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• BASIC RESEARCH •

Analysis of gene expression profile of pancreatic carcinoma using cDNA microarray

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Abstract

AIM: To identify new diagnostic markers and drug targets, the gene expression profiles of pancreatic cancer were compared with that of adjacent normal tissues utilizing cDNA microarray analysis.

METHODS: cDNA probes were prepared by labeling mRNA from samples of six pancreatic carcinoma tissues with Cy5-dUTP and mRNA from adjacent normal tissues with Cy3-dUTP respectively through reverse transcription. The mixed probes of each sample were then hybridized with 12 800 cDNA arrays (12 648 unique human cDNA sequences), and the fluorescent signals were scanned by ScanArray 3 000 scanner (General Scanning, Inc.). The values of Cy5-dUTP and Cy3-dUTP on each spot were analyzed and calculated by ImaGene 3.0 software (BioDiscovery, Inc.). Differentially expressed genes were screened according to the criterion that the absolute value of natural logarithm of the ratio of Cy5-dUTP to Cy3-dUTP was greater-than 0.69.

RESULTS: Among 6 samples investigated, 301 genes, which accounted for 2.38 % of genes on the microarry slides, exhibited differentially expression at least in 5. There were 166 over-expressed genes including 136 having been registered in Genebank, and 135 under-expressed genes including 79 in Genebank in cancerous tissues.

CONCLUSION: Microarray analysis may provide invaluable information on disease pathology, progression, resistance to treatment, and response to cellular microenvironments of pancreatic carcinoma and ultimately may lead to improving early diagnosis and discovering innovative therapeutic approaches for cancer.

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INTRODUCTION

The morbidity of pancreatic carcinoma has taken an upward trend all over the world. In occidental countries, the morbidity of pancreatic carcinoma has increased by 3 to 7 times in nearly thirty years, and pancreatic carcinoma has become one of the ten commonest malignant tumors. In China, the morbidity was 1.16/100 000 in Shanghai in 1963, and reached on 3.80/100 000 in 1974. Then, it took the 14th place of the morbidity of the malignant tumors, and jumped to fifth in 1984. The statistical results showed that it was 5.1/100 000, which was four times higher than that of twenty years before. In some medical centers, curative resections were given to minority of patients in early stage who were highly selected, and their five-year survival of these patients might even rise to 15 to 25 percent. But generally speaking, treatment of pancreatic cancer is still a serious challenge to us. The key problem to improve the current situation of treatment is to seek novel diagnostic markers, effective adjunctive therapy and mechanism of genesis and evolution of pancreatic cancer. Hence, more and more attention has been paid to the research on molecular pathology and related genes of pancreatic cancer.

Over the past decade, many studies involving pancreatic cancer have searched for cancer-causing gene. As a result, several cancer-related genes have been identified. DPC4, p53, and p16 are the three most frequently inactivated tumor suppressor genes. Other tumor suppressor genes that are altered in pancreatic cancer include BRCA2, ALK-5, MKK4, and STK11. Mutations of K-ras oncogene are commonly seen in pancreatic cancer, with it' s incidence as high as 90 %. Some other cancer-related genes, such as Her-2/neu, COX-2, VEGF have also been reported to be overexpressed in pancreatic cancer. Development and progression of pancreatic cancer is a very complicated process, so it is reasonable to predict that many other genes, as yet undiscovered, might be potential tumor markers or drug targets.

Microarray is the technique that a large number of cDNAs are arranged orderly on the carrier, such as glass chip or else in high density. Data are obtained by examining the signals of fluorescence, analyzed and compared by computer software. A large number of genes can be examined simutaneously, accurately, and effectively in one experiment. In this study, we have used a high-density cDNA microarray technique to assess the gene expression profile of pancreatic carcinoma versus adjacent normal tissue. Several genes, we identified, may be involved in pancreatic tumorigenesis as well as it' s potential clinical biomarkers that may be used to improve early diagnosis, and to constitute potential novel therapeutic targets.

MATERIALS AND METHODS

Materials

cDNA microarray slides used in this study were fabricated in United Gene Technique, Ltd. Briefly, each slide has 12 800 spots, containing 112 genes as negative control, such as ripe U2 RNA gene (8 spots), HCV coat protein gene (8 spots), spotting solution (96 spots); and 40 housekeeping genes as positive control. Each slide has 12 648 unique human cDNA sequences. Six samples of pancreatic carcinoma were obtained from patients undergoing pancreaticoduodenectomy in department of general surgery, Changhai Hospital, the Second Military Medical University. All cases were proved pathologically as carcinoma of pancreatic head. Normal tissues as control were taken from tissue adjacent to the cutting margin of the carcinoma and proved pathologically to be free from tumor invasion. Samples were snap-frozen in liquid nitrogen within 15 to 20 minutes after resection and then stored at -80 ℃.

Cy3-dUTP and Cy5-dUTP were purchased from Amersham Phamacia Biotech, Inc. and Oligotex mRNA Midi Kit from Quagen, Inc. ScanArray 3 000 scanner was manufactured by General Scanning, Inc. ImaGene3.0 software came from BioDiscovery, Inc.

Methods

Probe preparation Total RNA isolated from pancreas tissues and normal adjacent tissues by using modified single step extraction. Briefly, frozen tissues were crushed down and homogenized in solution D and 1 % mercaptoethanol. The supernatant was then extracted by phenol: chloroform (1:1) two times, and phenol: chloroform (5:1) and NaAc (PH=4.5) once. Afterward, the supernatant was precipitated in equal volume of isopropanol at -20 ℃ for 1.5 hours and was precipitated in LiCl for purification. Both kinds of mRNAs were purified using Oligotex mRNA Midi Kit. The fluorescent cDNA probes were prepared by reverse transcription and then purified, according to the protocol of Schena (Schena *et al*., 1995). The mRNA from normal tissue was labeled with Cy3- dUTP, and that from cancerous tissue with Cy5- dUTP. Then the two probes were mixed with equivalence, precipitated by ethanol, and resolved in 20 μl hybridization buffer (5×SSC + 0.4 % SDS, 50 % Formamide, 5×denhardt' s solution).

Hybridization Microarray slides were pre-hybridized in hybridization buffer, which contained 0.5 mg/ml denatured clupeine DNA, at 42 ℃ for 6 hours. After denatured at 95 ℃ for 5 minutes, the probes mixture were added on the pre-hybridized slides and sealed with cover glass. After hybridizing in HybChamber at 60 ℃ for 15 to 17 hours, the slides were subsequently washed in solutions of 2×SSC + 0.2 % SDS, 0.1×SSC+0.2 % SDS and 0.1×SSC respectively for 10 minutes, then dried at room temperature.

Scanning and analysis The slides were scanned by ScanArray 3 000 laser scanner at two wavelengths to obtain fluorescence intensities for both dyes (Cy3 and Cy5). The original value of each spot was normalized by the values of 40 housekeeping genes selected on the slides. The fluorescence intensities of Cy3 and Cy5 were analyzed and the ratios of Cy5 to Cy3 were calculated by ImaGene 3.0 software. The intensities of two fluorescent signals represented the quantities of two tagged probes. The ration of Cy5 to Cy3 of certain spot on the slides demonstrated that mRNA abundance of this gene expressed in cancerous tissues versus normal.

RESULTS

Verification of micrearray technique system

There were 12 800 spots on each slide, including 6 170 known genes and 6 478 unknown ones. In order to monitor entire process of microarray technique system, negative and positive control genes were set on the slides. They were ripe U2 RNA gene (8 spots), HCV coat protein gene (8 spots), spotting solution (96 spots), which served as negative control; and 40 housekeeping genes as positive control. In present study, the individual result of six hybridization showed that all of positive control signals are distinct, and all of negative control signals were very low. These prove the reliability of the data.

Table 1 Over-expressed genes in pancreatic carcinoma

Accession number	Gene name	Gene function	Ratio value
U06863	Alpha 1 (III) collagen	Extracellular metrix	33.89
X91148	Microsomal triglyceride transfer protein	Microsomal triglyceride transfer	21.89
AF017986	Secreted apoptosis related protein 1 (SARP1)	Repress apoptosis	20.32
Y00755	Extracellular matrix protein BM-40	Extracellular metrix	20.23
Z74616	Alpha2 (I) collagen	Extracellular metrix	12.52
D32039	Proteoglycan PG-M(V3)	Extracellular metrix	11.90
J03607	40-kDa keratin intermediate filament precursor	Extracellular metrix	11.62
AF144103	Novel chemokine family member with altered expression in human head and neck squamous cell carcinoma	Cytokine	9.93
AF141201	Ubiquitin fusion-degradation 1 protein (UFD1)	Protease	8.29
X02761	Fibronectin (FN)	Extracellular metrix	8.01
U06863	Follistatin-related protein precursor	Nucleoprotein	6.92
M14219	Chondroitin / dermatan sulfate proteoglycan (PG40) core protein	Extracellular metrix	6.65
M17783	Glia-derived nexin (GDN)	Protease inhibitor	6.49
U59877	Low-Mr GTP-binding protein (RAB31)	Signal transduction	5.97
AF000989	Thymosin beta 4 Y isoform (TB4Y)	Thymosin isoform	5.71
L20688	GDP-dissociation inhibitor protein (Ly-GDI)	Signal transduction	5.62
X57351	1-8D gene from interferon-inducible gene	Signal transduction	5.51
U18728	Lumican	Extracellular metrix	5.15
L02326	Lambda-like gene	Immunity correlation	4.77
M14144	Vimentin	Extracellular metrix	4.64
J05633	Integrin beta-5 subunit	Cell adhesion molecule	4.42
AF070523	JWA protein	-	4.32
M27749	Immunoglobulin-related 14.1 protein	Immunity correlation	4.28
S83308	Sry-related HMG box gene (SOX5)	Transcription factor	4.16
U05875	Interferon gamma receptor accessory factor-1 (AF-1)	Immunity correlation	3.92
M17733	Thymosin beta-4	Thymosin isoform	3.89
M20259	Thymosin beta-10	Thymosin isoform	3.75
M36501	Alpha-2-macroglobulin	Protease inhibitor	3.72
AF026977	Microsomal glutathione S-transferase 3 (MGST3)	Oxidation-reduction	3.62
M14630	Prothymosin-α	Nucleoprotein	3.45

Table 2 Under-expressed genes in pancreatic carcinoma

Accession number	Gene name	Gene function	Ratio value
U96628	Nuclear antigen H731-like protein	Tumor suppressor	0.16
U72649	B-cell translocation gene 2 (BTG2)	Tumor suppressor	0.18
Y15409	Putative glucose 6-phosphate translocase	Gluconeogenesis	0.18
Z21507	Elongation factor-1-delta	Translation factor	0.20
L13463	Helix-loop-helix basic phosphoprotein (G0S8)	Cell cycle correlation	0.21
S68805	L-arginine:glycine amidinotransferase	Amino transferase	0.22
AF041474	BAF53a (BAF53a)	Chromatin reformation	0.22
X05130	Prolyl 4-hydoxylase beta subunit	Hydroxylase	0.22
AF067855	Geminin	Cell cycle correlation	0.25
D87810	Phosphomannomutase	Glycometabolism	0.25
D28540	CDC10 homologue	Cell cycle correlation	0.27
L37368	RNA-binding protein	RNA metabolism	0.27
Y00711	Lactate dehydrogenase B (LDH-B)	Dehydrogenase	0.27
M24103	Trans-golgi network glycoprotein 51 (TGN)	-	0.29
M64930	Protein phosphatase 2A beta subunit	Signal transduction	0.29
X81197	Archain	Membranin	0.31
U75686	Polyadenylate binding protein	Signal transduction	0.32
AF133659	ATP-binding cassette 7 iron transporter (ABC7)	Iron transfer	0.32
M61832	S-adenosylhomocysteine hydrolase (AHCY)	Hydrolase	0.33
Z23064	HnRNP G protein	RNA-binding protein	0.34
X85960	TRK-T3 oncogene	Oncogene	0.35
L18887	Calnexin	Calcium binding protein	0.35
J02966	Mitochondrial ADP / ADT translocator	ATP / ADP transport	0.35
X78678	Ketohexokinase	Glycometabolism	0.35
M58460	75-kD autoantigen (PM-Sc1)	Autoantigen	0.35
L12711	Transketolase (tk)	Ribose metabolism	0.35
S75311	Glycosyl phosphatidylinositol (GPI)-linked glycoprotein CD24	Signal transduction	0.35
U46838	MCM105 protein	Cell cycle correlation	0.36
Y11312	Phosphoinositide 3-kinase	Signal transduction	0.36
U83908	PDCD4 (programmed cell death 4)	Tumor suppressor	0.36

Judgement of differentially expressed genes

Hybridization of each couple of probes repeated two times. The standard of determination for differentially expressed genes was that, the absolute value of natural logarithm of the ratio of Cy5 to Cy3 was greater than 0.69, that was to say change of gene expression was above 2 times, and the signal value of either Cy3 or Cy5 needed to be greater than 600.

Genes expressed differentially

Among 6 samples investigated, 301 genes, which accounted for 2.38 % of genes on the microarry slides, exhibited differentially expression at least in 5. There were 166 over-expressed genes including 136 having been registered in Genebank, and 135 under-expressed genes including 79 in Genebank. Some of these genes, which took superior places of differentially expression, were listed in Table 1 and Table 2.

DISCUSSION

There are altogether 215 previously reported genes differently expressed in cancer tissues in our research, including urokinase plasminogen activator surface receptor (uPAR)[1], glyceraldehyde-3-phosphate dehydrogenase (GAPDH), lumican[2], phospholipase A2[3], vascular cell adhesion molecule 1[4], which have been reported to play certain role in evolution of pancreatic carcinoma. However, the relationship between the majority of these genes and development of pancreatic carcinoma has not been covered in any study up to the present. These genes are involved in various cytobiological functions, such as signal transduction, transcription and translation, cytoskeleton, cell adhesion, extracellular matrix and matrix degradation, cell cycle and apoptosis, chromosome instability, tumor suppressor genes, enzyme, and "others". Moreover, some genes exhibit differently expression in pancreatic cancer in our study, as well as in other cancers. They are fibronectin[5], caltractin[6], glyceraldehyde-3-phosphate dehydrogenase (GAPDH)[7], lipocortin II, uPAR[8] in hepatic cancer; FN[9], glutathione peroxidase[10], phospholipase A2[11], thymosin beta-10[12], uPAR[13], uracil DNA glycosylase[14] in colorectal cancer; N-cadherin[15], uPAR[16], alpha-2-macroglobulin[17], caltractin[6], syntenin[18] in gastric cancer; phospholipase A2[19] in cholangiocarcinoma; fibronectin[20], glutathione peroxidase[21,22], prothymosin alpha[23,24], thymosin beta-10[25], uPAR[26], caltractin[6], GAPDH[27], proteoglycan PG-M(V3), syntenin[18], lumican[28] in mammary cancer and glutathione peroxidase[29], SPARC/osteonectin[29], thymosin beta-10[30], uPAR[31] in thyroid carcinoma. These data indicated that we have obtained the same results by using cDNA microarray as by other methods. Meanwhile, these confirmed the feasibility, accuracy and effectiveness of microarray as a method to investigate the expression profiles of pancreatic cancer. On the other hand, we might get the conclusion that genesis and progression of various neoplasms have some common mechanisms. Further study on these common mechanisms might lead us go into depth the knowledge of molecular biology of cancer, and find the key to the improvement of diagnosis and treatment of cancer.

Pancreatic carcinoma is one of the most malignant tumors, and is characterized by aggressive growth behavior and high incidence rate of recurrence. During proliferation of a primary tumor or the establishment of metastatic foci, there is

continuous remodeling of the extracellular matrix including various degrees of biosynthesis, reformation and degradation. Among over-expressed genes, several genes, such as alpha1 (III) collagen, extracellular matrix protein BM-40, alpha2 (I) collagen, proteoglycan PG-M (V3), fibronectin, chondroitin/ dermatan sulfate proteoglycan (PG40) core protein, lumican, vimentin, chondroitin sulfate proteoglycan versican V0 splice-variant, and versican V2 splice-variant core protein, component analysis related to extracellular metrix (ECM), take the superior places. Versican belongs to the family of large aggregating proteoglycans (PGs). In mammals, versican appears as four possible spliced variants, V_0, V_1, V_2, and V_3. It has been described that the versican-rich extracellular matrices exert an anti-adhesive effect on the cells, thus facilitating tumor cell migration and invasion. Besides decreasing cell adhesion, versican is also able to increase the cell proliferation. The study on melanoma has shown that this PG could serve as a good marker for primary malignant as well as metastatic lesion[32]. Lumincan is the member of the small leucine-rich proteoglycan (SLRP) family, whose members are known as keratocan, mimecan, decorin, biglycan, fibromodulin, epiphycan, osteoadherin, and lumican. SLRP proteins can modulate cellular behaviour, including cell migration and proliferation during embryonic development, tissue repair, and tumor growth. In breast cancer tissues, lumican mRNA is reported to be overexpressed. Furthermore, its high expression level was associated with high tumor grade, low estrogen receptor levels, and young age of patients[28]. It is also found that lumican is not synthesized by the exocrine components of the normal pancreas, but that these cells ectopically synthesize and secrete the lumican in cancer tissues, which may play a role in pancreatic cancer cell growth[2]. Extracellular matrix protein BM-40, an anti-adhesive protein, is proposed to modulate cell migration and vascular morphogenesis either by directly interacting with ECM proteins or by initiating a receptor-mediated signaling event. It may directly affect cell motility by inducing intracellular changes of cytoplasmic components; or indirectly promote cell migration by modulating the expression of proteolytic enzymes that degrade the ECM, such as collagenase, stromelysin and MMP-9. It may promote infiltration of tumor cells, serve as a cellular marker of invasion, and correlate to angiogenesis[33]. Fibronectin connects with the cancer cell via its receptors, including intergrins $\alpha_5\beta_1$ and $\alpha v\beta_3$. The abilities in adhesion to fibronectin and migration increased markedly, after hepatocellular carcinoma cells were transfected with H-ras oncogene[34]. In addition, fibronectin is the primary protein involved in the displacement of MMP-2 produced by adjacent normal cells to cancer tissues[35]. MMP-2 can associate with the cell surface through its COOH-terminal and hemopexin-like domain via a number of mechanisms, including binding to cell-associated collagen I and IV. In our present study, we also find that collagen I and IV overexpress in cancerous tissues. This may enhance the fibronectin-induced displacement of MMP-2, and facilitates invasion of cancerous cells. Vimentin is also a component of ECM. Enhanced expression of the vimentin is associated with high degree of motility, poor differentiation and metastasis of prostate carcinoma[36]. It has been shown that vimentin was immunohistochemically positive in basaloid squamous carcinoma of esophagus[37]. Moreover, uPAR and cathepsin O2 are also found to be over-expressed in our study. All of these changes endow pancreatic cancer with the trait of aggressive growth, and may be potential markers of invasion.

On the other hand, recurrence to chemotherapy after resection remains a major obstacle to the cure of pancreatic cancer. It is well known that cancerous cells are surrounded by an extensive stroma of ECM at both primary and metastatic sites, which contains, among other proteins, fibronectin, laminin and collgen IV. Adhesion of pancreatic cancer cells to these proteins confers resistance to apoptosis induced by standard chemotherapeutic agents. The study on small-cell lung cancer showed that β1-integrin-mediated cell adhesion to ECM proteins results in tyrosine phosphorylation, which, weaken persistent chemotherapy-induced DNA damage, prevents caspase activation and apoptosis[38]. Alpha-tubulin[39], lipocortin II and uracil DNA glycosylase[14] were also found to be contributed to resistance to chemotherapy. These genes over-express in pancreatic cancer in our study, and may play the same role. It is indicated that the regulation routes of these genes may be potential targets for treatment of pancreatic carcinoma.

There are still some genes that should be mentioned. Prothymosin alpha (PTA) is a nuclear protein that is present throughout the cell cycle. It has been shown that it binds histones in vitro and has been proposed to affect the chromatin state. PTA is expressed in various human tumor tissues of different origins, supporting the idea that PTA expression is required for tumor growth. Recent study has shown that in breast cancer, whose tumor with low or moderate PTA level demonstrated a statistically significant decreased rate of tumor recurrence and a statistically significant increased overall survival in comparison with those whose tumor had high PTA levels. It is proposed that PTA could be used as a predictor of the potential malignancy of breast tumors that might help to identify patients at high risk of fatality[23,24]. Fas-binding protein and secreted apoptosis related protein-1 (SARP-1) are proposed to repress apoptosis. SARP-1 may inhibit phosphorylation of liberated β-catenin and degradation by ubiquitin-protease system via Wnt signaling way. Subsequently, β-catenin accumulates in cytoplasm, and cell apoptosis is suppressed. SARP-1 thus promotes excessive proliferation and transformation. Fas-binding protein can arrest Fas-induced apoptosis. Thymosin beta-4 and Thymosin beta-10 are also up-regulated. These genes may serve as markers for measuring proliferation of pancreatic cancer cells.

There are two tumor suppressors, B-cell translocation gene 2 (BTG2) and programmed cell death 4 (PCDC4), which are down-regulated in cancer tissues. PDCD4 gene is homologous to the mouse gene (MA-3/Pdcd4/A7-1), which is associated with apoptosis and is shown to suppress tumor promoter-induced neoplastic transformation. The ORF of human PDCD4 encodes a protein of 458 aa with a predicted molecular size of 50.6 kDa. It has been demonstrated that PDCD4 protein inhibits neoplastic transformation and must be down-regulated for progression to occur[40]. BTG2, which is induced by p53, displays an antiproliferative activity in different cell types, such as fibroblasts and PC12 cells. It is well known that the control of the cell cycle plays an essential role in cell growth and in the activation of important cellular processes, such as differentiation and apoptosis. pRb and p53 are two molecules identified as key regulators of the cell cycle. Some suggestions came from a recent report, which showed that BTG2 interacted with a protein-arginine N-methyltransferase (Prmt1) by positively modulating its activity. Prmt1, in turn, has been found to bind the interferon receptors and to be required for interferon-mediated growth inhibition. A further investigation on BTG2 demonstrated that this gene inhibited G_1/S progression in an Rb-dependent manner and that this effect was correlated with its ability to inhibit cyclin D_1 expression. Furthermore, the impairment in the ability of BTG2 to lower cyclin D_1 levels, seen in consequence of mutations of the BTG2 molecule, correlated with the extent of impairment in growth arrest[41].

DNA replication is initiated at discrete sites on chromosomes through the coordinate action of a number of replication initiation factors. It is believed that a complex of proteins called

the origin recognition complex (ORC) associates with specific DNA sequences near origins of replication to recruit other replication initiation factors during the G_1 phase of the cell cycle. The other replication initiation factors, Cdc6p, Cdt1p, and the Mcm2-7p complex, associate with the origin sequence in an ORC-dependent reaction to form a pre-replicative complex (pre-RC). At the G_1-S transition, the activation of cyclin-dependent kinases leads to the recruitment of elongation factors, CDC45, DNA polymerases, and RPA to the pre-RCs at origins. The action of these replication elongation proteins leads to the initiation of DNA synthesis, the hallmark of S phase. Geminin interacts with Cdt1p and prevents the recruitment of the Mcm2-7p complex to origins during S, G_2, and early M phases of the cell cycle and thereby inhibits replication initiation, leading to the expectation that the protein acts as an inhibitor of cell proliferation[42]. The research on these under-expressed genes may render invaluable information to find novel targets for the treatment of pancreatic cancer.

In summary, though some genes have been missed in present study, the genes we have identified may be important to the tumorigenesis of pancreatic cancer, and potential to serve as tumor markers or drug targets.

REFERENCES

1 **Han H**, Bearss DJ, Browne LW, Calaluce R, Nagle RB, Von Hoff DD. Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. *Cancer Res* 2002; **62**: 2890-2896

2 **Ping Lu Y**, Ishiwata T, Asano G. Lumican expression in alpha cells of islets in pancreas and pancreatic cancer cells. *J Pathol* 2002; **196**: 324-330

3 **Kashiwagi M**, Friess H, Uhl W, Berberat P, Abou-Shady M, Martignoni M, Anghelacopoulos SE, Zimmermann A, Buchler MW. Group II and IV phospholipase A(2) are produced in human pancreatic cancer cells and influence prognosis. *Gut* 1999; **45**: 605-612

4 **Tempia-Caliera AA**, Horvath LZ, Zimmermann A, Tihanyi TT, Korc M, Friess H, Buchler MW. Adhesion molecules in human pancreatic cancer. *J Surg Oncol* 2002; **79**: 93-100

5 **Nejjari M**, Hafdi Z, Gouysse G, Fiorentino M, Beatrix O, Dumortier J, PourreyronC, Barozzi C, D'errico A, Grigioni WF, Scoazec JY. Expression, regulation, and function of alpha V integrins in hepatocellular carcinoma: an *in vivo* and *in vitro* study. *Hepatology* 2002; **36**: 418-426

6 **Tian DY**, Hayashi M, Yoshida T, Sakakura T, Kawarada Y, Tanaka T. Overexpression of caltractin gene in tumor-infiltrating lymphocytes. *Int J Oncol* 1998; **13**: 1135-1140

7 **Yamagata M**, Mori M, Begum NA, Shibuta K, Shimoda K, Barnard GF. Glyceraldehyde-3-phosphate dehydrogenase mRNA expression in hepatocellular carcinoma. *Int J Oncol* 1998; **12**: 677-683

8 **Zhou L**, Hayashi Y, Itoh T, Wang W, Rui J, Itoh H. Expression of urokinase-type plasminogen activator, urokinase-type plasminogen activator receptor, and plasminogen activator inhibitor-1 and -2 inhepatocellular carcinoma. *Pathol Int* 2000; **50**: 392-397

9 **Lopez-Conejo MT**, Olmo N, Turnay J, Lopez De Silanes I, Lizarbe MA. Interaction of fibronectin with human colon adenocarcinoma cells: effect on the *in vivo* tumorigenic capacity. *Oncology* 2002; **62**: 371-380

10 **Skrzydlewska E**, Stankiewicz A, Sulkowska M, Sulkowski S, Kasacka I. Antioxidant status and lipid peroxidation in colorectal cancer. *J Toxicol Environ Health A* 2001; **64**: 213-222

11 **Osterstrom A**, Dimberg J, Fransen K, Soderkvist P. Expression of cytosolic and group X secretory phospholipase A (2) genes in human colorectal adenocarcinomas. *Cancer Lett* 2002; **182**: 175-182

12 **Nimmrich I**, Erdmann S, Melchers U, Finke U, Hentsch S, Moyer MP, Hoffmann I, Muller O. Seven genes that are differentially transcribed in colorectal tumor cell lines. *Cancer Lett* 2000; **160**: 37-43

13 **Baker EA**, Bergin FG, Leaper DJ. Plasminogen activator system, vascular endothelial growth factor, and colorectalcancer progression. *Mol Pathol* 2000; **53**: 307-312

14 **Dusseau C**, Murray GI, Keenan RA, O'Kelly T, Krokan HE, McLeod HL. Analysis of uracil DNA glycosylase in human colorectal cancer. *Int J Oncol* 2001; **18**: 393-399

15 **Yanagimoto K**, Sato Y, Shimoyama Y, Tsuchiya B, Kuwao S, Kameya T. Co-expression of N-cadherin and alpha-fetoprotein in stomach cancer. *Pathol Int* 2001; **51**: 612-618

16 **Choi YK**, Yoon BI, Kook YH, Won YS, Kim JH, Lee CH, Hyun BH, Oh GT, Sipley J, KimDY. Overexpression of urokinase-type plasminogen activator in human gastric cancercell line (AGS) induces tumorigenicity in severe combined immunodeficient mice. *Jpn J Cancer Res* 2002; **93**: 151-156

17 **Allgayer H**, Babic R, Grutzner KU, Beyer BC, Tarabichi A, Schildberg FW, HeissMM. Tumor-associated proteases and inhibitors in gastric cancer: analysis of prognostic impact and individual risk protease patterns. *Clin Exp Metastasis* 1998; **16**: 62-73

18 **Koo TH**, Lee JJ, Kim EM, Kim KW, Kim HD, Lee JH. Syntenin is overexpressed and promotes cell migration in metastatic human breastand gastric cancer cell lines. *Oncogene* 2002; **21**: 4080-4088

19 **Wu T**, Han C, Lunz JG 3rd, Michalopoulos G, Shelhamer JH, Demetris AJ. Involvement of 85-kd cytosolic phospholipase A (2) and cyclooxygenase-2 in theproliferation of human cholangiocarcinoma cells. *Hepatology* 2002; **36**: 363-373

20 **Jiang Y**, Harlocker SL, Molesh DA, Dillon DC, Stolk JA, Houghton RL, Repasky EA, Badaro R, Reed SG, Xu J. Discovery of differentially expressed genes in human breast cancer using subtracted cDNA libraries and cDNA microarrays. *Oncogene* 2002; **21**: 2270-2282

21 **Perquin M**, Oster T, Maul A, Froment N, Untereiner M, Bagrel D. The glutathione-related detoxification system is increased in human breastcancer in correlation with clinical and histopathological features. *J Cancer Res Clin Oncol* 2001; **127**: 368-374

22 **Gorodzanskaya EG**, Larionova VB, Zubrikhina GN, Kormosh NG, Davydova TV, Laktionov KP. Role of glutathione-dependent peroxidase in regulation of lipoperoxide utilization in malignant tumors. *Biochemistry* 2001; **66**: 221-224

23 **Bianco NR**, Montano MM. Regulation of prothymosin alpha by estrogen receptor alpha: molecular mechanismsand relevance in estrogen-mediated breast cell growth. *Oncogene* 2002; **21**: 5233-5244

24 **Magdalena C**, Dominguez F, Loidi L, Puente JL. Tumour prothymosin alpha content, a potential prognostic marker for primarybreast cancer. *Br J Cancer* 2000; **82**: 584-590

25 **Santelli G**, Califano D, Chiappetta G, Maria Teresa Vento, Bartoli PC, Zullo F, Trapasso F, Viglietto G, Fusco A. Thymosin beta-10 gene overexpression is a general event in human carcinogenesis. *Am J Pathol* 1999; **155**: 799-804

26 **Gong SJ**, Rha SY, Chung HC, Yoo NC, Roh JK, Yang WI, Lee KS, Min JS, Kim BS, Chung HC. Tissue urokinase-type plasminogen activator receptor levels in breast cancer. *Int J Mol Med* 2000; **6**: 301-305

27 **Revillion F**, Pawlowski V, Hornez L, Peyrat JP. Glyceraldehyde-3-phosphate dehydrogenase gene expression in human breast cancer. *Eur J Cancer* 2000; **36**: 1038-1042

28 **Leygue E**, Snell L, Dotzlaw H, Troup S, Hiller-Hitchcock T, Murphy LC, Roughley PJ, Watson PH. Lumican and decorin are differentially expressed in human breast carcinoma. *J Pathol* 2000; **192**: 313-320

29 **Takano T**, Hasegawa Y, Matsuzuka F, Miyauchi A, Yoshida H, Higashiyama T, Kuma K, Amino N. Gene expression profiles in thyroid carcinomas. *Br J Cancer* 2000; **83**: 1495-1502

30 **Takano T**, Hasegawa Y, Miyauchi A, Matsuzuka F, Yoshida H, Kuma K, Amino N. Quantitative analysis of thymosin Beta-10 messenger RNA in thyroid carcinomas. *Jpn J Clin Oncol* 2002; **32**: 229-232

31 **Kim SJ**, Shiba E, Taguchi T, Tsukamoto F, Miyoshi Y, Tanji Y, Takai S, Noguchi S. uPA receptor expression in benign and malignant thyroid tumors. *Anticancer Res* 2002; **22**: 387-393

32 **Touab M**, Villena J, Barranco C, Arumi-Uria M, Bassols A. Versican is differentially expressed in human melanoma and may play a role intumor development. *Am J Pathol* 2002; **160**: 549-557

33 **Vajkoczy P**, Menger MD, Goldbrunner R, Ge S, Fong TAT, Vollmar B, Schilling L, Ullrich A, Hirth KP, Tonn JC, Schmiedek P, Rempel SA. Targeting angiogenesis inhibits tumor infiltration

and expression of the pro-invasive protein SPARC. *Int J Cancer* 2000; **87**: 261-268

34 **Wang Q**, Lin ZY, Feng XL. Alterations in metastatic properties of hepatocellular carcinoma cell following H-ras oncogene transfection. *World J Gastroenterol* 2001; **7**: 335-339

35 **Saad S**, Gottlieb DJ, Bradstock KF, Overall CM, Bendall LJ. Cancer cell-associated fibronectin induces release of matrix metalloproteinase-2from normal fibroblasts. *Cancer Res* 2002; **62**: 283-289

36 **Lang SH**, Hyde C, Reid IN, Hitchcock IS, Hart CA, Bryden AA, Villette JM, Stower MJ, Maitland NJ. Enhanced expression of vimentin in motile prostate cell lines and in poorly differentiated and metastatic prostate carcinoma. *Prostate* 2002; **52**: 253-263

37 **Zhang XH**, Sun GQ, Zhou XJ, Guo HF, Zhang TH. Basaloid squamous carcinoma of esophagus: a clinicopathological, immunohistochemical and electron microscopic study of sixteen cases. *World J Gastroenterol* 1998; **4**: 397-403

38 **Rintoul RC**, Sethi T. Extracellular matrix regulation of drug resistance in small-cell lung cancer. *Clin Sci* 2002; **102**: 417-424

39 **Kyu-Ho Han E**, Gehrke L, Tahir SK, Credo RB, Cherian SP, Sham H, Rosenberg SH, Ng S. Modulation of drug resistance by alpha-tubulin in paclitaxel-resistant humanlung cancer cell lines. *Eur J Cancer* 2000; **36**: 1565-1571

40 **Cmarik JL**, Min H, Hegamyer G, Zhan S, Kulesz-Martin M, Yoshinaga H, Matsuhashi S, Colburn NH. Differentially expressed protein Pdcd4 inhibits tumor promoter-induced neoplastic transformation. *Proc Natl Acad Sci U S A* 1999; **96**: 14037-14042

41 **Guardavaccaro D**, Corrente G, Covone F, Micheli L, Dagnano I, Starace G, Caruso M, Tirone F. Arrest of G_1 S progression by the p53-inducible gene PC3 is Rb dependent andrelies on the inhibition of cyclin D1 transcription. *Mol Cell Biol* 2000; **20**: 1797-1815

42 **Wohlschlegel JA**, Dwyer BT, Dhar SK, Cvetic C, Walter JC, Dutta A. Inhibition of eukaryotic DNA replication by geminin binding to Cdt1. *Science* 2000; **290**: 2309-2312

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