7), whose ligand is CCL21, is abundantly expressed in lymph node metastasis and promotes cancer progression. TNF-α is chronically produced at low levels within the tumor microenvironment, so we aimed to determine the relationship between TNF-α and the CCL21/CCR7 axis. First, human PCa cells were determined to express both TNF-α and CCR7. Low concentrations of TNF-α were next confirmed to induce CCR7 in PCa cells through phosphorylation of extracellular signal-regulated kinase. Finally, CCL21 was found to promote the migration of PCa cells *via* phosphorylation of the protein kinase p38. Our results suggest that TNF-α leads to the induction of CCR7 expression and that the CCL21/CCR7 axis may increase the metastatic potential of PCa cells in lymph node metastasis.

▲TOP

P-3219

Effects of endogenous IL33 and intratumoral administration of IL-33 in antitumor responses

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[purpose] Although IL33 has been studied for its role in the Th2 responses, recent reports suggest roles of IL33 for CD8 T cell. However, effects of either endogenous or intratumoral administration (i.t) of IL33 in antitumor responses is unclear. In this study, we examined them in syngeneic murine tumor transplantable models. [Results and Discussion] To determine roles of host endogenous IL33, Colon 26 (colon carcinoma) cells were inoculated into WT or IL33KO mice. In KO mice, tumor growth was accelerated. The % of IFN γ + CD8 T cells in spleen and TIL were decreased, whereas the % of Treg in spleen was decreased at day 21 in KO mice, suggesting that endogenous IL33 both positively and negatively regulates antitumor immunity. To examine effects of IL33 i.t, IL33 was injected into tumor site at days 0 and 7 in SCCVII (squamous cell carcinoma) model. Tumor growth was slightly delayed and CD8 T cell function in TIL was elevated by IL33 i.t. However, the % of Treg in spleen was increased and CD8 T/Treg ratio in TIL was not improved by IL33 i.t. Our results suggest that combination therapy that can suppress Treg induction may be able to further improve the therapeutic effect of IL33 i.t.

P-3220

Prognostic role of endogenous CXCL9 expression in intrahepatic cholangiocarcinoma

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【Background】CXCL9, an IFN-γ inducible chemokine, is reported to have versatile roles in tumor immunity and to affect the prognosis of various cancers, but few in intrahepatic cholangiocarcinoma (ICC). The aim of our study was to investigate the prognostic role of endogenous CXCL9 expression in ICC patients. 【Patients and methods】Consecutive 70 patients who underwent curative resection for ICC were enrolled. Endogenous CXCL9 expression was evaluated by immunohistochemistry (IHC). The number of tumor-infiltrating Th1, NK and Foxp3 positive Treg cells were also estimated by IHC.【Results】Patients were divided into two groups according to the IHC density of CXCL9 (high/low: 31/39 cases). Patients with high CXCL9 expression had better overall and recurrence free survival (RFS) vs. those with low CXCL9 expression. Multivariate analyses revealed high CXCL9 expression was an independent factor for prolonged RFS (p=0.048). In addition, high CXCL9 expression was significantly related with the much number of tumor-infiltrating Th1 and NK cells.【Conclusion】High CXCL9 expression was related with anti-tumor immunity leading to the favorable postoperative prognosis in ICC patients.

P-3221

sST2, a decoy receptor of the IL-33 receptor, enhances orthotopic tumor growth of murine pancreatic cancer cells

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[Purpose] Pancreatic inflammation is a major risk factor for pancreatic cancer. Pro-inflammatory cytokine IL-33 is suggested to be involved in this process, but its exact role in the control of malignant behavior of pancreatic cancer is obscure. Here we investigated the role of sST2, a decoy receptor of the IL-33 receptor (ST2L), in pancreatic cancer growth. [Methods] Mouse pancreatic cancer Panc02 orthotopic tumor model was used. sST2 expression was suppressed by shRNA. Chemokine/cytokine gene expression and angiogenesis was analyzed by a PCR array and CD31 staining, respectively. [Results] sST2 knockdown resulted in suppression of tumor growth with a decrease in vessel density, which was recovered by forced expression of sST2 and was not evident in IL-33 knockout mice. Cxcl3 was found to be downregulated in the sST2-knockdown tumors. Administration of SB225002, an inhibitor of the CXCL3 receptor CXCR2, into mice bearing orthotopic tumors inhibited angiogenesis and tumor growth. [Conclusion] These results suggest that sST2 enhances orthotopic tumor growth through inhibiting IL-33/ST2L axis and stimulating at least CXCL3/CXCR2-mediated angiogenesis in the tumor microenvironment.

▲TOP

P-3222

Withdrawn

No Abstract

P-3223

The effects of intratumoral glucocorticoid synthesis on tumor immune microenvironment in lung cancer

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Immune checkpoint target therapy for the patients with non-small cell lung cancer (NSCLC) has been developed. Its effects are largely determined by the status of programmed death ligand-1 (PD-L1) in carcinoma cells but in some cases no therapeutic effects observed despite marked PD-L1 expression in carcinoma cells. This study focused on glucocorticoid (GC) which exerted profound effects on immune system, and aims to clarify the correlation between intratumoral GC synthesis and tumor immune microenvironment in lung cancer patients. We performed cytokine array and RT-PCR analyses using A549 and LK2 treated by cortisol to elucidate the effects of GC on immune microenvironment. We then performed immunohistochemical analyses in 70 cases of NSCLC obtained by surgery to further explore the status of GC receptor (GR) and intratumoral glucocorticoid synthesis. Cortisol reduced the expression of IL-8, TGF-beta and PD-L1 in carcinoma cells examined, and GR and 11 β HSD1 were both identified in lung carcinoma cells. In conclusion, intratumoral production of cortisol could alter the status of tumor immune microenvironment in lung cancer.

P-3224

Hepatoma-derived growth factor contributes to stemness of pancreatic cancer cells

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Pancreatic cancer has been reported as a refractory disease by surrounding- and intra-tumor fibrotic reactions contributed by activated pancreatic stellate cells (PSCs). We previously demonstrated that hepatoma-derived growth factor (HDGF) was responsive to TGF-&beta1 in PSCs. HDGF functioned in anti-apoptosis of PSCs and synthesis and depositions of extracellular matrix proteins. We further found that secreted HDGF from PSCs could also contribute to cancerous sphere formation. However, the detailed mechanisms including HDGF receptor(s) and downstream effectors remain unclear. Following the prediction and using surface plasmon resonance technology, the results showed that HDGF could bind to the N-terminus of the identified novel HDGF receptor. Furthermore, the inactivation of the identified novel HDGF receptor could attenuate stemness sphere formation and HDGF-induced signaling pathways in pancreatic cancer cells. Taken together, we provided new insight and molecular mechanisms for highlighting the involvement of HDGF in anti-apoptosis of PSCs, turnover of extracellular matrix protein depositions, and stemness of pancreatic cancer cells.

▲TOP

[P-3239] P14-48 [English/Japanese]
Skin tumor and endocrine tumor

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Toru Takano / Dept. Metab. Med., Osaka Univ.

P-3239

Expression level of CDK4 in extramammary Paget's disease

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Extramammary Paget’s disease (EMPD) is a rare malignant tumor of the skin primarily in genitocrural region. CDK4/6 are crucial drivers of cell cycle progression by combination with cyclin D. CDK4/6 cooperated with cyclin D controls the cell cycle transition from the G1 phase into DNA synthesis phase by regulating the phosphorylation state of Rb. In vitro and vivo investigations, CDK4/6 inhibitors suppress the proliferation in many types of cancer cells. Recent approval of palbociclib, cyclin-dependent kinase (CDK) 4/6 inhibitor, drew significant attention for therapeutic use of breast cancer. Although EMPD shows biological similarity (not only HER2 but also hormonal receptors) to breast cancer, expression of CDK4/6 has not yet been examined in EMPD. In this study, we investigated the expression of CDK4 with EMPD and evaluated the clinical importance.

▲TOP

P-3240

The expression and biology of toll-like receptor 4 in squamous cell carcinoma of the skin

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Toll-like receptor 4 (TLR4) is a key regulator of the innate immune system. TLR4 is expressed not only in immune cells but also in carcinoma cells. It was shown that TLR4 expressed in normal epidermis and squamous cell carcinoma (SCC) of the skin. However, the expression and biological role of TLR4 are not fully elucidated. In this study, the expression and biology of TLR4 in SCC was examined by immunostaining of the cases of SCC, actinic keratosis (AK) and Bowen's disease (BD) and by in vitro experiments using a human SCC cell line HSC-1. The expression of TLR4 in SCC was significantly higher than those in AK and BD. Among the cases of SCC, the expression level of TLR4 appeared lower in poorly differentiated SCC than well-differentiated SCC. In HSC-1, reduction of TLR4 expression by siRNA accelerated the migration and invasion. The reduction is associated with the increase in CD44 expression and enhancement of filopodia formation in migrating cells. The reduction of TLR4 appeared to be associated with enhancement of malignant features in cases SCC and culture cell line. It is thus suggested that the expression of TLR4 is suppressive on the tumor biology of human SCC.

P-3241

Prognostic value of PD-L1 expression in Merkel cell carcinoma

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Merkel cell carcinoma (MCC) is a rare but highly malignant skin cancer. In spite of its well-known high malignancy, some cases have a good prognosis including cases of spontaneously regression. It has been suggested that T-cell-mediated immunity plays an important role in tumor regression and programmed death-ligand 1 (PD-L1) expression in cancer cells correlates with better clinical outcomes in contradiction to other solid carcinomas, e.g., malignant melanoma. To evaluate these immune response, we collected 64 specimens from 40 patients with MCC treated and followed in 6 facilities. The cohort included 15 males and 25 females with a median age of 79.8 (range 61-91). 5 cases showed spontaneously regression after biopsy. CD8+ lymphocytes as tumor-infiltrating lymphocytes in some cases and a wide variety of the strength of immune response factors including PD-L1 were observed. Statistically, the strength of PD-L1 expression in primary lesions and metastatic lesions was correlated with overall survival (linear regression analysis, P = 0.0114 : primary, P = 0.0043 : metastatic). Especially, PD-L1 expression in metastatic lesions can be a good marker to predict clinical outcomes.

P-3242

Pretreatment serum CTLA-4 is a potential biomarker of a risk of immune-related adverse events in metastatic melanoma

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Immune checkpoint inhibitors have drastically changed metastatic melanoma treatment strategies. However, these new immunotherapies have some problem to overcome. First, the development of biomarkers to predict their efficacy is needed. Second, that predicting the side effects are also required. By the immune system imbalance, these therapies also generate severe autoimmune toxicities, called immune-related adverse events that can potentially affect any tissue. It is important to develop biomarkers to predict severe immune-related adverse events for better management of melanoma treatment. Therefore, we investigated the pretreatment serum levels of soluble CTLA-4 whether it can be considered as a biomarker for immune-related adverse events. 38 patients with metastatic melanoma treated with immune checkpoint inhibitors were analyzed. The serum levels of soluble CTLA-4 in patients with immune-related adverse events were significantly higher than those of patients without them ($p<0.05$). These results suggested the possibility that soluble CTLA-4 could be a potential biomarker for immune checkpoint inhibitors in melanoma patients.

▲TOP

P-3243

Functional analysis of Delta-like-3 in the neuroendocrine cells of gastrointestinal tract

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(Background)Delta-like 3 (DLL3) is a member of Delta/Serrate/Lag2 (DSL) ligands in Notch receptors. Five DSL ligands (DLL1, DLL3, DLL4, Jagged1 and Jagged2) have been known in mammals and DLL3 has a specific structure in these DSL ligands.Recently, it has been reported that DLL3 is highly expressed on the cell surface of lung cancer such as small cell lung cancer. However, the functions of DLL3 in the neuroendocrine-gastrointestinal tracts are largely unclear. In this study, we examined the functions of DLL3 in the NEC cells of the gastrointestinal tracts. (Materials and Methods)　Expression levels of DLL3 were evaluated by Western Blotting analysis and RT-PCR. Also, we investigated the effects of DLL3 in siR-DLL3 treated NEC cells by MTT assay and several apoptosis analyses. (Results)High protein and mRNA expression of DLL3 were detected in NEC cells tested. Also, gene silencing of DLL3 induced growth suppression in NEC cells tested. Moreover, activation of apoptosis was observed in siR-DLL3-treated NEC cells. (Conclusion)DLL3 has the functions that promote growth of NEC cells of the gastrointestinal tracts. Now, we are investigating further mechanisms of DLL3 in NEC cells.

P-3244

Profiling the Tumour Immune Microenvironment in Pancreatic Neuroendocrine Neoplasms with Multispectral Imaging

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Aim: The aim in this study is to determine the difference of immune microenvironments between pNENs and pancreatic ductal adenocarcinomas (PDACs), and to elucidate the histology-dependent variability among pNENs.Methods: Tumour microenvironment of tumour tissue samples including 52 pNENs and 18 PDACs were comprehensively investigated, using multispectral fluorescent imaging system. The tumour-infiltrating lymphocytes (TILs), their PD-1 and PD-L1 expression were quantitatively analysed.
Results: A principal component analysis revealed that the tissue immune profile is related to tumour histology, with distinct groups being observed for NETs, NECs, and PDACs. While NECs and some PDACs had hot immune microenvironments with abundant TILs, NETs had a cold immune microenvironment with few TILs. Univariate analysis revealed that lymph node metastasis, grade, stage, PD-1^{high} T cells, and PD-$L1^{high}$ Type-II macrophages were predictors for RFS, while grade and PD-1^{high} T cells were prognostic factors for OS.
Conclusion: NEC has characteristic hot immune microenvironment, and our results support the thesis for WHO 2017 tumour classification criteria, which distinguish between G3 NETs and NECs.

▲TOP

[P-3249] P14-50 [English/Japanese]
Other Cancers

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Kiyoshi Yanagisawa / Div. Mol. Car., Nagoya Univ. Grad. Sch. Med.

P-3249

The outcomes of thymoma in patients undergoing preoperative chemotherapy or chemoradiotherapy followed by surgery

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【Objectives】Results of preoperative chemotherapy or chemoradiotherapy followed by surgery for locally advanced thymoma were analysed.【Methods】Data on 29 patients with thymoma underwent preoperative chemotherapy or chemoradiotherapy followed by surger were reviewed.【Results】The study population included 9 male and 20 female patients.The mean age was 49 years.The preoperative Masaoka stage was III in 12, IVa in 13, and IVb in 4.The histological type was B3 in 11 patients, B2 in 9, and others in 5. The mean tumor size was 8.0 cm.Three patients underwent chemoradiotherapy.The chemotherapy regimens were ADOC in 9, CBDCA+PTX in 6, CAMP in 5, and others in 9.The responses to chemotherapy were a PR in 11 patients, and SD in 18.Complete resection was achieved in 24 (83%) cases.There was no perioperative mortality; 6 patients (21%) developed postoperative complications.The 5- and 10- year overall survival rates were 100% and 85%, respectively.The 5- and 10- year disease-free survival rates were 81% and 52%, respectively.【Conclusions】Preoperative chemotherapy or chemoradiotherapy followed by surgery for locally advanced thymoma provides favorable long-term results.

▲TOP

P-3250

Comprehensive molecular analysis of 255 malignant plural mesotheliomas

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Malignant pleural mesothelioma (MPM) is a highly aggressive neoplasm that arises from mesothelial cells covering the surfaces of the pleura. Despite current development of diagnostic and therapeutic approaches, MPM prognosis remains poor. Here, we conducted the comprehensive molecular analysis of MPM and elucidated the molecular pathogenesis and ethnic specific genomic characteristics of MPM. We collected tumors, cell lines and corresponding peripheral blood samples from 81 Japanese MPM patients and performed the whole-exome sequencing (WES) and RNA-sequencing with the HiSeq 2000/2500 (Illumina). Together with the publicly available MPM genome data sets, we analyzed 255 MPM cases by WES, and 363 cases by RNA expression. Through mutation signature analysis, we observed contribution of alcohol drinking-related signature in Japanese cases, which frequency is correlated with alcohol consumption in Japanese cases with the inactive ALDH2 variant. Immune profiling analysis revealed that approximately 17% of MPMs showed high infiltration of CD8-positive T cell with PD-L1/2 high expression. These findings may lead to improved therapeutic and preventive strategies for mesothelioma.

P-3251

Gene expression analysis of the epithelioid and sarcomatoid mesothelioma derived from the same clone

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Background: Mesothelioma consists of epithelioid and sarcomatoid subtypes. Mechanism of histological differentiation is not known. Several studies described the difference of gene expression between them. They, however, compared gene expression in mesothelioma with different genetic backgrounds, and could not reflect specifically the difference in the expression. We showed that the cultured mesothelioma cell, NCI-H2452, monophasic and spindle-shaped in culture, differentiated into both of epithelioid and sarcomatoid type when transplanted into nude mouse. Method & Results: We did the single-cell-cloning of NIC-H2452 to rule out the possibility that the cells might be multiclonal, and transplanted the cloned-cell ($2x10^6$) into the mouse. We reproducibly obtained the biphasic mesothelioma in transplanted tumor. We retrieve the area of epithelioid and sarcomatoid part using the laser microdissection. Then we analyse gene expression pattern in them. Discussion: Histological differentiation was shown to be epigenetically controlled. We identify the genes affecting the morphology of mesothelioma derived from the same clone.

P-3252

Functional blockade of MUC1 can inhibit the progression of duodenum adenocarcinoma

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Background: Mucin1 (MUC1), a glycoprotein as a member of the mucin family, has been shown to be associated with poor prognosis in patients with several types of cancer. We verify the expression and the function of MUC1 in duodenal cancer. Materials and Methods: A human duodenal cancer cell line, HuTu80, is used for assessments. The expression of MUC1 and cell cycle were evaluated with flow cytometry. Proliferation, migration or invasion was assessed by MTT, scratch wound healing and matrigel invasion assay, respectively. All inhibitory experiments were evaluated with silencing by siRNA to MUC1 or anti-MUC1 peptide GO203.Results: Transduction of the MUC1 siRNA to HuTu80 decreased the expression of the MUC1 both in mRNA and protein levels. The inhibition of MUC1 expression resulted in the reduction of cells with S and M phases. Cell growth was inhibited by the addition of 50uM GO203 ($p<0.01$) as well as the siRNA treatment ($p<0.01$). Transduction of siRNAs also significantly reduced the migration ($p<0.01$) and invasive capacities ($p=0.04$). Conclusions: Interference of MUC1 reduces cell growth and invasiveness, suggesting that MUC1 is functionally associated with malignant potential.

▲TOP

P-3253

Clinicopathological study of small bowel adenocarcinoma at a single institution

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【Introduction】Small bowel adenocarcinoma(SBA) is rare. We aimed to clarify clinicopathological features of SBA. 【Objects and methods】All patients with histologically confirmed SBA from January 1997 to December 2017 were included in this study. We retrospectively collected and analyzed data from medical records. 【Results】A total of 21 patients (12 males and 9 females) were diagnosed with SBA. In all 21 patients, 5 patients had cancer histories, and 2 patients were treated with Crohn's disease. Median age at diagnosis was 62 year-old. Jejunum was the most common site(12 cases), and moderately differentiated tubular adenocarcinoma was the most common tissue(tub1/tub2/por/muc: 5/9/5/2 cases). In TMN classification of the AJCC cancer staging manual, stage ⅡA/ⅡB/ⅢB/Ⅳ were 5, 4, 2 and 10 cases respectively. In stage Ⅱ/Ⅲ, 6 patients had post-operative recurrence, and all T4 cases had postoperative recurrence. 【Conclusion】 At the time of diagnosis, SBA was in progress. All patients with T4 stage had high risk of post-operative recurrence, and thus postoperative adjuvant chemotherapy and careful follow-up are required. Further studies with a larger number are needed.

P-3254

Experience of chemotherapy for the patients with intestinal cancer complicated with inflammatory bowel disease

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Introduction: Chemotherapy for the patients with intestinal cancer complicated with inflammatory bowel disease (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) is expected to develop more severe adverse events due to the patients' inflammatory intestinal mucosa, or shorter residual intestine after surgery. Materials and Methods: Between April 2005 and March 2018, 7 CD and 7 UC patients with intestinal cancer experienced chemotherapy at our institution. The safety of chemotherapy were retrospectively evaluated. Results: Four patients received adjuvant chemotherapy and the others received palliative chemotherapy. In CD patients, almost all patients experienced diarrhea and symptoms were more severe in the case whose residual intestine were shorter. The adverse events in UC patients were relatively mild compared to CD patients. Exacerbation of IBD, which seems to be caused by chemotherapy, was not observed in any cases. Diarrhea, not myelosuppression or nausea, was the most serious adverse event for the patients, but they were controllable, and chemotherapy could be carried out safely.

▲TOP

[P-3261] P14-52 [English/Japanese]
Colorectal cancer: pathology

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Chu Matsuda / Dept. Gastroenterological Surg. Osaka Univ.

P-3261

Significance of CD204 positive tumor-associated macrophages in carcinogenesis of colorectal adenoma

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Background: We recently reported that CD204-positive tumor-associated macrophage (TAMs) were an independent risk factor in malignant transformation of gastric adenoma. However, the roles of TAM in adenoma-carcinoma sequence remain unclear in colorectum.Materials: Eighty-eight tubular or tubulovillous adenomas, removed via endoscopic mucosal resection between 2014 and 2017, were grouped into L (48 adenomas: mild atypia, 24; moderate atypia, 24) or H (40 adenomas: severe atypia, 21; intramucosal adenocarcinoma in adenoma, 19). Methods: Immunohistochemical studies were performed against a representative specimen of each lesion embedded in paraffin after formalin fixation. Results: Larger size, higher frequency of villous structure, loss of proliferation polarity, p53 expression, larger TAM numbers and larger microvessel density were detected with statistical significance in group H compared to group L. However, TAM number was not assessed as an independent risk factor in multivariate analysis. Conclusion: TAM may play a significant role in the malignant transformation of colorectal adenoma in association with angiogenesis, proliferative activity and p53 protein expression of the tumor

▲TOP

P-3262

MicroRNA expression profile correlated with lymph node metastasis in early colorectal cancer

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The purpose of this study is to identify the microRNAs (miRNA) expression profile in early (submucosal invasive) colorectal cancer (CRC) with lymph node metastasis. In two early CRC cases, the primary CRC elements and the lymph node metastasis elements were separately collected from formalin fixed paraffin embedded (FFPE) specimens by laser microdissection system. Total RNAs were extracted and then subjected to comprehensive miRNA expression analyses (Agilent Expression Array). Differentially expressed miRNAs in the lymph node metastasis elements, whose levels showed more than two folds changes in compared to those of the primary CRC elements, were picked up. miR-125b was obtained as the upregulated miRNA, while 25 miRNAs including miR-1228 were obtained as the downregulated miRNAs.

P-3263

Clinicopathological significance of ADAM28 expression in colorectal cancer

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We previously reported a novel mouse tumor model based on signature defects seen in many human serrated CRCs - CDX2 loss and BRAFV600E mutation. Through the validation of global gene expression profile including the mouse models and human CRCs, we focused on ADAM28 (A disintegrin and metalloproteinase 28) as a candidate of specific molecule for serrated adenocarcinoma. ADAM28 has two isoforms “membrane-anchored ADAM28 (ADAM28m) and secreted ADAM28 (ADAM28s)”. We examined the expression of both isoforms of ADAM28 using 11 CRC cases with BRAFV600E mutation and 13 CRC cases with KRAS codon 12/13 mutation and found that ADAM28m was specifically upregulated in BRAF mutant CRC cases, whereas ADAM28s upregulated in CRCs regardless of the presence of BRAF or KRAS mutation. Among 10 CRC cell lines (DLD-1, Lovo, SW48, SW480, SW837, SW1116, Caco2, RKO, HT-29, HCT-116), only HT-29, which has many phenotypic and genetic similarities to serrated CRC, expressed ADAM28m. nine of ten CRC cells, except for RKO, showed weak to modest expression of ADAM28s. The clinico-pathological significance of ADAM28s expression in CRC cases will be also reported.

P-3264

Protocadherin B9 is frequently overexpressed in human colorectal cancer

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Genes encoding transmembrane proteins expressed specifically in cancer cell may be ideal biomarkers for diagnosis. Previously, we showed that overexpression of Protocadherin B9, which was identified as a transmembrane protein, promotes peritoneal metastasis and correlates with poor prognosis in patients with gastric cancer. However, expression of Protocadherin B9 in colorectal cancer remains unclear. In the present study, we investigated the expression and function of Protocadherin B9 in colorectal cancer. Immunohistochemical analysis demonstrated that 57 (44%) of 130 colorectal cancer. cases were positive for Protocadherin B9. Protocadherin B9 expression was not correlated with TNM classification and Stage. There was no significant correlation between Protocadherin B9 expression and patient survival. Protocadherin B9 expression was also detected in high grade adenoma. Cell adhesion to fibronectin were significantly reduced by Protocadherin B9 knockdown. These results suggest that Protocadherin B9 may be a novel biomarker and therapeutic target for colorectal cancer.

▲TOP

P-3265

CDX2 expression between primary and metastatic sites in colorectal cancer in association with chemotherapy

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Background: Loss of CDX2 expression in colorectal cancers (CRCs) has been proposed as a predictive biomarker for not only prognosis but also response to chemotherapy. However, alterations in CDX2 expression during progression and response to chemotherapy remains unclear. We herein aimed to analyze the concordance of CDX2 expression between primary and liver metastases in association with chemotherapy.
Methods: A total of 144 consecutive patients with CRC treated at our hospital were included. Whole sections of primary and corresponding liver metastases were assessed for CDX2 expression.
Results: Primary CRCs exhibited heterogeneous CDX2 expression. Seven of the 144 CRCs in the cohort (4.9%) were CDX2-negative. The concordance rate of the CDX2 expression status in patients who did not receive chemotherapy was 100%. Moreover, the concordance rate in patients who received chemotherapy before both primary resection and liver metastasectomy was 100%.
Conclusion: CDX2 expression status was highly concordant between primary CRCs and corresponding liver metastases, independent of chemotherapy, suggesting that the CDX2 expression status in CRCs was not affected by metastasis or chemotherapy.

P-3266

Immunohistochemistry of LRP6 in colon cancer

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[Background] LRP6 (Low-density lipoprotein receptor related protein 6) is a key component of Wnt/β-catenin signaling pathway. It is reported that high expression of LRP6 was associated with malignant potential of breast cancer; however, there are only few reports of LRP6 expression in colon cancer. [Materials and Methods] In this study, we performed immunostaining of LRP6, and β-catenin acting at the downstream in 55 human colorectal and 8 esophageal cancer tissues. [Results] In the majority of CRC cases, nuclear LRP6 was noted in <10% tumor cells. Cytoplasmic LRP6 expression was generally weak. In contrast, β-catenin expression was abundantly observed. Thus, 49 of 55 cases displayed nuclear β-catenin in >50% tumor cells and 30 of 55 cases showed strong intensity at cytoplasm. On the other hand, when we examined human esophageal cancer tissues (8 cases) which rarely have APC and β-catenin mutation, we found that 5 of 8 cases showed high expression of LRP6 in the cytoplasm. [Conclusion] LRP6 expression was relatively low in colon cancer. This might in part be due to enhanced Wnt/β-catenin signal activity in colorectal cancer cells, as observed β-catenin accumulation.

P-3267

Development of novel therapeutic strategy targeting Histone acetyltransferases for colorectal cancer

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Histone acetyltransferases (HATs) are well known for epigenetic coactivator regulating transcription factor. Although one of HAT is associated with a variety of cancer progression, each interaction is still unclear in colorectal cancer. Therefore, in shRNA mediated triple knockdown examination, we found that the growth of colorectal cancer cell line HT-29 was suppressed through apoptotic cell death. Single and double knockdowns of each domains were not enough to suppress HT-29 growth, but one component of HAT was upregulated by the absence of others. This result suggests that comprehensive inhibition is critical for treatment of colorectal cancer. We picked a drug targeting this HAT domain from HTS screening. Under treatment of this drug, HT-29 growth was significantly suppressed (IC50 = 317 nM). As a result, inhibition of HAT protein is proceeding therapeutic method for colorectal cancer.

▲TOP

[P-3273] P14-54 [English/Japanese]
Colorectal cancer (1)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Yozo Suzuki / Dept. Gastroenterol. Surg. Osaka Police Hosp.

P-3273

eIF5 Mimic Protein 1 (5MP1) drives malignancy in colorectal cancer (CRC) by reprograming translation initiation of MYC

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Background: Translational reprogramming in cancer evolution is attracting attention. Our aim is to identify novel oncogene which promotes malignancy through this mechanism in CRC.Method: 5MP1 was detected as a candidate gene in CRC using a bioinformatics approach. To explore biological effect of 5MP1, proliferation and cell cycle assay were performed in vitro. In vivo effect of 5MP1 on tumor growth was assessed in xenograft mouse models. To explore downstream targets of 5MP1, Gene Set Enrichment Analysis (GSEA), RNA sequencing and translatomic analysis were performed. We assessed clinical significance of 5MP1 expression in 111 CRC cases in our hospital and 609 CRC cases in TCGA dataset.Result: 5MP1 was significantly and ubiquitously overexpressed in CRC tissues by chromosomal amplification. Overexpression of 5MP1 promoted proliferation and cell cycle progression of CRC cells in vitro and in vivo. 5MP1 induced c-Myc expression by altering translation initiation of MYC and increasing stable and oncogenic form of c-Myc protein. Patients with high 5MP1 expression had poorer prognosis. Conclusions: 5MP1 is a novel oncogenic regulator of translation as well as a prognostic marker in CRC.

▲TOP

P-3274

Fundamental study on the significance of expression of Double cortin-like kinase 1 in colorectal cancer

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IntroductionIt is reported that doublecortin like kinase1 (DCLK1) was colorectal cancer stem cell-specific molecules in mice (Nature Genetics, 2013). There are few reports that have its mechanisms such as relating to metastasis and invasion.Material and MethodWe established colorectal cancer cell lines (SW480, HCT116) silencing DCLK1 and investigated their effects on proliferation, migration and invasion, as well as E-cadherin levels, that related to EMT. The associations between DCLK1 expression in colorectal cancer specimens and clinicopathological features including prognosis were assessed by immunohistochemistry.ResultsDCLK1 upregulated E-cadherin expression and suppressed proliferation, migration and invasion in colorectal cancer cells. DCLK1 and Tribbles homolog3 (TRIB3) was suspected strong correlation by microarray analysis. Moreover, DCLK1 expression in colorectal cancer decreased with the progression of invasion and metastasis, and a high expression level of DCLK1 was correlated with a worse prognosis.ConclusionsDCLK1 has a tumor growth of function and may serve as a novel therapeutic target in colorectal cancer.

P-3275

The molecular characteristics of depressed colorectal cancer (CRC)

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AimThe development of endoscopy enables to detect “depressed CRC” categorized as de novo cancer, which invades massively and metastasizes in small size. We aim to clarify the molecular characteristics of depressed CRC by integrated analysis in comparison with protruded CRC which is considered to develop in adenoma-carcinoma sequence.MethodWe conducted whole exome sequence (WES) and RNA sequence in 8 cases of each submucosal invasive depressed CRC and protruded CRC.ResultThe rates of APC and TP53 mutations were higher in depressed CRC (100%, 75%) than in protruded CRC (62.5%, 62.5%). The rate of KRAS mutations was lower in depressed CRC (12.5%) than in protruded CRC (62.5%). The copy number amplifications (CNA) in chromosome 20p and 13pq were observed only in depressed CRC (87.5%, 37.5%). Gene Set Enrichment Analysis showed that the expression of genes related to Epithelial-Mesenchymal Transition (EMT) and angiogenesis were higher in depressed CRC than protruded CRC.ConclusionDepressed CRC is characterized by APC and TP53 mutations, high CNA, and the overexpression of EMT and angiogenesis genes. These molecular characteristics may lead to the malignant phenotype of depressed CRC.

P-3276

The clinicopathological significance of intelectin-1 in colorectal tumor

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In order to identify novel prognostic markers or therapeutic targets for colorectal cancer (CRC), we focused on intelectin-1 protein. Intelectin-1 is known to be secreted mainly from goblet cells. Recently, it has been reported that intelectin-1 can be a useful differential marker for pulmonary adenocarcinoma and malignant mesothelioma, but the significance in gastrointestinal cancer is unknown . An immunohistochemical analysis of intelectin-1 in 102 CRC samples demonstrated 29 (28%) CRC cases were positive. There was no correlation with clinicopathological factors, but in the survival rate, intelectin-1 positive CRC cases tend to show more favorable prognosis than negative cases. In addition, precancerous lesion including conventional adenoma, traditional serrated adenoma and SSA/P displayed decreased of expression of intelectin-1 although normal colorectal mucosa expressed intelectin-1 diffsely. These results indicate that intelectin-1 might play an important role in regulating colorectal tumorigenesis.

▲TOP

P-3277

Molecular alterations in colorectal adenomas and intramucosal adenocarcinomas defined by SNP arrays

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【Aims】We examined colorectal adenomas and intramucosal adenocarcinomas (IMAs) to develop a genome-wide overview of copy number alterations (CNAs).【Methods】We analysed CNAs using a SNP array of isolated tumour glands obtained from 55 colorectal adenomas ［35 low-grade adenomas (LGAs) and 20 high-grade adenomas (HGAs)］ and 30 IMAs. We examined whether frequent CNAs differed between LGAs and HGAs or HGAs and IMAs. Finally, we investigated the total lengths of the CNAs in LGAs, HGAs, and IMAs.【Results】Although no frequent CNAs were found in LGAs, the most frequent alterations of HGAs were gains of 7q11, 7q21 and 9p13 and loss of 5q. High levels of gains were detected at 13q, 7q, 8p, 20q, 7p, 18p and 17p in IMAs. Although no frequent alteration differed between LGAs and HGAs, significant differences of gains at 13q, 17p and 18p were found between HGAs and IMAs. Although the total lengths of all CNAs, copy number gains, and losses of heterozygosity were significantly greater in HGAs than in LGAs, no significant differences in the lengths of CNAs were found between HGAs and IMAs.【Conclusions】CNAs may play an essential role in early colorectal carcinogenesis.

P-3278

Identification of cancer-associated fibroblast-related genes in colorectal cancer

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We aimed to understand the molecular mechanism and to identify novel therapeutic targets in the tumor microenvironment of colorectal cancer (CRC). We performed RNA-seq analysis in stromal and epithelial cells isolated from surgically resected CRC tissues, and identified a set of genes upregulated in tumor stroma. By performing RT-qPCR of selected genes in clinical specimens, we identified AEBP1 as a novel CAF-related gene. Immunohistochemistry showed that AEBP1 was abundantly expressed in CAFs in CRC tissues. The Cancer Genome Atlas (TCGA) datasets revealed that higher expression of AEBP1 is associated with worse overall survival of CRC patients. Knockdown of AEBP1 suppressed proliferation of cultured CAFs. Conditioned medium from CAF with AEBP1 knockdown attenuated migration of CRC cells. Moreover, siRNA against mouse Aebp1 suppressed xenograft formation by human CRC cells in nude mice. Microarray analysis revealed that AEBP1 knockdown significantly altered gene expression signatures in CAF. Our results suggest that AEBP1 may play an important role in CRC development, and that it could be a potential therapeutic target.

P-3279

Genetic lineages of colorectal adenocarcinomas with and without adenoma components

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Aims. To know whether the colorectal carcinomas (CRCs) without an adenoma component can be discriminated from those with adenoma by DNA copy-number alteration (CNA) profile. Methods. Genomic DNAs were extracted from the adenoma (Ad), mucosal (Mu), invasive (In) and metastatic (Me) samples that were taken from FFPE tissue sections by laser microdissection. The samples were confirmed to be positive for MSH6 and PMS2. Array CGH data of 96 samples from 40 left-side CRCs were subjected to unsupervised hierarchical clustering. Results. Using ≥9-probe sized genes, the samples were divided into 5 clusters: gain-rich cluster 1 without Ad samples; loss-rich cluster 2 and other 3 clusters containing Ad samples. Chromosomal CNA profile of cluster 1 was characterized by scarce chromosomal CNAs except 13q+ and 20q+. Cluster 2 showed the greatest number of chromosomal CNAs, consisting of those partially sharing with Clusters 3-5 (7p/q+, 8p-, etc.) and those without sharing (8q+, etc.). Clusters 2-5 often showed very similar gene-level CNA pattern between Ad and Mu/In/Me components, though Ad had scarce chromosome-level CNAs. Conclusions. Cluster 1 may represent de novo CRCs without Ad components.

▲TOP

[P-3232] P14-47 [English/Japanese]
Brain tumor

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-3232

Midline glioma in adults : clinicopathological, genetic, and epigenetic analysis

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Among diffuse infiltrating brain tumors, those occurring in the thalamus, brainstem, and spinal cord are termed diffuse midline gliomas, among which tumors with H3K27M mutation have a particularly poor prognosis. Diffuse midline glioma H3K27M mutant is common in children, with few clinicopathological analyses in adult patients. EZH2 is associated with methylation of H3K27 (H3K27me), which is suppressed by H3K27M. Recent reports indicate that a high expression of EZH2 is associated with high grade glioma. We examined mutations or methylation of H3K27 and expression of EZH2 in adult cases. Histological grade and prognosis were correlated in adults. H3K27M was more common in cases with high histological grades. High EZH2 expression was associated with high histological grades and shorter survival. H3K27me was infrequently seen in high grade tumors. High grade glioma and H3K27M mutant showed high EZH2 expression and decreased H3K27me. Therefore, we suggest that EZH2 can be a therapeutic target for these tumors. Current research concerning alteration of the p16 gene is encouraging, so this was included in our discussion.

P-3233

Identification of molecular marker candidate by Ion Reporter exome sequencing in primary central nervous system lymphoma

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Exome sequencing for somatic mutation including SNVs and INDELs and CNV analysis are effective and valid methods for evaluating human cancers. Here we conducted target amplicon exome-sequencing on Ion Proton semiconductor sequencer. 27 samples of primary central nervous system lymphoma (PCNSL) were used for multiplex PCR amplification to obtain targeted coverages of the entire coding regions of 409 cancer-related genes. The average of the numbers of somatic mutations in each sample was 13.3. The most frequent mutations in 27 specimens were in PIM1, MYD88, CD79B, DST, IRF4, ERBB3, MYH11, DCC, and KMT2D. Somatic mutations of MYH11 were related to poor prognoses in PCNSL patients. The average of the ratios of nonsynonymous substitutions in each sample was 74.8%. CNVs were also duplicated and/or deleted from deep-sequencing in segmental genomic islands. Analyses of RTK/RAS/MAPK signaling and PTEN/PI3K/AKT proapoptotic pathway suggested that somatic activations and aberrations might be involved in a potential central oncopathway. The study provides a foundation for molecular targeted therapies based on genome diagnostics and prognosis in PCNSL.

P-3234

Novel therapeutic approach targeting NDRG1 and GSK3 β /AKT/S6 signaling against glioblastoma

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[Background] Glioblastoma (GBM) is the most aggressive brain tumor, and any therapeutic drugs and approaches has not yet contributed to elongate survival of GBM patients. N-myc downstream regulated gene 1 (NDRG1) is a differentiation-related gene of various organs including nervous systems. We previously reported that NDRG1 overexpression suppressed tumor growth and angiogenesis by gastric cancer and other tumor types. In this study, we further asked whether NDRG1 could be targetable for development of therapeutic drugs of GBM, and presents our findings as follows. [Result] [1]Enhanced NDRG1 expression in tumors was positively correlated with survival of GBM patients in our hospital; [2]NDRG1 overexpression suppressed both cell growth and activation of GSK3 β /AKT/S6 signaling, and conversely, NDRG1 knockdown induced stimulation of cell growth and GSK3 β /AKT/S6 signaling by GBM cells; [3]Treatment with drugs including an iron-chelating agent that augment NDRG1 expression suppressed cell growth, accompanied by G0/G1 arrest. [Conclusion] NDRG1 expression levels in tumors predict better outcomes of GBM patients. NDRG1 can be targetable for development of potent therapeutic drugs for GBM.

P-3235

The mechanisms of resistance to temozolomide in glioma cells

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Glioblastoma is one of the most aggressive brain tumor. One of the reasons of the worse prognosis is the acquisition of resistance to temozolomide (TMZ). TMZ is a DNA-methylating agent, delivering a methyl group to DNA (O6-guanine, N7-guanine and N3-adenine). The primary cytotoxic lesion, O6-methylguanine, mispair with thymine, leading to futile DNA mismatch repair (MMR), formation of double strand breaks and eventual cell death, in the absence of MGMT. To clarify the mechanisms of resistance to TMZ, several clones of TMZ-resistant U251 were obtained and analyzed. #3 resistant clone showed G2 arrest after TMZ exposure and this arrest was abrogated sooner compared to parental U251. TMZ did not induce G2 arrest in #8 clone. The ability of homologous recombination (HR) was increased in #3 clone, and by suppression of HR, #3 clone was resensitized to TMZ. The protein levels of MSH6, which was associated with MMR, was reduced in #8 clone. PARP inhibitor resensitized #8 clone to TMZ. Inhibition of HR or base excision repair was suggested to be a useful strategy to resensitize TMZ-resistant gliomas with higher HR or with MMR dysfunction to TMZ, respectively.

▲TOP

P-3236

Increase of mRNA levels of carnitine palmitoyltransferase 1C in human glioma cell lines administrated metformin

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In this previous annul meeting, we reported that both of Fatty acid synthase (FASN) and carnitine palmitoyltransferase 1C(CPT1C) immunohistochemically coexpressed in human gliomas, regardless of the genetic status of isocitrate dehydrogenase 1 (IDH1). FASN and carnitine palmitoyltransferase 1(CPT1) are related to fatty acid metabolism. CPT1C is a brain-specific isoform of CPT1. However, CPT1C is normally found only in the microsomal fraction of neurons, and shows a much lower carnitine acyltransferase activity than other CPT1s. Moreover, Metformin is a diabetes drug, also known as the anticancer agent. We examined the mRNA expression of FASN and CPT1C in human glioma cell lines at a medium administrated metformin used by a real-time reverse transcription polymerase chain reaction (RT-PCR). The mRNA of CPT1C increased according to concentration of the metformin (1mM, 5mM, 10mM, and 25mM) in human glioma cell lines. CPT1C might be related to a stress tolerance.

P-3237

CD24 enhances malignant features of glioblastoma

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Glioblastoma is high-frequent and extremely malignant brain tumor characterized by rapid growth, extensive invasiveness, and angiogenesis. Elucidation of the molecular mechanism of the malignant features and breakthrough in the treatment are highly desired. By our previous global analysis, we have identified CD24 as one of the genes involved in the invasive growth of glioblastoma cells. We used short hairpin RNA expressing retroviral vector to inactivate CD24 of U251 and YKG-1, human glioblastoma cell lines which express CD24 strongly. Stable knockdown of CD24 resulted in decreased invasiveness of glioblastoma cells in Matrigel invasion assays. The cDNA microarrays for transcriptional profiling revealed significantly reduced expression of several progression-associated genes and enhanced expression of suppression-associated genes in response to CD24 knockdown in both cells. Synergic or inversely correlative expressions of such genes were confirmed by real-time RT-PCR. Next, profiling of microRNAs of cells and exosomes are analyzed using miRNA microarrays. CD24 changes profiling of mRNA and miRNA both in cells and exosomes and may have a role in invasion of glioblastoma cells.

P-3238

A novel anticancer strategy targeting HMGB1/RAGE in glioblastoma using in silico and drug repositioning approaches

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Glioblastoma multiforms (GBM) are the most aggressive primary malignant brain cancers that are resistant to conventional radiation and chemotherapies. HMGB1, a chromosomal protein, functions as an extracellular signaling molecule during inflammation and tumor metastasis. It is known that HMGB1 interact with multiple cell surface receptors such as RAGE and TLRs. We have been studying the relationship of HMGB1/RAGE interaction and cancer cell growth in GBM. Here, our purpose is search of novel anticancer drugs targeting HMGB1/RAGE interaction for chemotherapy against GBM. We performed the screening of HMGB1/RAGE inhibitory candidates by in silico and drug repositioning (DR) approaches and evaluated them using in vitro HMGB1/RAGE binding (ELISA) and cell-based growth inhibition assays. We found that papaverine, an opium alkaloid, is a novel HMGB1/RAGE inhibitor, and that it significantly suppresses cell proliferation in human TMZ-resistant GBM cell line T98G. Furthermore, it was also revealed to inhibit tumor growth in the xenograft mouse model. These observations suggest that the HMGB1/RAGE inhibitor, papaverine, can provide a novel anticancer strategy against GBM.

▲TOP

[P-3245] P14-49 [English/Japanese]
Brain tumor and others

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-3245

Altered expression of ZEB1 correlates with invasiveness in glioma cell-lines, not with clinical prognosis

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The aim of the study was to investigate the effect of ZEB1 on biological behaviors in human gliomas cell lines and on patient survival data. To evaluate the role of ZEB1 in glioma invasiveness, cell invasion and migration capabilities were investigated after ZEB1 knockdown using siRNA. ZEB1 expression was examined in 89 human glioma samples by IHC. Comparison of ZEB1 expression in each histological grade, and its effect on glioma patient survival rates were also analyzed. ZEB1 knockdown resulted in significantly reduced cell invasion and migration in tumor cell lines. ZEB1 down-regulation also caused diminished expression of EMT related factors and genes by WB and RT-PCR. ZEB1 expression, however, did not correlate with WHO tumor grades, overall survival rates, and progression free survival. ZEB1 knockdown lead to decreased invasiveness and migration with reduced expression of EMT related factors and genes in glioma cell lines. However, ZEB1 expression in human glioma sample did not reveal a notable correlation with patient survival rates or clinicopathological variables.

▲TOP

P-3246

Mutation change after temozolomide treatment in primary glioblastoma

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[Introduction] Temozolomide (TMZ)-induced aberrant mutations cause resistance to the treatment for patients with glioblastoma (GBM) especially with methylated MGMT promoter. Here, we describe our molecular analyses of the paired samples from initial and recurrent tumors in 28 patients. [Methods] We collected paired GBM samples in patients who recurred after TMZ treatment. MGMT promoter methylation, mismatch repair (MMR) protein expression, and mutations of the cancer-related genes were analyzed by pyrosequencing, Western blotting, and Ion Ampliseq Cancer Hotspot Panel, respectively. [Results] Mutation acquisition of cancer-related genes was observed only in 11 (39%) patients. In those patients, MMR expression decreased significantly after treatment compared to mutation-stable patients. In addition, in contrast to MGMT unmethylated tumors, MGMT methylated tumors showed marked MMR inactivation (40% vs. 7.5%, p=0.078) and most of their acquired mutations were G:C to A:T at non-CpG sites. [Conclusions] We showed different types of the mutation acquisition after TMZ treatment according to MGMT status, providing further insights into the mechanism of TMZ resistance in primary GBM.

P-3247

Establishment of methotrexate-resistant primary central nervous system lymphoma cell lines and sensitivity to bortezomib

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Elucidation of the mechanisms by which primary central nervous system lymphoma (PCNSL) cells acquire resistance to methotrexate (MTX) may be indispensable to development of effective chemotherapy for MTX-resistant PCNSLs. We established two MTX-resistant cell lines (TK/MTX and HKBML/MTX) from PCNSL cell lines (TK and HKBML) and examined their sensitivity to bortezomib (BOR). MTX-resistant cell lines were established by exposure of the cell lines to increasing concentrations of MTX for about 4 months. Cytotoxic tests of MTX and BOR were conducted by MTT assay. IC_{50} of MTX in TK/MTX and HKBML/MTX were 4.1 μM and 22.9 μM, respectively, shown 6.9- and 28.7-fold higher resistance compared with the parent cell lines. In contrast, IC_{50} of BOR in TK/MTX was 0.26 μM, shown 1.6-fold higher sensitivity compared to TK. PCNSL cells acquired strong resistance to MTX, while the resistant cells showed high sensitivity to BOR. Therapeutic efficacy of BOR for MTX-resistant PCNSLs is warranted by further investigation of genes and enzymes related with MTX and BOR metabolism.

P-3248

Differential Expression of miRNA in Neuroblastoma Patients Using Next Generation Sequencing

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Introduction Neuroblastoma is one of common solid childhood tumors and outcome of the patients depends on tumor biology. Finding biomarker for neuroblastoma biology is important for the selection of treatment for this disease. This study aimed to find differentially expressed miRNA in plasma and tissue samples of neuroblastoma patients using next generation sequencing. miRNAs found could become potential biomarker in the future management of this disease. MethodWe extracted RNAs from 32 pairs of plasma and tissue samples of 32 neuroblastoma patients. We then prepared small RNA libraries and quantify them. 17 pairs of plasma-tissue libraries were sequenced on hiSeq 2500. Ten pairs has raw reads more than 1 million for each sample. Fold change for each of the 10 pairs was analysed and common dysregulated miRNA in samples belong to same stage were identified. ResultsWe found 87 miRNA upregulated and 17 miRNA downregulated in M stage plasma samples of neuroblastoma patients. ConclusionIn M stage neuroblastoma patients, 87 miRNA were found to be upregulated and 17 miRNA were downregulated. These dysregulated miRNA have potential as diagnostic biomarker but needed futher investigation.

▲TOP

[P-3255] P14-51 [English/Japanese]
Colorectal cancer: prognostic factor (1)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

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P-3255

Identification of high risk factors for stage II colorectal cancer

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Background:
The role of adjuvant therapy is controversial for the stage II colorectal cancer (CRC) patients. Adjuvant chemotherapy may be applied for patients with high risk stage II CRC, however, exact poor prognostic factors have not been identified.
Methods:
Data were retrospectively reviewed from patients with stage II CRC who underwent surgery between 2007 and 2011 at two Japanese institutions. We analyzed overall survival (OS) and relapse-free survival (RFS) according to various factors.
Results:
In this study, 497 patients with stage II CRC were identified.On the multivariate analysis, pT4 (HR 3.33 (95% CI, 1.97-5.65)), colorectal obstruction (HR 2.64 (95% CI, 1.46-4.79)), poorly differentiated carcinoma (HR 2.73 (95% CI, 1.34-5.59)) and age ≥70 (HR 2.09 (95% CI, 1.28-3.43)) were significantly poor prognostic factors for OS, and pT4 (HR 2.87 (95% CI, 1.85-4.46)) and colorectal obstruction (HR 2.09 (95% CI, 1.24-3.55)) were significantly poor prognostic factors for RFS.
Conclusions:
Colorectal obstruction and pT4 are independent poor prognostic and relapse factors for stage II CRC. Adjuvant chemotherapy might be feasible for stage II CRC with colorectal obstruction and pT4.

▲TOP

P-3256

Influence of KRAS mutation on prognostic impact of systemic inflammation in metastatic colorectal cancer patients

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BackgroundThe influence of KRAS genotypes on these markers in mCRC patients remains unclear. We aimed to evaluate the association between pretreatment systemic inflammation and outcome according to KRAS genotypes in mCRC patients.MethodsFive systemic inflammation markers, neutrophi to lymphocyte ratio (NLR), prognostic nutritional index (PNI), Glasgow prognostic score (GPS), systemic inflammatory index (SII) and controlling nutritional status (CONUT), were retrospectively calculated in 252 mCRC patients who received chemotherapy. Patients were categorized into the high or low groups based on their median index values.ResultsMultivariable COX regression analyses revealed that high-NLR, SII and CONUT, and low-PNI were significant predictors of shorter OS in all patients. These correlations were confirmed more clearly in in KRAS-wt patients, but not in KRAS-mut patients except PNI. The interaction test revealed that KRAS genotype may exert a marginally significant influence in the prognostic impact of NLR (p for interaction= 0.063).ConclusionThe prognostic significance of systemic inflammation markers may be more useful in KRAS-wt mCRC patients.

P-3257

Noncoding RNA H19 Regulates Oncogenic Signaling in Colorectal Cancer

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Introduction Accumulating evidence suggests that long noncoding RNAs (lncRNAs) have essential roles in cancer initiation and progression, and deregulated lncRNA expression is found in a variety of cancer types. However, the clinical significance of lncRNAs in colorectal cancer (CRC) remains largely unknown. Material and Methods For the clinical dataset, we analyzed gene expression and clinical data from the Cancer Genome Atlas Project for CRC patients. We used two additional CRC cohorts for validation. To identify the functional involvement of H19 in CRC, we used an integrative approach combining unbiased microarray analysis, experimental validation, and bioinformatic analysis of the clinical data. Results We identified that H19 is the lncRNA most significantly associated with the survival of CRC. Based on functional studies, H19 loss of function reduces CRC malignant phenotypes. An unbiased approach to analyze essential networks mediating H19 function revealed a mechanism by which H19 regulates RB E2F signaling and CDK8 β catenin signaling. Conclusions We demonstrated the clinical and biological significance of H19 in CRC, and discovered its effect on oncogenic signaling.

P-3258

A study of prognostic nutritional index and recurrence risk factors for colorectal cancer after curative resection

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*Background*Nutritional condition is reported to correlate with the prognosis of cancer. Prognostic nutritional index (PNI) is calculated by variables of preoperative total lymphocyte count and C-reactive protein, and it relates to postoperative complications and overall survivals of gastrointestinal cancers. Here, we examined the clinicopathological risk factors including PNI for tumor recurrence and disease-free survival (DFS) in colorectal cancer (CRC).*Methods*We examined 169 CRC patients who underwent curative resection from October 2011 to December 2012 in our hospital. We evaluated PNI and the other clinicopathological factors for the recurrence. We set 46 as the cut-off value of PNI and classified into high PNI (≧46) and low PNI (＜46) groups.*Results*DFS rate was significantly lower in the low PNI group (average, 40.5 months) than the high PNI group (average, 49.2 months) (p=0.006). A multivariate analysis revealed that PNI, venous invasion and tumor location were significant independent risk factors for the recurrence.*Conclusion*The low PNI was a risk factor for the recurrence in CRC. By integrating these risk factors, we report a novel model to predict the recurrence.

▲TOP

P-3259

An analysis of prognostic factors in patients with synchronous and metachronous metastatic colorectal cancer

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Background; Whether there is a difference in the prognostic factors of synchronous metastatic colorectal cancer (mCRC) and metachronous mCRC is unknown. Aim; The aim of this study is to analyze the clinicopathological prognostic factors between the synchronous and metachronous mCRC. Patients and Methods; A total of 144 patients who received only systemic chemotherapy at our department from January 2008 and December 2015 were classified as synchronous mCRC and metachronous mCRC. Overall survival (OS) and clinicopathological prognostic factors were analyzed between groups. Results; The median OS was 24.2 months in patients with synchronous mCRC and 26.3 months in patients with metachronous mCRC (p=0.637). In patients with synchronous mCRC, tumor sideness (right-sided), pT4, and high CA19-9 value before chemotherapy correlated with overall survival. In patients with metachronous mCRC, tumor differentiation correlated with overall survival. Conclusions; Classification of patients according to the synchronous or metachronous presentation of mCRC is prognostic. These results may support the selection of more effective chemotherapy.

P-3260

Enhanced PAICS expression is associated with favorite prognosis in stage Ⅲ colorectal cancer patients

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【Purpose】PAICS (Phosphoribosylaminoimidazole carboxylase) is an important bifunctional enzyme in de novo purine biosynthesis. We investigated PAICS expression in colorectal cancer (CRC) and analyzed the association between PAICS expression and clinicopathological factors.【Methods】PAICS mRNA expression in 83 CRC patients was examined by realtime RT-PCR. We investigated PAICS expression immunohistochemically in 258 CRC patients and analyzed the association between PAICS expression and clinicopathological factors and prognosis. Knockdown of PAICS expression was performed by a siRNA method using CRC cell lines.【Results】PAICS mRNA expression in CRC tissue was significantly enhanced when compared to that in normal tissue. Positive PAICS expression was detected in 55% cases. In stage Ⅲ CRC patients, patients with positive PAICS expression had good prognosis as compared to those with negative expression (p=0.015). Knockdown of PAICS expression acquired the ability of cellular invasion.【Conclusion】PAICS expression was up-regulated in approximately 55% CRC, and higher level of PAICS expression might serve as a good prognostic marker in stage Ⅲ CRC patients.

▲TOP

[P-3268] P14-53 [English/Japanese]
Colorectal cancer: prognostic factor (2)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(B)/7F Lobby/701+702, Osaka International Convention Center Room P(B)

Taishi Hata / Dept. GI Surg. Grad. Sch. of Med., Osaka Univ.

P-3268

Expression and function analysis of syntenin-1 in colorectal cancer

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【Background & purpose】Syntenin-1 is tandem PDZ domains.High expression of syntenin-1 has been reported to be a poor prognostic factor in several kinds of cancers, except colorectal cancer.The purpose of this study were to examine the expression and function of syntenin-1 on colorectal cancer.【Methods】Resected specimens of consecutive 139 cases of colorectal cancer were analyzed by immunohistrochemistry using anti syntenin-1 antibody.We silenced syntenin-1 in three kinds of microsatellite stable colon cancer cell lines using shRNA and performed proliferation,migration and chemosensitivity assays.【Results】92 cases were low expression,and others were high.In overall survival and relapse free survival, high expression group was poor prognosis($p<0.01$) and syntenin-1 expression was risk factor in multivariate analysis($p<0.01$).Syntenin-1 silenced cell lines showed no difference in growth,but significantly decreased cell migration.The sensitivity to oxaliplatin increased with significant difference.【Conclusion】Our result suggests that high expression of syntenin-1 may be a useful prognosis factor and that syntenin-1 is involved in cell migration and sensitivity to oxaliplatin.

▲TOP

P-3269

Prognostic efficacy of the Lymphocyte-to-Monocyte Ratio in patients with curative colorectal cancer resection

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Background: Recent evidence, although limited, suggests that the preoperative the lymphocyte-to-monocyte ratio (LMR) may be prognostic in colorectal cancer (CRC). Objective: The present study aimed to investigate the prognostic value of the LMR in patients with CRC undergoing curative resection. Methods: We performed a retrospective analysis of 207 consecutive patients with stage I to III colorectal cancer who underwent curative surgery between 2010 and 2011. Results: In this analysis, cutpoint of 2.75 were identified for the LMR. The median follow-up was 61.1 months. Versus the high-LMR group, the low-LMR group manifested a significantly shorter OS, and DFS ($p < 0.01$ and $p < 0.01$). In multivariate analysis of all patients, high-LMR was associated with better OS (hazard ratio (HR) 0.502, 95% confidence interval (CI): 0.25-1.00, P = 0.04) independent of age > 70 (P < 0.01), Female (P = 0.02), N positive (P = 0.02), and better DFS (HR 0.543, 95% CI: 0.30-0.98, P = 0.04), independent of age > 70 (P < 0.01), Female (P = 0.01), preoperative serum CA19-9 > 37 (P = 0.03). Conclusions: The LMR affects long-term outcomes after curative colorectal cancer resection.

P-3270

Fusobacterium nucleatum in colorectal cancer liver metastasis and patient prognosis

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Background: Accumulating evidence links the intestinal microbiota and colorectal carcinogenesis. Fusobacterium nucleatum has been shown to promote colorectal tumor growth and inhibit antitumor immune responses. Emerging evidence demonstrates an enrichment of Fusobacterium species in colorectal cancer live metastasis (CRLM).
Aim: To evaluate an association of the amount of Fusobacterium nucleatum in CRLM with patient survival.
Method: Genomic DNA was extracted from CRLM and adjacent normal liver tissues. We measured the amount of Fusobacterium nucleatum DNA in 84 CRLM tissues using a quantitative polymerase chain reaction assay.
Result: Fusobacterium nucleatum was detected in 9 patients (11%) in CRLM and 5 patients (7.5%) in adjacent normal liver tissues. Compared to Fusobacterium nucleatum-negative cases, Fusobacterium nucleatum-positive cases was associated with shorter overall survival in Kaplan-Meier analysis (P = 0.031).
Conclusions: The amount of Fusobacterium nucleatum DNA in CRLM is associated with shorter survival, and may potentially serve as a prognostic biomarker.

P-3271

Molecular staging using OSNA in colorectal cancer

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[Background & Aim]Accurate evaluation of lymph node (LN) status is important for prediction of prognosis. One-step nucleic acid amplification (OSNA^{TM}) assay, which measures cytokeratin (CK) 19 mRNA expression level in whole LN, provides molecular judgement for LN metastasis equivalent to 5mm interval H&E staining. In this study, we and colleagues investigated the clinical impact of OSNA positive cases. [Methods]Patients with cN0 and cN1 colorectal cancer (CRC) in 11 Japanese representative medical institutes were enrolled. All LNs were examined by H&E staining and half of the LN was examined by OSNA assay. The 3-year disease-free survival (DFS) of each cohort was analyzed.[Results]We analyzed 195 patients. Of the patients with node-negative CRCs, only one was OSNA positive at stage I (2%: 1/50), and 11 were OSNA positive at stage II (15.7%：11/70). OSNA-positive stage II cases had much lower 3-year DFS rate than OSNA negative ones (P=0.005) and only OSNA status was a significant prognostic factor for 3-year DFS in stage II CRC cases.[Conclusion]OSNA assay is useful for selecting high-risk patients with stage II CRC.

▲TOP

P-3272

Tumor expression of Activin A is associated with clinical outcome in patients with colorectal cancer

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Background: Activin signaling has been reported to have a critical role in cancer cachexia. We examined the relationship between Activin A expression and clinical outcomes in patients with colorectal cancer.Methods: We analyzed 129 primary colorectal tumors, which were curatively resected in our institution between 2008 and 2012. The expression level of Activin A was measured in tumor tissue by quantitative RT-PCR and immunohistochemical staining. The skeletal muscle amount in the L3 region were measured on preoperative CT.Result: RT-PCR analysis showed that expression of Activin A was significantly higher in tumor area compared with those in normal. Patients in low skeletal muscle group had a significantly shorter overall survival (OS) than those in high skeletal muscle group (5-year OS: 73.6% vs 50.8%, $p<0.05$). In addition, patients with Activin A high-expressed tumor showed a significantly shorter OS than those with activin A low-expressed tumor, though without statistical significance (5-year OS: 64.9% vs 41.9%, $p=0.084$).Conclusion: Our results suggests that high Activin A expression leads to cancer cachexia and associated with poor prognosis in colorectal cancer patients.

▲TOP

[P-3287] P14-56 [English/Japanese]
Colorectal cancer: clinical study

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Norihisa Saeki / Okinawa Pref College Nursing

P-3287

Risk factors for bleeding in patients receiving prophylaxis with Enoxaparin after colorectal cancer surgery

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[Background] The aim of this study was to identify the risk factors for bleeding complications in patients who underwent VTE prophylaxis with Enoxaparin (ENP) after colorectal cancer surgery.[Methods] Records of 382 patients who underwent VTE prophylaxis with intermittent pneumatic compression and ENP after colorectal cancer surgery between April, 2014 and March, 2017 were reviewed. The patient characteristics, surgical procedures and laboratory data were examined to establish risks for bleeding complications using an univariate and multivariate logistic regression model.[Results] Sex (Male/Female), median age and BMI (kg/m^2) were 209/173, 68, 22.1, respectively. The number of laparoscopic surgeries, median operation time (min) and blood loss (ml) were 362, 190.5, 10, respectively. Incidence of bleeding events was 12.6%. In univariate analysis, preoperative ALT level (IU/L)>20, operation time (min)>250 and blood loss (ml)>50 were associated with bleeding events. However, multivariate analysis identified was no independent risk factor.[Conclusion] We did not find any risk factors for bleeding in patients who received ENP for VTE prophylaxis after colorectal cancer surgery.

▲TOP

P-3288

Negative-Pressure Wound Therapy for perineum surgical wound of rectal cancer patients after chemo-radiation therapy

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Extirpation of the rectum, anus, and other pelvic organ results in a large and fixed pelvic dead space. Infection in this cavity after abdominoperineal resection (APR) or total pelvic exenteration (TPE) is one of the most feared complications results in longer post-operative hospital stay. Neoadjuvant Chemoradiotherapy (CRT) for rectal or anal cancer is a risk factor for delay of wound healing. We applied negative-pressure wound therapy (NPWT) for perineal wound of patients having APR or TPE after neoadjuvant CRT and evaluated the usefulness of the NPWT to preventing infection in the pelvic dead space retrospectively.11 patients had neoadjuvant CRT and APR of TPE after 2008 in our hospital. After 2015, we introduced NPWT. 6 patients had normal wound management (control group) and 5 patients had NPWT (NPWT group). Wound dehiscence occurred in 3 patients in control group, but no patients had wound dehiscence in NPWT group (p = 0.04). Average postoperative hospital stay was 66.8 days in control group and 47.6 days (p = 0.13). Wound healing was significantly better in NPWT group. NPWT can be a good therapeutic alternative for patients having APR or TPE after CRT.

P-3289

Laparoscopic surgery for malignant colorectal obstruction after SEMS

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To assess the safety and feasibility of bride to surgery (BTS) using laparoscopic surgery (Lap) after Endoscopic self-expandable metallic stents (SEMS) for malignant colorectal obstruction, we evaluate the results of clinical and pathological outcomes about 25 ceases of BTS-Lap from 2012 to 2016 in our department. Tumor locations of these patients; A/T/D/S/RS, were 3/2/3/13/4. All cases of endoscopic SEMS were successfully inserted to obstruction. Median interval day from SEMS to BTS-Lap was 15 days. The median operation time was 247 minutes and median bleeding was 48.5 ml. Twenty-four cases were performed primary anastomosis. There is no mortality and the morbidity was 16%. Hospital stay after Lap was 11.5 days. We further verified pathological findings that the well or moderate tubular adenocarcinoma（tub1/tub2） was 96%, 24 cases. T3 (Sub-serous invasion) cases were 76%, 19 cases and positive lymph-node rates were 56%, 14 cases. Stage Ⅳ was 12%, 3cases and curative surgery was 88%, 22 cases. Our results showed high primary anastomosis rate, and the morbidity and hospital stays were also feasible outcomes compared to laparoscopic surgery without obstruction.

P-3290

Usefulness of surgical navigation for RPS colorectal cancer operation

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It is difficult to get a sense of touch and top overview for laparoscopic colorectal cancer surgery. Especially, Reduced port surgery(RPS) included single incision Laparoscopic surgery(SILS) is more difficult to do them.We induced navigation surgery from 2005 and RPS operation from 2009.We have been used 3D navigation image for preoperative simulation.In addition, surgery is performed confirming the local anatomy in real time by using this image as navigation during operation. Because if it becomes possible to clearly and stereoscopically grasp the variation of the blood vessel visually, bleeding with accidental blood vessel damage can be avoided. In addition, if we can grasp the position of the lymph nodes suspected of metastasis before the operation, efficient lymph node dissection is possible. Laparoscopic surgery using navigation is an effective method for performing safe and accurate surgery, and it also leads to reduced stress of the surgeon and shortened operation time. At our institution, we are actively implementing safe RPS surgery using this navigation.

▲TOP

P-3291

Effect on clinical outcomes of waiting times for neoadjuvant hyperthermo-chemo-radiation in rectal cancer

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The aim of this study is to initiate a debate on the methodological problems related to measuring patient delay in cancer studies, and to whether there is an association between time to diagnosis, treatment and pCR.Materials and MethodsA retrospective study was performed of 34 patients with stage Ⅱ to Ⅳ resected rectal cancer between May 2012 and June 2016. We measured the waiting time from the day of PETCT to treatment start as a system-related delay time. Intervals from date of PETCT to chemoradiation and thermic treatment were compared.Results1. Proper waiting time depended on tumor sizes.2. Small tumors showed longer waiting time than large tumors; GTV 28.0, 43.7, and 78.6 cm3, 120.4, 67.0, and 12.9 days, respectively. 3. HCRT modality for rectal cancer can be speculated to maximize control in patients with GTV<90cm3. The maximum delay of PETCT to chemoradiation start was 90 days or less.ConclusionThe large tumors needed to reduce time to radiation initiation, and also follow to hyperthermia treatment than small tumors. We believe that efforts to improve the timely delivery of appropriate precise treatment to individual cancer patient is advisable.

P-3292

Factors affecting sentinel lymph node identification rate for lower rectal cancer patients

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OBJECTIVE: The aim of this study was to evaluate clinical and pathological factors affecting lateral pelvic sentinel lymph node (SLN) identification in patients with rectal cancer. BACKGROUND: We have been reported feasibility of lateral pelvic SLN biopsy in patients with lower rectal cancer. Indication for SLN biopsy is still unclear. METHODS: Patients with rectal cancer were enrolled. SLN biopsy were planned in 192 patients. Indocyanine green was injected around the tumor, and the lateral pelvic lymph node was visualized with a near-infrared camera system. Univariate and multivariate analysis assessing factors affecting SLN identification were performed. RESULTS: The lateral SLNs were identified in 176 (92%) of the 192 patients. Among factors evaluated, age (over 60 years old) and tumor location (more than 30mm above the dentate line) were increasing the likelihood of failure to identify the SLN. Clinical T or N stage, tumor differentiation, and lymphovascular invasion did not significantly affect the SLN identification rate. CONCLUSIONS: There are no independent factors found to impact SLN identification. Further evaluation of the predictors of SLN identification is required.

▲TOP

[P-3300] P14-58 [English/Japanese]
Colorectal cancer: metastasis of CRC

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Hiroki Ochiai / Surg., Kitasato Univ., Kitasato Inst. Hosp.

P-3300

Metastasis mouse model using patient derived from colorectal cancer organoids

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Metastasizing to other organs is a major cause of human death associated with colorectal cancer. To investigate metastasis process, we produced the mouse model for colorectal cancer by orthotopic transplantation of patient derived organoids. For this purpose, the matched organoids from primary, liver metastasis and recurrence lesion of same patient were established. No significant differences in pathogenic mutations or drug responses to chemotherapeutic agents were noted among the organoids. The mouse model enables the evaluation of their metastatic ability in vivo. The organoids marked by fluorescent protein and luciferase gene were injected under rectal mucosa on prolapse, so the over time process was visualized by in vivo imaging system. At 15 weeks after transplantation, lung metastases were observed only in recurrence organoids mouse models (4/4 mouse models), but not in primary or metastasis models (each 0/5 mouse models). The results indicate that the recurrence lesion has higher metastatic capacity than the primary and metastasis lesion in vivo. The orthotropic transplantation of patient derived organoids provided us the valuable opportunity to investigate the metastasis.

▲TOP

P-3301

Investigation of the influcence of Fusobacterium on colon carcinogensis and development -establish microinjection model-

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The relationship between H.pylori and gastric cancer is well known. H.pylori eradication therapy is an important strategy for prevention of gastric cancer. On the other hand, Fusobacterium nucleatum (F.nucleatum) which is Gram-negative and anaerobic bacillus is detected at high rate from colon cancer, suggesting that F.nucleatum is related to colon carcinogenesis and progression. However, few reports have examined the detailed mechanism. In this study, we constructed a microinjection model to examine the effect of bacterial infection on carcinogenesis and progression, using colon organoids established from endoscopic specimens and F.nucleatum isolated from clinical specimens.
Endoscopic specimens were 2mm square, but it was possible to establish organoids from normal mucosa, polyp and cancer at a high rate. When F.nucleatum was injected into organoids using the microinjection method, phenotype such as cell proliferation speeds and organoid morphology were changed. In gene expression analysis, cyclrin D1, bclx and c-mic were enhanced. We will also report the other gene analysis and result of transplantation organoids into nude mice subcutaneously.

P-3302

miR-487b may suppress metastasis of CRC progression through inhibition of KRAS

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Recent studies have shown that miRNAs are involved in the progression of colorectal cancer (CRC). The aim of this study is to identify a novel miRNA that especially relates to liver metastasis and to explore the underlying mechanism. Differentially expressed miRNAs were analyzed using microarray, in primary CRC tumors without metastasis (n=16), those with liver metastasis (n=12), and liver metastatic lesions (n=8). We found that miR487b level decreased in liver metastatic lesions. Survival analysis indicated that high expression of miR-487b was associated with better prognosis. In vitro studies were also performed to investigate the functional significance of miR487b in human CRC cell lines. miR487b showed an inhibitory effect on cell proliferation and invasion of CRC cells. miR487b downregulated KRAS and inhibited its downstream signal pathways, and the luciferase reporter assay revealed that miR487b directly targeted LRP6, a receptor for WNT/β-catenin signaling. Our data suggest a possibility that miR487b may suppress metastasis of CRC progression through inhibition of KRAS.

P-3303

Protein components of maple syrup as a potential source to develop novel anti-cancer drugs for colorectal cancer

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Background: Colorectal cancer (CRC) is a leading cause of death among cancer patients. Thus, effective CRC therapy is necessary for saving CRC patients. Maple syrup is popular natural sweetener in the world. We previously reported that maple syrup treatment to CRC cells showed suppression of cell proliferation and invasion via inhibition of Akt activation. In this study, we performed identification of the active ingredients in maple syrup. Method: We fractionated maple syrup based on molecular weight by ultrafiltration. Then, protein components in the high molecular fraction of maple syrup were isolated by ammonium sulfate precipitation method. Cell growth, migratin and invasion assay of CRC cells that were treated the obtained protein componets of maple syrup were performed. Results: CRC cells that were treated protein components of maple syrup showed significantly lower growth rate than control cells. Moreover, cell migration and invasion was also inhibited by protein components treatment. Conclusion: Protein component in maple syrup might inhibit cell proliferation, migration and invasion of CRC cells and be a useful source to develop novel anti-cancer drugs for CRC treatment.

▲TOP

P-3304

TS in CRC predict the response of 5-FU and oxaliplatin-based preoperative chemotherapy for liver metastases

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Background: In patients with colorectal liver metastases (CRLM), predictive markers for response to preoperative chemotherapy are lacking. We evaluated expression of thymidylate synthase (TS) and excision repair cross-complementation group 1 (ERCC1) as predictive markers. Methods: Twenty-four patients with CRLM were included in the study. Tumor responses were evaluated using the tumor regression grade (TRG) and Response Evaluation Criteria in Solid Tumors (RECIST) methods. TS and ERCC1 expression in paired CRLM and primary lesions was assessed by immunohistochemistry. We analyzed correlations between (i) TS and ERCC1 expression and the response evaluated by TRG and RESICT, and (ii) TS and ERCC1 expression in matched pairs of primary tumor and CRLM. Results: The response based on TRG and RECIST were significantly associated with TS expression in the primary tumor (P = 0.0137, and P = 0.0272, respectively). No correlations were detected between marker expression in the primary lesion and in CRLM for either TS or ERCC1. Conclusion:TS expression in the primary tumor is a predictive marker of preoperative chemotherapy response for CRLM.

P-3305

Prediction of Chemotherapy Response to Metastatic Hepatic Colorectal Cancer by Peripheral Ring Enhancement on CT

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Prediction of chemotherapy response is a crucial for clinically management of patients with hepatic metastatic tumors.A total of 48 consecutive colorectal cancer (CRC) patients with hepatic metastasis treated by with or without hepatic resection were retrospectively reviewed and the factors, including the tumor peripheral ring-enhancement (R-E) on CT and chemotherapy response, were analyzed. Additionally, tumor angiogenesis was microscopically evaluated by micro vessel counting (MVD). The overall response rate for the liver metastasis with R-E on CT was 64 % (23/36), whereas the rate without R-E cases was 25% (3/12), with statistically significant differences. The survival rate between with and without liver resections of the all R-E positive cases was not different, whereas the rate with liver resection cases was longer than that without the resection of the all R-E negative cases. Microscopic examination revealed that the R-E appearance on CT of metastatic tumor was associated with the angiogenesis by MVD. The R-E on CT appearance would be associated with the angiogenesis and predict chemotherapy response. The combination of liver resection would further improve the survival.

P-3306

Usefulness of H-classification and oligometastases as prognostic factors in patients with colorectal liver metastases

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Background: Benefits and limitation of the surgical resection of colorectal liver metastases (CRLM) remain unclear. Reliable prognostic factors are needed for appropriate patient selections for the surgical indication. Methods; A total of 327 patients with CRLM from 2000 through 2012 were included in this study. Patients were treated with either surgical resections or chemotherapies (non-resected). Clinical outcomes were examined based on both H-classification (H1, H2, H3) and the concept of oligometastases. Oligo- / polymetastases were defined as a rate thresholds of < / > 2.3 new metastases per year, respectively in this study.Results; The 5-year OS rates in H1 (98), H2 (28), and H3 (13) groups were 56.7%, 40.3% and 0%, respectively. In the non-resected group, H1 (102), H2 (27), H3 (56) groups were 54%, 39% and 12%. Patients with H3 hepatic metastases had poor outcomes in both resected and non-resected groups. The 5-year OS rate of patients with oligo- (52) and polymetastases (27) were 84% and 66%, respectively ($p<0.05$).Conclusion: Our results suggested that H-classification and oligometastases criteria were useful to predict outcomes of patients with CRLM.

▲TOP

[P-3313] P14-60 [English/Japanese]
Colorectal cancer (2)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Mutsumi Fukunaga / Dept. Surg., Hyogo Prefectural Nishinomiya Hospital

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P-3313

Pterostilbene inhibits cancer stem cells by increase of oxidative stress

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Pterostilbene (PTE) is a natural analog of resveratrol and has examined antitumoractivity against various types of cancer. We assessed the effect of PTE on cancer stemcells. Mouse colon cancer CT26 cells were treated with high (100 μM) and low (10 μM)concentration PTE. We investigated the gene expression of cancer stem cell markersnucleostemin and kip2. The gene expressions were decreased by PTE at bothconcentrations. However, only high dose PTE increased hemeoxygenase-1 expression,an oxidative stress marker. An oxidative stress was examined by 4-hydroxynonenallevels. It was increased by PTE in a dose-dependent manner. High dose PTE inhibitedcell growth by 20%, which was restored by necrostatin-1 and vitamin E. Based on theabove results, it was suggested that high concentration PTE was increased oxidativestress and impairs cancer stem cells via necroptosis

▲TOP

P-3314

A Src-YAP module promotes colonic tumorigenesis

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The important effector of the Hippo pathway, YAP, is strongly activated in the majority of colorectal cancer. However, the mechanism by which YAP is activated in colorectal cancer remains unknown. Here, we found that in addition to β-catenin, loss of tumor suppressor adenomatous polyposis coli (APC) activates Src family kinases (SFKs), YAP and STAT3, which are simultaneously activated in 60-70% of human colorectal cancer specimens. We also found that up-regulation of IL-6 signal transducer (IL-6ST/gp130), which is induced by YAP activation, causes the activation of SFKs, YAP and STAT3, and YAP activation is Src-dependent in APC-deleted intestinal organoids. The combination treatment of SFK and JAK inhibitors results in regression of established mouse colorectal tumors. These results suggest that these signaling pathways might be attractive therapeutic targets in human colorectal cancer.

P-3315

Chronological analysis of combination of serum microRNA expression in colorectal cancer

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Background: Serum microRNAs (miRNAs) have been shown to be biomarkers in colorectal cancer (CRC), but have not been applied in a clinical setting. We evaluated the clinical effectiveness of serum miRNAs expression in CRC using periodically gathered serum.Materials and Method: We obtained 400 serum samples, which gathered periodically, from 71 patients with stage II/III CRC and from 72 healthy control. Previously-reported miRNAs were quantified by microRNA microarray analysis.Results: In no recurrence cases, preoperative miR-25-3p, 1246 expression was significantly higher than postoperative time point. We observed trend of miR-25-3p, 1246 expression re-elevation before recurrence. The sensitivity and specificity of miR-25-3p, 1246 and combination for detection of CRC were 27%, 45%, 52% and 90, 91, 92% (AUC=0.737, 0.749, 0.780). The sensitivity of CEA is 38% and the specificity is 94%. The sensitivity and specificity of combination was higher than of CEA. Conclusion: Combination of serum miRNA are promising biomarker for CRC with high sensitivity and specificity. Next we are going to identify novel microRNAs which has higher sensitivity and specificity using our microarray analysis.

P-3316

The clinical implication of O6-methylguanine DNA methyltransferase in rectal cancers

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Concurrent chemoradiotherapy (CCRT) followed by surgery is now the standard of treatment for advanced rectal cancers. The DNA repair enzyme, O6-methylguanine-DNA methyltransferase (MGMT), has been reported to be involved in colorectal carcinogenesis. However, the clinical impact of MGMT expression on rectal cancers remained unclear. In this study, after examining tumor tissues from 172 patients with rectal cancer undergoing neoadjuvant CCRT, high MGMT expression was significantly correlated with advanced post-treatment tumor and node stages, and inferior tumor regression grade. Notably, high MGMT expression significantly appeared as an adverse predictor not only for recurrence-free survival (RFS) but also overall survival (OS). Moreover, high MGMT expression maintained an independent prognostic predictor for shorter RFS and OS. After combination treatment with irradiation and MGMT inhibitor, the rates of colony formation were decreased, and both annexin-V and gamma H2AX positive cells were increased in colorectal cancer cells, as compared with irradiation alone. These findings suggest novel therapeutic approaches targeting MGMT for rectal cancers.

▲TOP

P-3317

Theranostics with hybrid liposomes in the orthotopic graft model mouse of colorectal cancer

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Inhibitory effects of hybrid liposomes (HL) composed of vesicular (DMPC) and micellar ($C_{12}(EO)_{25}$) molecules without drugs on the growth of various tumor cells have been obtained in vitro, in vivo, and for clinical applications without any side effects. This study aimed to elucidate the therapeutic effects and ability of HL to detect cancer in an in vivo orthotopic graft mouse model of colorectal cancer with HCT116 cells for the use of HL as theranostic agents. Intravenously administered HL caused a remarkable reduction in the relative cecum weight in an orthotopic graft mouse model of colorectal cancer. A decrease in tumor size in the cecal sections was confirmed by histological analysis using HE staining. TUNEL staining indicated an induction of apoptosis in HCT116 cells in the mouse model of colorectal cancer. The accumulation of HL encapsulating a fluorescent probe (ICG) was observed in HCT116 cells in the in vivo colorectal cancer model following intravenous administration. These data indicate that HL can accumulate in tumor cells in the cecum of the orthotopic graft mouse model of colorectal cancer for a prolonged period of time, and inhibit the growth of HCT116 cells.

P-3318

Fluid shear stress promotes the growth of cancer cells in cell clusters of colorectal cancers

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[Backgrounds] Circulating tumor cells are exposed to fluid shear stress (FSS) by blood flow. While circulating cancer cell clusters are reportedly more responsible for metastasis than single cells, most of the studies have been done on single cells. We have recently reported that disruption of the architecture of cancer spheroids promotes the stemness and the growth of colorectal cancer. Here, we investigated the contribution of FSS on the stimulated growth of cell clusters, comparing with single cells. [Experiments] Colorectal CTOS (Cancer Tissue-Originated Spheroid), a patient-derived 3D culture system, was used as a cancer cell cluster model. FSS stimulated growth of CTOS but not of single cells. Gene ontology analysis showed that “response to wound” and “membrane region” genes were induced by FSS. Indeed, membrane damage by electroporation stimulated the growth of CTOS. in addition, knockdown of ‘Gene X’, a membrane repair related gene induced by FSS, reduced the growth stimulation of CTOS by FSS. [Conclusions] FSS stimulated the growth of cancer cell clusters, possibly via membrane damage and repair process.

▲TOP

[P-3325] P14-62 [English/Japanese]
Biliary tract cancer (2)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Kunihito Gotoh / Dept. Gastroenterol. Surg., Osaka Univ., Sch. Med.

P-3325

Combination radiotherapy with NF- κ B inhibitor enhances the antitumor effect of gallbladder cancer cells

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【Background】Radiotherapy has been reported as a neoadjuvant therapy for patients with locally advanced nonresectable gallbladder cancer (GBC), but its effect is limited. Nuclear factor kappa B (NF-κB) is activated in cancer cells and involved in tumor growth and invasion. NF-κB is activated by radiotherapy, causing treatment tolerance. We previously reported that nafamostat mesilate (NM) inhibited NF-κB activation and induced antitumor effects in pancreatic cancer. We hypothesized that NM may inhibit radiation-induced NF-κB activation and enhanced the antitumor effect of radiotherapy for GBC cells.【Methods】We assessed NF-κB activity, cell viability and quantitative apoptosis of human GBC cells treated with radiation alone (5Gy), NM alone or combination of radiation and NM.【Results】As compared with radiation group, combination group had lower NF-κB activity ($p<0.01$), reduced cell viability ($p<0.01$), and increased apoptosis ($p<0.01$). 【Conclusion】NM suppressed radiation-induced NF-κB activation and enhanced the antitumor effect on gallbladder cancer cells.

▲TOP

P-3326

Intratumoral T-cell heterogeneity in resected biliary tract cancer and implication of survival

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Backgrounds:
We found IL6 and TGF-b expressed with intratumoral heterogeneity of biliary tract cancers (BTC); We hypothesized that cytokine heterogeneity in BTC induced heterogenetic differentiation of T-cells , and aimed to clarify this heterogeneity, the clinicopathological features, and implication for survival after surgical resection.
Methods:
We performed immunohistochemistry for Foxp3 and IL17 in 127 resected BTCs from 2000 to 2012 and examined heterogeneity, the related clinicopathological features, and overall survival (OS) after surgery.
Results:
(1) The average count of Foxp3+ cells was higher at center and that of IL17+ cells was higher at periphery, but there was not statistically different.
(2) We divided to two groups according to Foxp3 or IL17 positive reactivity (high and low groups). The analysis of OS related to T-cell heterogeneity revealed that 5-year OS at in Foxp3-high at center was poor and 5-year OS in IL17-high at periphery was poor. Conclusions:
As our hypothesis, Foxp3+ T cells highly accumulated at center, IL17+ T cells slightly accumulated at peripheral, and both Foxp3+ at center and IL17+ at periphery were prognostic factors.

P-3327

Effects of fatty liver on development of intrahepatic cholangiocarcinoma

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Fatty liver accelerates hepatic inflammation, fibrosis and hepatocellular carcinoma development, but it is unknown whether fatty liver affects development of intrahepatic cholangiocarcinoma (IHCC). We previously found bile ductular proliferation and IHCC lesions in an experimental mouse model of a carcinogen-induced colorectal cancer. In the present study, we used obese and diabetic C57BL/KsJ-db/db (db/db) and control C57BL/6 mice, and they were administered the carcinogen in order to establish a novel mouse IHCC model and to investigate the effects of obesity and fatty liver on IHCC development. As expected, db/db mice showed significant body weight gain and fatty liver compared to control. The extent of bile canalicular epithelial hyperplasia with atypia significantly progressed, and the incidence and tumor area of IHCC markedly increased in db/db mice. These results suggest that fatty liver can exacerbate bile ductular proliferation and promote IHCC development. In addition, the novel animal model appears useful to investigate the mechanism of intrahepatic bile duct carcinogenesis and the chemoprevention of this malignancy.

P-3328

Roles of biliary tract cancer cell-derived exosomes in tumor angiogenesis

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Introduction; Tumor-derived exosomes, which contain microRNAs(miRs), were shown to be involved in tumor microenvironment, tumor angiogenesis, metastasis, and tumor immunity. We explored roles of the human gallbladder cancer cell-derived exosomes, particularly focusing the effect of the exosomes on capillary formation. Methods; Exosomes are isolated from culture media of gallbladder cancer cell lines NOZ and G-415 by means of ultracentrifugation. We examined whether the tumor-derived exosomes affect angiogenesis by employing HUVEC culture systems. Results and Summary; In the presence of the exosomes in the medium, HUVEC declined its tube formation on Matrigel, but did not change its proliferation. Because we previously discovered that the exosome contained several anti-angiogenic miRs by miRNA array analysis, we hypothesized that the miRs repressed the vascular network formation. We next applied HUVEC tube formation assay with miRNA mimics. miR-494 mimic inhibited the tube formation. The results suggest that gallbladder cancer cell-derived exosomes may inhibit tumor angiogenesis, through transmission of these miRs into endothelia.

▲TOP

[P-3280] P14-55 [English/Japanese]
Colorectal cancer (2)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

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P-3280

Universal screening with microsatellite instability testing in Japanese patients with colorectal cancer

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<Background>Microsatellite instability (MSI) testing facilitates screening for Lynch syndrome (LS), and also helps to predict the efficacy of immune checkpoint inhibitors (ICIs). However, the frequency of MSI -High is unclear in Japanese colorectal cancer (CRC) patients.
<Purpose>To evaluate the frequency and patient characteristics of MSI-High in Japanese CRC patients.
<Methods>Examination of detailed family history, screening using the revised Bethesda guidelines and Amsterdam criteria II, and MSI testing were done in a consecutive series of 30 patients with newly diagnosed CRC (NDCRC) who visited our institution from December, 2017 to April, 2018.
<Results>MSI-High was found in 5 patients (16.7%). Four of them did not meet both the revised Bethesda guidelines and Amsterdam criteria II. Furthermore, in this MSI-High group, CRC arose predominantly in the right side of the colon, in contrast to the microsatellite stable group (n=25) ($p<0.01$). There were no significant differences in age, sex, stage, or pathological findings.
<Conclusion>The frequency of MSI-High in Japanese NDCRC patients will be approximately the same as that of Western CRC patients.

▲TOP

P-3281

Prognostic factor of Dipeptidyl peptidase 9 expression in patients with colorectal cancer

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Background: Dipeptidyl peptidase 9 (DPP9) is a member of a family of ubiquitous atypical serine proteases, which mediate diverse physiological and pathological processes beyond incretin degradation, such as cell migration, tumor cell invasion, and metastasis. However, the biological function in human cancer is largely unknown. Methods: This study included 196 patients who underwent surgery for CRC. DPP9 expression was examined by real-time reverse transcription-polymerase chain reaction and expression levels were evaluated with various clinical parameters. We also examined the potential role of the DPP9 inhibitor, vildagliptin, for the treatment of CRC using a CRC cell line and primary cultured CRC cells in vitro.Results: The patient group with hHigh DPP9 expression was associated with significantly worse overall survival than the group with low DPP9 expression, according to univariate and multivariate analyses. Conclusions: DPP9 expression is a risk factor for poor prognosis in patients with CRC. DPP9 inhibitors might be useful therapeutic agents for CRC.

P-3282

The association of PLXND1 and epithelial-to-mesenchymal transition in colon cancer

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Background:PLXND1 is a cell membrane single penetrating receptor, involved in invasion and metastasis in various cancer. There were few reports showing the relationship between colon cancer and PLXND1. There was a report that the number of distant metastases had decreased when knocked down of PLXND1 in colon cancer. There are many unclear points about the function of PLXND1. There is a report that PLXND1 and epithelial to mesenchymal transition (EMT) are related in ovarian cancer. Object:To determine whether PLXND1 is involved in EMT in colon cancer. Result: (1) The Expression of EMT markers (E-cadhelin, Vimentin, Snail, ZEB1) was examined by qRT-PCR by sorting colon cancer cell lines (SW480, HCT116, CaCO2, HT29) into PLXND1 negative cells and positive cells using fluorescence activated cell sorting. The expression of the EMT markers (Vimentin, Snail, ZEB1) showed significant increase in PLXND1 positive cells compared to PLXND1 negative cells. (2) PLXND1 was knocked down in the cell line with the high expression of PLXND1, the expression of EMT markers (Vimentin, Snail, ZEB1) decreased. Conclusion:These results suggested the association between PLXND1 and EMT in colon cancer.

P-3283

Microsurface structures are associated with mutational intratumoral heterogeneity in colorectal tumors

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Intratumoral heterogeneity (ITH) of genetic alterations affects cancer diagnosis and treatment. We aimed to clarify the relationship between surface microstructures (pit patterns) of colorectal and genetic ITH. A total of 711 biopsy specimens were obtained from respective pit pattern areas of 477 colorectal lesions. Colorectal tumors with multiple pit patterns exhibited higher frequencies of KRAS and/or TP53 mutations than tumors with a single pit pattern. In tumors with multiple pit patterns, mutations were observed as public (common to all areas) or private (specific to certain areas), and private KRAS and/or TP53 mutations were often variable and unrelated to the pit pattern grade. Notably, invasive CRCs frequently exhibited public TP53 mutations, even in adenomatous areas, which is indicative of their early malignant potential. Targeted sequencing revealed additional public and private mutations in tumors with multiple pit patterns, indicating their single clonal origin. Our results demonstrate that intratumoral variations in the surface microstructure may represent molecular subclones in early colorectal lesions and may be predictive of the malignant progression.

▲TOP

P-3284

Epitranscriptome Methyl-reader Protein YTHDF1 Via Regulation of c-Myc Facilitates Colorectal Cancer Progression

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To facilitate the individualized medicine in colorectal cancer (CRC), it is necessary to study genome sequence but also epitranscriptome (ETR) information, i.e., the methylation of RNA. Recent studies indicated RNA modification is a promising target for cancer detection, diagnosis, and treatment. We here studied the cancer genome atlas (TCGA) database showing significant roles of ETR, in which N6-methyladenosine marking is the most common modification, and found that Ythdf1, but not other families, plays a role in cancer phenotypes as a reader protein of ETR in CRC. Immunohistochemical study revealed tumors with strong staining of Ythdf have significantly poorer prognosis. Chromatin immunoprecipitation-sequence profiling analysis combined with subsequent knockdown experiment indicated that oncogenic transcription factor c-Myc regulated expression of Ythdf1. We confirmed ETR control is associated with proliferation and reduces sensitivity to therapeutic agents such as 5-FU and L-OHP. In summary, the present study demonstrates that ETR regulation under the control of c-Myc plays an important role in cancer progression and suggests a novel target for precision medicine in CRC.

P-3285

Effects of LPA receptors on the acquisition of malignant properties in colon cancer cells treated with anticancer drug

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Lysophosphatidic acid (LPA) signaling via G protein-coupled LPA receptors regulates a variety of malignant properties. In this study, we investigated whether LPA receptors regulate cellular functions in colon cancer cells treated with anticancer drugs. To obtain the long-term anticancer drug treated cells, DLD1 cells were treated with fluorouracil (5-FU) and cisplatin (CDDP) (DLD-5FU and DLD-CDDP cells, respectively). The expressions of ABC transporter genes were elevated in DLD-5FU and DLD-CDDP cells. In cell motility assay, DLD-5FU cells indicated the high cell motile activity, compared with DLD1 cells. The elevated cell motile activity was suppressed by LPA1 knockdown. LPA4 and LPA6 knockdown enhanced the cell motile activity of DLD-5FU cells. On the other hand, the cell motile activity of DLD-CDDP cells was reduced. In colony assay, DLD-5FU cells formed the large sized colonies, while no colony formation was observed in DLD1 and DLD-CDDP cells. The colony formation was inhibited by LPA1 knockdown. These results suggest that LPA receptor signaling is involved in the acquisition of malignant properties in DLD1 cells treated with anticancer drugs.

P-3286

Expression and functional analysis of DSG1 in colorectal cancer

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Spheroid colony formation is an effective model for characterization of cancer stem cells. Previously, we performed microarray analysis in spheroid body-forming and parental cells and found that expression of Desmoglein1(DSG1) was upregulated in gastric spheroid colonies. Desmoglein is cadherin family member that mediates cell-cell adhesion in desmosomes. Desmosomal cadherins are also involved in epithelial cell proliferation and tumorigenesis. However, the significance of DSG1 in CRC has not been analyzed. In the present study, we have analyzed the expression and distribution of DSG1 in human CRC cases. Immunostaining demonstrated that 52 out of 100(52%) CRC cases were positive for DSG1. Expression of DSG1 was associated with T classification and survival in patients with CRC. The growth of DSG1 siRNA-transfected CRC cells was significantly slower than the growth of negative control siRNA-transfected CRC cells. In addition, both the number and size of spheres from CRC cells were significantly reduced upon DSG1 siRNA-transfection compared with negative control. These results suggest that DSG1 is involved in tumor progression and plays an important role in cancer stem cells of CRC.

▲TOP

[P-3293] P14-57 [English/Japanese]
Colorectal cancer: chemotherapy

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Kazumasa Minami / Dept. Radonc., Osaka Univ., Grad. Sch. Med.

P-3293

Comparing the responses of wild-type and mutant k-ras colorectal cancer tumors to interleukin-6 receptor antibody

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Interleukin-6 receptor antibody (IL-6RAb) has been demonstrated to inhibit the tumor growth in k-ras mutated colorectal cancer. Here, we further compared IL-6RAb effect in vivo to the xenograft of wild-type HT-29 and mutant k-ras SW480 cells. HT-29 and SW480 cells were inoculated subcutaneously in nu/nu mice. The tumor growth were suppressed in IL-6RAb treated mice compared with untreated mice in both HT-29 and SW480 xenografts since the tumor shrinking was only found in SW480 xenograft. Angiogenetic vessels in tumors were in the untreated mice, whereas only peripheral vessels could be found in both IL-6RAb treated tumors. The growth signaling were downregulated in the tumor cells of IL-6RAb treated mice.IL-6RAb could suppress tumorigenesis and angiogenesis in the xenograft of HT-29 and SW480 cells, indicating its potential application on the treatment of mutated more than wild-type k-ras CRC.

▲TOP

P-3294

CD44/CD133-Positive Colorectal Cancer Stem Cells Are Sensitive to Trifluridine

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Cancer stem cells (CSCs) are recognized to be involved in disease recurrence in patients with metastatic colorectal cancer, but no effective anticancer agents targeting CSCs are currently available. Because trifluridine (FTD)/tipiracil (TPI) therapy is used for refractory colorectal cancer that is considered to show an enriched CSC phenotype, we sought to determine whether FTD might be effective against CSC-like cells in which CD44 and CD133 are highly expressed. Markedly higher sphere formation was observed with CD44high/CD133high DLD-1 cells than with CD44low/CD133low cells, and relative to unsorted cells, CD44high/CD133high cells displayed increased 5-fluorouracil resistance but not FTD resistance (2.2- and 1.2-fold change in IC50) in proliferation assays. FTD treatment disrupted sphere formation by DLD-1 cells, and in DLD-1 cells exposed to long-term FTD treatment, the CD44high/CD133high population was lower than that in the parental cell line. Notably, these FTD-treated cells showed diminished sphere-forming ability and decreased tumor-forming potential in vivo. FTD treatment is effective against CSC-like cells, and long-term FTD exposure could lead to CSC depletion.

P-3295

Inhibition of HER3 and MET as combination targeted therapy in human colorectal cancer

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Colorectal cancer is one of the leading causes of human cancer death all over the world. Therefore, it is necessary to establish novel diagnosis and therapy for colorectal cancers. We have recently found that expression levels of HER3 and MET proteins were positively correlated on colorectal cancer cell lines by flow cytometry analysis. In the present study, we have investigated whether co-inhibition of HER3 and MET can be an effective therapeutic strategy for colorectal cancer. Co-stimulation of HER3 and MET by neuregulin-1 (NRG) and hepatocyte growth factor (HGF), a ligand of HER3 and MET, respectively, potentiated the growth of SW1116 colorectal cancer cells, as compared with each treatment alone. Furthermore, simultaneous treatment with NRG and HGF increased the phosphorylation of ERK, while this effect was abolished by anti-HER3 monoclonal antibody and PHA665752 MET inhibitor. Co-inhibition of HER3 and MET caused a reduction of cell growth in vitro, and a delay in tumor growth in xenograft model. These findings suggest that HER3 and MET are involved in the growth of colorectal cancers, and are potential therapeutic target molecules. Collaborators: H Okuno, T Imaida

P-3296

Significance of monitoring VEGF signals in blood during treatment of colorectal cancer patients

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(Background) Anti-VEGF drugs are used in colorectal cancer (CRC) treatment. VEGF signals may change during chemotherapy but monitoring real time changes hasn't been conducted. (Methods) Blood monitoring was carried out to track changes in the status of VEGF ligands (A, C, and D) in CRC patients. Expression was determined by ELISA using patients' plasma. We evaluated differences before and after thermotherapy, and differences with or without bevacizumab (Bmab). (Results) VEGF monitoring was performed in 67 metastatic CRC patients under chemotherapy. Regarding Bmab therapy, VEGF-A with Bmab is significantly higher than without Bmab. Regarding VEGF level, patients with high VEGF-D had a significantly worse prognosis than those with low VEGF-D. Moreover, the group of low VEGF-D with Bmab has better prognosis than without Bmab. On the other hand, there is no difference in high VEGF-D group regardless of Bmab. (Conclusions) High VEGF-D patients might be candidate another anti-VEGF drug than Bmab. We must continue monitoring VEGF. Monitoring VEGF signals could provide useful information to evaluate treatment response and optimal drug selection.

▲TOP

P-3297

New predictive marker for anti-EGFR therapy in metastatic colorectal cancer with wild-type KRAS

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BACKGROUND. Since the KRAS mutation is not responsible for all metastatic colorectal cancer (mCRC) patients with resistance to anti-epidermal growth factor receptor (EGFR) monoclonal antibody (MoAb) therapy, new predictive and prognostic factors are actively being sought.METHODS. We retrospectively evaluated the efficacy of anti-EGFR MoAb-based therapies in 91 patients with mCRC according to KRAS, BRAF and PIK3CA mutational status as well as PTEN and MET expression.RESULT. Patients with MET overexpression showed lower DCR (P = .040) and shorter PFS (P = .018) when compared to patients with normal MET expression. In multivariate analysis, the BRAF mutation and MET overexpression were identified as independent factors for shorter PFS among patients with wild-type KRAS (BRAF; P = .004; MET; P = .046). The BRAF mutation was also identified as an independent factor for shorter OS among patients with wild-type KRAS (P = .001). CONCLUSION. Our data point to the usefulness of MET overexpression, in addition to BRAF mutation, as a new predictive marker for responsiveness to anti-EGFR MoAbs in mCRC patients with wild-type KRAS.

P-3298

Prospective-retrospective biomarker analysis of T-CORE0801: DNA methylation status in anti-EGFR treatment against mCRC

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We previously reported that, in addition to the RAS mutation status, DNA methylation status of metastatic colorectal cancer (mCRC) is a predictive marker of anti-epidermal growth factor receptor (EGFR) treatment. However the result has not been confirmed in other cohort.Of 43 cases registered in the T-CORE0801 trial, which is a phase II trial of irinotecan plus anti-EGFR antibody for mCRC and was conducted before the approval of the RAS mutation test, 30 cases available were conducted for DNA methylation analysis by Infinium Methylation EPIC array. Then the association with updated clinical outcomes for anti-EGFR treatment and DNA methylation status was retrospectively analyzed. The 30 cases were classified to 3 groups: RAS mutant (n=15), RAS wild and highly methylated CRC (HMCC, n=7) and RAS wild and low methylated CRC (LMCC, n=8). The response rate to anti-EGFR treatment in RAS mutant, HMCC and LMCC were 0%, 0% and 27%, respectively. The median progression-free survival in RAS mutant, HMCC and LMCC were 67, 56 and 156 days, respectively. This prospective-retrospective analysis supported predictive value of DNA methylation status for response to anti-EGFR treatment in mCRC.

P-3299

The impact of adjuvant chemotherapy completion on prognosis of stage III colorectal cancer

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[Introduction] The duration of adjuvant chemotherapy (aCTx) for colorectal cancer is 6 months. IDEA collaboration study could not prove noninferiority of 3 months duration of oxaliplatin-based aCTx compared with 6 months duration. Thus, the duration of aCTx might be related to the prognosis of patients who received aCTx. This study was undertaken to investigate the relation between completion of aCTx and prognosis. [Methods and Patients] We retrospectively analyzed patients who received aCTx postoperatively with Stage III. The completion of aCTx was defined as the completion of duration of planned aCTx. We divided patients into two group, aCTx completion group (Comp group) and aCTx interruption group (non-Comp group). [Results] From 2008 to 2015, 221 patients received aCTx. The completion rate was 76.0% and the 5-year DFS and OS was 76.3% and 90.2%. We could not find significant difference of the prognosis between the groups, however, in stage IIIb patients, or oxaliplatin-based aCTx patients, the RFS tended to be bad in non-Comp group. [Conclusion] We compared the prognosis of stage III patients who received aCTx.

▲TOP

[P-3307] P14-59 [English/Japanese]
Colorectal cancer (1)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

Kazuhiro Morishita / Dept. of Med Sci., Fac. of Med., Univ. of Miyazaki

P-3307

Withdrawn

No Abstract

▲TOP

P-3308

The chemopreventive effect of combination treatment of aspirin and metformin for colorectal carcinogenesis

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The most precise chemoprevention agent for colorectal cancer (CRC) is aspirin. However, the chemoprevention effect of aspirin is not so strong and has some adverse effect. We previously analyzed the chemopreventive effect of low dose metformin in rodent model and conducted clinical trial for adenoma prevention, and revealed good response. Then we analyzed the chemopreventive effect of combination treatment of aspirin and metformin for colorectal carcinogenesis. We obtain normal colorectal tissue, adenoma and cancer from CRC patients by colonoscopy and cultured organoid in the matrigel. Then we treated both or with or without aspirin and metformin and analyzed the chemoprevention effects. Combination treatment significantly suppressed cell proliferation in both in normal tissue, adenoma and cancer compared with single treatment of aspirin or metformin. Combination treatment significantly activated AMPK and suppressed mTOR pathway. The chemopreventive effect of combination treatment for colorectal carcinogenesis is stronger than that of single use of each agent. The combination treatment of aspirin and metformin is one of the option for the CRC chemoprevention.

P-3309

Investigating the regulatory mechanisms of the extracellular vesicle secretion in colorectal cancer cells

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Cancer-derived extracellular vesicles (EVs) dictate its microenvironmental cells which support the growth of tumor cells, suggesting the indispensable roles of EVs in cancer progression. Thus, the mechanisms of EV secretion might contribute the regulation of EV-mediated cancer progression, however, its mechanism is still unclear. The purpose of this study is to elucidate the mechanisms of EV secretion in cancer cells. To reveal this, miRNAs, which are known to involve in cancer progression, were employed. It has been well known that miRNAs regulate almost all of the genes, thus the regulation of EV secretion by miRNAs could be expected. Combined with ExoScreen, which is ultra-sensitive detection method of EV, miRNA-based screening was performed in colorectal cancer cell line HCT116, which is already known to regulate its microenvironment by EVs. Through this screening, we found several miRNAs which regulate the EV secretion negatively and positively. These miRNAs have not been previously reported for the involvement in cancer progression. The targets of these miRNAs were identified, and our results provided the new insight into the mechanisms of cancer progression mediated by EVs.

P-3310

Molecular imaging of colorectal tumor targeting epidermal growth factor receptor (EGFR)

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Background & Aim: Colorectal cancer (CRC) ranks one of the highest leading causes of cancer-related death worldwide. We conducted molecular imaging targeting EGFR for evaluating therapeutic efficacy and the possibility of endoscopic detection of colorectal tumors. Methods: 1) The number of EGFR was quantified by flow cytometry using FITC-labeled anti-EGFR antibody (EGFR-Ab). 2) Subcutaneous tumors were induced in the nude mice and were treated by 5-FU. Images were recorded with IVIS Spectrum. 3) Colon adenocarcinogenic model rats were created by injecting azoxymethane. Colonoscopy was performed after spraying Alexa Fluor (AF)-labeled EGFR-Ab. Results: 1) Significant correlation was observed between the number of EGFR and fluorescence intensity in cell lines. 2) Fluorescence intensity of AF-EGFR-Ab was strong in LIM1215 tumor. Tumor growth was suppressed by 5-FU and treated tumor tissue showed necrosis, which was corresponded to fluorescence intensity. 3) In vivo animal endoscopy targeting EGFR showed strong fluorescence at the site of polyp. Conclusion: Molecular imaging of EGFR can differentiate colorectal tumors and can help evaluating the efficacy after chemotherapy in CRC.

▲TOP

P-3311

Analysis on stem, basal and neuroendocrine markers in human colorectal cancers resected after chemoradiation therapy

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Therapy resistance is the most serious problem in managing cancer patients. Recent study showed that cellular plasticity is involved in therapy resistance. In this study, we analyzed phenotypic changes in human colorectal cancer specimens resected after chemoradiation therapy (CRT), compared with untreated cancer specimens. [Materials and Methods] Phenotypic markers, including stem, basal, and neuroendocrine markers, were immunohistochemically analyzed in thirty cases of human colorectal cancer specimens, and compared with thirty cases of untreated specimens. The RNAscope assay was also used in investigating Lgr5 expression. [Results] There was no significant change in stem cell markers, including Lgr5, BMI1, YAP/TAZ, EZH2 and SOX2 between CRT specimens and untreated ones. Expressions of basal and luminal markers also showed no significant changes. In contrast, expression of NEUROD1, a neuroendocrine marker, significantly increased in CRT specimens, compared with the untreated specimens. [Conclusion] Neuroendocrine differentiation may be induced after CRT in human colorectal cancer. We will add some in vitro data and discuss the significance of this finding.

P-3312

Hibiscus delphinidin-rich extract induced apoptosis via target AMPK in Colon Cancer Cells

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Colorectal cancer (CRC) is the third most common malignant cancer in Taiwanese. Hibiscus sabdariffa L. (Malvaceae) contain a high level of flavonoids, such as anthocyanins. Previous evidence supports that Hibiscus delphinidin-rich extract (HDE) can reduce tumorigenesis and possess antioxidant and anti-carcinogenesis functions. In this report, We found that apoptosis was induced by HDE in LoVo cells, by examination of morphological changes, DAPI assay, the propidium iodide(PI) flow cytometric assay and mitochondrial membrane potential. Further, in order to investigate the expression of apoptosis-related proteins were analyzed by Western blotting. After 36 h treatment, HDE abrogated the expression of anti-apoptotic Bcl-xl protein and enhanced the levels of pro-apoptotic tBid and Bax proteins followed by cytochrome c release and caspase-3 activation. These findings suggest that HDE is a strong potential agent for the treatment of colon cancer since it induced apoptosis through the activation of caspase activity in LoVo cells. HDE exerts more significant cancer chemopreventive effect and may be helpful for clinical use in the future.

▲TOP

[P-3319] P14-61 [English/Japanese]
Biliary tract cancer (1)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(C)/8F Lobby/801+802, Osaka International Convention Center Room P(C)

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P-3319

Cellular senescence and inhibitory effects of PRIMA-1MET in cholangiocarcinoma

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Cellular senescence is an irreversible growth arrest. It is mediated by p53-dependent and -independent pathways in the response to various stresses. Moreover, cellular senescence also suppresses tumorigenesis. Approximately 50% of cholangiocarcinoma (CCA) have a p53 mutation in relation to the resistance of cancer cells to conventional chemotherapy. Recently, prosenescent agents such as PRIMA-1^{MET} has been used for restoration of p53 activity which induces cellular senescence and apoptosis leading to inhibition of cancer cell growth and metastasis. Here we investigated the expression of $p16^{INK4a}$ and p21 in human CCA tissues and the effects of PRIMA-1^{MET} on CCA cells. Our data showed that the high expression of $p16^{INK4a}$ and p21 was associated with longer survival rate of CCA patients. Moreover, we found that PRIMA-1^{MET} inhibited CCA cell growth via induction of cellular senescence followed by apoptosis, which demonstrated as the increase of $p16^{INK4a}$, p21 and Bax/Bcl-2 ratio. In addition, PRIMA-1^{MET} inhibited CCA cell migration. Thus, Induction of cellular senescence is a good prognostic factor and PRIMA-1^{MET} can be used as a potential anticancer drug in CCA treatment.

▲TOP

P-3320

Pro-apoptotic activity of asiatic acid against human cholangiocarcinoma cells

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Asiatic acid, a triterpene found in Centella asiatica (L.) Urban, exerts a wide variety of biological effects including antioxidant, anti-inflammatory and anti-cancer activities. Cholangiocarcinoma (CCA), a malignancy of bile duct epithelial cells, is a leading cause of death in Thailand. As current treatments for CCA are not sufficiently effective, there is still an urgent need to find effective strategies for prevention and treatment of CCA. This study sought to assess the anti-cancer effects of asiatic acid on two human CCA cell lines (KKU-156 and KKU-213) through measurement of cell viability using SRB assay, detection of apoptotic cells by flow cytometry and analysis of mRNA expression levels of apoptosis-related genes by real-time PCR. The results showed that asiatic acid efficiently suppressed CCA cellular viability via induction of apoptosis as evidenced by microscopic observation of apoptotic vesicles, down-regulation of anti-apoptotic genes (BCL2 and Survivin/BIRC5) and increased early and late apoptotic cells. These findings shed a light on the application of the plant-derived asiatic acid in CCA prevention and treatment.

P-3321

Overexpression of secretory leukocyte protease inhibitor promotes the invasion of cholangiocarcinoma

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Cholangiocarcinoma (CCA) is a highly invasive tumor with poor prognosis. Secretory leukocyte protease inhibitor (SLPI), is a secretory protein, It has been shown to be involved in the host defense during the inflammatory response and associated with many diseases. However, the expression and the functional role of SLPI in CCA are still unknown. We found respective high to low expression of SLPI in CCA cell lines and in immortalized cholangiocyte, which correlated with their metastatic potential as determined by in vitro Transwell invasion and wound healing assays. Our findings suggested that SLPI is involved in the development of the metastatic phenotype of the cholangiocyte. To test if this is the case, we overexpressed SLPI in MMNK-1 and assessed the phenotypes of the overexpressing cell line compared with the control cells. We found that cell proliferation, migration, invasion and MMP 2 & 9 secretion were significantly enhanced. Altogether, our findings demonstrated that SLPI plays an important role in tumor progression associated with cell growth and metastasis of CCA, and that SLPI has the potential to serve as a novel therapeutic target of CCA.

P-3322

Sorafenib induces cytotoxicity in cholangiocarcinoma cell lines by inhibiting Akt signalling

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North-eastern Thailand is known for its prevalence in a rare cancer called Cholangiocarcinoma (CCA). It has been established that the cancer is manifested from parasitic liver-fluke Opisthorchis viverinni infection. Early stage diagnosis for CCA is difficult until the tumor advances aggressively. This limits the options for suitable treatment. Sorafenib, a multikinase inhibitor, effectively treats hepatocellular, renal and thyroid cancers, by inducing cell death by inhibiting cell signaling. CCA cells were speculated to respond to this treatment due to their upregulated activity of receptor tyrosine kinase signaling, such as EGFR and VEGFR, which are known targets of Sorafenib. In this investigation, using MTT assay, we showed CCA cell lines, HuCCA-1, KKU-100, KKU-M055, RBE and TFK, were sensitive to 48-hour Sorafenib treatment. Western Blot analyses with 24-hour Sorafenib treatment were conducted to observe reduced signaling of phospho-Akt and increased signaling of phospho-ERK1/2. Further experiments will be performed to include drug treatments in combination with clinical standard therapy Cisplatin and Gemcitabine.

▲TOP

P-3323

Gadd45beta regulates viability and metastasis of cholangiocarcinoma cells

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Cholangiocarcinoma (CCA) is a lethal malignancy of the bile duct epithelium with extremely poor prognosis. Growth Arrest and DNA Damage-inducible-beta (Gadd45&b;) is a stress sensor shown to be involved in cancer cell growth, apoptosis, cell cycle control and DNA repair. Gadd45 β expression was dysregulated in many cancers, functioning either as a tumor promoter or a tumor suppressor. Here, we showed that tumor specimens from CCA patients expressed high level of Gadd45 β and this was associated with metastasis. Silencing of Gadd45 β gene in HuCCA-1 cell line markedly reduced cell viability and Akt/protein kinase B (Akt/PKB) phosphorylation, but not p38 MAPK nor ERK1/2 phosphorylation. Moreover, in vitro invasion and migration were significantly reduced, which paralleled a decrease of EMT markers, namely, slug, vimentin, Claudin-1 and ZO-1, and an increase of E-cadherin. Our results suggest that Gadd45 β regulates the metastatic properties of the CCA cells mediated by EMT pathway and may serve as a potential therapeutic target for CCA treatment.

P-3324

Bile microbiota changes in hepatobiliary diseases

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Bacterial infection plays role in hepatobiliary diseases including the common bile duct stone (CBD) and cholangiocarcinoma (CCA) patients in worldwide. The present study aims to search microbiome in the bile of CCA compared to CBD patients using metagenomics next-generation sequencing. The results revealed that the most common bacteria in bile of CCA patients were Enterococcus, Klebsiella, Pseudomonas and Stenotrophomonas, which their OUTs number obviously increased when compared to CBD. On the other hand, OUTs number of Synergistetes and Fusobacteria trended to reduce significantly in CCA. Nested PCR revealed that v3-v4 16srRNA analysis of Helicobacter genus was found in both CBD and CCA groups. Helicobacter genus-positive bile samples were identified for 41.6% and 40% of CBD and CCA, respectively. In conclusion, the results indicate that microbiome alters in the bile of CBD and CCA patients and alteration of microbial community may play role for CCA development, which its needs further investigation.

▲TOP

[P-3335] P14-64 [English/Japanese]
Pancreatic cancer (1)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

Koji Umeshita / Div.Health Sci., Osaka Univ.Med.Sch.

P-3335

Identification of Novel Biomarkers for Gemcitabine Resistance in Pancreatic Cancer

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Pancreatic cancer (PC) is a highly malignant disease that represents the fourth leading cancer-related death worldwide. Gemcitabine has been used the first therapy in all stages in PC, however, its resistance is frequently occurred. Therefore, it is urgent to find biomarkers and therapeutic targets to overcome drug resistance in PC. In this study, our purpose is to identify novel genes which are related with gemcitabine resistance in PC. First, to establish gemcitabine resistant mouse model, L3.6pl cells were injected into pancreas and then gemcitabine was treated. After 8 weeks treatment of gemcitabine, the cancer cells were isolated, and analyzed gene profiling between control and drug resistant cells by RNA sequencing. Additionally, the RNA sequencing data were merged with TCGA data set, which were upregulated genes in drug non-responder compared to responder. From these results, we finally found 15 drug resistance-related genes. Among them, knockdown experiment of two genes indicated increase of sensitivity for gemcitabine in PC cells. In conclusion, these results suggest that the two genes might be used as gemcitabine resistant diagnostic markers and therapeutic targets in PC.

▲TOP

P-3336

High Tenascin C perineural expression is a poor prognostic factor associated with local recurrence in pancreatic cancer

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Background: Tenascin C (TNC), an extracellular matrix glycoprotein, has been reported to be expressed mainly in cancer stroma and associated with poor prognosis in various types of cancer. The aim of this study is to clarify correlations between TNC expression and clinicopathological factors in pancreatic ductal adenocarcinoma (PDAC).Method: A total of 78 resected patients with PDAC were enrolled in this study. TNC expression was examined immunohistochemically on paraffin-embedded sections. TNC perineural expression in the invasive front of PDAC was defined as high or low, by comparing with that in adjacent non-cancerous tissue in the same section. The relationships between TNC expression and clinicopathological features were retrospectively analyzed. Results: High TNC perineural expression was seen in 30 patients and associated with perineural invasion (p=0.008), pT3≤(p=0.021) and locoregional recurrence (p=0.002). High TNC perineural expression was significantly associated with worse disease free survival rates by multivariate analysis (p=0.023).Conclusion: High TNC perineural expression is a potential predictable marker for poor prognosis associated with locoregional recurrence.

P-3337

MLN2238 inhibit pancreatic cancer proliferation by attenuating Warburg effect

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Though significant progress has been made in the availability of treatment strategies, pancreatic cancer remains a disease of high mortality rates. Therefore, there is an urgent need for new drugs and understanding of the molecular mechanisms of drug resistance. In our study we demonstrated that proteasome inhibitor MLN2238 can reduce pancreatic cancer cell proliferation and attenuated the Warburg effect. Moreover, the sensitivity of pancreatic cancer cell to MLN2238 is dependent on KRAS mutational status. Knocking down the mutant KRAS will increase the sensitivity to MLN2238. The mechanism of MLN2238 regulating Warburg effect is also dependent on KRAS mutational status. Taken together, our novel findings suggest KRAS mutation plays a critical role in MLN2238 caused inhibiting of proliferation and the Warburg effect in pancreatic cancer.

P-3338

Expression of an ATP-grasp superfamily enzyme under hypoxia

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Most solid tumors contain areas of reduced oxygen concentration, hypoxia, where the cells show more resistance to both chemo- and radiotherapy. To find out responsible genes for survival under hypoxia we employed pancreatic cancer cell lines and analyzed gene expressions by oligo DNA microarray that were activated under hypoxic conditions. The candidates included an ATP-grasp superfamily enzyme, whose inducible expression was presented by qRT-PCR for at least four cell lines. Stronger expression was observed when charcoal-stripped serum was used and more without serum, suggesting its transcription is additively affected by starvation. We raised an antibody and showed that also its protein synthesis is induced. Gene knockout MIA PaCa-2 cell lines were established using CRISPR/Cas9 system. Transplantation of these cells to mice resulted dramatically smaller xenografts compared to the wild-type cells, though they did not show a significant growth defect in in vitro cell culture studies. This implies that this enzyme would have a role in developing cancer in in vivo microenvironments. The enzyme and its products could be utilized for cancer diagnosis and a target for therapy.

▲TOP

P-3339

The immunotherapy potential of SANN- JHONG- KUEY-JIAN- TANG in pancreas cancer cells

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Pancreatic cancer remains a challenging disease in worldwide. Sann- Jhong- Kuey- Jian- Tang (SJKJT), a traditional medicinal prescription, has been used to treat lymphadenopathy and exhibits cytotoxic activity in many types of human cancer.The recent research indicates that cancer cells can induce immune cells start "immuno-suppression" message conduction, the checkpoint blockade message conduction by "immuno-suppression" blocking; even leading to immune cells in the tumor can be activated to complete the task of killing cancer cells. The main therapeutic target is CTLA-4 and PD-1 expression in both immune T cell surface receptor inhibition, when CTLA-4 and PD-1 with the respective ligand binding, inhibition of T cell immune response messages conduction will be started and lead to functional activity of T cells decrease. In the present studies showed that the human pancreatic cancer cell BxPC-3, MIAPaCa-2 and PANC-1 were treated by SJKJT can inhibit the expression of CTLA-4 and PD-1. In the future, SJKJT may be used in adjuvant therapy for pancreatic cancer patients.

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[P-3347] P14-66 [English/Japanese]
Pancreatic cancer (3)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

Atsushi Miyamoto / Dept. Surg., Natl. Hosp. Organization Osaka Natl. Hosp.

P-3347

Pancreatic KRAS and TP53 oncogenes cooperatively activate ARF6-AMAP1 pathway to drive malignancy and immune evasion

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KRAS and *TP53* mutations are the major driver oncogenes of pancreatic ductal adenocarcinoma (PDAC). However, molecular mechanisms by which these oncogenes drive tumor malignancy still remain largely elusive. A series of our studies have demonstrated that ARF6-AMAP1 pathway is at the core driving invasion and metastasis of different types of cancers, if overexpressed and hyper-activated. Receptor tyrosine kinases (RTKs) activate ARF6, in which mevalonate pathway (MVP) is essential. Here we show that *KRAS* and *TP53* oncogenes generate and help activating the ARF6 pathway. In this process, *KRAS* promotes eIF4A-dependent *ARF6* mRNA translation and also eIF4E/mTORC1-dependent *AMAP1* mRNA translation, while *TP53* promotes ARF6 activation by the induction of PDGFR and the activation of MVP. ARF6-AMAP1 pathway, as well as *KRAS/TP53* oncogenes, furthermore promoted PD-L1 recycling and cell surface expression, in which MVP, eIF4A, eIF4E/mTORC1 and MVP are all essential. Thus, the cooperation between eIF4A/4E-dependent translation and MVP has emerged as a link by which pancreatic driver oncogenes promote malignancy and immune evasion, via empowering ARF6-AMAP1 pathway.

▲TOP

P-3348

Scavenger receptor CD36 can predict prognosis in patients with pancreatic cancer

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Background: CD36, a class B scavenger receptor, has been investigated for the relationship with several cancers.Aim: To examine the clinical significance of CD36 expression in pancreatic ductal adenocarcinoma (PDAC).Materials and Methods: 56 PDAC patients who underwent radical resection with preoperative chemo-radiation therapy from 2007 to 2012. CD36 expression of their specimens was evaluated by immunohistochemistry. In vitro, CD36 expression of PDAC cell lines (MiaPaCa2 and GEM-resistant MiaPaCa2 (Mia-GR)) were assessed by PCR. Chemo-sensitivity assay was performed to confirm effect of combination therapy with gemcitabine (GEM) and CD36 inhibitor.Results: (1) The overall survival of CD36 strongly positive patients (n=40) was significantly poorer than that of CD36 weakly positive patients (n=16) ($p<0.05$). (2) The expression of CD36 was observed in Mia-GR cells significantly higher than in MiaPaCa2 ($p<0.01$). (3) By chemo-sensitivity assay, the combination therapy with GEM and CD36 inhibitor showed significantly higher cytotoxic effect in Mia-GR cells than GEM alone.Conclusion: CD36 could be a predictive marker and a valuable target for improvement of survival in PDAC patients.

P-3349

Dysregulation of lncRNAs located at the HOXA locus in metastatic pancreatic ductal carcinoma

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Recent studies have revealed that long non-coding RNAs (lncRNAs) play pivotal roles in the development and progression of cancer. In this study, we aimed to clarify the role of lncRNAs in the metastasis of pancreatic ductal adenocarcinoma (PDAC). To this end, we screened for lncRNAs overexpressed in metastatic PDAC using The Cancer Genome Atlas (TCGA) database. By comparing the expression levels of respective exons, we identified a series of 222 exons (corresponding to 150 genes) which are potentially associated with the PDAC metastasis. Among them, we noted that multiple lncRNAs are located at the HOXA cluster, and we selected HOXA11-AS for further analysis. HOXA11-AS is overexpressed in PDAC cell lines, and knockdown of HOXA11-AS suppressed PDAC cell proliferation, suggesting its potential oncogenic function. We are currently proceeding functional analysis of HOXA11-AS in PDAC, and we will discuss its biological and clinical implications.

P-3350

Comparison of morphology and stroma ratio between orthotopic and subcutaneous xenograft models of pancreatic cancer

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Pancreatic cancer tissue contains large amount of stroma, and patient-derived xenograft (PDX) models are useful tools to examine the cancer microenvironment, though the stroma of PDX is replaced by mouse-derived stromal cells. Thus, we are establishing PDX models of pancreatic cancer rich in stroma to study the cancer-stromal interaction. Surgical specimens were subcutaneously or orthotopically implanted into NOG mice, and transplanted. Rates of orthotopic engraftment were similar with those of subcutaneous one. Ratio of cancer to stroma markedly increased in xenografts compared to the surgical specimen, but well-/moderately differentiated types of xenografts reconstituted stroma derived from mouse tissue. The xenograft tumors showed heterogeneity similar to original tumor tissue. Stroma ratio in xenografts depended on the degree of differentiation, and there were no apparent differences in histology and stroma ratio between orthotopic and subcutaneous xenografts. Remarkably, only orthotopic xenografts destroyed normal mouse pancreatic parenchymal tissue and spread metastatically to lungs, spleen and livers. Changes of the heterogeneity during passages are also now being examined.

▲TOP

P-3351

A novel mechanism of miR-216a regulating a hyaluronan-degrading enzyme KIAA1199/CEMIP in PDAC

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Hyaluronan (HA) is involved in cancer progression by promoting cell migration and invasion. Recently, a novel HA-degrading enzyme, KIAA1199 (also termed CEMIP), has been identified as overexpressed in various cancers. We reported overexpression of KIAA1199 in pancreatic ductal adenocarcinoma (PDAC). in association with shorter survival. A previous study has shown that KIAA1199 expression was regulated by a microRNA, miR-216a. We therefore investigated a possible correlation between KIAA1199 and miR-216a in PDAC cell lines.We analyzed KIAA1199 mRNA expression and miR-216a expression in PDAC cell lines and a non-neoplastic human pancreatic duct epithelial cell line (HPDE). We investigated KIAA1199 mRNA expression and cell migration by introducing miR216a mimic into PDAC cell line with a high KIAA1199 expression.All PDAC cell lines showed decreased miR-216a expression compared with HPDE. PDAC cells transfected with miR-216a mimic showed decreased KIAA1199 expression in association with reduced migration ability.In PDAC cells, reduced expression of miR-216a may be involved in enhanced expression of KIAA1199, which subsequently contributes to increased aggressiveness.

P-3352

The role of transcriptional factors FOXM1/KLF4 in glucose metabolism during EMT of pancreatic cancer

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Tight junction molecule claudin-1 is expressed in various types of epithelial cells. We previously reported that claudin-1 was closely related with epithelial-mesenchymal transition (EMT) of pancreatic cancer (PDAC). Cancer is one of metabolic diseases, PDAC is also associated with impaired glucose metabolism. The abnormality of transcriptional factors Forkhead Box M1 (FOXM1) and Kruppel-like factor 4 (KLF4) is observed in PDAC tissue. In this study, we investigated the regulation of FOXM1 and KLF4 during EMT by using PDAC cell lines (PANC-1, HPAC) and normal human pancreatic duct epithelial (HPDE) cells. In PDAC cell lines cultured with low glucose medium, FOXM1 was decreased, and KLF4 and claudin-1 were increased compared to high glucose medium. Furthermore, the epithelial barrier function was enhanced, and cell invasion and migration were suppressed. To investigate glucose metabolism in EMT of PDAC cell lines, the profiles of mitochondrial respiration and glycolysis were measured by using extracellular flux analysis. These marked differences of glucose metabolism among PDAC cell lines and HPDE cells may lead to development of a novel therapeutic method for PDAC.

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[P-3329] P14-63 [English/Japanese]
Biliary tract cancer (3)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

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P-3329

Inflammatory cytokine crosstalk progress cancer malignant potency in biliary tract cancers

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Backgrounds:
We previously showed IL6 induced anti-apoptotic effect in biliary tract cancers (BTC) via Mcl-1. Herein, we explored the relationship between IL6 and other cytokines and effects on cancer malignant potency.
Methods:
We investigated IL6, TGF-b, and implication for cancer invasion, EMT, and chemoresistance (CR) using 5 BTC cells and resected specimen.
Results:
(1) IL6 induced TGF-b (mRNA and protein) as crosstalk, which induced malignant cycles; invasion, EMT (spindle shape and expression of n-cadherin and vimentin), and CR for gemcitabine (Gem, GR). IL6, TGF-b, and n-cadherin were stained at the invasion front in resected BTC. Survival of MzChA1 mouse were longer than GR-MzChA1 mouse. These cytokines also related to T-cell differentiation.
(2) This malignant cycles was halted by SMAD regulation; siSMAD4 and HDAC inhibitor (Vorinostat) for SMAD inhibition. siSMAD4 and Vorinostat sensitized for Gem in GR cells. Combination of Gem and Vorinostat prolonged GR-MzChA1 mouse survival than Gem alone ($P < 0.01$).
Conclusion:
Inflammatory cytokine crosstalk induces cancer malignant potency and effect on the prognosis. HDAC inhibitor may halt or reverse this malignant potency.

▲TOP

P-3330

New approach for development of human-derived advanced biliary cancer cell lines

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Most patients with biliary cancer are found inoperable at the initial diagnosis. Although organoid culture enables long-term propagation of primary cells in a physiological setting, there has been no modality to obtain tumor cells for culture, in a non-invasive manner from such patients. To address this issue, we aimed at establishing methods for organoid culture of tumor cells from bile duct juice. We conducted organoid culture of bile duct juice collected by therapeutic ERCP from 84 patients. Obtained organoids were characterized in histology, immunochemistry, KRAS mutation and tumorigenesis in immunodeficient mice. Overall success rate for organoid culture was 89% in malignant cases. Organoids could be also obtained from resected tumors and benign cases. Histologically, tumor-derived organoids resembled original tumors. KRAS mutation rate was 36%, and immunopositivity for p53 and HER2 was 31% and 20%, respectively. Organoids developed subcutaneous tumors in 45%. In conclusion, this is the first report on successful organoid culture of bile-derived advanced biliary cancer. This technique will likely contribute to development of companion diagnosis and novel therapeutics.

P-3331

LCK Regulates YAP Tyrosine Phosphorylation and Nuclear Localization in Cholangiocarcinoma Cells

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Nathan W. Werneburg, Matthew C. Hernandez, Lin Yang, Ayano Kabashima, Petra Hirsova, Lavanya Yohanathan, Carlos Sosa, Mark J. Truty, George Vasmatzis, Gregory J. Gores, Rory L. Smoot.The Hippo Pathway effector Yes-associated protein (YAP), a transcriptional co-activator, is implicated in cholangiocarcinoma (CCA) pathogenesis. We examined YAP regulation by tyrosine phosphorylation in human and mouse CCA cell lines, as well as patient-derived xenograft (PDX) models. YAP was phosphorylated on tyrosine 357 (Y357) in both CCA cell lines and PDX models. SRC family kinase (SFK) inhibition with dasatinib resulted in loss of YAPY357 phosphorylation, promoted its translocation from the nucleus to the cytoplasm, and reduced YAP target gene expression. Targeted siRNA experiments identified LCK as the SFK most potently mediating YAPY357 phosphorylation. Likewise, inducible CRISPR/Cas9 targeted LCK deletion decreased YAPY357 phosphorylation and its nuclear localization. Finally, dasatinib displayed therapeutic efficacy in PDX models. These studies demonstrate a targetable, LCK-mediated YAP tyrosine phosphorylation pathway in CCA regulating YAP's nuclear retention and oncogenic activity.

P-3332

The efficacy of oncolytic virus therapy using G47Δ in mouse biliary tract cancer models

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In this study, we evaluated the efficacy of G47Δ, a triple-mutated oncolytic HSV-1, in mouse biliary tract cancer (BTC) models using various administration routes. When injected intratumorally in athymic mice bearing subcutaneous tumors of HuCCT1 (cholangiocellular carcinoma) or NOZ (gallbladder cancer), G47Δ significantly suppressed the tumor growth in both models. To test a direct liver injection, orthotopic liver tumors were generated in athymic mice by implanting HuCCT1 cells into the hepatic subcapsular space. When G47Δ was injected into the liver parenchyma nearby spotted tumors, G47Δ treatment tended to prolong the survival of mice, but not significantly. In the NOZ peritoneal dissemination model, intraperitoneal G47Δ administration significantly prolonged the survival. A plaque inhibition assay using serially diluted bovine bile proved that G47Δ was easily inactivated in the presence of 1% v/v bile. In conclusion, G47Δ exhibits efficacy for BTC when administrated intratumorally or intraperitoneally. However, neither direct liver injection nor intrabiliary administration is likely a suitable route for G47Δ.

▲TOP

P-3333

Establishment of mouse gall bladder mouse model using organoid cell line

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Gallbladder cancer (GBC) is a relatively rare neoplasm worldwide. The experimental tools to analyze molecular biology of GBC are limited. According to COSMIC, a big database of mutated gene in human cancers, the most frequently altered gene in GBCs is TP53, followed by KRAS.Thus,we aimed to develop mouse GBC organoid cell line with TP53 inactivation and Kras activation.We established organoid cell lines from the gall bladder of Lox-Stop-Lox(LSL)-KrasG12D mouse.The cells were transduced Cre recombinase to activate Kras.Then, we introduced TP53 knockout with CRISPER CAS9 system. We used double nickase plasmids for induction of TP53 knockout to decrease cutting off-target sites. After single cell cloning, we confirmed the recombination LSL site and the loss of guide RNA target site.The knockout of TP53 protein was confirmed by western blotting.These GBC organoid cell lines could create subcutaneous tumor in wild type mice with normal anti-tumor immunity.The condition of orthotopic transplantation is currently being studied. Our mouse GBC organoid cell line enable to analyze molecular biology of GBC in vivo including anti-tumor immunity of host mouse in detail.

P-3334

Intraoperative frozen section diagnosis of bile duct margin for extrahepatic cholangiocarcinoma

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Usefulness of intraoperative frozen section diagnosis (FSD) of bile duct margin (BDM) for extrahepatic cholangiocarcinoma was investigated in 74 consecutive patients (100 BDMs) from 2012 to 2017 with surgery during which FSD was performed. The diagnosis was classified into either negative, borderline, or positive. Postoperative comparison between FSD and permanent section diagnosis (PSD) revealed that concordance rate was 68.0% in the total layer (TL), 69.0% in the epithelial layer (EL), and 98.0% in the subepithelial layer (SL). Discordance was observed in 31 ELs and two SLs. Alteration from borderline to negative was the most frequent (20/31 ELs). Patients with positive margin in TL and EL by FSD demonstrated a significantly worse local recurrence-free survival (RFS) compared with patients with borderline and negative margins, which revealed comparable local RFS, suggesting that epithelial borderline might be regarded substantially as negative. When classifying the status of EL either as negative or positive, concordance rates between FSD and PSD in TL, EL, SL were 95.0%, 93.0%, and 98.0%, respectively, and FSD was reliable enough for pathological diagnosis.

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[P-3340] P14-65 [English/Japanese]
Pancreatic cancer (2)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

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P-3340

Leucine rich alfa 2 glycoprotein enhances cytokines inducing endothelial mesenchymal transition in pancreatic cancer

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Background/Aims:The levels of inflammatory-related molecule leucine-rich alpha-2- glycoprotein (LRG), which enhances the TGF-β1-induced phosphorylation of Smad proteins, were elevated in patients with pancreatic ductal adenocarcinoma (PDAC). Given that TGF-β/Smad signaling is considered a key role of epithelial-mesenchymal transition (EMT), which can cause metastasis, invasion, and/or chemo-resistance, we aimed to clarify the mechanism underlying LRG-related EMT in PDAC.Methods:We constructed LRG-overexpressing PDAC cells (Panc1/LRG). We evaluated the expression of LRG, morphology, EMT-related molecules in PDAC cells. Results:The expression of LRG in PDAC cells were very low, but under inflammatory cytokines, LRG production was increased in PDAC cells. A spindle-like shape was visualized more often than other shapes in Panc1/LRG with TGF-β1 exposure. The expression of E-cadherin decreased and that of vimentin increased by TGF-β1 exposure in Panc1/LRG. Invasion increased with TGF-β1 stimulation of Panc1/LRG. Conclusion: Inflammatory cytokines induced LRG production, and LRG promoted EMT by enhancing TGF-β/Smad2 signaling in PDAC.

▲TOP

P-3341

Regulation of malignant mechanisms in pancreatic cancer using exosomal microRNAs in healthy blood samples

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Pancreatic ductal adenocarcinoma (PDAC) has a poor prognosis and multidisciplinary treatments have still remained in unsatisfactory results. It is necessary to elucidate the mechanisms related to the malignant transformation of PDAC and identify new therapeutic targets for the disease. In this study, we focused on the microRNAs (miRNAs) encapsulated in exosomes in the blood of healthy people and analyzed the mechanisms of the malignant transformation of PDAC. Exosomes were extracted from 16 serum samples collected from healthy people using an exosome extraction reagent and then we assessed the proliferative ability of a pancreatic cancer cell line by adding the exosomes extracted. Microarray analysis on the serum exosome-encapsulated miRNAs was performed for the two groups (three cases in each group), and then candidate miRNAs and target genes are currently being identified. It was suggested that exosomes in the blood of healthy people might contain factors that increase the proliferation ability of pancreatic cancer cells. Analysis on the functions of exosome-encapsulated miRNAs using microarray analysis might contribute to the development of a new treatment for PDAC.

P-3342

CK14 and CK17 are partially expressed in microaggregate form of invasive cells of pancreatic cancer

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It has been reported that Cytokeratin (CK) 14 and CK17 expression is associated with oral and bladder cancers. However, the significance in pancreatic cancer is not well understood. We examined the expression of CK14, CK17, MMP3/10 and Differentiated embryonic chondrocyte gene (DEC) 2/BHLHE41 in primary and metastasis lesions of pancreatic cancer in 2 autopsy cases. CK14, CK17 and MMP3/10 are partially expressed in primary tumor spindle cells, which may be microaggregate form of invasive cells. On the other hand, they were little expressed in non-tumor pancreatic ducts. We also found that positive immunostaining of CK14, CK17 and MMP3/10 were observed in metastatic lesions. Taken together, CK14, CK17 and MMP3/10 may have possible association with tumor progression. We previously showed that DEC2 was little expressed in tumor cells. We found DEC2 expression decreased in both primary and metastastic tumor cells compared to non-tumor cells. We further examined whether DEC2 affected cytokeratin expression and found that DEC2 overexpression suppressed CK17 expression. These results suggest that DEC2 may be a negative regulator of CK17 in pancreatic cancer.

P-3343

Elucidation of the mechanism involved in cancer progression of pancreatic cancer (PC) -related gene, ASAP2

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Background: ASAP2 is an effector for ADP-ribosylation factor6 (Arf6) by stably binding to GTP-Arf6. Arf6 promotes cancer invasive, proliferative, and angiogenic activity in various cancer types. Here we investigated the mechanism involved in cancer progression of ASAP2 as a PC-related gene.Methods: We analyzed public datasets of PC (178 RNA-sequencing data from The Cancer Genome Atlas, 36 gene expression array data from GSE 15471, and 45 gene expression array data from GSE 28735) and examined the ASAP2 expression in PC by immunostaining. To explore biological effect of ASAP2, proliferation, cell cycle, and migration assay were performed in vitro.Result: mRNA expression of ASAP2 is higher in PC compared to normal tissue and the high expression group had a poor prognosis. Immunostaining showed high expression of ASAP2 in PC cells. Depletion of ASAP2 by siRNA showed a significant decrease of colony formation ability, cell proliferation, cell cycle progression, and migration activity of PC cells.Conclusions: ASAP2 contribute to malignant phenotype of PC by activating migration activity and proliferation through promotion of cell cycle progression possibly via activation of Arf6.

▲TOP

P-3344

Involvement of lysophosphatidic acid receptors in the promotion of malignant properties in pancreatic cancer cells

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Lysophosphatidic acid (LPA) is a simple biological lipid and mediates various cellular responses through the binding of G protein-coupled LPA receptors (LPA1 to LPA6). The aim of this study was to investigate an involvement of LPA receptors in the promotion of malignant properties in pancreatic cancer cells. To generate the long-term cisplatin (CDDP) treated (PANC-CDDP) cells, PANC-1 cells were treated with cisplatin (CDDP) for approximately 6 months. In motility and invasive assay, PANC-CDDP cells indicated the high cell motile and invasive activities, correlating with LPAR1 and LPAR3 expressions. The cell motile and invasive activities of PANC-CDDP cells were significantly suppressed by LPA1 and LPA3 knockdown. In addition, we established the highly invasion (PANC-M6) cells from PANC-1 cells. The cell invasive activity of PANC-M6 cells was approximately 15 times higher than that of PANC-1 cells. LPAR2 expression was markedly elevated in PANC-M6 cells, while LPAR3 expression was reduced. Our results suggest that LPA signaling via LPA receptors is involved in the regulation of malignant properties during tumor progression in PANC-1 cells.

P-3345

Identification of cancer restraining CAF

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Cancer-associated fibroblasts (CAFs) promote cancer progression through multiple mechanisms. Recently, observations from genetically engineered mouse cancer models and a clinical test have idicated the possibility that there exist CAFs that restrain cancer progression. The nature and identity of the cancer-restraining CAFs, however, have been unknown. Here we show that CAFs that express Meflin, a glycosylphosphatidylinositol (GPI)-anchored protein constitute a CAF population that is distinct from α-SMA-positive cancer-promoting CAFs. The infiltration of Meflin-positive CAFs in cancer stroma correlated with favorable prognosis of pancreatic cancer patients, which was consistent with the finding that Meflin-deficiency led to a marked progression of pancreatic cancer in mouse models. Interestingly, Meflin deficiency led to the development of a poorly differentiated type of pancreatic cancer accompanied by more desmoplastic stroma. These data help to understand the heterogeneity of CAFs, which may be useful for the development of therapeutic strategies to specifically target cancer-promoting CAFs.

P-3346

The role of HABP2 (Hyaluronan Binding Protein 2) in migration and EMT of pancreatic cancer cells

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We previously reported a number of evidence supporting an enhanced processing of hyaluronan (HA) in pancreatic ductal adenocarcinoma (PDAC). However, the mechanisms underlying the accelerated HA processing remain poorly understood. Recently, HABP 2 (Hyaluronan Binding Protein 2), one of the extracellular serine proteases, has been reported to play a role in promoting migration of cancer cells in response to LMW-HA. The role of HABP2 in pancreatic cancer biology is unknown. We therefore analyzed the expression and function of HABP2 in PDAC cells. To examine the function of HABP2 in PDAC, We established HABP2 knockout clone using the CRISPR-Cas9 system and HABP2 overexpression clone using gene transduction. We investigated cell migration in association with epithelial-mesenchymal transition (EMT) in these clones. Knockout of HABP2 resulted in decreased cell migration, increased CDH1 mRNA expression, and decreased CDH2 and vimentin mRNA expression. Conversely, forced expression of HABP2 resulted in the opposite to those with knockout clones of HABP2. These findings suggest that HABP2 is overexpressed in some PDAC cells and is involved in enhanced migration possibly through EMT.

▲TOP

[P-3353] P14-67 [English/Japanese]
Pancreatic cancer (4)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(D)/10F 1010+10-1&2, Osaka International Convention Center Room P(D)

Yasuhiko Tomita / Dept. Pathol. Int. Univ. Health Welfare Sch. Med.

P-3353

Crizotinib inhibits peritoneal dissemination of pancreatic cancer in a xenograft mouse model

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Peritoneal dissemination is frequently complicated in pancreatic cancer, which is associated with poor prognosis. MET is associated with the progression of pancreatic cancer, therefore, we evaluated the effect of a MET inhibitor (crizotinib) on peritoneal dissemination of pancreatic cancer. Crizotinib induced growth inhibition of 8 pancreatic cancer cells with the IC_{50} range from 1.4 to 4.3 μM. Invasion of pancreatic cancer cell (SUIT2) was suppressed in vitro at a concentration of 1.0 μM, which is enough for the inhibition of MET phosphorylation. This effect on cell invasion was recapitulated also by the reduction of MET expression in SUIT2 with siRNA. Crizotinib also inhibited RhoA activation in addition to MET phosphorylation. We further evaluated the effect of crizotinib on peritoneal dissemination of pancreatic cancer in vivo. Crizotinib reduced tumor burden and ascites accumulation due to development of peritoneal dissemination after inoculation of SUIT2. Taken together, crizotinib may be a potent drug for treating peritoneal dissemination of pancreatic cancer by the suppression of HGF/MET signaling and RhoA activation.

▲TOP

P-3354

Analysis of exosome secretion from pancreatic cancers by knockout of tetraspanin genes

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We have recently reported relationship of the surface expression between cancer cells and exosomes secreted from various human cancer cells. Pancreatic cancer is the most fatal malignancy in that 5-year survival rate is less than 8%, and efficient standard therapy is not established. Therefore, understanding of molecular heterogeneity in addition to oncogenic mechanisms of pancreatic cancers is important in both diagnosis and therapy for pancreatic cancers. Exosomes are vesicles originated from the eukaryotic endosomal system, and are released extracellularly in almost all types of mammalian cells including cancer cells. Tetraspanin family proteins (CD9, CD63, CD81), known as the exosome markers, are membrane proteins with 4 transmembrane domains, however, functions other than the role as exosome markers remain unsolved. In this context, we have established CRISPR/Cas9-based knock-outed (KO) cells of tetraspanin genes, and analyzed possible mechanisms of the secretion of exosomes from cancer cells. We expect these studies will lead to the breakthrough of diagnosis and therapy on pancreatic cancers. Collaborators: Kazunori Yoshikawa, Tesshin Fujioka, Souta Takemoto, Kazue Masuko

P-3355

MAST4 expression correlates with gemcitabine resistance in pancreatic ductal carcinoma

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To study gemcitabine resistance in pancreatic ductal adenocarcinoma (PDAC), we established a gemcitabine (GEM)-resistant cell line, MIA-G, which was derived from MIA-PaCa-2 (MIA-P) cells by continuous GEM treatment. MIA-G cell did not acquire resistance to 5-FU or cisplatin. A gene expression profiling between MIA-G and MIA-P cells showed that the most increased expression in MIA-G was MAST4 (microtubule-associated serine/threonine kinase family 4) compared to MIA-P cells. MAST4 knockdown abrogated GEM resistance in MIA-G cells. In MIA-G cells, protein levels of AKT3 and FoxO3 were correlated with upregulation of MAST4. These findings suggest MAST4 might induce in GEM resistance through AKT pathway.

P-3356

NF-κB signaling contributes to the expression of PD-L1 in pancreatic cancer

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BACKGROUND: Potential applications of immune checkpoint antibodies in various types of cancers are being evaluated in preclinical and clinical settings. PD-L1 expression is reported to be an important biomarker for the treatment. However, the regulation of PD-L1 in pancreatic cancer is still unclear.
METHODS: Human and murine pancreatic cancer cells were treated with or without chemotherapeutic agents and TNFα. NF-κB inhibitor was used to analyze the importance of NF-κB signaling for PD-L1 expression. Murine pancreatic cancer cells (EPPK1) and IKKβ-deleted cells (EPPK1$^{\delta IKK\beta}$) were transplanted subcutaneously into wild type mice.
RESULTS: We found that PD-L1 was expressed in most of the cell lines. In Panc1 cells, PD-L1 expression was increased by the treatment of 5-FU and TNFα. Basal and TNFα induced PD-L1 expression were inhibited by NF-κB inhibitor. EPPK1$^{\delta IKK\beta}$ tumors were significantly small size and weight as compared to EPPK1 tumors.
CONCLUSION: NF-κB signaling contributes to the expression of PD-L1 in pancreatic cancer. Downregulation of PD-L1 expression by the inhibition of NF-κB signaling can be one of the therapeutic option for treating pancreatic cancer.

▲TOP

P-3357

High expression of ARHGEF2 is associated with poor prognosis in Patients with Pancreatic ductal adenocarcinom

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Background: ARHGEF2 is one of the RHO guanine exchange factor (RHOGEF). RHOGEF is a microtubule-associated guanine nucleotide exchange factor for the Rho family of small GTPases.Methods: The cohort of 102 patients who underwent curative resection for PDAC from GSE21501 was used to assess the association with molecular pathways and the prognosis of patients. Pathway analysis was performed by GSEA (gene set enrichment analysis) using HALLMARK geneset.Result: In GSE21501 (n=102), the patients were separated into high- (upper 25%, n=25) and low- (lower 75%, n=77) ARHGEF2 group. High-ARHGEF2 group had significantly poor overall survival than low-ARHGEF group (p = 0.0004). Moreover, high ARHGEF2 group was associated with high lymph node metastasis (p=0.0012). The GSEA analysis revealed that the high ARHGEF expression was significantly associated with “HALLMARK-MYC-TARGET-V2” geneset (p=0.04). Also high ARHGEF expression was significantly associated with CDC25A (p=0.006), suggesting that ARHGEF promote cell cycle via MYC signaling.Conclusion: High expression of ARHGEF2 is associated with poor patients' prognosis, and may promote cell cycle via MYC signaling in PDAC.

▲TOP

[P-3358] P14-68 [English/Japanese]
Pancreatic cancer (5)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Hirofumi Akita / Dept. of Gastroenterological Surg., Osaka Univ. Grad. Sch. of Med.

P-3358

Influence of preoperative chemoradiotherapy to the feasibility of adjuvant chemotherapy in pancreatic cancer patients

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[Purpose] In order to decrease the postoperative recurrence rate of pancreatic ductal adenocarcinoma (PDAC), postoperative therapy has been shown indispensable. On the other hand, preoperative therapy may be an additional strategy; however, the influence of preoperative therapy to postoperative therapy remains obscure. This retrospective study evaluates the feasibility of postoperative therapy after preoperative chemoradiotherapy (CRT).[Methods] The subjects were 99 consecutive patients who underwent pancreatectomy for PDAC in our hospital. As a preoperative therapy, 28 received gemcitabine (GEM) and 40 Gy radiation (G-CRT group), and 32 received GEM, S-1, and 50.4 Gy radiation (GS-CRT group). Thirty-nine patients who underwent surgery alone were also evaluated (SA group).[Results] In the G-CRT, GS-CRT, and SA groups, the completion rates were 86 % (19/22), 88 % (22/25), and 82 % (23/28), respectively; and adverse event frequencies were 36 % (8/22), 28 % (7/25), and 43 % (12/28), respectively. No significant difference was found among the three groups.[Conclusions] Preoperative CRT was demonstrated to be safe and did not compromise the feasibility of postoperative chemotherapy.

▲TOP

P-3359

Ionizing radiation enhances migration of pancreatic cancer cells through promoting hyaluronan metabolism

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Hyaluronan (HA) is a major component of extracellular matrix and provides favorable microenvironment for cancer progression. We have shown that HA, a low-molecular-weight HA (LMW-HA), enhances migration of pancreatic adenocarcinoma (PDAC) cells. Radiotherapy is one of the major treatment options for PDAC. However, We hypothesize that HA, especially LMW-HA, is involved in the acquisition of malignant phenotype in PDAC after irradiation.PDAC cells were treated with ionizing radiation (5Gy, 10Gy). Concentration of HA was measured in the conditioned media using ELISA. Expression of mRNA of HA synthesizing and degrading enzyme was examined using quantitative real time RT-PCR. Proliferation and migration of cells were also examined after irradiation. Ionizing radiation increased the concentration of HA (including LMW-HA) in the conditioned media in cell lines. The increased concentration of HA was associated with increased mRNA expression of HA synthesizing and degrading. Radiation enhanced migration of PDAC cells, while inhibiting their proliferation. These findings suggest that radiation enhances migration of PDAC cells possibly through activating HA synthesis and degradation.

P-3360

The association between cellular senescence of cancer associated fibroblasts and tumor progression in pancreatic cancer

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Background: We focused on the Cav-1, as a senescence marker, expression in cancer-associated fibroblasts (CAFs) in patients with pancreatic cancer (PC). Methods: A total of 157 consecutive patients with PC underwent curative resection between January 2004 and December 2016 is enrolled. First, we investigated the relationship between the expression of Cav-1 in CAFs and patients' clinicopathological factors. Second, we established the CAFs-cell lines from the resected tissues of patient with PC, and we underwent co-culture of PC-cell lines and CAFs-conditioned medium (CM). Results: The high level of Cav-1 expression group counts of 49 patients (31%), and the low level of Cav-1 expression group counts of 108 patients (69%). The high Cav-1 expression group had significantly worse outcomes both in overall (p=0.0062) and disease-free survivals (p=0.0017). Co-culture assay of MIAPaCa-2 and CAFs-CM treated with Cav-1 knockdown showed less invasive ability of MIAPaCa-2 compared to that with control (p<0.0001). Conclusion: Our results suggested that Cav-1 expression in CAFs in PC is associated with cancer invasiveness and patients' poor prognosis.

P-3361

Characteristics of E-cadherin high and low expressed human pancreatic cancer cell lines cultured in 2D and 3D-cultures

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Epithelial to mesenchymal transition (EMT) contributes to carcinogenesis, metastasis and anti-cancer drug resistance in cancer. In this study, we compared characteristics of E-cadherin high (PK-1 cells) and low expressed pancreatic cancer cells (PANC-1 cells) under adherent and sphere-forming conditions. Low-vacuum scanning electron microscopy showed that PK-1 formed round to oval spheres, while PANC-1 formed grape-like spheres, cultured in the ultra-low attachment plates. Immunocytochemical analysis using cell blocks showed that AE1/3 was localized in adherent cells and spheres of both cells. Vimentin was expressed in adherent cells and spheres of PANC-1, but not in PK-1. In contrast, CK7 and CA19-9 were localized in adherent cells and spheres of PK-1, but not in PANC-1. After the addition of TGF-beta 1, E-cadherin mRNA was decreased in PANC-1, while it was not altered in PK-1. In summary, these findings indicate that there are differences of 3D-morphology, EMT-related protein expression and EMT induction between E-cadherin high and low expressed pancreatic cancer cells. Heterogeneity of pancreatic cancer cells may contribute to difficulty of diagnosis and treatment of the cancer.

▲TOP

P-3362

Autophagy marker LC3 serves as a prognostic marker in pancreatic cancer after neoadjuvant chemoradiotherapy

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Objectives; To investigate the relationship between autophagy-related protein LC3 expression and clinical outcome of patients with pancreatic cancer receiving preoperative chemoradiotherapy.Patients and methods; we used data and specimen of 50 patients performed curative resection for pancreatic cancer after neoadjuvant chemoradiotherapy between 2007 to 2012. We performed immunohistochemistry and analysed clinicopathological factors between highly expression group and low expression group.Results; Clinicopathological bachgrounds were not different between LC3 High group (n=14) and LC3 low group (n=36). Kaplan-Meier survival analysis showed significant difference between 2 groups (LC3 high vs low) in overall survival (p=0.0378) and in disease-free survival (p=0.023). According to univariate analysis in overall survival, LC3 high expression and tumor size (>20mm) were significant prognostic factor. On the other hand, according to univariate analysis in disease-free survival, LC3 high expression and tumor size, and ly(+) were significant factors.Conclusions; LC3 expression could serve as a prognostic marker in patients with pancreatic cancer receiving preoperative chemoradiotherapy.

P-3363

Mitochondrial functions are indispensable to survival of pancreatic cancer cells during glucose deprivation

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Glucose deprivation (GD) is a hallmark of tumor microenvironment, especially in pancreatic cancers with hypovascular features. Thus, identification of indispensable factors to survival of pancreatic cancer cells during GD could lead to the development of effective chemotherapy. We here investigated the roles of mitochondria on survival and adaptive response of pancreatic cancer cell lines, including PANC-1 and MIA-PaCa-2, during GD. We established mitochondrial DNA-deficient PANC-1_ ρ 0 and MIA-PaCa-2_ ρ 0 cells. Compared to their parental cell lines, both ρ 0 cells exhibited the vulnerability to GD. PANC-1_ ρ 0 and MIA-PaCa-2_ ρ 0 cells also had much lower oxygen consumption rate, an indicator for mitochondrial respiration, and failed to induction of unfold protein response, including ATF4 up-regulation and ATF6 cleavage. This is consistent with GD-selective cancer cell killing by rotenone, a mitochondrial complex I inhibitor. These results indicate that mitochondrial functions could be indispensable to survival and adaptation of pancreatic cancer cells during GD. Collaborators: Shuhei Takei and Yuri Tani (JFCR)

▲TOP

[P-3371] P14-70 [English/Japanese]
Pancreatic cancer (7)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Tadafumi Asaoka / Dept. Gastroenterological Surg., Grad. Sch. of Med., Osaka Univ.

P-3371

5-FU/Gemcitabine resistance is associated with epitherial-mesenchymal transition in pancreatic cancer cell lines

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Pancreatic cancer has a poor prognosis. In addition to the high recurrence rate and the difficulty of early detection, chemoresistance is an important factor associated with the malignancy. However, the molecular mechanisms of chemoresistance in pancreatic cancer is unclear. To investigate acquired chemoresistance in pancreatic cancer, we tried to establish chemoresistant (CR) cell lines from the PANC-1, MIA PaCa-2, BxPC-3 and AsPC-1 by long-term exposure to doses of 5-FU and gemcitabine, which corresponded to the approximate EC50 as determined by MTT assay in parental cells. Alteration of proteins expression related to EMT and some oncogenes between CR cell lines and parental cells was tested in Western blotting, it has been revealed that the alteration depends on cell lines / drug - specific manners. Moreover, CR cell lines showed unique-morphological changes. These results indicated that there are several mechanisms in chemoresistance including EMT and activated proliferation signals, depending on tumor characteristics and types of anti-cancer drugs. Further studies are needed to optimize the efficacy of chemotherapy and improve chemosensitivity for pancreatic cancer.

▲TOP

P-3372

TNF-&alpha derived from infiltrating macrophages promote PD-L1 expression and leads to poor pancreatic cancer prognosis

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Cancer immunotherapy using anti-PD-1/PD-L1 antibodies has received considerable attention in recent decades. However, molecular mechanism underlying PD-L1 expression in pancreatic ductal adenocarcinoma (PDAC) cells has not been clearly elucidated. We investigated the clinical significance and regulatory mechanism of PD-L1 expression in PDAC cells. We performed immunohistochemistry assay with consecutive paraffin section from 228 PDAC patients. Survival analysis revealed that high PD-L1 expression was significantly associated with poor prognosis in patients with PDAC. Moreover, PD-L1 expression in PDAC cells was positively correlated with macrophage infiltration in tumor stroma of human PDAC tissues. In addition, PDAC cells were co-cultured with human monocyte derived macrophages and were subjected to real-time PCR. Finally, we identified that infiltrating macrophage-derived tumor necrosis factor (TNF)- α up-regulated the expression level of PD-L1 via the NF- κ B pathway in PDAC cells. We concluded that PD-L1 expression in PDAC cells is promoted by TNF- α derived from tumor-infiltrating macrophages, leading to poor prognosis for patients with PDAC.

P-3373

Functional analysis of cancer stem cell marker CXCR4 in pancreatic cancer

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Objective: CXCR4 is one of cancer stem cell markers (CSCM) in PaCa and we have reported that CXCR4 was a key regulator of invasive ability (Int. J Cancer 2009), but resistance to chemoradiotherapy has not been elucidated. Functional analysis of CXCR4 was performed for new treatment of pancreatic cancer. Methods and Results: (1) CXCR4 in chemoresistance: We established gemcitabine resistant (Gem-R) PaCa and DNA microarray was performed. The log2 ratio of CXCR4 is 1.23. The expression of CXCR4 in Gem-R was enhanced by addition of Gem. (2) Radiation resistance (RR) and CXCR4: RR PaCa was prepared and the expression change of CXCR4 was compared (5.32). (3) CXCR4 as a target for novel therapeutic agents: 1) NF- κ B inhibitor: Since CXCR4 is regulated by NF- κ B, Zerumbone, which inhibited NF- κ B, suppressed the expression of CXCR4, so that the invasive ability of PaCa was decreased. 2) CXCR4 antagonist: Gem-R tumor was reduced by addition of the CXCR4 antagonist in nude mice model. Conclusion: CXCR4 was involved in chemo- and radio-resistance in PaCa. So, it was suggested that NF- κ B inhibitors and CXCR4 antagonists could become new molecular targeted therapeutic agents for PaCa.

P-3374

Complement factor B is identified as a secreted protein in pancreatic cancer by comprehensive secretome analysis

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The interaction between cancer cells and stroma cells through the secreted proteins plays important roles in pancreatic cancer (PDAC) progression. To identify key secreted proteins involving in PDAC progression, we performed comparative analyses of the supernatant obtained from medium culturing mouse PanIN cells and PDAC cells, using Stable Isotope Labeling by Amino acid Cell culture with click chemistry, and LC-MS/MS. Total 195 proteins were identified as secreted proteins differentially expressed. Among 20 proteins secreted more than 2-fold in PDAC cells, we focused on complement factor B (CFB) for further analyses. The stromal CFB expression in 113 resected PDAC tissues were analyzed by immunohistochemistry. Dividing into two groups based on the staining intensity, high expression group (n=75) showed significantly higher hematogenous recurrence (p=0.0083), and shorter overall survival time (p=0.014) compared to low expression group (n=38). In multivariate analysis, tumor size (p=0.047), venous invasion (p<0.001) and CFB expression (p=0.011) showed as independent prognostic factors. In the present study, the functions of CFB in PDAC have been assessed in in vitro experiments.

▲TOP

P-3375

Novel gemcitabine derivative responsive to ROS in pancreatic cancer

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Reactive oxygen species (ROS) induce the oxidative stress in cancer, but not non-cancerous cells in tissue microenvironment. Given previous reports indicated that the technology targeting ROS can improve the specificity of reagents, we developed the novel gemcitabine derivative (Gd) responsive to hydrogen peroxide, a subtype of ROS. The Gd can be activated by tumor tissues with anaerobic condition, while it will be less toxic to non-cancerous parts in pancreas, compared to the original GEM (oG). To study the usefulness of Gd, we performed the cell growth (MTT) and apoptosis (Annexin V and Caspases 3 and 7) assays, showing that tumor suppressive effect was equivalent between Gd and oG. Xenograft model of immunodeficient mice indicated that the effect of Gd was not inferior to oG, which were administered in vivo. Importantly, the suppressive effect on hematopoietic cells in bone marrow was significantly less in Gd, compared with oG. The present study demonstrated that Gd showed anti-tumor effect equivalent to oG, and less adverse effects, suggesting the feasibility that the ROS-targeting modification can improve the usefulness of cytotoxic chemotherapeutic reagents.

▲TOP

[P-3383] P14-72 [English/Japanese]
Lung cancer (2)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Takashi Kijima / Div. Resp. Med., Dept. Int Med., Hyogo College of Med.

P-3383

Degradation of tumor suppressor BHLHE41 by ubiquitin-proteasome pathway in lung adenocarcinoma

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BHLHE41 a helix-loop-helix transcription factor and has been reported to be involved in circadian rhythm, proliferation, and epithelial-to-mesenchymal transition. However, its effects on tumor progression are controversial and roles in lung adenocarcinoma remain unclear.We explored the genes associating with prognosis of non-small lung cancer using TCGA database and found that the prognosis of patients harboring lung adenocarcinoma with high BHLHE41 expression was longer than that with low BHLHE41 expression.BHLHE41 transfected A549 cells grew more slowly than parental cells on dishes and in soft agar. This result supported that BHLHE41 is tumor suppressor in lung adenocarcinoma. We investigated regulatory mechanism of BHLHE41 protein expression since BHLHE41 protein expression was not correlated its mRNA in some of the transfected cells clones and found that BHLHE41 was targeted for proteasome dependent degradation by ubiquitination. This result suggests that the ubiquitin-proteasome pathway suppress BHLHE41 expression and associate with malignant progression in lung adenocarcinoma. (collaborator: Toshiyuki Nagata)

▲TOP

P-3384

Functional analysis of MOB1 in resectable lung adenocarcinoma

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Introduction: Downregulation or mutation of MOB1, a core component of the Hippo pathway, have been recognized in various human tumors. However, the roles of MOB1 in lung adenocarcinoma are largely unknown. We investigated the relationship between MOB1 expression and prognosis of lung adenocarcinoma patients.Method: MOB1 expression was immunohistochemically studied in 205 surgically resected lung adenocarcinoma. The association between expression of MOB1 and clinicopathologocal parameters was evaluated. Results: MOB1 expression was significantly associated with vascular invasion (p=0.0005). However, p-TNM stage was not significantly associated with MOB1 expression (p=0.3151). In MOB1 high expression group, the disease-free survival was significantly shortened compared to the MOB1 low group (p=0.0161), but no significant difference was observed in the overall survival. Conclusions: This study suggests that MOB1 expression in lung adenocarcinoma is a risk factor for early recurrence, and further studies are required on the effect of MOB1 on tumor growth or vascular invasion.

P-3385

Dissection of the function of CD109 in lung adenocarcinoma

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CD109, a glycosylphosphatidylinositol-anchored glycoprotein, is a member of the α2-macrogloblin/C3, C4, C5 family of thioester-containing proteins.CD109 has been reported to be a component of the TGF-β1 receptor system, and negatively regulates TGF-β1 signaling. We previously demonstrated that CD109 is highly expressed in several types of human malignant tumors including malignant melanoma, breast cancer and glioma, and is associated with poor prognosis. Reportedly, CD109 is highly expressed also in lung cancer tissue, but its functions remain unclear. In this study, we examined expression level of CD109 protein in human lung adenocarcinoma tissue and investigated the functions of CD109 using lung cancer cell lines.

P-3386

Analysis of Arl4c during the carcinogenesis of lung adenocarcinoma

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Small GTP-binding protein ADP-ribosylation factor (Arf)-like protein 4c (Arl4c), of which expression is induced by the Wnt/β-catenin and Ras-MAPK signaling, has been shown to be highly expressed in lung adenocarcinoma. The upregulation of Arl4c was also observed in Atypical Adenomatous Hyperplasia, which is a pre-cancerous stage. However, the detailed mechanism of the involvement of Arl4c in the initial process of lung carcinogenesis has been elusive. To analyze the role of Arl4c in the processes of lung carcinogenesis, mutated KRAS (KrasG12V) and/or Arl4c were introduced into the normal human small airway epithelial cell (SAEC). Cell proliferation of SAEC expressing KrasG12V and Arl4c (SAEC/KrasG12V/Arl4c) was remarkably higher than that of cells expressing either KrasG12V or Arl4c alone. Phosphorylation of ERK1/2 was also upregulated in SAEC/KrasG12V/Arl4c. E-cadherin and N-cadherin expression were decreased and increased, respectively, in SAEC/KrasG12V/Arl4c, dependent on MAPK signaling. These results suggest that coexpression of KrasG12V and Arl4c promotes proliferative capacity and induces epithelial to mesenchymal transition through the enhanced activation of MAPK pathway.

▲TOP

P-3387

KRAS, NRG1 mutations and copy number alterations in non-TRU lung adenocarcinomas with interstitial pneumonia

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We herein investigated a KRAS mutations, NRG1 translocations, and their copy number alterations in non-TRU lung adenocarcinomas (LADC) that developed in patients with idiopathic interstitial pneumonia (IIP). Tumors in patients with IIP (10 cases), and those in patients without IIP (15 cases) were subjected. Mutations in KRAS codon 12 were analyzed by the direct sequencing. NRG1 translocations were analyzed by FISH. KRAS and NRG1 copy number were analyzed by FISH. KRAS mutations were detected in 8 out of the 10 IIP LADCs, 8 out of the 15 non-IIP LADCs. NRG1 gene mutations were detected in none of the 10 IIP LADCs, 2 out of the 15 non-IIP LADCs. KRAS copy number gains were detected in 4 out of 10 IIP LADCs, 2 out of the 15 non-IIP LADCs. NRG1 copy number gains were detected in none of 10 IIP LADCs, 2 out of the 15 non-IIP LADCs. The result suggested the IIP-associated non-TRU LADCs are different from usual non-TRU LADCs in the profiles of genetic alterations.

P-3388

Fibroblasts induce epithelial cell senescence via extracellular vesicles in age-related lung diseases

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Lung cancer and idiopathic pulmonary fibrosis (IPF), which is a chronically progressive and lethal fibrosing interstitial lung disease, share common risk factors and are closely related age-related lung diseases. Although epithelial cells in IPF can serve as a possible origin of carcinoma development, the nature of the pathogenic molecular mechanism remains obscure. Extracellular vesicles (EVs) are about 100 nm in diameter and released by a variety of cells into their environment. EVs contain biomolecules such as microRNAs (miRNAs) and now have found to serve as the cell-to-cell communicator. Here, we investigated the involvement of EV-mediated intercellular communication between lung fibroblasts (LFs) and bronchial epithelial cells (BECs) in regulating epithelial cell senescence during IPF pathogenesis. We found several miRNAs in LF-EVs induced mitochondrial dysfunction and ROS accompanied by cellular senescence in BECs through direct regulation of tumor suppressor genes of lung cancer. These results indicate that EVs between epithelial cell and lung fibrosis play a key role in the pathogenesis of lung cancer and IPF.

▲TOP

[P-3395] P14-74 [English/Japanese]
Lung cancer (4)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Takeshi Yoshida / Dept. Med. Oncology, Kindai Univ. Faculty of Med.

P-3395

mRNA expression profile specific to micropapillary element in EGFR-mutated lung adenocarcinoma

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[Background] Our recent study demonstrated that malignant grade of EGFR-mutated lung adenocarcinoma (LADC) is determined by proportion of micropapillary element. [Purpose] To uncover the potential molecular basis of micropapillary element, we here investigated mRNA expression profile specific to micropapillary element in EGFR-mutated LADC.[Materials and Method] Micropapillary and other elements were separately collected from frozen tissue sections of 3 EGFR-mutated LADCs by the laser capture microdissection system. Total RNAs were extracted and then subjected to comprehensive RNA expression analyses with U133 gene chip microarray. Differentially expressed genes in the micropapillary elements, whose levels showed more than 3 folds changes in compared to those of the other elements, were picked up.[Results] KIAA1324, CEACAM7, MMP12, and so on, were obtained as the upregulated genes, while COL4A3, CYR61, WIF1, and so on were obtained as the downregulated genes. It is suggested that these differentially expressed genes could be related to the highly malignancy activity of micropapillary element.

▲TOP

P-3396

Subclassification of patients with lung adenocarcinoma harboring EGFR-activating mutations by gene expression profiling

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Background: Since response to EGFR-TKIs in these patients is not uniform, we aimed to identify these molecular subgroups in patients with lung adenocarcinoma harboring EGFR-activating mutations, by gene expression profiling (GEP) data.Methods: Surgically resected tumor samples from 294 patients with lung adenocarcinoma were collected with informed consent and subjected to whole-exome sequencing and GEP. Statistical evaluation of GEP data was conducted using principal component analysis (PCA) and orthogonal projection to latent structures discriminant analysis (OPLS-DA). Results: Patients with EGFR-activating mutations were divided into two clusters (C1 and C2) by the evaluation of those expression profile with PCA. The genes relevant to cell cycle progression, DNA repair and tumor immunity were selected, and which have contributed to the differences between two clusters. Patients classified into C1 showed the significant association with poor differentiation, relapse and smoking history. Conclusions: These results demonstrate that those patients can be further stratified into two additional molecular subgroups by assessing the expression level of genes above mentioned.

P-3397

Activation of the FGF2-FGFR1 pathway is associated with resistance to pemetrexed in lung cancer cells

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Pemetrexed (MTA) is a folate antimetabolite used for treating non-small cell lung cancer. To elucidate the mechanisms of MTA resistance in lung cancer, we established MTA-resistant sublines in PC9 (mutant EGFR) and H1993 (wild-type EGFR) adenocarcinoma cell lines (PC9-MTA, H1993-MTA). Gene expression profile comparison by microarray analyses identified enhanced fibroblast growth factor 2 (FGF2) and FGF receptor 1 (FGFR1) expression. ERK phosphorylation was increased in PC9-MTA but decreased in H1993-MTA along with decreased downstream signaling molecule phosphorylation. A morphological change from epithelial to spindle-shape together with increased mesenchymal marker protein expression was observed in H1993-MTA. SiRNA-mediated FGF2 knockdown partially restored MTA sensitivity in both lines, whereas an anti-FGFR1 inhibitor restored MTA sensitivity in PC9-MTA. Although thymidylate synthase strongly facilitates the development of MTA resistance, our results revealed the involvement of the FGF2-FGFR1 pathway in MTA resistance in lung cancer cells and suggested that cellular function alterations induced by FGF2-FGFR1 pathway activation might depend on the innate feature of cancer cells.

P-3398

Expression of intratumoral PD-L1 and intratumoral CD4+ T cell, CD8+ T cell, and FOXP3+ T cell in lung cancer

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Background: Individual tumor microenvironments vary according to the immune evasion process of each cancer tissue. $FOXP3^{+}CD4^{+}$ Treg cells recognizing tumor-specific shared antigens maintain the immunological self-tolerance. However, the precise conditions and diagnostic value of these immunological factors remain uncertain and specific biomarkers predictive of response have not yet been identified. Design: PD-L1, CD4, CD8 and FOXP3 expression in tumor cells and tumor infiltrating lymphocytes were examined by IHC in 45 cases with advanced lung cancer treated with nivolumab or pembrolizumab. Results: The objective response rate was 47%, and it was significantly correlated PD-L1 positivity with CD8 TILs ($p<0.0001$). Cases with PD-L1 overexpression showed consistently dense CD8+ TILs, even in subgroup analyses according to histological subtype, stage, age and smoking status. In cancer-associated stroma, CD4+ cells and FOXP3+ positive cells were detected. Conclusion: Overexpression of PD-L1 with CD8+ TILs was associated with a favorable response to treatment with nivolumab and pembrolizumab. In contrast, PD-L1- with low CD8+ TILs and FOXP3+ TILs was not associated with favorable response.

▲TOP

P-3399

The Link between Tumor Promoting Fibrous Microenvironment and Immune Microenvironment in Stage I Lung Adenocarcinoma

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Background: Podoplanin-positive cancer-associated fibroblasts (PDPN+ CAFs) play an important role in cancer progression, but the correlation between fibrous and immune microenvironment has not been clarified.
Patients: 174 cases with stage I lung adenocarcinoma (LA) were analyzed. We evaluated PDPN+ CAFs and immune-related cells; CD 204-positive tumor-associated macrophages (CD204+ TAMs), CD8+ T cells, and FOXP3+ T cells in cancer stroma by immunohistochemical staining method. By analyzing the gene expression profiles of LA (n=442), we compared the expression level of immune-regulatory cytokines according to the PDPN expression.
Results: The number of CD204+ TAMs was significantly higher (P<0.001), and CD8/FOXP3 T cell ratio was significantly lower in PDPN+ CAFs cases (P=0.027). The same result could be obtained within the same tumor in PDPN+ CAFs area than in PDPN- CAFs area. Microarray analysis revealed PDPN expression-high group had significant higher levels of immune-regulatory cytokines.
Conclusion: LA with PDPN+ CAFs is typified by an immunosuppressive microenvironment, suggesting the close link between tumor promoting fibrous microenvironment and immune microenvironment.

P-3400

Correlation between glycosyl transferase gene expression and poorer outcome in advanced lung adenocarcinoma

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Background:Correlation between different glycosylation patterns and clinicopathological features is still unclear in cancer. We investigate differing glycosylation in advanced lung adenocarcinoma (ADC) tissues.Methods:Formalin-fixed and paraffin-embedded biopsy specimens were obtained from 62 patients with lung ADC. Solubilized glycoprotein and mRNA was extracted from microdissected tumor cells. Expression levels of 44 lectins were analyzed by lectin array. mRNA expression levels of MGAT3, MGAT4a, MGAT5, FUT7, and FUT8 were analyzed. Results:Expression levels of Datura stramonium lectin, Solanum tuberosum lectin, and Aspergillus oryzae lectin were significantly higher in drug-resistant ADCs (P = 0.036, 0.0005, and 0.035, respectively). Overexpression of MGAT4a and MGAT5 mRNA was detected in 7/62 (11.3%) and 3/62 (4.8%) of ADCs. The prognosis of patients with MGAT4a and/or MGAT5 positive ADC was worse than those with negative ADC (P = 0.018, log-rank).Conclusion:Different glycosylation of N-glycan in lung ADC tissues were suggested. MGAT4a and/or MGAT5 overexpression may correlate with a poorer response to standard chemotherapy and poorer outcome in advanced lung ADCs.

▲TOP

[P-3407] P14-76 [English/Japanese]
Osteosarcoma

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Tadashi Hasegawa / Dept. Surg. Pathol., Sapporo Med. Univ. Sch. Med.

P-3407

Eribulin inhibits lung metastasis

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RINT

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A new chemotherapeutic agent, eribulin, has been approved for the treatment of advanced breast cancer and malignant soft tissue sarcoma. The results of clinical trials so far indicated that eribulin elongates overall survival period rather than disease free survival period, suggesting that the effect of eribulin may prevent metastasis. To confirm this hypothesis, we examined the inhibitory effect eribulin on lung metastasis and its mechanism using mouse osteosarcoma cell line, LM8.We investigated the effect of eribulin on LM8 in *in vitro* 2D and 3D experiments. Higher concentration than IC_{50} of eribulin induced cell cycle arrest and apoptosis. Lower concentration of eribulin suppressed microtubule elongation, filopodia formation, cell migration, and directionality during migration. These concentration of eribulin also reduced number and size of colonies in collagen 3D culture system. In mouse experiments, eribulin significantly reduced the number of lung metastatic foci and circulating tumor cells. These results claimed that the dynamic changes in cytoskeleton by low concentration of eribulin sustain its anti-metastatic effect aside from cell killing effects.

▲TOP

P-3408

The CpG Island Methylator Phenotype is A Potential Therapeutic Target in Osteosarcoma

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Epigenetic alterations are frequently observed in various cancer types, but its involvement in the development of osteosarcoma remains unclear. Here, we conducted a genome-wide DNA methylation analysis using Infinium HumanMethylation 450 bead array. Ten of 28 osteosarcomas showed the CpG island methylator phenotype (CIMP), but the number of aberrantly methylated genes was quite smaller than those in gastrointestinal cancers with CIMP. Genes involved in hormone metabolism, neurological function, skeletal system morphogenesis, and cell proliferation were aberrantly methylated in primary osteosarcoma. Patients with CIMP tended to have shorter disease-free survival than those without (P = 0.116). Growth inhibitory effect of DNA demethylation therapy on osteosarcoma was also observed in vitro and in vivo. Cell proliferation of four osteosarcoma cell lines was suppressed to mean 43% (4-81%) by treatment of 0.1 μM of 5-aza-dC. The volume of xenograft tumor was decreased to 29% by administration of 2 mg/kg of 5-aza-dC. These indicated that targeting aberrant DNA methylation could be a novel therapeutic strategy for osteosarcoma.

P-3409

Anti-osteosarcoma effect of the survivin inhibitor YM-155 in vitro and in vivo by induction of the ER stress response

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Osteosarcoma (OS) is the most common, primary malignant bone tumor. Although the multimodal therapy greatly improved the prognosis, more than 30% of patients still cannot get long-term survival. Therefore, novel therapeutic options for refractory cases should be urgently developed. Previously, we developed an OS mouse model by overexpressing c-MYC in bone marrow stromal cells (BMSCs) derived from Ink4a/Arf knockout mice. We isolated highly tumorigenic cells (designated AXT cells). Inoculation of AXT cells into syngeneic C57BL/6 mice results in the development of lethal OS with metastatic lesions in various organs, including lung, which mimics human osteoblastic osteosarcoma. To obtain the novel candidate agents for OS, we performed drug screening and found that the survivin inhibitor YM-155 strongly suppressed AXT cell growth by blocking cell cycle progression. Notably, YM-155 does not exhibit toxicity for normal BMSCs. Treatment of YM-155 induces endoplasmic reticulum (ER) stress and activation of Akt, p38Mapk and Erk. Single treatment of YM-155 attenuated OS tumor growth in vivo. These findings suggest that YM-155 becomes a potential therapeutic option for OS.

P-3410

Oncolytic adenoviral therapy with p53 transactivation induces profound immunogenic cell death in osteosarcoma

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Immunogenic cell death (ICD) is a form of cell death that activates antitumor immune response. Recent reports have demonstrated that some chemotherapeutic agents and radiation induce ICD through activation of tumor suppressor p53. However, p53 inactivation has been frequently observed in more than half of osteosarcomas. We previously developed a tumor-specific replication-competent oncolytic adenovirus, OBP-301, and modified OBP-301 (OBP-702) that induces the wild-type p53 gene. In this study, we assessed the antitumor effect and ICD induction ability of OBP-301 and OBP-702 in human osteosarcoma cells. We used 3 human osteosarcoma cells with different p53 status, U2OS (p53 wild-type), MNNG/HOS (p53-mutant), and SaOS-2 (p53-null). OBP-702 suppressed the cell viability of all human osteosarcoma cells more strongly compared to OBP-301. Moreover, OBP-702 induced more profound ICD with ATP release in all human osteosarcoma cells compared with OBP-301. Interestingly, OBP-702 induced more ATP release than chemotherapeutic agents and radiation even in p53-wild type U2OS cells. Our data suggest that OBP-702 is a promising strategy to induce ICD in osteosarcoma independent of p53 status.

▲TOP

P-3411

Mutational profiling and copy number analysis suggest homologous recombination deficiency in osteosarcoma

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Background: Homologous recombination deficiency (HRD), mainly due to inactivation of BRCA1/2, causes many structural variations, copy number alterations, and a specific mutational signature (signature 3) in breast and ovarian cancer genomes. We assessed genomic alterations in osteosarcoma (OS) from the viewpoint of HRD. Methods: We performed whole-exome sequencing (WES) analysis of 53 sarcomas (including 5 OSs) from adolescent and young adults (AYAs) and SNP array analysis of additional 17 OSs from children and AYAs. Results: In the WES analysis, the signature 3 contributions and LOH scores (a kind of estimate of HRD) of 5 OSs were higher than those of the other 48 AYA sarcomas ($P < 0.01$, respectively), and were correlated to each other ($r = 0.64$). In the SNP array analysis, the LOH scores of 17 OSs were as high as those reported in breast and ovarian cancers, and the scores of good responders to neoadjuvant chemotherapy were higher than those of poor responders ($P < 0.05$). Conclusions: Our results suggest that HRD underlies high genomic alterations in OS and that HRD scores may become predictive biomarkers for OS treatment.

P-3412

Clinical and Functional Significance of Single Intracellular and Extracellular Onco-microRNA in Osteosarcoma

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Osteosarcoma (OS) is the most common primary malignant bone tumor, and mainly occurs in children and adolescents. We previously identified highly expressed extracellular miR-25-3p, which was identified also in OS patients' serum. In this study, we investigated functional and clinical significance of intracellular and extracellular miR-25-3p in OS. miR-25-3p expression levels were negatively correlated with clinical prognosis ($p=0.004$), which was revealed by analysis of 45 human OS tissue specimens. We identified DKK3 as a direct target of miR-25-3p and found that DKK3 expression was positively correlated with clinical prognosis ($p=0.022$). MiR-25-3p promoted tumor growth, invasion, and drug resistance, which was consistent with DKK3 silencing in OS cells. In addition, miR-25-3p was embedded in OS-derived exosomes, which promoted capillary formation and the invasion of normal vascular endothelial cells. These results demonstrated that oncomiR-25-3p promotes OS progression in both the intracellular and extracellular space with clinicopathological relevance, indicating its potential as a novel diagnostic and therapeutic tool for novel clinical management.

▲TOP

[P-3418] P14-78 [English/Japanese]
The development of novel therapies for soft tissue sarcomas

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Yoshiyuki Suehara / Dept. Orthopedic Surg., Juntendo Univ.

P-3418

Anti-tumor effects of Eribulin mesilate on clear cell sarcoma cell lines

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Clear cell sarcoma (CCS) is an aggressive soft-tissue sarcoma characterized by melanocytic differentiation. Eribulin mesilate (eribulin) is a novel, non-taxane, synthetic microtubule inhibitor, and the therapeutic efficacy on CCS is still unknown. In this study, we investigated mechanisms of anti-tumor activity of eribulin on four CCS cell lines. Eribulin had antiproliferative effect against CCS cells both in vitro and in vivo. Intriguingly, eribulin upregulated melanin synthesis and melanocytic differentiation markers including MITF and TYR. In addition, eribulin-induced vascular remodeling was identified by increased microvessel density and by reduced HIF-1α in CCS xenograft model. Degradation assay showed that the stabilization of MITF protein by eribulin caused the augmentation of MITF protein levels. Phosphorylation of ERK1/2, which promotes degradation of MITF, was inhibited by eribulin per se and by subsequent elimination of tumor hypoxia. Taken together, we first demonstrate that eribulin has potent antitumor activity and promotes melanocytic differentiation via inhibition of ERK1/2 in CCS cell lines.

▲TOP

P-3419

The histone deacetylase inhibitor LBH589 inhibit undifferentiated pleomorphic sarcoma growth via downregulation of FOSL1

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Undifferentiated pleomorphic sarcoma (UPS) is the second most frequent soft tissue sarcoma. Because of its resistance to chemotherapy, UPS patients are treated with surgical resection and complementary radiotherapy. Since standard chemotherapy has not been established, unresectable or metastatic cases result in a poor prognosis. In this study, we investigated the potential effects and mechanisms of an HDAC inhibitor, LBH589, against UPS cells. We confirmed that LBH589 exhibits potent antitumor activities in four human UPS cell lines and IC50 values ranged from 7 nM to 13 nM. A mouse xenograft model showed that LBH589 treatment suppressed tumor growth. FACS analysis showed that LBH589 induced apoptosis and G2/M cell cycle arrest. RNA microarray identified the FOSL1 gene as a downregulated gene in response to LBH589 in UPS cells. While knockdown of FOSL1 decreased UPS cell proliferation, overexpression induced cell proliferation. Our results show that LBH589 could be a potent chemotherapeutic agent in the treatment of UPS and downregulation of FOSL1 is associated with its antitumor mechanism.

P-3420

Investigating the expression and regulation of NKG2D ligands to control metastasis in synovial sarcoma

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Synovial sarcoma is one of the rare soft-tissue tumors but relatively common in adolescents and young adults. Metastasis to the distant organ, most of the case is lung, frequently occurs in patients with SS and it causes poor prognosis, thus controlling metastasis is thought to be the better way to improve outcome. Nevertheless, it is still challenging issue because the mechanisms underlying metastasis is complex systems including cellular characteristics, response to microenvironment and relationship to the immune system. We here focus on expression and regulation of MICA/B, the cell-surface ligands which are recognized by immune-receptor, NKG2D, on natural killer cells NK cells. We found MICA/B is expressed in Aska-SS but not in Yamato-SS. MICA/B expression in Aska-SS was sensitive to Twist1 expression, hypoxia and floating condition which are known as a trigger of the metastatic cue. MICA/B mRNA expression and protein stability were sensitive to HDAC inhibitors and N-Glycosylation inhibitor, respectively. Correspondently, the expression of one glycosyltransferase was low in Yamato-SS. Perhaps MICA/B regulatory mechanism is a point to potentiate metastasis in synovial sarcoma.

P-3421

Identification of potential immunohistochemical markers for liposarcoma based on proteomic analysis using FFPE tissue

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Recent technical improvements in both mass spectrometry and protein extraction have made it possible to use formalin-fixed paraffin-embedded (FFPE) tissues for proteome analysis. In this study, comparable proteome analysis of FFPE tissues revealed multiple candidate marker molecules for differentiating atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDL) from lipoma. We identified 181 unique proteins for ALT/WDL. Of the identified proteins, we focused on CCDC180 and LRRC4 as candidate markers of ALT/WDL. We showed that CCDC180 and LRRC4 immunohistochemistry clearly stained tumor cells of ALT/WDL and dedifferentiated liposarcoma (DDL) and could differentiate them from lipoma with high accuracy. We also used cell biological methods to further examine the expression of the candidate marker molecules in liposarcoma cells. In liposarcoma cells, knockdown of CCDC180 and LRRC4 inhibited cell proliferation. CCDC180 inhibited cell migration, invasion and apoptosis resistance in WDL cells. These results indicated that LRRC4 and CCDC180 are novel immunohistochemical markers for differentiating ALT/WDLS.

▲TOP

P-3422

A Case Report: Experience of using Larotrectinib against pediatric soft tissue sarcoma with LMNA-NTRK1 fusion gene

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Background: NTRK1, 2 and 3 encode tropomyosin-related kinase (TRK) proteins TRKA, TRKB and TRKC, respectively. NTRK rearrangements occur in a broad range of solid tumors and are oncogenic by leading to TRK autophosphorylation. Larotrectinib is an oral, potent and selective inhibitor of TRK. Here we report on the first experience in Japan with larotrectinib to treat a patient with recurrent low-grade LMNA-NTRK1 fusion soft tissue sarcoma. Case: The patient is an 8-year-old female child. At the age of 6, she underwent resection of a soft tissue tumor that developed in the right brachialis. Eight months after surgery, local recurrence was suspected by MRI examination with slow enlargement over the next 8 months. Since re-operation likely required amputation, the patient was started on larotrectinib provided under compassionate access at a dose of 100 mg BID. Complete remission was achieved at three months. Response is still sustained on treatment after 5 months without surgery. There were no adverse events attributed to larotrectinib. Summary: Larotrectinib showed excellent outcomes in this Japanese soft tissue sarcoma patient.

▲TOP

[P-3364] P14-69 [English/Japanese]
Pancreatic cancer (6)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Hidenori Takahashi / Dept. Surg., Osaka International Cancer Institute

P-3364

Concomitant IPMN in pancreatic ductal adenocarcinoma is a predictive factor for new cancer in the remnant pancreas

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Objective: To determine the factors predicting subsequent development of pancreatic ductal adenocarcinoma in the remnant pancreas (PDAC-RP) after partial pancreatectomy for pancreatic ductal adenocarcinoma (PDAC).
Methods: We retrospectively reviewed a consecutive series of 379 patients with PDAC treated by partial pancreatectomy, and 14 patients (3.69%) were PDAC-RP. Clinicopathological variables were compared between PDAC-RP and non PDAC-RP.
Results: In univariate analysis, concomitant intraductal papillary mucinous neoplasm (IPMN), cancer location (body - tail), lower T factor in UICC were correlated with development of PDAC-RP. Multivariate analysis revealed concomitant IPMN to be an independent predictive factor related to the PDAC-RP. Histologically, PDAC concomitant with IPMN showed increased number and density of PanIN in the background pancreas.
Conclusions: Concomitant IPMN in PDAC was an independent predictive factor for the development of new PDAC in the remnant pancreas probably due to increased number and density of PanIN lesions in the background pancreas.

▲TOP

P-3365

Subclinical peritoneal dissemination detected by RT-PCR in preoperative treatment strategy for pancreatic cancer

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Background:. Peritoneal recurrence is a major recurrence pattern after surgery for pancreatic cancer (PC) following preoperative chemoradiation therapy (CRT), even in patients with negative peritoneal lavage fluid cytology. Detection of CEA-mRNA by RT-PCR is reported to be useful for evaluating subclinical tumor cell dissemination in peritoneal lavage fluid. Methods: Patients with PC treated with preoperative gemcitabine-based CRT and subsequent surgery were enrolled. CEA-mRNA was detected in the peritoneal lavage fluid at laparotomy using RT-PCR. Recurrence patterns and survival were evaluated in association with the CEA-mRNA status in the peritoneal lavage fluid. Results: The peritoneal lavage fluid from 57 of the 237 patients (24%) was CEA-mRNA (+). The CEA-mRNA (+) patients had a significantly higher incidence of peritoneal recurrence than the CEA-mRNA (-) patients (36% vs. 15%; $p < 0.001$). The 5-year survival rates of the CEA-mRNA (+) and (-) patients were 31% and 51%, respectively ($p = 0.037$). Conclusions: A CEA-mRNA (+) status was associated with a significantly increased incidence of peritoneal recurrence in patients with PC treated with preoperative CRT.

P-3366

Clinicopathological relevance of SMAD4 and RUNX3 in pancreatic cancer

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SMAD4/DPC4 is considered to play primary roles in tumorigenesis and progression of pancreatic cancer. Runt-related transcription factors (RUNX) are important regulators of lineage-specific gene expression in developmental pathways. RUNX3 also functions as a tumor suppressor in some kinds of cancers through TGF-beta, Wnt, and other signaling pathways. A published report has indicated that RUNX3 and SMAD4 coordinately regulate the balance between cancer cell proliferation and dissemination in genetically engineered mouse models. We examined the relevance of genetic and expression state of SMAD4 and RUNX3 in clinicopathological features of 104 patients who received surgery for pancreatic cancer. We found that retain of SMAD4 expression in primary pancreatic cancer tissues was significantly associated with their metastatic recurrences. We also found that the diffuse expression of RUNX3 and loss of SMAD4 was significantly associated, however, its clinicopathological relevance was not elucidated. These results suggest that retain of SMAD4 may promote the metastatic recurrence in pancreatic cancer.

P-3367

Molecular surgical margin analysis of pancreatic cancer surgeries

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R0 tumor resection should be one of the critical prognostic factors of pancreatic cancers. However, histological diagnosis of surgical margin is sometimes unreliable due to tissue shrinkage and skipped lesion. We applied our 'molecular surgical margin analysis' to evaluate the precise surgical margin status of pancreatic surgeries. Quantitative methylation-specific PCR (QMSP) assay for three representative pancreatic cancer-specific methylation markers (CD1D, KCNK12, PAX5) were established and validated. These markers of tumor tissues were significantly higher than adjacent normal tissues. Then, they were applied to prospectively collected surgical margin imprinting samples (n=13) from histologically margin negative specimens. Molecular surgical margin analysis revealed four positive cases. Among them, one case relapsed, two cases were likely to relapse. Whereas, only one case relapsed in nine margin negative cases. Although we still need to follow-up the cases for a year or more, invisible cancer cells on the surgical margin might have an association with early tumor recurrence after the surgery.

▲TOP

P-3368

Homogeneity of the replacement growth pattern in liver metastasis of pancreatic cancer

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Background: The replacement growth pattern (RGP), in which metastatic cells infiltrate replacing hepatocyte show poor prognosis and resistance to anti-angiogenic therapy in patients(pts) with liver metastasis (LM) from malignant tumor. There is little knowledge of the homogeneity of RGP in LM from PCa.Methods: Resected specimens of treatment-naive LM from PCa were evaluated histologically. The tumor-liver interface was investigated for evaluation of the homogeneity of RGP. The relative fraction of RGP with the length of＞ 5% of the total length of the interface was estimated. The relative fraction of RGP over 80% was defined as the homogenous RGP. The prognostic value of a homogenous RGP was also evaluated. Results: Fourteen resected hepatic specimen from 14 PCa pts were evaluated. RGP was observed in 9 pts. The relative fraction of RGP was 100% in 7 pts, 70% in 1 pt and 5% in 1 pt. A homogeneous RGP was found in 78% of the 9 LMs showing RGP and in 50% of all LMs. The median overall survival in the homogeneous RGP group was shorter than that in the non RGP group (7.5 vs 16.5 months). Conclusion: RGP was the homogenous morphological factor of LM and related poor prognosis in PCa.

P-3369

miR-296-5p inhibits apoptosis and enhances invasion through the down-regulated BOK in unresectable pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy, but early diagnosis is disturbed by the absence of novel biomarkers. MicroRNAs (miRNA) are noncoding RNAs involved in initiation and progression of cancers. We analyzed whether certain miRNAs could serve as a biomarker for the prognosis of PDAC and uncover the uncharacterized miRNAs function in the pancreatic carcinogenesis. Expressions of 2042 miRNAs were profiled in 13 micro tissues of PDAC by endoscopic ultrasound-fine needle aspiration, and compared to the survival periods of 13 patients with chemotherapy. Higher expression of miR-296-5p, known as a cancer-promoting miRNA, could predict worse survival of 13 patients. Bioinformatically, we identified Bcl2-related ovarian killer (BOK), a pro-apoptotic gene as a target of miR-296-5p, and overexpression of miR-296-5p suppressed BOK levels. In addition, overexpression of miR-296-5p suppressed Caspase-9 activation, and enhanced cell invasion with epithelial-mesenchymal transition in PDAC cells. These results provide novel role of miR-296-5p on the regulation of BOK in PDAC, and tissue measurements of miR-296-5p have predicted the poor prognosis in clinical use.

P-3370

Radiogenomics: Association between imaging features and p53 expression level and survival in pancreatic cancer

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Introduction: Pancreatic cancer is a highly lethal cancer with no established markers of survival. And existing prediction factor rely mainly on post-resection data and are of limited utility in preoperative management. Radiogenomics(RG) is an emerging field of research that seeks to correlate imaging features(IF) with molecular profiles. We investigated an association between p53 expression and survival for pancreatic ductal adenocarcinoma(PDAC) patients, and between p53 expression and IF resulted from Radiomic analysis of CT preoperatively performed for PDAC patients. Materials and Methods: A retrospectively maintained database in Chiba Cancer Center identified 113 patients resected for PDAC between January 2013 and December 2017. We obtained the specimens and measured levels of p53 by immunohistochemistry. We extracted some IF by 3D gray level co-occurrence matrix from CT, and analyzed IF such as c, entropy, energy, cluster shade, etc. Result: It was suggested that in PDAC, p53 was a poor prognostic factor, and there was a relationship between IF and p53 by RG. Conclusion: RG suggested that IF might predict p53 expression and survival in PDAC.

▲TOP

[P-3376] P14-71 [English/Japanese]
Lung cancer (1)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

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P-3376

S100A11 facilitates the migratory, invasive, and proliferative capacity of NSCLC

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Non-small cell lung cancers (NSCLCs) make 85% of lung cancers and adenocarcinomas are the most common type. Currently our understanding of the molecular signatures that drive the progression of NSCLC is incomplete. The S100 protein family comprises 25 proteins and several of them have been implicated in the pathogenesis of NSCLC, however, the role of S100A11 is not fully understood. We used doxycycline regulated knockdown of S100A11 to uncover its role in the cellular function of H1975 cells. Knockdown of S100A11 significantly reduced the migratory as well as invasive capability of H1975 cells. Confocal microscopic analyses suggested a dysregulation in cytoskeletal organization. Further, CCK-8 assay showed high reduction in the proliferative capacity of H1975 cells. Moreover, in vivo tumorigenic assay resulted in decreased tumor volumes in mice administered with doxycycline. Together, we report that S100A11 is an important molecule for the tumorigenic properties of H1975 cells and it may serve as a potential therapeutic target to attenuate the progression of lung adenocarcinomas.

▲TOP

P-3377

Identification of the lead compounds targeting Src and their underlying mechanisms in lung cancer progression

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The tyrosine kinase Src plays an important role in cancer progression and has been considered as a target molecule for drug development. This study is aimed to identify the compounds that can target Src and suppress tumorigenesis and metastasis in NSCLC. We first screened the potential compounds from NCI (40,000 molecules) compound library by molecular docking approach. Seventeen candidate compounds were selected and subjected to evaluate their efficacy by ELISA assay. Among which, 11 compounds are efficient in reducing phosphor-Src over 60%. One of the candidates, NSC-91580, was further used in Western blot and in vitro and in vivo functional assays. We found that NSC-91580 can significantly decrease the phosphorylation of Src and its downstream molecules including STAT3 and FAK in a dose-dependent manner. Moreover, NSC-91580 could suppress cancer cell colony formation, migration and invasion ability in a dose-dependent manner, as well as tumor growth in vivo. Furthermore, combination treatment of NSC-91580 with Iressa showed a synergistic effect on inhibiting lung cancer cell proliferation. These findings will contribute to the development of anti-cancer drugs for lung cancer.

P-3378

Discovery and characterization of a novel long non-coding RNA in lung cancer

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Lung cancer is the most common cancer and the leading cause of cancer deaths in Japan. Long non-coding RNAs (lncRNAs) have been increasingly recognized as a key player in various physiological and pathological processes, including tumor formation and metastasis. The aim of the current study was to identify novel lncRNAs involved in development of lung cancer. Through in silico analysis of publically available RNA-seq dataset of lung squamous cell carcinoma (LUSC), we found 61 lncRNAs whose expression levels increased >100-fold in LUSC. PCR detection of couples of those lncRNAs in human lung cancer cell lines allowed us to identify S180122, a yet uncharacterized lncRNA, which is strongly up-regulated in lung cancer. Loss-of-function experiment indicated that S180122 significantly promoted cell migration and invasion, but had little effect on cell proliferation. Investigation on its subcellular location is in progress. In summary, our data reveal a role of the novel lncRNA, S180122, in progression of lung cancer, but further studies are needed to determine more details of its underlying mechanism.

P-3379

The role of OGFOD1 in the growth of lung cancer

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OGFOD1 (2-oxoglutarate and Fe (2)-dependent oxygenase domain containing protein 1) is one of the hydroxylases, which is suggested that it works as a cell cycle regulator in cancers, or a translational regulator against many stress stimuli. However, its substrates and mechanisms of activation have been still unknown. Here, we investigated its expression patterns mainly in lung cancers by immunohistochemical staining, and using some lung cancer cell lines, we analyzed changes of these proliferation abilities or gene expression patterns after knockdown of OGFOD1 gene. As a result, we confirmed that OGFOD1 expressed in lung cancer, especially strongly in poor differentiated types. And OGFOD1 knockdown led some lung cancer cell lines to reduce these proliferation activities, inducing some changes of these cancer associated genes' expression levels. Therefore, it is considered that OGFOD1 plays an important role in lung cancer progression through regulating some cancer associated genes.

▲TOP

P-3380

DLL3 regulates migration and invasion of small cell lung cancer

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Background: Delta-like protein 3 (DLL3) is a ligand of Notch, which is reported to be a tumor suppressor in small cell lung cancer (SCLC). Previous studies suggest that DLL3 might be associated with neuroendocrine tumorigenesis through inhibition of Notch signaling unlike other activating ligands. However, little is known about function of DLL3 in SCLC. Methods: We used small interfering RNA (siRNA) to down-regulate the expression of DLL3 in SCLC cell lines. Anchorage-dependent and anchorage-independent cell growth were measured by MTT assay. Migration and invasion were assessed by transwell assay. Results: The suppression of DLL3 by siRNA resulted in the slight inhibition of cell growth of H82 cells in both anchorage-dependent and anchorage-independent cell proliferation. The depletion of DLL3 prevented migration and invasion and downregulated Snail expression in H69 and H82 cell lines. The expressions of Notch1 and Hes1 were also downregulated by DLL3 knockdown in both SCLC cells. Conclusion: DLL3 promotes the migration and invasion in SCLC cells by modulating Notch1 and Snail.

P-3381

Expression of Delta-like protein 3 and its regulation in patients with small cell lung cancer

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Objective: Rovalpituzumab tesirine, Delta-like protein 3 (DLL3)-targeted antibody-drug conjugate is expected to be the first targeted therapy for small cell lung carcinoma (SCLC). However, prevalence of DLL3 as well as association with clinical characteristics and its regulation in SCLC patients have been unclear. Method: immunohistochemical (IHC) staining for DLL3 were performed in 63 patients with SCLC. The correlation of clinical characteristics to status of DLL3 expression was compared. In addition, transcription factors to regulate DLL3 expression were comprehensively examined by using public database. Result and conclusion: Among total 63 patients, 52 patients (83%) were positive for DLL3 expression, with 20 patients (32%) being positive in at least 50% of cancer cells. DLL3 expression was not associated with any of the characteristics examined. Based on data of cell line encyclopedia (CCLE), we found two transcription factors, ASCL1 and NeuroD1. Analyses of 49 SCLC cell lines in CCLE revealed that DLL3 expression is significantly associated with elevated ASCL1 and low to null expression of NeuroD1.

P-3382

Evaluation of PD-L1, DLL3, and EZH2 expressions in small cell lung cancer

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One useful candidate for small cell lung cancer (SCLC) therapy is immune checkpoint blockade therapy targeting programmed death-1 (PD-1) and its ligand PD-L1. Furthermore, Rovalpituzumab tesirine (Rova-T), a delta-like protein 3 (DLL3)-targeted antibody-drug conjugate, and enhancer of zeste homologue (EZH2) inhibitor are expected to be the first targeted therapy for SCLC. In the current study, we conducted to evaluate PD-L1, DLL3, and EZH2 expressions in SCLC tumors to find a candidate responder to those therapies. We have performed immunohistochemical (IHC) staining for PD-L1, DLL3, and EZH2 in 20 patients with SCLC and compared the clinicopathological features and IHC staining intensity. We found that one of the 20 patients (5.0%) showed positive PD-L1 expression in the metastatic tumors, as well as in the primary lung tumor. DLL3 was highly expressed in 14 of the 20 patients (70%) and positive EZH2 was found in 17 of the 20 patients (85%). None of these cases had any correlation with age, gender, smoking, stage or treatment. IHC staining found candidate responders for anti-PD-L1/ PD-1 immunotherapy, Rova-T therapy, or EZH2 inhibitory therapy.

▲TOP

[P-3389] P14-73 [English/Japanese]
Lung cancer (3)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Nagio Takigawa / Dept. General Int. Med. 4, Kawasaki Med. Sch.

P-3389

Circulating PD-L1+ Exosomes As Prognostic And Predictive Biomarkers In pulmonary lymphoepithelioma-like carcinoma

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Pulmonary lymphoepithelioma-like carcinoma (LELC) is an Epstein-Barr virus (EBV)-associated epithelial neoplasm. It is not clear whether PD-L1 carried by circulating exosomes correlates with clinical outcomes in pulmonary LELC. Plasma exosomes were isolated using the exosome-specific dual-patterned immunofiltration (ExoDIF). Plasma exosomes were incubated with activated $CD69^+$ human $CD8^+$ T cells and/or PD-1 inhibitor. 51.8% pulmonary LELC patients harbored PD-$L1^{high}$ exosomes, and 54.2% showed PD-$L1^+$ tumor cells in tumor tissue with 93% concordance between tissue and plasma exosomes. Blocking of PD-$L1^+$ exosome signaling to PD-1^+ T cells attenuated immune suppression. After treatment of PD-1 inhibitor, there was a significant correlation ($r = 0.87$; $P = 0.003$) between synchronous changes of PD-L1 level in plasma exosome and tumor size. Detection of plasma PD-$L1^{high}$ exosomes at the end of treatment was also a significant prognostic worse factor of overall survival ($p < 0.001$). Monitoring PD-L1 expression by circulating exosomes is a valuable tool to assess prognosis and tumor response in patients with pulmonary LELC. PD-1 inhibitors deserve further evaluation in pulmonary LELC.

▲TOP

P-3390

Genomic and immunological profiling of pleomorphic carcinoma of the lung

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Background: Pulmonary pleomorphic carcinoma (PPC) is a rare and aggressive subtype of lung cancer containing both carcinomatous and sarcomatous components.The two components were morphologically different. Patients and Methods: Eleven PPC formalin-fixed paraffin-embedded surgical samples were collected from 2006 to 2016 in National Cancer Center Hospital. We separated the samples to carcinomatous and sarcomatous components by macro dissection and performed target sequence, gene expression analysis and immunohistochemistry (IHC) of immune cell markers for both components. Tumor mutation burden (TMB) was compared to 280 lung adeno carcinoma (LUAD) samples. Results: The TMB of PPC was higher than that of LUAD (the mean 19.3 vs. 7.9 mutations/megabase, p =5.0E-7). The majority of gene mutations (81.6%) were shared between both components. The gene expressions of the major immunological genes were similar between both components. More than half of samples (54.5%) highly expressed of PD-L1 IHC between both components. Conclusions: Both components of PPC develop with sharing the same genomic alterations and immunological features.

P-3391

The clinical significance of autophagy in patients with non-small cell lung cancer

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Background: Recently, autophagy was reported to play an important role in cancer. The role of autophagy in non-small cell lung cancer (NSCLC) has not been well clarified. So we investigate the clinical significance of autophagy in NSCLC. Method:Tumor tissues from 67 patients underwent operation were analyzed. The status of autophagy was evaluated by the intratumoral expressions of the marker p62 with immunohistochemistry.The Ki-67 proliferation index was also investigated by immunohistochemistry. Results:Among the 67 tissues, 23 tumors (34.3%) were autophagy-high. No significant relation was observed between the autophagy status and the other patient variables.The Ki-67 index was significantly higher in the autophagy-high tumors than low tumors(P=0.0291). The 5-year survival rate for patients with autophagy-high tumor was significantly worse than low tumors (P=0.0005). A Cox regression analyses also demonstrated autophagy status to be a significant prognostic factor (P=0.0021).Conclusion: The high expression of autophagy evaluated by p62 was associated with the tumor proliferation in NSCLC. The autophagy status can be used as a poor prognosis in NSCLC.

P-3392

The clinical significance of glutamate/cystine antiporter SLC7A11/xCT in non-small cell lung cancer

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Back ground: Cell-surface markers should be useful for developing novel targeted imaging probes or therapeutics for personalized treatment of cancer. The glutamate/cystine antiporter solute carrier family 7 member 11 (SLC7A11, also called xCT) is overexpressed in several cancers. The role of SLC7A11 in non-small cell lung cancer (NSCLC) has not been well clarified. Method: Tumor tissues from 40 patients underwent operation with NSCLC were analyzed. The SLC7A11 expression was evaluated with immunohistochemistry. The percent of positive tumor cell was scored. Results: The percentage of SLC7A11 positive tumor cells varied greatly (median=60.0%; mean±SD=48.6±27.4%) in the NSCLC tumors. Among the 40 NSCLCs, 22 tumors (55.0%) were SLC7A11-high. No significant relation was observed between the SLC7A11 status and the patient variables. Furthermore, the 5-year survival rate for patients with SLC7A11-high tumor was significantly worse than that for patients with autophagy-low expression tumors (67.6% vs. 93.8%, P=0.041). Conclusion: The Cell-surface SLC7A11 expression is the poor prognostic factors for NSCLC and may become a potential target for targeted imaging probes or new therapy.

▲TOP

P-3393

MCL-1 expression of non small cell lung cancer is prognostic factor

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Backgroud: MCL-1 is an important member in the pro-survival Bcl-2 family.We investigated the significance for MCL-1 expression of non small cell lung cancer (NSCLC).
Methods: Tumor tissues from 80 patients underwent R0 resection with NSCLC without any neoadjuvant therapy were analyzed. The intratumoral expressions of MCL-1 and Ki-67 were evaluated with immunohistochemistry in the tumor nucleus.Apoptotic index(AI) was detected by TUNEL.
Results: The percentage of MCL-1 positive tumor nucleus varied greatly. A sample was classified as MCL-1 high tumor if >25% of the nucleus in the tumor exhibited positive stainings. 36(45.0%) were MCL-1 high status. The 5-year survival rate for patients with MCL-1 high tumors was significantly worse than that for patients with MCL-1 low tumors (68.3% vs. 93.1%, p=0.0057). The Ki-67 index was significantly higher in the MCL-1 high tumor than that in MCL-1 low tumor (19.7±16.3% vs. 8.4±11.1%, p=0.005). There was no significant relation between the AI and MCL-1 status.Multivariate analysis demonstrated MCl-1 expression to be a significant prognostic factor (HR, 4.069; p=0.0405)
Conclusions: MCL-1 expression is prognostic factor in NSCLC.

P-3394

Relationship between expression of S100A10 and prognosis in lung sqaamous carcinoma

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S100A10 is one of the members of the S100 protein family and its upregulation is reported in many types of tumors. In lung cancer, association between upregulation of S100A10 and poor prognoses was reported only in adenocarcinomas. We pursued the possibility of significance in other subtypes of lung cancer; squamous cell carcinoma (SCC) was analyzed in this study. We first examined S100A10 protein expression by immunohistochemical staining in resected specimens from 81 patients with lung squamous cell carcinoma and observed that 27 (33.3%) of 81 tumors showed overexpression, mainly in cell membrane at the invasive front. Expression levels significantly associated with higher pathological TNM stage (P=0.029), tumor size (P=0.009), lymphatic invasion (P=0.005), lymph node metastasis (P<0.0001), and poorer prognosis (P=0.038). Our present results suggest that high S100A10 expression plays an important role in tumor progression mainly caused by lymphatic invasion and metastasis in patients with lung squamous cell carcinoma.

▲TOP

[P-3401] P14-75 [English/Japanese]
Lung cancer (5)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Izumi Nagatomo / Dept. Respiratory Med. and Clin. Immunol., Osaka Univ., Grad. Sch. Med.

P-3401

The effectiveness of afatinib, a second-generation EGFR-TKI, for NSCLC patients in clinical practice

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Introduction: Afatinib is a second-generation tyrosine kinase inhibitor and has shown clinical activity in patients with EGFR-mutated lung adenocarcinoma. Methods: In 41 NSCLC patients who received afatinib, we retrospectively assessed the administration status and effectiveness of afatinib. Results: Of all 41 patients, 6 (14.6%) started low-dose administration due to an older age and low performance status, and 27 (65.8%) experienced dose reduction over the course of treatment due to side effects, The median time to treatment failure (TTF) was 9.6 month. Four patients tolerated the treatment well and continued it for more than two years. Nine patients had uncommon EGFR mutations, including four one-point mutations, three compound point mutations and two exon 20 insertions. The median TTF of these 9 patients was 8.0 months. The response rate of the 7 patients with uncommon mutations who showed an evaluable response was 71.4 %. One patient with progressive disease had exon 20 insertion. Conclusion: These results suggest that afatinib may be beneficial for EGFR mutated NSCLC patients, especially for those with uncommon mutations other than exon 20 insertion.

▲TOP

P-3402

Serum C-reactive protein as a predictive factor for responses to EGFR-TKIs in patients with EGFR-mutated NSCLC

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Background: Epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) shows significant efficacy in patients with EGFR mutated non-small cell lung cancer (NSCLC). A recent study reported that high plasma concentrations of interleukin (IL)-6 are associated with a worse response to EGFR-TKIs in these patients. Serum C-reactive protein (CRP) widely measured in clinical practice is induced by IL-6. This study was conducted to assess whether serum CRP levels were associated with therapeutic responses to EGFR-TKIs. Methods: We retrospectively reviewed the medical records of 82 patients with EGFR mutated NSCLC who received EGFR-TKI therapy. The association of serum CRP levels with clinical factors was tested. Results: High serum CRP levels (> 1.0 mg/dL) prior to EGFR-TKI therapy were observed in 22 patients (27%). Patients with high CRP levels had lower objective response rates ($p = 0.016$), and showed reduced time to treatment failure (median 5.8 vs. 20.7 months; $p < 0.001$) and overall survival (median 14.2 vs. 47.3 months; $p < 0.001$). Conclusions: Serum CRP levels prior to EGFR-TKI therapy may predict therapeutic responses to EGFR-TKIs in patients with EGFR-mutated NSCLC.

P-3403

Exploration of serum biomarkers predicting clinical outcome and irAEs in advanced NSCLC patients treated with nivolumab

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PD-1/PD-L1 blockade elicits unprecedented clinical benefit in non-small lung cancer (NSCLC). However, additional biomarkers are needed to predict clinical benefit and immune-related adverse events (irAEs). Here, we conducted a serial evaluation of multiple serum proteins in advanced NSCLC patients treated with nivolumab. Thirty-eight patients were registered in the study between January 2016 and March 2017 at Wakayama Medical University Hospital and included in the final analysis. Among 57 serum proteins, serum follistatin level was significantly lower in PR patients than non-PR patients at baseline. Serum IL-8 and HGF levels were significantly changed between baseline and week 4 in PR patients than non-PR patients. Serum follistatin and IL-8 levels were correlated with longer PFS at baseline and both at baseline and week 4, respectively. Levels of G-CSF and Rantes were significantly higher at week 4 and Leptin at week 4 was significantly lower in irAE patients than those in non-irAE patients, respectively. Early changes of these proteins may have the potential to predict clinical benefit and irAEs from nivolumab treatment in advanced NSCLC.

P-3404

Retrospective analysis for stool abnormality on the efficacy of immune checkpoint inhibitors in patients with NSCLC

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【Background】Cancer immunotherapy has being developed as a promising alternative strategy for advanced NSCLC. However, it is still needed to develop the novel biomarker to optimize use of ICIs. Recently, tumor mutation burden and local infiltration of TIL were reported as potential biomarkers for it. More recent report showed gut microbiota regulates the condition of host immunity. 【Materials and methods】We retrospectively enrolled 34 patients with advanced NSCLC who treated with ICIs. The median age was 70 years, the histological subtypes were adenocarcinoma in 20 patients, squamous cell carcinoma in 10 patients, other types in 4 patients, and 23 patients were smokers. The association between stool abnormality and efficacy of ICIs were investigated retrospectively. We defined the patients with constipation or use of laxative before treatment of ICIs as the group of stool abnormality.【Result】In the group of without stool abnormality, disease control rate was 64.3%, whereas disease control rate was 33.3% in the group of stool abnormality ($P=0.0008$).【Conclusion】The stool abnormality has the possibility of predictive biomarker for the clinical benefit of ICIs in NSCLC.

▲TOP

P-3405

Rare case of pulmonary carcinosarcoma characterized by neuroendcrine, myogenic, and chondrogenic differentiations

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Carcinosarcoma is a clonal tumor developing thorough sarcomatoid change in a carcinoma. We present an extremely rare case of pulmonary carcinosarcoma characterized by components showing pluripotency with neuroendocrine, myogenic, and chondrogenic differentiations immunohistochemically. There was transition between carcinomatous and sarcomatous components. Immunohistochemical expressions suggesting immortality were shown in both of the carcinomatous and sarcomatous components, namely completely loss of p53, diffuse and strong expression of p16, and heterogeneous expression of PTEN. There were also a few cell groups in sarcomatous component that immunohistochemically expressed aldehyde dehydrogenase 1 family member A1 (ALDH1A1) or c-kit suggesting cancer stem-cells like cells. This case suggests that epithelial mesenchymal transition (EMT) played a key role for tumor cells having immortality and pluripotency like cancer stem-cells.

P-3406

A Resected Case of Synchronous Multiple Lung Cancer(Squamous Cell Cancer And Adenomarcinoma) in The Same Pulmonary Lobe

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We report a resected case of synchronous multiple lung cancer (squamous cell carcinoma and adenocarcinoma) in the same pulmonary lobe. A 80-year-old woman was pointed out a lung nodule in right lower lobe on chest CT by primary care physician. She was referred to our hospital to investigate the lung shadow. Brinkman index:200. No abnormal findings were observed in blood biochemical examination and tumor marker.Chest X-ray showed a nodule in the right middle lung field.Chest CT showed a 15mm tumor shadow in the right lung S6, and a 15mm ground-glass opacity(GGO) around a cavity with thin wall in the same lobe S8.Histpathological diagnosis of abnormal shadow in S6 was diagnosed as squamous cell carcinoma by transbronchial biopsy, but S8 could not be made. FDG-PET showed abnormal uptake in S6 tumor with a maximal standardized uptake value of 9.11, and no abnormal uptake in S8 lesion was observed. With preoperative diagnosis was squamous cell carcinoma in right lower lobe (cT1bN0M0), we performed right lower lobectomy. The right S6 tumor was histopathologically diagnosed as squamous cell carcinoma(pT1bN0M0) and the right S8 cavitary lesion with GGO as adenocarcinoma (pT1bN0M0).

▲TOP

[P-3413] P14-77 [English/Japanese]
Lung cancer (6)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Masakuni Serizawa / Drug Discovery & Development Div. Shizuoka Cancer Ctr. Res. Inst.

P-3413

Murine pulmonary vascularization changes in a metastatic lung mouse model using micro-CT

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Lung metastasis is one of the deadliest forms of cancer and is a major therapeutic burden with poor survival rates. Approximately 30% of all cancer deaths are caused by lung metastasis. A number of studies have shown how metastatic foci influence the pulmonary vascularization. In our previous study, we suggested that systemic chemotherapy based on the EPR effect might be ineffective in the early stages of lung metastasis. Here we show pulmonary vascularization changes that occur in a mouse metastatic lung model using micro-CT. FM3A-Luc and KM-Luc/GFP cells were intranodally inoculated into the subiliac lymph node (SiLN) of MXH10/Mo-lpr/lpr mice and inoculated SiLNs were dissected on day 3 post-inoculation in order to create the experimental model. Bioluminescence imaging revealed that tumor cells in the lung were activated after dissection of tumor bearing SiLN. No luciferase activity was detected near the region of the tumor bearing SiLN after dissection. Histopathology revealed that metastatic foci were present in the pulmonary artery.

▲TOP

P-3414

Early Detection of Lung Adenocarcinoma in Low Dose Computed Tomography by 3D Reconstruction

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Lung cancer is the leading cause of death among all malignancies in Taiwan and southen Asia. Individuals who received low dose computed tomography (LDCT) had a 20% mortality reduction compared with individuals receiving X-ray scan. To date, existing studies using LDCT for early detection of lung cancer remains wit poor specificity. This poster presents a computational methodology for early detection of lung adenocarcinoma using LDCT. The 3D nodule structure is reconstructed from piles of 2D LDCT images. This preprocess provides novel features not or hard to be found in the 2D images. 3D tumor-disappearing rate is calculated as a novel feature for distinguishing partial solid tumors. These features are collectively used to train a classification algorithm. The developed method is applied on a clinical cohort and controls for demonstrating the sensitivity and specificity.

P-3415

Gene expression changes by cancer-stroma interaction in a patient-/cell line-derived xenograft model of lung carcinoma

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To evaluate effects of new therapeutic agents, preclinical models resembling patient tumors are needed. Among our originally-established lung cancer patient-derived xenografts (PDXs), one PDX with EGFR-mutation was found to be highly metastatic to the lungs. Significantly, in this case, two kinds of cancer cell lines from the xenograft and its lung-metastasis tissue and primary-cultured cancer cells and fibroblasts from the corresponding surgical specimen were also established. Gene expression profiles revealed that gene X was found to be expressed in the primary tumor and PDX tissues, but quite low or no expression was observed in the above cancer cell cultures. However, co-culture of cancer cells with fibroblasts elevated gene X expression. Subcutaneous injection of the cancer cells into immune-deficient mice developed cell-derived xenografts (CDXs). Expectedly, in the CDX tissue, gene X expression was confirmed. Thus, a PDX/CDX was suggested to provide preclinical models reflecting cancer cells-to-fibroblasts interaction. To clarify the importance of gene X for maintenance and/or growth of tumor tissues, the effect of gene X knockout is now being examined.

P-3416

Mesothelin-positive proliferative lesions in the lung of N-bis(2-hydroxypropyl)nitrosamine (DHPN)-treated rats

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Background: We previously reported that mesothelin-positive alveolar proliferative lesions were observed in rats treated with multi-wall carbon nanotube administered by the intratracheal instillation, and they were diagnosed as early stage squamous metaplasias. The present study was conducted to assess the expression of mesothelin in lung proliferative lesions induced by N-bis(2-hydroxypropyl)nitrosamine (DHPN). Methods: Male F344 rats (6 weeks old, n=20) were treated with 0.1% DHPN in the drinking water for 2 weeks, kept for 30 weeks and killed for the histological examination.Results and Discussion: Histologically, alveolar hyperplasias, adenomas and adenocarcinomas were observed in 18 rats surviving at the end of the study with the incidences of 18/18, in alveolar hyperplasia, 6/18 and 3/18, respectively. Among them, 6/18 alveolar hyperplasias, 2/6 adenomas and 1/3 adenocarcinomas were positive for the expression of mesothelin. The total number of hyperplasias in 18 rats was 619, among which 8 lesions (1.53%) were positive fort the expression of mesothelin. Detailed histopathological evaluations and their comparison with the data of MWCNT-induced lesions are in progress

▲TOP

P-3417

A Comparison of Commercially Available Next Generation Sequence Assays for cell-free DNA Analysis

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Liquid Biopsy is generating significant attention because clinical use of analytical tests to detect somatic mutations in cell-free DNA (cfDNA) is increasing. Especially, recent advantages in Next Generation Sequence (NGS), molecular barcoding enables to correct PCR errors bioinformatically and quantify the number of target molecules, which have allowed to read rare variants with high specificity and sensitivity. Interestingly a few NGS kits for cfDNA analysis have been on market last 12 months and all kits noted ≧0.5%　sensitivity even though each kit uses different barcoding strategies. Here, we pick Oncomine Lung cfDNA Assay (Thermo Fisher Scientific Inc.) and AVENIO ctDNA Targeted Kit (Roche Diagnostics) to exam their capabilities and potentials with Horizon Discovery Reference Standards and clinical samples. To conclude, both kits were detected variant alleles consistently at >1% frequency with high reproducibility and would be a good option for the future clinical practice.

▲TOP

[P-3423] P14-79 [English/Japanese]
The pathogenesis for soft tissue sarcomas

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(E)/12F Lobby, Osaka International Convention Center Room P(E)

Miwa Tanaka / Div. Carcinogenesis, Cancer Inst, JFCR

P-3423

Myxofibrosarcoma is characterized by frequent abnormalities in TP53 and increased genetic instability

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Myxofibrosarcoma (MFS) is a rare subtype of sarcomas in the elderly, whose genetic basis is poorly understood. We analyzed a total of 44 cases with MFS by whole-genome sequencing (WGS), whole-exome sequencing (WES), and/or immunohistochemistry (IHC). The data were analyzed by combining those from The Cancer Genome Atlas cohort (WES, n=17). Longitudinal samples were also analyzed in 6 cases. A median of 44.0 mutations/sample was identified in WES, where most frequently mutated genes included *TP53* (34.4%), *ATRX* (14.8%), and *RB1* (4.9%). Combining copy number alterations (n=35) and abnormal IHC staining (n=13), *TP53* was affected in as many as 91.8% cases (n=56). WGS revealed numerous somatic structural variations (a median of 179.0/sample), including complex abnormalities, suggesting extensive genetic instability. In longitudinal analysis, relapse samples had increasing numbers of mutations (odds ratio 1.6, p=0.03). In all cases, *TP53* lesions were present at the time of diagnosis, while most of the other lesions were subclonal and acquired during the clinical course. In summary, MFS is characterized by frequent abnormalities in *TP53*, leading to increased genetic instability.

▲TOP

P-3424

Investigation of the molecular mechanisms underlying the bone metastasis of myxoid liposarcoma

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Myxoid liposarcoma (MLS) accounts for about 30% of all liposarcoma. While chemotherapy and radiotherapy improved the survival rate of MLS patients, 30% of MLS patients developed the distant metastasis. Unlike soft tissue sarcomas that tend to metastasize to the lungs, MLS metastasizes to unusual extra-pulmonary sites such as abdomen and bone. For understanding the molecular mechanisms underlying MLS bone-metastasis, in vivo animal models are essential. Therefore, in the present study, we sought to establish a mouse model for bone-metastasis of human MLS. After the preparation of four MLS cell lines expressing firefly luciferase and GFP reporter genes by lentivirus vectors, we injected these cell lines into NOD/SCID mice through the tail arteries to isolate the cells that infiltrate and grow in the femur regions. Using this in vivo selection, we succeeded the establishment of bone-metastatic MLS cell lines. Thus, our study could provide a unique in vivo model for the bone-metastasis of MLS that enables us to explore the mechanisms of bone metastasis and evaluate the potential therapies for MLS patients.

P-3425

Multinodularity of Solitary Fibrous Tumor; A Background of Dedifferentiation

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Solitary fibrous tumors (SFTs) are soft tissue tumor of intermediate malignancy. Dedifferentiation is known as a high-grade transformation of SFT, characterized by sarcomatous overgrowth, high-grade nuclear atypia or heterogeneous component. About one percent of SFTs may present dedifferentiation and poorer clinical course, while the background of dedifferentiated SFT remains to be clear. Therefore, we investigated the clinicopathological and histopathological background of dedifferentiated SFT. We reviewed 140 SFT cases. NAB2-STAT6 fusion gene was detected in 55 of 108 available cases. Histopathologically, 104 of 140 primary tumors showed incomplete fibrous capsule, 39 showed multinodular structure, 32 showed fibrous septa, 16 showed infiltrative borders and 6 showed dedifferentiated areas. Statistically, multinodular structure was related with dedifferentiation (p=0.009). Fusion gene pattern was not statistically related with prognosis and dedifferentiation. In conclusion, we considered that multinodular structure may be a pre-dedifferentiated condition and a potential utility as clinical marker of worse clinical course.

P-3426

Roles of lysophosphatidic acid receptors in cellular functions by anticancer drug treatment in fibrosarcoma cells

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Lysophosphatidic acid (LPA) is an extracellular biophysical lipid which interacts with at least six subtypes of G protein-coupled LPA receptors (LPA1 to LPA6). In the present study, to investigate an involvement of LPA receptors in cellular functions induced by anticancer drug in fibrosarcoma cells, the long-term cisplatin (CDDP) treated (HT-CDDP) cells were generated from HT1080 cells. Expressions of LPA receptor genes were changed in HT-CDDP cells, compared with HT1080 cells. Since LPAR2 and LPAR6 gene expressions were elevated in HT-CDDP cells, LPA2 and LPA5 knockdown cells were established from HT-CDDP cells. The cell motile activity of HT-CDDP cells was reduced by LPA2 knockdown. In contrast, LPA5 knockdown enhanced the cell motile activity of HT-CDDP cells. In soft agar colony formation assay, HT-CDDP cells formed the large sized colonies. The colony formation activity of HT-CDDP cells were inhibited by LPA2 knockdown. These results suggest that LPA signaling via LPA receptors may play an important role in the regulation of cellular functions by the long-term anticancer drug treatment in HT1080 cells.

▲TOP

P-3427

Molecular genetic analysis of CIC-rearranged sarcoma

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Genomic rearrangements of CIC gene account for a subset of small round cell sarcomas. Although CIC-rearranged sarcomas have been conventionally viewed as Ewing-like sarcomas, recent studies have clarified the significant clinical differences between CIC-rearranged sarcomas and EWSR1-rearranged Ewing sarcomas. In this study, we performed high-throughput RNA sequencing analysis of 13 round cell sarcomas using FFPE samples to diagnose CIC gene fusions in detail. Four cases of them were negative for both CIC and EWSR1 rearrangements by break-apart FISH, although closely resembled CIC-rearranged sarcomas on morphological and immunohistochemical grounds. In all 13 cases, CIC-DUX4 fusion transcript was detected including the four CIC FISH-negative ones. The CIC breakpoints were located within exon 20 in all but 1 case, in which the fusion occurred within exon 15 of CIC. The breakpoints in DUX4 were more variable within the open reading frame. The breakpoint junction commonly contained microhomology sequences. These analyses help molecular diagnosis and genotype-based therapy of sarcoma.

▲TOP

[P-3428] P24-1 [English/Japanese]
Cancer epidemiology (1)

2018 / 9 / 29 (Sat) 16:00-16:45 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Motoki Iwasaki / Natl. Cancer Ctr., Ctr. Pub. Hlth. Sci., Div. Epi.

P-3428

Plasma 25-hydroxyvitamin D concentration and subsequent risk of total and site-specific cancers in a Japanese population

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Although preclinical studies have fairly consistently supported the chemopreventive properties of vitamin D in carcinogenic processes, evidence linking vitamin D to the prevention of cancer in humans is inconsistent. Here we evaluated the association of plasma 25-hydroxyvitamin D concentration, the accepted marker of vitamin D status, with the risk of total and site-specific cancer in a large case-cohort study consisting of 3301 incident cancer cases and 4044 random subcohorts within the Japan Public Health Center-based Prospective Study cohort. The results showed that a higher vitamin D concentration was associated with a lower risk of overall cancer, with the multivariable-adjusted hazard ratios (HRs) for the highest quartile compared to the lowest quartile of 0.78 (95% CI 0.67-0.91), P_{trend}=0.001. Among the findings for cancer of specific sites, an inverse association was found for liver cancer, with the corresponding HRs of 0.45 (0.26-0.79), P_{trend}=0.006. More importantly, none of the cancer endpoints examined showed increased risk associated with higher vitamin D concentration. These findings support the hypothesis that vitamin D has protective effects in carcinogenesis.

▲TOP

P-3429

Oral hygiene and the survival of head and neck cancer

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Poor oral hygiene is an established risk factor of head and neck cancer (HNC); however, its role in the survival of HNC is unclear. We investigated the association between oral hygiene habits, including regular dental visits, frequency of tooth brushing, and use of dental floss, and the overall survival (OS) of HNC using interview data collected from 740 HNC patients. We found that poor oral hygiene was significantly associated with a worse OS of HNC (hazard ratio (HR) = 1.38, 95% confidence interval (CI): 1.03-1.86). Furthermore, this relationship was modified by the single nucleotide polymorphism, rs11536889, of TLR4. A strong association between poor oral hygiene and a worse survival of HNC was observed among those with the CG or CC genotype (HR = 2.32, 95% CI: 1.41-3.82) but not among those with the GG genotype (HR = 0.95, 95% CI: 0.65-1.40). Overall, our results indicated that poor oral hygiene, a risk factor of HNC, may also be a prognostic factor of HNC. More investigations are needed to determine the biological mechanisms to explain the worse HNC survival associated with poor oral hygiene.

P-3430

Association between functional polymorphism of PD-L1 with its protein expression and prognosis of gastric cancer

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Expression of programmed death ligand 1 (PD–L1) was associated with a higher response rate to anti–PD–L1 treatment of gastric cancer (GC) patients. In this study, we aimed to analyze the relationship between functional polymorphisms of PD–L1, PD–L1 protein expression and GC prognosis. We collected 728 blood samples of patients who underwent radical gastrectomy. Functional polymorphisms of PD–L1 (rs822336, rs866066, rs822338 and rs2297136) were genotyped using MassARRAY technology. The expression of PD–L1 protein was detected by immunohistochemical method in 157 patients of them. Patients carrying rs822336 CC genotypes lived longer than patients with GG+CG genotypes (HR: 0.504, 95% CI: 0.283–0.897). AA+AG genotype of rs2297136, which located in 3'utr of PD–L1 was correlated with PD–L1 protein expression (P=0.013) and served as an independent factor of better prognosis in patients without postoperative chemotherapy (HR: 0.348, 95% CI: 0.125–0.968). Comprehensive evaluation of PD–L1 polymorphisms and protein expression could help predict the survival in patients with gastric cancer.

P-3431

The epidemiology of gastric cancer in the era of H. pylori eradication: a nation-wide registry-based study in Taiwan

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Gastric cancer is a common cancer in Asia. Helicobacter pylori (HP) infection is an important risk factor for gastric adenocarcinoma and lymphoma. HP eradication has been shown to decrease the risk of gastric adenocarcinoma. We analyzed the incidence and survival of gastric cancer using data from the Taiwan Cancer Registry, a nation-wide population-based cancer registry, from 1996 to 2013 by histologic subtype. The overall incidence of gastric cancer decreased from 15.97 per 100,000 in 1996 to 11.57 per 100,000 in 2013. The most common histologic subtype of gastric cancer was adenocarcinoma, followed by lymphoma, and sarcoma. The best survival was observed in patients with sarcoma, followed by lymphoma, neuroendocrine tumor and adenocarcinoma. The incidence of adenocarcinoma significantly decreased from 13.56 per 100,000 in 1996 to 9.82 per 100,000 in 2013 (P<0.0001). In contrast, the incidence of mucosa-associated lymphoid tissue lymphoma and diffuse large B cell lymphoma increased. The disparity of the incidence trends in HP-associated adenocarcinoma and lymphoma highlighted the importance of risk factors other than HP for the development of gastric lymphoma.

▲TOP

P-3432

Precancerous lesions and cervical cancer in women aged 20 to 60 years in the West and North of Phayao Province, Thailand

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In Thailand, cervical cancer is the first type of cancer in women. The most common cause is HPV infection. Recently, the cancer is increasingly found in the patients aged lower than 30 years. The coverage screening and early detection leading to the effective cure and reduce the progression of cancer. This study aimed to detect the precancerous lesion and cervical cancer in women aged between 20 and 60 in the West and North regions of Phayao Province, Thailand. 20,137 Pap smears were collected from 2010-2014. The population were separated into six groups are aged less than 20 years, 20-30 years, 31-40 years, 41-50 years, 51-60 years and over 60 years. 222 cases of abnormal lesion (1.10%) were detected. Of the 222 abnormal cases, 219 (98.65%) cases with precancerous lesion and 3 (1.35%) cases with cancer including ASC-US 64 (28.83%) cases, ASC-H 13 (5.86%) cases, LSIL 87 (39.19%) cases, HSIL 55 (24.77%) cases, SCC 2 (0.90%) cases and adenosquamous carcinoma 1 (0.45%) case. Twenty one (9.46%) cases found in aged lower than 30 years. In conclusion, the screening in young aged population will increase coverage of risk women and provide the effective treatment of the early diagnosis.

P-3433

Smoking cessation and subsequent risk of cancer: A pooled analysis of eight population-based cohort studies in Japan

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We aimed to assess the effect of cessation of smoking on the risk of total cancers and smoking-related cancers using pooled data from eight population-based prospective cohort studies in Japan. Cancer risks in men with 21+ years of smoking cessation before baseline were found to decrease to the same level as never smokers for total cancer (never smokers: reference; former smokers with 21+ years since smoking cessation: HR1.01; 95%CI: 0.91-1.11). Even men who are heavy smokers (more than 20 pack-years) reported a reduced risk of total cancer (never smokers: reference; former smokers with 21+ years since smoking cessation: HR1.06; 95%CI: 0.92-1.23). In women, the risk of total cancer did not differ from that of never smokers after 11 years of smoking cessation before baseline (never smokers: reference; former smokers with 11+ years since smoking cessation: HR0.96; 95%CI: 0.74-1.23). Our study suggests that longer duration of smoking cessation may attenuate the risk of cancer in both men and women. Collaborators: Sugawara Y, Tsuji I, Mizoue T.

P-3434

IGF and IGFBP and incidence of malignant neoplasms in a nested case-control study

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IGF1 is a potent mitogen but BP3 binds and inhibits IGF1. To elucidate the relationship those and the risk of carcinogenesis, we analyzed associations of serum levels of IGF1 and BP3 with incidence of all malignant tumors, including hematopoietic and solid tumors, in a prospective case-control study nested in the JACC Study. A baseline survey was conducted from 1988 and 39,242 subjects donated blood samples. Those who had been diagnosed as tumors by 1997 were regarded as cases. We randomly selected 2 or 3 controls, matching for gender, age, and area. 1349 cases and 4012 controls are eligible for this study.After controlling for alcohol intake, BMI, and smoking, participants with high total-BP3 and free-BP3(molar difference of (BP3 - IGF1)) had the risk of future neoplasms (p for trend= 0.004 and 0.009, respectively), but those with IGF1 not. Limiting subjects to those followed for 3 years weakened the negative associations of total- and free-BP3, whereas the positive relation of free-IGF1(molar ratio of IGF1/BP3) was revealed (OR= 1.076; p for trend= 0.036).Our findings might suggest that serum IGF1 and BP3 are related to future risk of malignant neoplasms in Japanese.

▲TOP

[P-3435] P24-2 [English/Japanese]
Cancer epidemiology (2)

2018 / 9 / 29 (Sat) 16:45-17:30 Room P(F)/3F Lobby, RIHGA Royal Hotel Osaka Room P(F)

Yusuke Takahashi / Dept. Surg., Osaka InterNatl. Cancer Institite

P-3435

Dietary acrylamide intake and risk of breast cancer in Japanese women

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Background: Acrylamide is a probable human carcinogen and it forms in foods during high-temperature cooking. However, the association between dietary acrylamide and risk of breast cancer in Japanese women is unclear.
Methods: We conducted a population-based prospective study (JPHC study) and 48,910 women aged 45-74 years were included in this study. Dietary acrylamide intake was calculated using a food frequency questionnaire and energy adjustment was conducted by residual model. Cox proportional hazards regression models were used to estimate hazard ratios and 95% confidence intervals.
Results: Total of 792 breast cancers were diagnosed during 15.4 years of follow up. Dietary acrylamide intake was not associated with the risk of breast cancer: HRs (95%CI) compared with the lowest tertile was 1.00 (0.84-1.18) in middle tertile and 0.95 (0.79-1.14) in the highest tertile, p-trend=0.58). Further, when stratified analyses were conducted by confounding factors, we did not detect any significant association.
Conclusion: Dietary acrylamide intake was not associated with the risk of breast cancer in Japanese women.
Acknowledgement: Ling Zha and Rong Liu contributed to data analysis.

▲TOP

P-3436

Premature mortality due to cancers among working-age population evaluated by years of potential life lost

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[Objective] This study aimed to investigate the status of premature deaths from cancers among the working-age population in the last two decades in Japan. [Methods] Vital statistics for the period 1995-2014 were used. Age-standardized years of potential life lost (YPLL) among the working-age population (age between 15-64 years) were calculated for all cancers or common sites of cancer (i.e. lung, stomach, esophagus, large intestines, liver, pancreas, breast, and uterus). [Results] The decline in the YPLL rate calculated by simple regression analysis was 20.9 and 10.0 years (/year/100,000 population) for males and females, respectively. The reduction in YPLL due to stomach cancer largely accounted for the improvement in premature deaths for both sexes. Liver cancer was the second candidate for males. Other common cancers contributed more or less to the reduction, except pancreas cancer for both sexes, esophagus and uterus cancers for females. [Conclusion] Premature deaths from cancer among the Japanese working-age population has considerably improved in the last two decades. However, measures for certain cancers such as pancreatic and cervical cancers are required in the future.

P-3437

The trends in esophageal and stomach cancer screening of 9404 alcoholic men during 1993-2017

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We performed endoscopic screening with esophageal iodine staining in 9401 alcoholic men (30-79 yrs, 54±11 yrs), and detected head and neck cancer (HNC) in 1.1% of them, esophageal cancer (EC) in 3.2% of them, and stomach cancer (SC) in 1.0% of them by their initial screening. A history of HNC, EC, SC, and gastrectomy was found in 0.5%, 1.0%, 3.8%, and 10.2% of them, respectively. The EC detection rate in HNC-/EC-free men was 3.3%, 3.6%, 3.9%, 2.6%, and 1.6%, respectively, in 1993-7, 1998-2002, 2003-7, 2008-12, and 2013-17, and the SC detection rate in SC-/gastrectomy-free men was 1.4%, 1.2%, 1.1%, 0.3%, and 0.7%, respectively. Decreased trend in pack-years and increased trend in age were found during 1993-2017. When 1993-1997 period was used as a reference, the age- and pack-years-adjusted odds ratio of each period was 1.04, 1.07, 0.71, and 0.44 , respectively, for EC detection (trend p=0.002), and 0.76, 0.65, 0.15, 0.36, respectively, for SC detection (trend p=0.001). The NHC detection rate did not change during the period. EC detection as well as SC detection has decreased in alcoholic men in the recent decade, which cannot be explained by the changes in their age and smoking.

P-3438

Japanese SEER Program: Requirements for Nationwide Cancer Epidemiological Studies Based on the National Cancer Registry

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Objectives
In United States, the Surveillance, epidemiology, and End Results（SEER） program has been conducted since 1970 and many cancer epidemiological studies have been published. In Japan, the nationwide population-based cancer registry started in 2016. However, because only 26 items are collected, more information such as genome is needed for new types of research.
Methods
In 2017 we visited Utah Cancer Registry (UCR) to understand collecting and management of database. We compared the difference between US and Japan and considered the requirements for the same program in Japan.
Results
UCR was funded by SEER program and employed tumor registrars. Data was controlled by rules of data standards, training to the registrars, and accreditation of the registry. The way of data disclosure was limited under Utah health code and agreement of Utah department of health. To start the same program in Japan, 11 requirements were extracted, those were categorized into 3 problems such as employment of staff, data quality control, and enough budget to continue.
Conclusion
Nationwide support is needed to start the same program in Japan and model project is important to solve the problems actually.

▲TOP

P-3439

Social Capital of Cancer Awareness and Prevention via EWOM

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Our research concerns the aspects of EWOM (electronic word-of-mouth) in relation to cancer awareness and prevention. Notably, we focus on the types of information leading to cancer awareness and prevention as well as social capital relationships between the information source (senders) and readers (recipients). Recently, it has been known in various fields of psychology that both information type and social capital affect how information recipients recognize and react to EWOM information, but almost no literature exists in the field of EWOM information regarding cancer awareness and prevention. We have taken a survey, and by stochastic methods, have identified how certain types of information and how certain social capital relationship have more impact on information recipients than others. Outcomes of our research will enable both cancer awareness and prevention organizations to better reach and communicate to citizens, attaining greater level of awareness and prevention.

P-3440

Outlook for Japan's International Cooperation in Cancer Care: Analyzing Trends Towards the Realization of the SDGs

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BackgroundAgainst the backdrop of international efforts towards achieving the SDGs, a major and urgent issue for cancer care is to ensure access to affordable treatment for all. As a step towards realizing this objective, the World Health Assembly (WHA) Cancer Resolution that was adopted in May 2017 calls for strengthened collaboration among various stakeholders in order to realize effective medicines and affordable access to cancer care.ObjectiveTo seek to identify a direction for enhancing corporate commitments and forming public-private partnerships that involve various international organizations, with a view to improving access to cancer treatment in developing countries, where approaches to cancer in the context of global health have yet to be fully formulated.Method Analysis of documents issued by international stakeholders relating to the WHA Cancer Resolution, as well as the outcomes of relevant meetings and interviews with stakeholders.ConclusionBased on current international trends, develop an outlook for the role that Japan can play in international cooperation in cancer care and address any challenges.

▲TOP

[Public Open Seminar] CL [Japanese]
Public Open Seminar

2018 / 9 / 29 (Sat) 15:30-17:50 Public Open Seminar/Hall A+B, Knowledge Capital Congrès Convention Center, Second Basement, North Building, Grand Front Osaka Public Open Seminar

Masaki Mori / Dept. Gastroenterological Surg. Osaka. Univ.、Takuro Nakamura / Div. Carcinogenesis, Cancer Inst, JFCR

Public Open Seminar

Shinichi Yachida
Dapt. Cancer Genome Informatics, Grad. Sch. Med., Osaka Univ.

No Abstract

Public Open Seminar

Koji Tamada
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No Abstract

Public Open Seminar

Hideyuki Saya
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No Abstract

▲TOP