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Meeting Report

Recent Progress in Cancer Therapeutics

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From November 11 to 13, 2008, the Chulabhorn Research Institute welcomed approximately 450 scientists working in experimental and clinical cancer research at the Chulabhorn Convention Center, Bangkok, Thailand. Prof. Dr. HRH Princess Chulabhorn, Conference and Scientific Chairperson, together with the cochairpersons Dr. Enrico Mihich (USA) and Dr. Kurt S. Zänker (Germany) and with the Secretary General, Dr. Khunying Mathuros Ruchirawat, designed a scientific program at the cutting edge of "Recent Progress in Cancer Therapeutics." The meeting was under the joint sponsorship of the American Association for Cancer Research (USA), the Chulabhorn Research Institute (Thailand), the Fritz-Bender-Foundation (Germany), and Mrs. Sybille Bartels-Hetzler (KPMG Hamburg, Germany).

A Satellite Symposium on "Trends in Innate Immunity: the Complement Cascade Proteins in Transplantation, Cancer and Hereditary Angioedema" sponsored by the Network Complement Related Diseases (NCRD, Luzern, Switzerland) preceded the main meeting.

Kurt S. Zänker (Witten, Germany) gave a short introduction into innate immunity, which increasingly gains renewed interest, particularly because it became apparent that it is an evolutionary ancient defense system. The phylogenetically ancient innate immune response attacks infectious DNA/RNA carriers from the moment of first contact and is the fundamental defensive weapon of unicellular and multicellular organisms. Already the arthropods have a cellular and humoral-based immune competence, which is mediated by hemocytes, which resemble in development and function the cells of the mammalian myeloid lineage. In mammalians, a broad variety of different cell types exists that support the innate immune response. Neutrophils, macrophages, dendritic cells, natural killer cells, and eosinophilic granulocytes are important cellular components of the innate immune response. The short- and long-distance communication between these cells and the regulatory links to adaptive immunity take place by messenger molecules, such as cytokines, chemokines, neurotransmitters, and hormones.

Recommended literature: *Trends in Innate Immunity* (2008). A Egesten, A Schmidt, and H Herwald (Eds.). *Contrib Microbiol*. Karger, Basel, Switzerland. vol 15.

Gilles Blancho (Nantes, France) spoke about the important role of the innate immunity, with special reference to the complement system in transplantation. Organ transplantation has become a major therapeutic strategy to overcome organ failure. The alloimmune response was considered for a long time as the almost unique *in vivo* phenomenon to control acceptance and function of the graft; however, it turned out that organ preservation, cold and warm ischemia, and the activation of danger signals initiating a fast innate immune response, including complement response, which concomitantly might start the adaptive immune response, are important parameters that determine graft survival or rejection.

Recommended literature: LeBas-Bernadet St and Blancho G (2008). Current cellular immunological hurdles in pig-to-primate xenotransplantation. *Transpl Immunol*. Oct 24 (Epub ahead of print).

Marco Cicardi (Milan, Italy) spoke about the acquired deficiency of the C1 inhibitor. Angioedema due to the acquired deficiency of the first component of human complement (C1-INH) is a rare syndrome usually identified as acquired angioedema (AAE). The clinical features are subcutaneous, nonpruritic swelling without urticaria, involvement of the upper respiratory tract, and partial obstruction of the gastrointestinal tract causing abdominal pain. During the past 30 years, the group observed 34 patients with AAE for a median follow-up period of 8 years. Ten of the 34 patients with AAE had no apparent hematological disease at diagnosis or during follow-up. Eight of them had anti–C1-INH autoantibodies and two had autoantibodies and a nonhematologic malignancy. However, 11 of these patients presented non–Hodgkin lymphoma. The clinical coexistence of AAE and B-cell malignancy or nonmalignant B-cell proliferation and pathogenic autoimmune responses suggest that the etiopathogenesis of AAE is dominated by an impaired control of B-cell proliferation; therefore, patients with AAE should be closely monitored for lymphoproliferative diseases.

Recommended literature: Cicardi M, Zingale LC, Pappalardo E, Folcioni A, and Agostoni A (2003). Autoantibodies and lymphoproliferative diseases in acquired C1-inhibitor deficiencies. *Medicine (Baltimore)* **82**, 274–281.

A general discussion, led by **M. Schata** (Cologne, Germany), on the functional role of complement in oncology closed the symposium. It was discussed that pattern recognition receptors of innate immune cells detect changes in glycosylation, which are concurrent with tumor development and signals for complement activation and tumor cell lysis. However, to avoid complement-mediated tumor cell lysis and elimination, many tumor types overexpress membrane-bound complement inhibitors (CD46, CD55, and CD59) as well as factor H. Moreover, some tumor types secrete matrix metalloproteinases, which can cleave

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C3b and liberate the deposited C3 fragment from tumor cells, thereby preventing the efficient recognition by phagocytes and promoting metastasis formation.

The main symposium started with the opening keynote lecture delivered by **Prof. Dr. HRH Princess Chulabhorn Mahidol**. The focus of HRH Princess Chulabhorn Mahidol's lecture was on genetic alterations in nasopharyngeal carcinoma (NPC) in the Thai population. Her Royal Highness also emphasized the influence of living environment on the risk of cancer development with an example of school children, living in Bangkok, who have high levels of DNA adducts and impaired DNA repair mechanisms, thus having a higher risk in developing cancer compared with children living in rural regions. In Southeast Asia, esophageal cancer, liver cancer, and, above all, NPC, are prevalent. Nasopharyngeal carcinoma has a marked geographical and ethnic distribution; it is rare among whites in Western Europe and North America. The disease is predominantly endemic in Southeast Asia and southern China (30-50 cases/100,000/year). Nasopharyngeal carcinoma is a multifactorial disease, and Epstein-Barr virus infection, environmental factors, and genetic susceptibility are widely recognized as risk factors. Genetic alterations in p53, exon 8, codon 273 have been found in 33% of NPC patients, and therefore, detection of this p53 polymorphism may be a useful tool for screening of early NPC. A molecular and clinical study is ongoing in Chiang Mai and Bangkok to support this hypothesis.

Stephen Baylin (Baltimore, MD, USA) presented epigenetic modifications as a major regulator of eukaryotic gene expression and aberrant epigenetic silencing of gene expression, which contributes to tumorigenesis. Both regional DNA methylation and global chromatin packaging are interrelated partners that function in concert to control gene transcription. In colon cancer tumor specimens, they found more than 300 genes hypermethylated and around 30 genes of the functional extracellular matrix hypermethylated at the same time; hypermethylation means silencing of genes and has concomitantly a dramatic influence on the performance and disease outcome of the patients. *Hic-1* gene codes for a transcription factor, regulated by p53, and was found to be hypermethylated but not mutated. Aging, chronic injury and inflammation, and cell stress give rise to malignant clonal expansion, when so-called "error zones of genes" are hypermethylated. The visionary therapy options are as follows: 1) reversal of gene silencing, 2) reexpression of key genes, and 3) correction of key pathways. Drugs that kill tumor cells cause likely regrowth of tumors, whereas drugs killing tumor stem cells will likely eradicate the tumor.

Recommended literature: Baylin SD (2008). Epigenetics and cancer. In *Molecular Basis of Cancer*, 3rd ed. G Mendelsohn (Ed.). Saunders Elsevier, Philadelphia, PA. pp. 57–65.

David W. Goodrich (Buffalo, NY, USA) talked about the role of Thoc1 in prostate cancer. A problem in gene expression is that the transcription initiation is imperfect; however, regulation of RNA processing events through ribonuclear particles (mRNPs)–driven mechanism may be important for coordinating the expression of functionally related genes. His group investigates the potential role of regulated mRNP biogenesis linked to carcinogenesis. They characterized the effects of Thoc1 loss on a mouse model of prostate cancer. Thoc1 encodes an essential component of the evolutionary conserved TREX complex, proteins accompanying the elongating RNA polymerase II during transcription of some genes, and facilitate mRNP assembly and the recruitment of RNA processing factors. It seems Thoc1 is required for prostate tumorigenesis initiated by Rb1 and p53 mutation. Preliminary data suggest that Thoc1 may also be important in human prostate cancer. Because cancer cells seem to be particularly sensitive to Thoc1 loss, Thoc1 and the TREX complex may provide novel targets for cancer therapy.

Recommended literature: Li Y, Lin AW, Zhang X, Wang Y, Wang X, and Goodrich DW (2007). Cancer cells and normal cells differ in their requirement of Thoc1. *Cancer Res* **67**, 6657–6664.

Frank Thévenod (Witten/Germany) reported the role of the Wnt/β-catenin signaling pathway in renal cancer induced by the environmental contaminant cadmium. His group found out that exposure of kidney proximal tubule cells to cadmium induces the expression of the proto-oncogene *c-Myc*, the cell cycle regulator *cyclin D1*, and the multidrug-resistant P-glycoprotein (*Abcb1*) owing to the cadmium-dependent release of β-catenin from adherens junctions. β-Catenin serves as a coactivator of the lymphoid enhancer factor/T-cell factor (TCF) DNA-binding protein family. Using a luciferase reporter plasmid for lymphoid enhancer factor/TCF binding sites, his group found that cadmium increases luciferase activity in proliferating proximal tubule cells, whereas in quiescent confluent epithelia, cadmium reduces basal luciferase activity. This suggests that cadmium transactivates survival genes in proliferating cells only, which may be caused by the predominance of TCF-activating molecular forms of β-catenin. In contrast, in confluent cells, β-catenin–α-catenin dimers may be released from adherence junction complex to inactivate transcription. Using a luciferase reporter assay, Thévenod et al. also found that cadmium-induced release of β-catenin also activates PITX2, a bicoid-related homeodomain factor that regulates cell cycle control genes. Unexpectedly, PITX2 was found to induce *Abcb1* expression. Hence, cadmium triggers β-catenin–dependent transcriptional responses of TCF and PITX2 to upregulate proliferation and cell survival genes to promote carcinogenesis. Further characterization of both pathways may ultimately contribute to the development of strategies for cancer prevention and novel cancer therapeutics.

Recommended literature: Thévenod F, Wolff NA, Bork U, Lee WK, and Abouhamed M (2007). Cadmium induces nuclear translocation of *b*-catenin and increases expression of *c-myc* and *Abcb1a* in kidney proximal tubule cells. *Biometals* **20**, 807–820.

Peter A. Jones (Los Angeles, CA, USA) continued on the epigenetics of cancer addressing the future of epigenetic therapy. It becomes increasingly clear that the epigenetic silencing of growth-regulating genes by methylation of CpG islands located in the promoters of developmental genes represents a common pathway for their inactivation. DNA methylation patterns are somatically heritable over a lifetime. In principle, there are three pathways silencing tumor suppressor genes: 1) adopting a chromatin configuration that is associated with increased binding of methyl binding proteins (nucleosome remodeling), 2) increasing the methylation of lysine 9 residue of histone H3, and 3) increasing methylation of cytosine residues in human DNA. These pathways can be interfered and rapidly reversed by transient treatment with 5-aza-2′-deoxycytidine. However, gene expression is not permanent because methylation tends to reoccur in the promoter regions. More recently, the group has focused on the stable cytidine analog, zebularine, which is incorporated into DNA and forms covalent bond with DNA methyltransferase enzymes. The drug also results in a strong knockdown in the level of active DNA methyltransferase protein and showed reexpression of silenced tumor suppressor genes in human tumor xenografts growing in nude mice. For chronic treatment of human cancers, the clinical problems of dosages and time of application, the resilencing of activated suppressor genes, and the drug instability have to be solved.

Recommended literature: Jones PA and Baylin SB (2002). The fundamental role of epigenetic events in cancer. *Nat Rev Genet* **3**, 415–428.

Alain Sarasin (Villejuif, France) spoke about the overexpression of some DNA repair pathways associated with metastasis risk in melanoma patients. Using a collection of 83 frozen human primary cutaneous melanomas, his group determined their genome-wide gene expression profiles. Alain's group identified a signature of 254 genes allowing to predict with a high probability distant metastasis-free survival as well as overall survival at 4 years; most of the genes were correlated with thickness or the tumor. Using a newly developed bioinformatic tool to analyze the differential gene expression by looking at whole biological pathways, the following most significant pathways associated with progression to metastasis were found: 1) DNA replication and 2) DNA repair pathways. It was found that 46 genes of the DNA repair pathway were associated with metastatic progression and poor prognosis. Few genes were directly involved in nucleotide excision repair, base excision repair, and mismatch repair. This overexpression of repair genes explains nicely the extraordinary resistance of metastatic melanoma to chemotherapy and radiotherapy.

Recommended literature: Sarasin A and Kauffmann A (2008). Overexpression of DNA repair genes is associated with metastasis: a new hypothesis. *Mutat Res* **65**, 49–55.

Feyruz Rassool (Baltimore, MD, USA) talked about the inhibition of double-strand break (DSB) repair and alternative nonhomologous end-joining (NHEJ): potential therapeutic targets in chronic myeloid leukemia (CML) with resistance to imatinib. Her group found that genomic instability in myeloid malignancies may be driven by a combination of ongoing constitutive DNA damage coupled with increased frequencies of the error-prone NHEJ, which results in improper repair of DSBs. BCR-ABL fusion tyrosine kinase in CML induces high levels of radical oxygen species that generate DSBs. The CML cells repair DSBs by aberrant NHEJ that is characterized by large DNA deletions. Imatinib that targets BCR-ABL is the criterion standard of therapy in CML patients. However, it has become clear that patients become resistant to imatinib and other tyrosine kinase inhibitors, such as dasatinib and nilotinib. Therefore, there is an urgent need for alternative therapeutic targets for patients resistant to tyrosine inhibitors. The main proteins involved in alternative NHEJ, which include DNA ligase IIIα, x-ray repair cross-complementing gene 1 protein, DNA ligase I, poly (ADP-ribose) polymerase, and Werner-RecQ helicase have the potential to be novel therapeutic targets in CML patients that have acquired resistance to imatinib.

Recommended literature: Rassool FV, Gaymes TJ, Omidavar N, Brady N, Beurlet S, Pla M, Reboul M, Lea N, Chomenne C, Thomas NSB, et al. (2007). Reactive oxygen species: DNA damage and error-prone repair: a model for genomic instability with progression in myeloid leukemia. *Cancer Res* **67**, 8762–8771.

Robert A. Weinberg (Cambridge, MA, USA) delivered a keynote address on the mechanisms of malignant progression. He gave a magnificent overview on the mechanisms of malignant progression, extending the Vogelstein model of carcinogenesis by addressing four major questions with respect to metastasis formation: 1) is the development of metastases a selective or adaptive process? 2) what are rate limiting determinants of metastases?, 3) do cancer cells learn to colonize while in the primary tumor (cell-of-origin) or at the sites of dissemination?, and 4) does a partial or complete epithelial-mesenchymal-transition program underlie the invasive/metastatic phenotype of all high-grade human tumors? He spoke on cancer stem cells and, most importantly, on evidences of the redifferentiation of non–stem cells into cancer stem cells. This has the tremendous implication in cancer chemotherapy because both cell types, cancer stem cells and non–stem cells, have to be eradicated therapeutically to prevent tumor regrowth and metastasis formation. This concept is an answer to why cancer is so difficult to treat. The race to identify stem cells and cancer stem cells in all different kinds of tumors is now open. The findings and molecular discoveries of differentiation and redifferentiation processes of cancer stem cells and non–stem cells will undoubtedly revolutionize our understanding of how cancer develops.

Recommended literature: Weinberg RA (2007). *The Biology of Cancer*. Taylor & Francis, Garlan Science.

Li Yang (Nashville, TN, USA) addressed transforming growth factor β (TGF-β) as a regulator of the tumor microenvironment. Transforming growth factor β is known to work as both a tumor suppressor and a tumor promoter; therefore, it is most important to find out what factors mediate this switch in function and when this switch occurs. Loss of TGF-β signaling decreases tumor latency, enhances metastasis formation, and is associated with the recruitment of Gr-1/CD11b–positive cells to the tumor microenvironment. In early tumor development, TGF-β activates smad signaling, whereas in late tumor development, there is non-smad signaling predominant. Interestingly, the point of preseeding of myeloid cells (Gr-1/CD11b–positive) is at premetastasis lesions.

Recommended literature: Yang L and Moses HL (2008). Transforming growth factor β: tumor suppressor of promoter? Are host immune cells the answer? *Cancer Res* **68**, 9107–9111.

Frank Entschladen (Witten, Germany) reported on a metastasis-promoting function of the neurotransmitter norepinephrine, investigated in nude mice. His group provided evidence for a newly discovered interaction of the tumor with the nervous system in analogy to neoangiogenesis. Tumor cells release nerve growth and nerve guidance factors by which the tumor establishes its own innervation, a process termed *neo*-neurogenesis and, as overall related to cancer more specifically, cancer-associated neurogenesis. Norepinephrine, as one neurotransmitter, senses the expressed β_2-adrenoreceptors at the surface of the tumor cells, thereby initiating a signal cascade through adenyl cyclase and protein kinase A to activate the transcription factors cAMP-response element binding protein, activating transcription factor 1, and nuclear factor κ B, which promote proliferation and metastasis formation. The major strength of his presentation is the novelty combining experimental, translational, and epidemiological research and that the neuroparacrine microenvironment of a tumor opens a new gateway for therapeutic approaches.

Recommended literature: Zaenker KS and Entschladen F (Eds.) (2007). *Neuronal Activity in Tumor Tissue*. In *Progr Exp Tumor Res*. Bertino JR (Series Ed.). Karger, Basel, Switzerland. Vol. 39.

Niramol Savaraj (Miami, FL, USA) spoke about amino acid deprivation, autophagy, and apoptosis. The triple A's—as targets in cancer therapy. She stressed that within the classic biochemical pathways of glucose, amino acid, and fatty acid metabolisms, a few key enzymes and substrates can be targeted therapeutically because of the differences in activities between tumor and normal cells. Individuals with metastatic melanoma have a poor prognosis, and many human melanomas are auxotrophic for arginine, and arginine is not an essential amino acid in humans; this auxotrophy might be therapeutically exploited. A novel formulated amino acid–degrading enzyme, arginine deiminase (ADI), was used to lower plasma arginine in melanoma patients with advanced disease. Autophagy is a lysosomal-based pathway, which leads, when in excess, activated to cell death. From experimental data

with melanoma cells, it was evident that ADI can induce cell death through autophagy and apoptosis. Within phases 1 and 2 studies with ADI, 6 of 24 patients with advanced melanoma responded to treatment (partial remission, 5/25; complete remission, 1/24). Elimination of all detectable plasma arginine in patients with metastatic melanoma was well tolerated and may be effective in the therapy for metastatic melanoma.

Recommended literature: Savaraj N, You M, Wu C, Marini M, Kuo T, Wangpaichitr M, Bomalaski L, and Feun G (2008). Arginine deprivation for the treatment of advanced melanoma: Clinical results/correlation with argininosuccinate synthetase (ASS) expression. *J Clin Oncol* **26**, Abstract. 2019.

Alberto Mantovani (Milan, Italy) gave a talk on inflammation and cancer because there is broad spectrum of evidences on the interaction of inflammatory processes and cancer, e.g., 1) colitis may by associated with cancer by TIR8, 2) D6 acts as a gatekeeper for inflammatory CC chemokines, 3) increased damage of colon mucosa in D6−/− mice by chronic inflammation gives rise to increased numbers of tumors, 4) pancreatic tumor cells expressing the fractalkine/neurotactin CX3C receptor1, when engaged, are protected from apoptosis and are forced to migrate, and when expressed in pancreas carcinoma *in vivo*, the tumor shows neuronal invasion; CX3CR1 expression correlates with malignancy of tumors. He explained the pathways through nuclear factor κ B, Stat, and hypoxia-inducing factor of inflammatory cells, leading to cancer-related inflammation and chronic inflammatory microenvironment of cancer. For inflammatory cells, he considered macrophages as key orchestrators of chronic inflammation responding to genetic and functional programs. M1 macrophages, which are classically activated by microbial products and interferon, are potent effector cells that kill microorganisms and tumors. In contrast, M2 macrophages tune inflammation and adaptive immunity, promote cell proliferation by producing growth factors and products of the arginase pathway, and express scavenger receptors, promote angiogenesis and tissue remodeling and repair. M2 polarization of phagocytes represents the dark side of the immune competence in respect to proinflammatory human tumor programs.

Recommended literature: Mantovani A (2005). Cancer inflammation by remote control. *Nature* **435**, 752–753.

Soldano Ferrone (Pittsburgh, PA, USA) introduced a *Listeria*-based vaccine against high molecular weight–melanoma-associated antigen (HMW-MAA), also known as melanoma chondroitin sulfate proteoglycan. This antigen is expressed on the cell surface of melanoma cells, cancer stem cells, and tumor-activated pericytes and has a restricted distribution in normal tissues. A fragment of HMW-MAA (residues 2160-2258) was cloned in frame to the first 441 residues of the Listeriolysin O protein to be secreted by a recombinant *Listeria monocytogenes* (Im-LLO-HMW-MAA-C). Immunization with this vaccine was able to impede tumor growth of early established B16F10 HMW-MAA tumors in mice, and both CD4- and CD8-positive T cells were required for therapeutic efficacy. In conclusion, this novel immune therapeutic approach with a *Listeria*-based vaccine against HMW-MAA can trigger cell-mediated immune response to this antigen and can also target pericytes reducing these cells in numbers in the tumor vasculature, which are important for tumor angiogenesis.

Recommended literature: Maciag PC, Seavey MM, Pan ZK, Ferrone S, and Patterson Y (2008). Cancer immunotherapy targeting the high molecular weight melanoma-associated antigen protein results in an anti-tumor response and reduction of pericytes in the tumor vasculature. *Cancer Res* **68**, 8066–8075.

Michael Rossbach (Singapore, Singapore) introduced Dicer-deficient mouse embryonic stem cells that were rescued with the two Dicer proteins from *Drosophila melanogaster*, dcr-1 and dcr-2. This resulted in a structure and function analysis of Dicer with the potential of specifically rescuing either the miRNA or the siRNA pathway. Having embryonic stem cells on hand, that now either process miRNA or siRNA helps to study the involvement of individual small RNA in tumorigenesis. Three of the best-understood mRNA, namely, lin-4, let-7, and bantam, regulate cell division and differentiation. In cancer, numerous miRNA are altered, and in let-7 mutants, the cells fail to terminally differentiate and keep on dividing. In lung cancer cells, let-7 expression was found to be significantly lower than in normal lung tissue. The loss of the miRNA regulation of the *ras* oncogenes could lead to *ras* overexpression. In contrast, overexpression of let-7 results in a down-regulation in *ras* expression—a vision for new strategies in lung cancer therapy. For the development of new therapies to target cancer cells and cancer stem cells, which are chemoresistant, miRNA could push stem cells to become more differentiated and less tumorigenic through switching off particular genes.

Recommended literature: Rossbach M (2007). *Dissecting RNA Mediated Gene Silencing Pathways In Murine Embryonic Stem Cells* [dissertation]. University Witten/Herdecke, Germany.

Kurt S. Zänker (Witten, Germany) summarized the meeting and referred to the Opening Address by Prof. Dr. HRH Princess Chulabhorn Mahidol that "this meeting has been organized to inaugurate the Chulabhorn Cancer Center and to carry forward the spirit of cooperation and collaboration. It should serve to further encourage and enhance our endeavors in an area of vital importance to the health and well being of human mankind." These meeting days and the results were well embedded within this aforementioned scientific and social scope.