Cancer Chemother Pharmacol (2006) 58: 279–282
DOI 10.1007/s00280-005-0170-9

MEETING REPORT

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# Molecular targets of cancer chemotherapy

Received: 30 November 2005 / Accepted: 1 December 2005 / Published online: 24 December 2005


This International Symposium was held in Trieste, Italy from September 26 to 28, 2005 under the joint sponsorship of the International Union Against Cancer (UICC, Geneva), the Fritz-Bender-Foundation (Munich), the Associazone Italiana di Oncologia Medica (AIOM), Regione Friuli Venezia Giulia, University of Trieste, Ordine del Medici Chirurghi e Odontoiatri della provincia di Trieste and Azienda per i Servizi Sanitari Nr.1 triestina. The Symposium was organized by E. Mihich (Buffalo, USA), K.S. Zänker (Witten, Germany) and G. Mustacchi (Trieste, Italy).

The presentations concerned four areas of experimental and two areas of clinical cancer research: (1) genetic and epigenetic mechanisms of cancer cell regulation, (2) molecular nuclear controls, (3) signal transduction pathways, (4) cell environment interactions and (5) clinical strategies I and II.

## Session I: genetic and epigenetic mechanisms of cancer cell regulation

M. Rossi (Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY, USA) spoke about approaches for high-throughput detection of molecular events in cancer. Traditionally, position cloning approaches have been used to identify genes involved in tumor progression but this gene-by-gene approach has been time consuming and relatively unsuccessful. Whole genome screens are now being developed to speed up the process of tumor-related gene identification. Although transcripts from mutant genes may be generated, they are often rapidly degraded by the intracellular nonsense mediated decay (NMD) process which has evolved to eliminate mutated/non-allowed mRNA species and prevent their translation. The recent development of arrays of bacterial artificial chromosomes that can be used for comparative genome hybridization (aCGH) has greatly improved the resolution for defining small copy number abnormalities in cancer cells. Combining the data from NMD studies, gene expression studies and aCGH provides a powerful approach for the identification of the driver genes associated with the copy number abnormalities seen in tumor cells.

P.A. Jones (University of Southern California, Keck School of Medicine, Los Angeles, CA, USA) discussed the fundamental role of epigenetic events in carcinogenesis. The abnormal methylation of CpG islands located near the transcriptional start sites of human genes play a major role; the methylation of cytosine residues in these regions is associated with alterations in chromatin structure including the binding of methylated DNA binding proteins and changes in the state of modification of histone residues in nucleosomes. Aberrantly methylated CpG islands can be detected with a high degree of sensitivity making some of these changes also suitable as potential biomarkers. Methylation is also used for long-term epigenetic silencing of X-linked and imprinted genes. Dnmt 1 is one of the most important enzymes involved in gene silencing. The enzyme exhibits an amazing mode of action. It flips out the cytosine from the DNA-helix, puts on the methyl-group and allows the methylated cytosine to swing back. Drugs such as 5-aza-2′-deoxycytidine for DNA, 5-azacytidine for RNA and zebularine (1-(beta-D-ribofuranosyl)-1,2-dihydropyrimidine-2-one) can reverse DNA-methylation changes and reactivate gene expression.

J.E. Walter, (Universität des Saarlandes, FR8.2 Genetik, Saarbrücken, Germany) addressed the DNA-methylation reprogramming in development and disease. With the completion of the human genome we are

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facing the tremendous challenge to understand the complexity of differential usage of the genetic information during development and in disease context. Genomes are not static blueprints of biological information. Their activity is modulated by dynamic epigenetic programs to form a multitude of epigenomes reflecting the various cellular identities of an organism. The nature of epigenetic codes on chromosomes is complex and mediated by a not yet very well understood interplay of several epigenetic chromatin marks: (1) differential histone modifications, (2) RNA-mediated chromatin changes and (3) DNA-methylation. Particularly the generation of totipotent/pluripotent stem cells and their correct differentiation is apparently a reflection of a coordinated epigenetic reprogramming. Totipotent stem cells are "whitewashed" from most epigenomic marks to regain genetic totipotency. Such phases of major epigenetic reprogramming are occurring in primordial germ cells and the zygote.

## Session II: molecular nuclear controls

F.J. Rauscher (The Wistar Institute, Philadelphia, PA, USA), spoke about regulating gene expression via histone modification: roles in development and disease. Historically, much focus has been placed in the past on defining the functions of DNA binding proteins which bind to and regulate specific sets of target genes. The past few years has seen an explosion of research on the regulation of chromatin structure as a primary mechanism of gene regulation. In particular, the post-transcriptional modification of core histone tails via phosphorylation, acetylation/deacetylation, methylation and ubiquination has emerged as a key event. He has been studying ZBRK 1, a zinc finger protein which binds to the BRCA 1 protein and appears to target both activation and repression of transcription of specific genes. Repression is mediated by the transcriptional corepressor protein KAP-1, which has the capability to both bind to sequence-specific DNA-binding subunits (KRAB domain-containing Zinc Finger proteins) and recruit complexes containing histone de-actylases (NuRD), histone methylases (SETDBA1)) and heterochromatin proteins (HP1s). Loss of HP1 expression is seen in advanced metastatic breast cancer suggesting that a global loss of silencing mechanisms contributes to tumor progression.

W. Krek (Institute of Cell Biology, ETH Zürich, Zürich, Switzerland), stressed the issue of cell-cycle signaling networks and cancer. The decision of a cell whether to proliferate or arrest are made during the G1 phase of the cell cycle. During this period many disparate inputs including diverse metabolic and environmental cues as well as dictums from neighboring cells are integrated and interpreted and decisions are made regarding whether to reproduce, differentiate or die. A central role herein plays the F-box protein Skp2 in the regulation of cell proliferation. Skp2 has properties of an oncoprotein and is found overexpressed in various human cancers. The best documented function of Skp2 is that of a substrate recognition component of an E3 ubiquitin protein ligase complex that targets key cell cycle regulator proteins including the cyclin-dependent kinase (CDK) inhibitor p27 and the transcription factors E2F1, c-myc and FOXO1 for ubiquitination. Recently, it was discovered that Skp2 can also assemble SCF-like complex that is based on a novel RING finger protein of the RBCC motif family.

D. Goodrich (Roswell Park Cancer Institute, Buffalo, NY, USA) spoke on transcriptional elongation, RNA processing and the Rb tumor suppressor pathway. Co-transcriptional loading of RNA processing factors onto nascent RNA facilitates efficient gene expression. Mechanisms responsible for coupling transcription and RNA processing are not well defined, but the *Saccharomyces cerevisiae* TREX provides an example. TREX is composed of the subcomplex THO that associates with RNA polymerase II and is required for normal transcriptional elongation. THO associates with proteins involved in RNA splicing and export to form the larger TREX complex. Human Rb-associated protein Thoc1/p84N5 associates with elongation RNA polymerase II and the RNA splicing and export factor UAP56 in intact cells. Depletion of Thoc1/p84N5 causes transcriptional elongation defects and associated cellular phenotypes similar to those observed in THO deficient yeast. There is evidence that Rb may influence the function of such complexes.

## Session III: signal transduction pathways

J. Kann (OSI Pharmaceuticals, Farmingdale, NY, USA) talked about kinase signaling in colon cancer. A complex network of signaling pathways plays a key role in colorectal multi-step carcinogenesis. Recent studies have shown that the p110 $\alpha$-subunit of PI3K is mutated in approximately one third of 234 colorectal cancers tested with mutations correlating with later stages of tumorigenesis. This implicates that PI3K signaling pathway has a central role in colon cancer and inhibition of key kinases in the PI3K signaling axis should impact tumor survival.

K. Nilsson (Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Sweden) addressed the insulin-like growth factor-1 receptor (IGF-1R) signaling pathway in multiple myeloma (MM). Probably, the most important cytokines that regulate the proliferation and survival/apoptosis in MM are interleukin-6 and IGF-1, both of which have been shown to act in a paracrine and autocrine manner. IL-6 seems to be preferentially a growth factor but will also stimulate survival and inhibit apoptosis; IGF-1 is a stronger stimulator of survival as IL-6 and is less potent as a growth factor. It could be demonstrated that a molecule downstream of IGF-IR, mTOR, the mammalian target of rapamycin, is an adequate target in MM cell lines and

fresh biopsy cells to induce apoptosis in clinically achievable concentrations. Recently, members of the cyclolignan family have been shown to inhibit selectively the receptor tyrosine kinase activity of the IGF-1R $\beta$-chain. The effects of the cyclolignan picropodophyllin (PPP) and deoxypodophyllotoxin (DPPT) were studied in vitro, using a panel of nine MM cell lines and freshly purified tumor cells from MM patients, and, in vivo, using the 5TMM mouse MM model. Both IGF-1 RTK inhibitors effectively inhibited growth in all MM cell lines by increasing apoptosis and by inducing G2/M-arrest. In the mouse model, treatment of MM cells with the selective IGF-1 RTK inhibitors decreases proliferation and increases survival of the animals. Importantly, IGF-1, IGF-2, insulin and IL-6 did not reduce the inhibitory effects of PPP.

F. Entschladen (Institute of Immunology, University Witten/Herdecke, Germany) reviewed the formation of metastases as a multi-step process which notably requires the migration of tumor cells at two stages: the tumor cells must actively detach and emigrate from the primary tumor until reaching blood or lymph vessels, where they are disseminated. Secondly, the tumor cells have to emigrate from their carrier route and invade into tissues and organs to settle and proliferate in order to form secondaries. He addressed the question, why tumor cells switch on the locomotory machinery and what are the cues? The first, slower and less active type of migration is initiated by integrin signaling. The second, faster and more active type of migration is initiated by soluble signal substances. A plethora of substances of different classes has been shown to induce migration, e.g., cytokines, hormones, neurotransmitters and chemokines. The most potent regulators for migration are those binding to G protein-coupled receptors (GPCRs). The engagement of GPCRs by chemokines or neurotransmitters leads to the activation of heterotrimeric G proteins and the dissociation of the $\alpha$-subunit from the $\beta\chi$-subunit of these proteins, which both activate distinct signaling pathways–pathways leading to a path! Norepinephrine (NE) mediates its promigratory effect via $\beta$2-adrenoreceptors. NE is the most potent inducer of migration so far investigated in different tumor cell lines. NE leads to an upregulation of the collagen receptor $\beta$2-integrin, to a downregulation of the metastasis inhibitors gelsolin and (myristoylated alanine-rich C kinase substrate) MARCKS, which sums up to a metastatogenic tumor cell type.

## Session IV: cell environment:interactions

J. Pahler (Comprehensive Cancer Center, UCSF, San Francisco, USA and University of Turin) spoke about the constituent cell types mediating tumor angiogenesis as multi-targets. Infiltration of innate immune cell types into early neoplastic lesions helps activate the angiogenic switch in previously quiescent vasculature. Among the leukocyte-supplied effectors of the angiogenic switch are enzymes that modify the tissue microenvironment, including matrix metalloproteases-9, cysteine cathepsin proteases and heparanase; one consequence of their actions is mobilization of the angiogenic inducer VEGF-A. Once activated, the angiogenic tumor vasculature is sustained in part by chronic VEGF signaling from the tumor cells, and in part by pericytes, a vascular support cell, whose association is maintained by PDGF signaling.

P.H. Krammer (Tumorimmunology Program, Division of Immunogenetic, German Cancer Research Center, Heidelberg, Germany), describes the CD95(APO-1/FAS) signaling in respect to different diseases. CD95, a member of the tumor necrosis factor (TNF) receptor superfamily induces apoptosis upon receptor oligomerization. The receptor and its ligand are important for apoptosis of peripheral T cells for downregulation of an immune response and also for peripheral T cell tolerance. The CD95 death system also plays a role in destruction of liver tissue; in hepatitis cytotoxic T lymphocytes might us the CD95 system to kill infected hepatocytes. In Morbus Wilson copper overload leads the upregulation of CD95 ligand that may finally contribute to acute liver failure. In hepato-cellular carcinoma chemotherapeutically treated patients the CD94 receptor and ligand are upregulated and may contribute to apoptosis of the tumor.

A. Mantovani (Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy) characterized macrophages as versatile, plastic cells which respond to microenvironmental signals with distinct functional programs. Recent evidence suggests that differential modulation of the chemokine system integrates polarized macrophages in pathways of resistance against, or promotion of, microbial pathogens and tumors, immunoregulation, tissue repair and remodeling. Polarized type of macrophages and tumor infiltrating macrophages (TAM) are key components of inflammatory circuits which promote tumor progression.

## Sessions V and VI: clinical strategies

D. Small (Johns Hopkins University, School of Medicine, Baltimore, MD, USA) targeted FLT3 as a molecular structure for therapy in leukemia. FLT3 is a receptor with tyrosine kinase activity mutated in one of three acute myelotic lymphoma (AML). The mutation constitutively activates the tyrosine kinase activity of FLT3. There is evidence that the mutated FLT3 protects the cells from apoptosis. A number of studies have demonstrated that the presence of FLT3 activating mutations portends a poor prognosis for AML patients. Several potent, highly selective FLT3 inhibitors have been discovered as evidence by $IC_{50}$ of 2–3 nM against FLT3 autophosphorylation and a lack of inhibition of KIT, FMS and PDGFR at drug concentrations below 500 nM. These inhibitors result in inhibition of proliferation, induction of apoptosis and overcome blocks to

differentiation into cell lines transformed with FLT3 mutations isolated from patients. The use of antibodies generated against FLT3 is another molecularly targeted approach which suggests being useful in treating acute leukemias expressing FLT3.

S. Menard (Molecular Targeting Unit, Department of Experimental Oncology, Istituto Nazionale Tumore, Milan, Italy) selected HER2 as a specific target for therapy. HER2 overexpression in human breast carcinoma increases tumor cell proliferation, vessel formation, and/or invasiveness. HER2 overexpression has also been associated with sensitivity to anthracyclins and resistance to endocrine therapy, suggesting that tyrosine kinase receptor and hormone receptor pathways represents two major proliferation pathways exclusively active in breast carcinomas; one sensitive to chemotherapeutic drugs and the other to antiestrogens. However, a consistent number of HER2-positive tumors are not responsive to HER2-driven therapy, indicating the need for a better understanding of the mode of action of this new biological drug in vivo. While preclinical studies suggest antibody-dependent cellular cytotoxicity as the major mechanism, determination of NK activity at time of treatment remains mandatory, especially in patients with immunosuppressive drugs.

R. Danesi (Division of Pharmacology and Chemotherapy, Department of Oncology, University of Pisa, Italy) spoke about pharmacogenetic strategies for clinical development of target-specific anticancer agents. The variability of tumor response to chemotherapy is a topic of major interest in current clinical cancer research. The next generation of anticancer treatments might be tailored according to the molecular alterations identified in tumor cells of individual patients. However, before these alterations can be exploited from a therapeutic point of view, it is necessary to understand how such alterations influence the cellular pathways that control sensitivity to chemotherapeutic agents; pharmacogenetics and pharmacoproteomics will be instrumental in developing optimal chemotherapeutic regimens for cancer patients. The bcr-abl inhibitor imatinib and the EGFR inhibitor gefitinib are promising examples of molecules involved in tumor responses when combined with anticancer chemotherapy.

F. Caligaris-Cappio (Universita Vita-Salute San Raffaele, Dipartimento di Oncologia Istituto Scientifico Universitaria San Raffaele, Milano, Italy) introduced chromic lymphatic leukemia (CLL) as a model to develop new clinical strategies for indolent lymphoid malignancies. There are three key questions whose answer may help fostering new clinical strategies: (1) through which molecular pathways the microenvironment exerts its influence on the malignant clone? (2) which are the relationships between proliferation and defective apoptosis? and (3) is there any evidence for a role of antigenic stimulation. CLL cells are often highly dependent on the microenvironment and their functional capacity is influenced by external stimuli, e.g., antigen stimulation and stromal support. Proliferating CLL cells differ from circulating resting cells in terms of expression of several molecules, including apoptosis regulators like survivin, chemokines like CCL-17 and CCL-22 or proliferation-related genes like Ki67. The expression of CD38 and of the T-cell associated tyrosine kinase ZAP-70 has also a prognostic significance, the absence of CD38 and of ZAP-70 identifying patients with better prognosis. CLL heterogeneity has been demonstrated at functional level by the presence or absence of responses to stimulation via different cell surface receptors such as the B-cell receptor, CD5 and CD40. The emerging concept is the analysis to find out the different signal competences of CLL cells from patients in order to reveal the overall malignant cell capacity.

K.S. Zänker (Institute of Immunology, University Witten/Herdecke, Witten, Germany) gave a summary and thanked all participants. Many junior scientists working in the field of molecular medicine, were particularly convinced that the conference was at the cutting edge of molecular targeting of cancer chemotherapy and that it provided significant new information to be worthy of broad distribution.