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Editorial: June 2008

It is a great pleasure in this issue to announce the winner of the 2008 Pezcoller Foundation – AACR International Award for Cancer Research, Axel Ullrich, Managing Director Max Planck Institute of Biochemistry in Martinsried, Germany.

This year's Selection Committee was composed of Pier Giorgio Natali of Regina Elena in Rome, as Chairperson, Mina Bissell, Distinguished Scientist Lawrence Berkeley National Laboratory, USA; Julius Celis Professor and Scientific Director Danish Cancer Society Institute of Cancer Biology, Copenaghen; Mario P. Colombo Head, Gene Therapy Unit, Ist. Nazionale Tumori, Milano; James R. Downing Chairman and Member, Department of Pathology St. Jude Children's Research Hospital, USA; Albert J. Fornace, Jr. Department of Biochemistry and Molecular and Cellular Biology Lombardi Comprehensive Cancer Center at Georgetown University, USA; Tona M. Gilmer Global Director, Biological Sciences Oncology Translational Research, USA; Michael R. Stratton Deputy Director, Cancer Genome Project Wellcome Trust Sanger Institute, UK with the assistance of Marge Foti for the AACR and Gios Bernardi for the Foundation.

"2008 Pezcoller Foundation-AACR International Award for Cancer Research"
Trento, May 9 2008
Dr Gios Bernardi, President of the Pezcoller Foundation
and the winner, Prof. Axel Ullrich PhD, Max Planck Institute of Biochemistry,
Department of Molecular Biology, Martinsried - Germany

The Committee met in Trento (Italy) in December 2007 to select the winner.

Dr. Axel Ullrich has been named the recipient of the 2008 award for his pioneering work in the translation of molecular genetic research into biomedical applications. His fundamental discoveries in signal transduction research led to novel insights into the genomic determinants that promote cancer progression and to the development of novel cancer treatments including the FDA approved therapeutics Herceptin and SUTENT/Sunitinib.
Throughout his career in the most diverse scientific environments Dr. Ullrich has demonstrated unsurpassed

leadership and vision. His unique focus of the genetics and biology of growth factor receptors has kept him at the forefront in the discovery of oncogenes as well as the translation of basic scientific knowledge into clinical applications. The AACR and the Pezcoller Foundation are proud to honour his achievements as a rare example of uncompromising search for excellence and innovation in cancer research.

Axel Ullrich gave the important Pezcoller Lecture in San Diego at the annual AACR Meeting in April. He also gave the Korsmeyer Lecture in Padova at the VIMM Venetian Institute of Molecular Medicine to honour the memory of the late Stanley Korsmeyer who received our Award in 2004.
On May 9 Axel Ullrich was given the Award in an impressive ceremony in the historical Buon Consiglio Castle in Trento.

We are also pleased to announce the twentieth Pezcoller Symposium, to be held in Trento in June, to which we intend to give a unique relevance, having a look back on the achievements of items discussed in previous Symposia. The theme is "Molecular biology of cancer: 20 years of progress punctuated by the Pezcoller Symposia".
Of special relevance are the outstanding positions held in the field by the speakers: James Allison, Allan Balmain, Alberto Bardelli, Stephen Baylin, Mina Bissell, Lewis Cantley, Federica Cavallo, Paolo Comoglio, Carlo Croce, Fabrizio d'Adda di Fagagna, Napoleone Ferrara, Guido Forni, Raffaella Giavazzi, William Kaelin, Wilhelm Krek, David Livingston, Enrico Mihich, Pier Paolo Panfolfi, Giuseppe Pelicci, Stefano Piccolo, Marco Pierotti, Bruce Ponder, Jacques Pouyssegur, Carol Prives, William Sellers, Tadatsugu Taniguchi, Karen Vousden, Zena Werb.

Therefore we are pleased to present in this issue the speakers abstract.

Gios Bernardi MD
The Pezcoller Foundation President
and Editor of the Journal

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20th Pezcoller Symposium

MOLECULAR BIOLOGY OF CANCER: 20 YEARS OF PROGRESS PUNCTUATED BY THE PEZCOLLER SYMPOSIA

June 11-13, 2008 – Trento, Italy

ABSTRACTS OF ORAL PRESENTATIONS

Role of microRNAs and other non-coding RNAs in the pathogenesis of human cancer

Carlo M. Croce
The Ohio State University, Comprehensive Cancer Center, Columbus, Ohio, USA

MicroRNA alterations are involved in the initiation and progression of human cancer. The causes of the widespread differential expression of miRNA genes in malignant compared with normal cells can be explained by the location of these genes in cancer-associated genomic regions, by epigenetic mechanisms and by alterations in the microRNA processing machinery. MicroRNA expression profiling of human tumors has identified signatures associated with diagnosis, staging, progression, prognosis and response to treatment. In addition, profiling has been exploited to identify microRNAs genes that may represent downstream targets of activated oncogenic pathways or that are targeting protein coding genes involved in cancer.

Vaccine for cancer prevention: mining the breast cancer transcriptome to identify new oncoantigens

Federica Cavallo
Molecular Biotechnology Center, Department of Clinical and Biological Sciences, University of Turin, Turin, Italy

The immune response elicited by therapeutic vaccinations against tumor antigens is faced with a diffuse tumor burden (1, 2) and a negative setting of the immunoregulatory mechanisms (3-5). The reaction elicited can lead to tumor shrinkage. However, remissions frequently end with the recurrence of tumors that escape immune control by down-regulating their expression of the target antigen and the glycoproteins of the major histocompatibility complex (MHC) (6).
It is becoming increasingly clear that despite their marginal efficacy in the cure of established tumors antitumor vaccines can successfully inhibit the progress of initial neoplastic lesions. Recent work in my lab has shown that vaccines targeting overexpressed molecules directly involved in tumor progression (oncoantigens) provide an effective and long-lasting protection since the chance a tumor will evade the immune reaction are reduced. The induction of a specific immune response against oncoantigens expressed by early neoplastic lesions constitutes a fresh scenario wherein immunity may be much more effective (2). Clinical exploitation of vaccines for tumor prevention requires definition of the optimal conditions for its translation to humans and the identification of new genetic and epigenetic targets in the microenvironment associated with tumor progression in order to design an appropriate antigen-specific vaccine.
We have applied genome-wide transcriptional analysis to look for tumor- and microenvironment-associated oncoantigens suitable for preventive vaccination. Neoplastic tissues from cancer-prone transgenic mice were taken at various weeks of age to track tumour progression from atypical hyperplasia to invasive cancer. Total RNA

was extracted and gene expression profiles were generated using genome-wide mouse arrays. Microarray data were then studied with a consolidated pipe-line (7). Oncoantigens with low expression in normal human tissues and high and homogeneous expression in human cancer were retained for further evaluation (7). Their subsequent validation in mouse vaccination experiments is envisaged. We feel that this procedure provides a basis for the rational design of cancer vaccines for clinical trials (7). We have used the mammary glands of BALB/c mice transgenic for the rat Her-2/neu oncogene (BALB-neuT mice) (8, 9) to generate a list of new oncoantigens as potentially suitable targets for anti-tumor vaccination (10). We are now looking to see whether DNA vaccination against these oncoantigens elicits in an effective anti-tumor response in BALB-neuT mice.

Central and peripheral tolerance of these oncoantigens by the immune system occurs because they are self-antigens. Central tolerance cannot be altered. Peripheral tolerance, on the other hand, can be interfered with by opposing the production of suppressor cytokines and changing the function of regulatory T cells (Treg) (3). We are therefore evaluating the effects of antibody-mediated Treg depletion and RNA interfering targeting specific molecules in immune suppression concomitant with DNA vaccination against the oncoantigens.

Finally, in view of the role played by miRNAs in cancer progression, the characterization of their expression profile during carcinogenesis may yield new information. This may have a pivotal role in the design of fresh combinatorial treatment modalities. Changes in miRNA expression during the transition from preneoplastic to neoplastic lesions were detected by means of a method similar to that we have used to identify transcripts associated with neuT-driven DNA vaccination (11). We examined three situations: i) normal hyperplasia (2 weeks pregnant BALB-c mice), ii) atypical hyperplasia (10 weeks old BALB-neuT mice) and iii) neoplastic lesion (19 weeks old BALB-neuT mice). Total RNA was extracted from the all 10 mammary glands of 4 animals for each group and miRNAs changes were evaluated with LNA microarrays (EXIQON A/S, Denmark). Linear model analysis was performed to look for a subset of probes differentially expressed by the glands of 10- and 19-week-old mice compared to pregnant BALB-c mice. Principal component analysis (PCA) of 169 miRNAs indicated a clear difference between those expressed in the tumors and those expressed in the mammary glands of the pregnant BALB/c females. By contrast, only slight differences were found between atypical hyperplasia and neoplastic lesions. It would thus seem that miRNA disregulation in the mammary gland of BALB-neuT females is a very early event that is stably maintained during tumor progression.

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Targeting Hypoxia-induced Tumor Metabolism

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During embryonic development or in the context of tumor expansion, growing cells rapidly outstrip the supply of nutrients. Although cells sense and respond to variations in concentrations of all nutrients, oxygen sensing has emerged as a central control mechanism of vasculogenesis and energy metabolism. At the heart of this regulatory system is the Hypoxia-Inducible Factor, HIF, which interestingly controls, among other gene products, the expression of VEGF-A and Angiopoïetin-2 (Ang-2), two key angiogenic factors. This finding has therefore placed the hypoxia-signaling pathway at the forefront of nutritional control. HIF can induce a vast array of gene products controlling glycolysis, intracellular pH (pHi), angiogenesis, cell migration and invasion, and so has become recognized as a strong promoter of tumor growth. This pro-oncogenic feature is only one facet of the dual action of HIF. Besides being a 'guardian' of oxygen homeostasis, HIF is capable of inducing pro-apoptotic genes leading to autophagy and cell death, which can be features of hypoxic tissues and tumors. In the context of this meeting, we will highlight some of the HIF-induced markers that participate in tumor resistance to nutrient-depleted and acidic microenvironment. First we will show that the two HIF-induced 'BH3-only'-proteins (BNIP3, BNIP3L), in contrast to the current believe, do not trigger cell death but tumor cell survival by inducing autophagy. Second we will show how tumor cells by expressing two HIF-dependent membrane-bound carbonic anhydrases, CAIX and CAXII, acidify the extracellular milieu, and ensure a more alkaline pHi favoring migration and survival to the acidic tumor microenvironment. Finally we will show that additional HIF-regulated targets controlling intracellular pH (MCT-4, NHE1) could be exploited to enforce tumor regression by collapsing ATP levels.
***Keywords**: Hypoxia signaling, HIF, BH3-only proteins, autophagy, metabolism, pH regulation, ion transporters, necrotic cell death, tumor microenvironment.*

Molecular mechanisms of cellular senescence

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Early tumorigenesis is associated with the engagement of the DNA-damage checkpoint response (DDR). Cell proliferation and transformation induced by oncogene activation are restrained by cellular senescence. It is unclear whether DDR activation and oncogene-induced senescence (OIS) are causally linked. Here we show that the expression of an activated oncogene (H-RasV12) in normal human cells, results in a permanent cell cycle arrest caused by the activation of a robust DDR. Experimental inactivation of DDR abrogates OIS and promotes cell transformation. DDR and OIS are established after a hyper-replicative phase occurring immediately after oncogene expression. Senescent cells arrest with partly replicated DNA and with DNA replication origins having fired multiple times. In vivo DNA labelling

and molecular DNA combing reveal that oncogene activation leads to augmented numbers of active replicons and to alterations in DNA replication fork progression. Therefore OIS results from the enforcement of a DDR triggered by oncogene-induced DNA hyper-replication. Senescence is also associated with a global heterochromatinization of nuclear DNA. These senescence associated heterochromatic foci (SAHFs) are enriched in histone H3 di-tri methylated on lysine 9 (H3K9m) and HP1 proteins and High mobility group A (HMGA) proteins are also known to be essential structural components of SAHFs. Our most recent results on the interplay between DDR activation and oncogene-induced heterochromatinization will be presented.

The IRF family transcription factors in oncogenesis and immunity

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Cytokines have gained much attention in biological sciences and medicine over the last several decades. My career in research on cytokines began with the characterization of the human fibroblast IFN gene (now referred to as IFN-β) and elucidated its primary structure. In collaboration with Dr. Charles Weissmann and colleagues, we subsequently demonstrated in 1980 that IFN-α and IFN-β genes constitute a gene family, the first of the numerous cytokine gene families to be identified later. We also identified and characterized a human interleukin gene, the IL-2 gene and generated recombinant IL-2, thereby enabling the study of the molecular basis of lymphocyte proliferation. The availability of these recombinant cytokines has made their clinical applications in the treatment of cancer and other diseases, and their use in studies of molecular signaling mechanisms possible.
Host defense consists of two main aspects, namely, immune response to invading pathogens and suppression of tumor development. A family of transcription factors, interferon regulatory factors (IRFs), was originally identified in the context of IFN gene regulation and it has recently gained much attention in terms of its critical role in linking these two aspects of host defense. We demonstrated, in collaboration with other groups, the important and broad functions of IRF-1 in the regulation of interferon responses and other immune functions such as the differentiation of NK and CD4 T cells. Our achievements in the study of IRFs in oncogenesis include the studies demonstrating that IRF-1 regulates cell cycle arrest in cooperation with the tumor suppressor p53. These findings originally established important links between IRFs in oncogenesis and immunity. We also adduced evidence that IFN signaling contributes to tumor suppression via p53 induction, helping to explain the mechanism underlying the antitumor actions of IFNs. The importance of the IRF family members has been further corroborated by the recent demonstration of their key roles, in particular IRF3, IRF5, IRF7, in Toll-like receptor (TLR) signaling in innate and adaptive immune responses. More recently, we found new facets of IRF5. IRF5 is critical in antiviral immunity by inducing apoptosis, or altruistic suicide, of virus-infected cells. It is also critical for the induction of apoptosis, but not in cell cycle arrest, in response to DNA damage and it functions as a tumor suppressor by acting on a pathway that may be distinct from the well-known tumor suppressor p53. Moreover, IRF5 plays a critical role in the Fas-death receptor siginaling. These results provide a new link in the transcriptional network underlying immunity and tumor suppression.
It is remarkable that studies of the IFN-α/β system significantly contributed to our understanding of the mechanisms underlying cytokine action, immune responses and oncogenesis. Indeed, the critical roles of the Janus family of protein tyrosine kinases (JAK kinases), signal transducers and activators of transcription (STATs), and IRFs have all been identified in the context of IFN induction and action, and their broad functions in other biological systems are widely appreciated by now. As for the future prospects, we envisage that a further understanding of the versatile functions of IRFs should

also provide important insights into improvements of therapeutic interventions for numerous diseases such as cancer and autoimmunity.

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Polygenic susceptibility to breast cancer and its practical implications

Bruce Ponder[1], Paul Pharoah[1,2], Doug Easton[2], Alison Dunning[1], Dennis Ballinger[3], David Cox[3] and the International Breast Cancer Association Consortium.
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We reported (Nature 447: 1087-1093, 2007) 5 common allelic variants that confer susceptibility to breast cancer, identified in a genome wide association study. Others have confirmed some of these associations, and reported additional loci. In aggregate, these common variants account for roughly 5% of the total inherited component of breast cancer risk (by comparison, mutations in BRCA1 and BRCA2 each account for about 8%). The common allelic variant of greatest effect, which confers increased expression of the gene FGFR2, has a frequency of 0.38 in the UK population and confers a relative risk of breast cancer of 1.28 in heterozygotes, and 1.60 in homozygotes, compared to the wild-type homozygote. The results of our study, and of others published subsequently, suggest that no common allele will be found that has a larger effect on breast cancer susceptibility than the FGFR2 allele.

It has been suggested that, since the common susceptibility alleles are individually of such small effect, their identification has no practical application in risk estimation, to select women who might most benefit from screening or prevention. Our analysis suggests this is not the case.

In the UK National Health Service (NHS) mammographic screening is offered to all women from the age of 50. This is essentially an absolute risk criterion based on age: the average 10 year risk of breast cancer at age 50 in the UK is 2.3% If this 10 year risk were adopted as the criterion for eligibility for screening, application of even the few common susceptibility alleles so far identified might substantially alter screening policy. The 10% of women at least risk, based on this polygenic profile, will never reach a 2.3% in 10 year risk by age 75, while the 10% at greatest risk will reach this threshold at age 36. Incorporating other risk factors such as family history and mammographic density (largely independent of the susceptibility alleles identified to date) would further refine the risk distribution and estimates of individual risk. There are of course logistical, economic and social factors to be factored in; but these results suggest that for breast and for other common cancers, improvements in screening strategy based on the incorporation of polygenic susceptibility may soon be possible.

Role of the dynamic tumor microenvironment in neoplastic progression

Mikala Egeblad, Andrew J. Ewald and Zena Werb
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Leukocytes are recruited to tumors can be hi-jacked to stimulate tumor progression. we used four-color, long-term, fluorescent imaging in living mice to gain an understanding of leukocyte behavior during mammary tumor progression. Using mice expressing GFP in myeloid, dendritic, or T cell populations cross-bred with the MMTV-PyMT mammary carcinoma mouse model, we

were able to investigate the migration of the leukocytes in different tumor microenvironments. T cell migration was highest along blood vessels, whereas both myeloid and dendritic cells were most motile at the tumor-stroma border. Deeper within the tumors, we observed little migration of macrophages and other cells, although newly recruited myeloid cells extravasated and infiltrated the tissue. Intravenously injected fluorescent dextran identified a dextran-ingesting, low-migratory myeloid subpopulation, that were identified as alternative activated (M2) macrophages by marker analysis. Myeloid cell infiltration and migration was dramatically increased in mice challenged with fluorescently labeled necrotic tumor debris. The myeloid cells initially exhibited random migration and did not localize the debris. However, once a first cell made direct contact with the necrotic debris, other cells changed direction within minutes and migrated towards it. Targeted deletion of MMP-9, a major secreted protein of myeloid cells in tumors, resulted in decreased angiogenesis and altered tumor differentiation, but increased number of tumors, although the overall rate of tumor initiation and metastasis were similar. These data reveal that behavior of leukocytes varies with the microenvironment and that there are functional subpopulations of these cells.

Cancer mutations and targeted therapies

Alberto Bardelli
The Laboratory of Molecular Genetics, Institute for Cancer Research and Treatment, University of Torino-Medical School, Candiolo (TO) and FIRC Institute of Molecular Oncology, Milan, Italy

It is now clear that cancer has a genetic basis and that mutations affecting the sequences of specific genes are the hallmark of this disease.
This knowledge has already had a dramatic impact in the clinical arena. First, many studies have demonstrated that tumours can be diagnosed and classified on the basis of their genetic profiles. Second, the pattern of genetic alterations present in individual cancers can, at least in part, be used to predict their clinical outcome. Third, the success of cancer drugs designed to target the molecular alterations underlying tumorigenesis has proven that genetic alterations are legitimate targets for therapy.

We have exploited cancer mutations using two complementary and interconnected approaches. On one side we coupled genetic alterations in genes involved in kinase-mediated signaling with the clinical response to molecularly targeted therapies. On the other we have generated tumor progression models closely recapitulating the genetic alterations present in human cancer using an innovative technology that allows targeted homologous recombination in human cells.

Overall this approach is providing new insights into the pathogenesis of cancer and has led to results relevant for therapeutic intervention.

Gene Networks and control of cancer susceptibility

Allan Balmain,
UCSF, University of California, San Francisco, USA

Studies of mouse models of human cancer have demonstrated the existence of multiple germline polymorphisms (SNPs) that influence cancer susceptibility through their effects on tumor multiplicity, size, or the probability of malignant progression. Such genetic modifiers, each of which in isolation has a weak effect, are likely to affect the response of the host animal to environmental (carcinogen) insult, as well as the intrinsic cellular processes involved in tumor formation. Statistical analyses have shown that a major component of cancer susceptibility is due to strong interactions between genetic modifiers. Characterization of the genetic architecture of these low penetrance genetic variants would provide important tools for recognition of individuals at risk of cancer development, and for prevention strategies. Using mouse interspecific crosses, we

have identified a number of genetic loci that confer increased or decreased risk of development of cancers of the skin, lung, or lymphoid system, and in some cases the causative genes and polymorphisms are known. Bioinformatics analysis tools have now been developed to construct SNP and gene expression networks associated with phenotypes, such as inflammation, that are involved in cancer susceptibility. These new approaches offer substantially greater power than classical methods for identification of the factors that contribute to cancer susceptibility.

Understanding Plasticity in Tissue Specificity and Cancer

Mina J. Bissell
Lawrence Berkeley National Laboratory, Berkeley, CA, USA

Much has been learned about tissue specificity and cancer since I attended my first Pezcoller symposium more than a decade ago. The field of microenvironment has been born 'officially' and is now an accepted and exciting area of endeavor. I wrote a detailed summary of our findings last year in an article for the Pezcoller Journal upon receiving the Pezcoller Foundation-AACR International Award for Cancer Research. I will not reiterate the background again.

I refer the interested readers to that article and references within. In this talk I will outline the outstanding questions about the relation of tissue specificity and cancer and will discuss some recent work on human breast stem cells done in collaboration with Mark LaBarge of my laboratory and Ole Petersen's laboratory in Denmark.

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The Role of PI 3-Kinase in Cancer

Lewis C. Cantley
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Activating mutations in phosphoinositide 3-kinase (PI 3-kinase) and loss of function mutations in PTEN, the phosphatase that degrades the product of this enzyme, are among the most common events in solid tumors. We have been investigating the signaling network surrounding PI 3-kinase that regulates cell growth and cell survival. In addition, we have generated mice with mutations in PI 3-kinase genes and are investigating the effects of these alterations on formation of endogenous tumors. Tumors that develop in mice due to expression of mutated forms of phosphoinositide 3-kinase or of the downstream protein kinase, AKT have increased expression of genes involved in glucose uptake and metabolism, and thus can be visualized with ^{18}F-FDG-PET. Based on these studies, opportunities for pharmaceutical intervention in this pathway to treat cancers will be discussed.

The Era of the Cancer Epigenome: Exploring Its Components and Origins

Stephen B. Baylin
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, The Johns Hopkins University, School of Medicine Baltimore, Maryland USA

The past decade, including presentations at these Pezcoller meetings, has seen growing evidence that cancer is not only a genetic disease, but an epigenetic one as well. In other words, in addition to disruption of gene function through classic mutations and other abnormalities in base sequence in cancer, there are heritable changes, not dependent on altering primary DNA sequence, which produce abnormal gene expression patterns which are fundamental to tumorigenesis. In fact, at this meeting, I will put forth the possibility that the abnormal genetic steps underlying the most common forms of human cancer cannot play out their potential unless given the opportunity by the abnormal "epigenetic set" of the tumor and that the latter arises during cancer initiation and continues to deepen during progression. In turn, these epigenetic abnormalities may involve conversion of a normally plastic, to a heritably stable, gene transcriptional repression which locks in gene expression patterns of embryonic type cells and thus, in tumorigenesis, hinders stem/progenitor cells from normal commitment.

The most studied epigenetic aspect of cancer, and the fundamental abnormal epigenetic "set" alluded to above, is DNA hypermethylation of CpG islands in gene promoter regions and aberrant transcriptional repression of the genes involved. Epigenomic profiling of human cancers, including ongoing work in the NIH/NCI genome atlas project (TCGA), indicates that a patient's given cancer harbors several hundred such genes and many are now documented to be important for development – and, thus, to be genes required to be held in a low, poised expression state to preserve cell stem/progenitor status at the expense of preventing cell commitment to defined lineages or more mature cells. Mouse knockout studies of one such gene, which is frequently DNA hypermethylated, but not mutated, at pre-invasive stages of multiple common human tumor types will be used to illustrate that DNA hypermethylation and associated tight epigenetic gene silencing can help foster the very earliest steps in cancer formation.

The numbers of genes simultaneously DNA hypermethylated in a given cancer is reminiscent of the numbers of CpG island containing genes marked, in embryonic stem and progenitor cells, by the key, long term, silenc-

ing protein complex, polycomb (PcG). Many such embryonic genes have promoters with "bivalent" chromatin constituted by simultaneous presence of the repressive PcG catalyzed, histone modification, H3K27me3 and the trithorax catalyzed, activating marks, H3K4me2 and 3. In colon cancer cells, tiling array studies indicate that hundreds of DNA hypermethylated genes, with virtually no basal expression, have such promoter bivalent chromatin, which becomes especially apparent when the genes are induced to DNA de-methylate and are re-expressed, often at a low transcription level. Compared to the ~ 10% of all embryonic cell genes marked by PcG, ~50% of the DNA hypermethylated genes in the colon cancer cells are among these embryonic genes. Thus, the PcG complex may regulate a key state of chromatin which is vulnerable to abnormal DNA methylation in stem/progenitor cells, especially during chronic cell renewal in cancer risk states. This methylation, in turn, may be fundamental in initiation of abnormal clonal expansion at such sites and for the recently observed embryonic stem cell-like expression patterns in human cancers. As such, the DNA methylation has great potential for exploitation as a biomarker system and as a target for strategies to prevent and treat cancer.

Ubiquitin-mediated control of TGFβ signaling

Stefano Piccolo
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TGFβ is a pleiotropic cytokine regulating a vast array of biological processes, from embryonic development to tissue renewal and homeostasis. In tumorigenesis, TGFβ plays a dual role, acting as a tumor suppressor in early stages but turning into a promoter of malignancy in advanced cancers.
TGFβ ligands bind to cognate receptors leading to Smad2/3 phosphorylation and nuclear translocation. Here, the Smad2/Smad4 complex acts as transcription factor to regulate the expression of TGFβ target genes. The simplicity of this signal transduction mechanism contrasts sharply with the complexity of elicited biological responses. Ubiquitination of TGFβ receptors and Smads is a leading mechanism to keep under tight control cell responsiveness and to regulate signal intensity and duration. This regulation ultimately unfolds cell-type or context dependent transcriptional programs. Several E3 ubiquitin ligase impinging on TGFβ signaling have been identified; so far, the research in this field considered ubiquitination according to its canonical role in protein degradation; however, in recent years several other mechanisms emerged by which ubiquitination can regulate protein function, including regulation of subcellular localization/endocytic trafficking, protein-protein interactions and activity. The main appeal of regulative-ubiquitination is its reversibility, as indicated by the existence of a whole family of ubiquitin-proteases (DUBs) in the genome, whose functions are only now starting to emerge. Yet, nothing is known on DUBs that regulate TGFβ responsiveness.
At this meeting, I will present the result of an unbiased loss-of-function screen to identify these enzymes and show the cloning and mechanisms of action of a DUB for Smad4.

Promoter Selectivity by the p53 Tumor Suppressor Protein

*Carol Prives, Rachel Beckerman, Melissa Mattia, Lynn Biderman, Andrew Zupnick and Tomoaki Tanaka**
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The p53 tumor suppressor protein is a sequence specific transcriptional regulator whose target genes control many cellular processes including cell cycle, cell death and senescence. One challenge is to determine how and when p53 selects its target promoters. Our group has approached this in different ways and our work indicates several mechanisms are at play.

1. *We used a modified ChIP-mass-spectrometry protocol to identify proteins co-associated with p53 at pro-apoptotic genes. This approach yielded a number of candidates including the nuclear export factor hCAS/Cse1L. We found that cells depleted of hCAS/Cse1L are defective in undergoing p53 mediated apoptosis and show impairment of activation of select p53 target genes. Delving into this further we showed that p53 and hCAS/Cse1L interact independently with those promoters whose expression was affected by hCAS/Cse1L downregulation. Further hCAS/Cse1L and p53 cooperate to reduce long-range tri-methylation of histone H3 K27, a known repressive mark in chromatin. Our results therefore identify hCAS/Cse1L as a novel co-regulator and selectivity factor for p53.*
2. *We have also been examining the roles of key lysines in the C-terminus of p53 by introducing select mutations that either conserve charge (K/R) or mimic acetylation (K/Q). Our results indicate that lysines in a specific region of the C-terminus regulate a shift of p53 between cell cycle arrest and apoptosis and may do so by selectively regulating key p53 targets.*
3. *In another study we have found that when cells are arrested in S phase p53 is selectively impaired in inducing target genes. One key target of p53, the p21 cyclin dependent kinase inhibitor, normally expressed at high levels after many forms of DNA damage, is only weakly induced when S phase is blocked. Our results show that the main defect is in elongation of p21 RNA. We are working to identify the signaling pathways and molecules that are responsible for this replication checkpoint dependent elongation defect.*
4. *The p53 protein has two transactivation sub-domains within its N-terminus, TAD I (residues 20-40) and TAD II (residues 41-60). TAD I is clearly the dominant transactivation domain because mutation of two TAD I amino acids (L22Q and W23S) abrogates most of the transcriptional activity of p53. We have discovered that caspase 2 activation is required for cell death initiated by either wild-type or $p53^{Q22/S23}$. Remarkably, although $p53^{Q22/S23}$ is known to be defective in transcriptional activation of numerous p53 target genes, it can induce expression of pro-apoptotic targets including PIDD and AIP1 at least to the same extent as wild-type p53. Further, RNAi silencing of PIDD, previously shown to be required for caspase 2 activation, suppresses apoptosis by both wild-type p53 and $p53^{Q22/S23}$. These data indicating that some targets such as PIDD do not require TAD are supported by our observation that treating cells with Nutlin that blocks Mdm2 binding to p53, results in significant increased activation of numerous p53 target genes with the marked exception of PIDD. Thus, therapeutic agents such as Nutlin can still allow p53 to activate some of its transcriptional targets.*

Functions of p53 in prevention and response to stress

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The Beaston Institute for Cancer Research, Switchback Road, Glasgow, UK

p53 is a tumor suppressor protein with potent cell cycle arrest and apoptotic functions, both of which contribute to the inhibition of malignant development. Most cancer cells show loss of p53 function, and there is evidence that reactivation of p53 is more likely to induce apoptosis in tumor cells than their normal, unstressed counterparts. This differential response underlies the hope that p53 may be an effective therapeutic target. The choice of response to p53 is determined – in part – by differential activation of gene expression by p53, and this is regulated by the availability of transcriptional cofactors such as the ASPP family. Modulation of ASPP activity in cells can profoundly affect the response to p53, and we have been investigating which genes are regulated in a p53 – and ASPP– dependent manner, to drive death or survival in response to stress.
Understanding what regulates the choice of response to p53 is important when considering reactivation of p53

as a tumor therapy, and how to achieve maximum differential between the death of cancer cells and survival of normal cells. Interestingly, in addition to the induction of pro-apoptotic targets, p53 can induce expression of several proteins that show anti-apoptotic activity. It is clear that p53-target genes can be differentially activated and so the balance of death and survival signals induced by p53 may be critical in determining the ultimate response to p53 activation. We have found a novel p53-target gene – TIGAR - that encodes a protein with similarity to the phosphatase domain of the bifunctional enzyme PFK-2/FBPase-2, one of the principal regulators of glycolysis. Expression of TIGAR may enhance the oxidative branch of the pentose phosphate pathway, hence conferring resistance to oxidative stress by enhancing NADPH production which provides the necessary reducing equivalents to restore reduced glutathione (GSH) levels. Therefore, ectopic expression of TIGAR protects cells from both ROS and p53-induced cell death, and TIGAR appears to belong to a group of p53-inducible genes that contribute to the survival of cells undergoing oxidative stress. The survival functions of TIGAR suggest that deregulated expression could contribute to the genesis of cancers. In support of this proposal, our preliminary studies have found over-expression of TIGAR in a number of cancer cell lines (regardless of p53 status) and in a significant proportion of malignant colon cancers.

Biological Properties of Cancer Stem Cells

A.Viale[1], G. Bonizzi[1], A. Cicalese[1], F. DeFranco[1], C. Pasi[1], S. Pece[1], A. Orleth[1], P.P. DiFiore[1,2], P.G. Pelicci[1,2].
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Recent findings support the concept that cells with the properties of stem cells (SC) are integral to the development and perpetuation of several forms of human cancer, and that eradication of cancer stem cells (CSC) may be essential to achieve cancer cure. However, direct proof of these concepts is still lacking, mainly due the scarcity of appropriate model systems. We are characterizing the biological differences between normal and transformed SCs. SCs are defined by their abilities to generate more SCs ('self-renewal') and to produce cells that differentiate. One mechanism by which SCs accomplish these two tasks is asymmetric cell division, whereby each SC divides to generate one daughter with SC fate and one that differentiates. SCs, however, possess the ability to expand in number, as it occurs during development and in adulthood after injury or disease. This increase is not accounted by asymmetric divisions, in which only one daughter cell maintains SC identity. Recent findings in C.elegans and Drosophila indicate that SCs can also generate daughter cells that are destined to acquire the same fate (symmetric cell division). On the other hand, SC quiescence is critical to maintain tissue homeostasis after injury. We will present our recent findings showing increased symmetric divisions of CSCs in breast tumors (due to inactivation of the p53 tumor suppressor) and dependency of leukemia development on quiescent leukemia SCs (due to transcriptional up-regulation of the cell cycle inhibitor p21 by leukemia-associated fusion proteins). Our findings suggest that that asymmetric divisions of stem cells function as a mechanism of tumor suppression, that SC quiescence is critical to the maintenance of the transformed clone and that symmetric divisions of SCs permits its geometric expansion.

Targeting the PI3K/mTOR pathway in cancer

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Targeted therapeutics directly interdicting the activity of key oncogenic proteins in cancer have now demonstrated a high degree of clinical activity in diverse sets of human malignancies including common adult epithe-

lial cancers such as HER2+ breast cancer and EGFR mutant lung adenocarcinomas; aggressive sarcomas such KIT and PDGFR mutant gastrointestinal stromal tumors; and smoldering diseases such as BCR-ABL+ chronic myelogenous leukemia. These examples underscore the possibility of directing therapy based on molecular genetic alterations in human cancer. The PI3K/mTOR pathway is frequently constitutively dysregulated in human cancers through activating mutations in PIK3CA and in upstream receptor tyrosine kinases, and through inactivating mutations in the tumor suppressor genes PTEN, TSC1, TSC2 and LKB1. The high frequency of such genetic alterations has made targeting either mTOR or PI3K itself a high priority. RAD001 is an orally available mTOR inhibitor while BEZ235 and BGT226 are orally available dual PI3K/mTOR inhibitors. Each agent is currently in clinical development. The preclinical development of these novel therapeutics and the ongoing clinical development will be discussed.

The von Hippel-Lindau Tumor Suppressor Protein: Oxygen Sensing and Cancer

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Inactivating germline mutations of the von Hippel-Lindau tumor suppressor gene (VHL) cause VHL disease, which is characterized by an increased risk of hemangioblastomas, clear cell renal carcinomas, and pheochromocytomas in an allele-specific manner. Somatic VHL mutations are also common in sporadic hemangioblastomas and clear cell renal carcinomas. The VHL gene product, pVHL, is part of a ubiquitin ligase complex that targets the alpha subunits of the heterodimeric transcription factor HIF for polyubiquitylation. Other functions of pVHL include regulation of the primary cilium, microtubule stability, extracellular matrix formation, integrin function, receptor endocytosis, atypical PKC activity, and NFκB activity. The interaction of pVHL with HIFα requires that HIFα be hydroxylated on either (or both) of two conserved prolyl residues by members of the EglN (also called PHD) family of prolyl hydroxylases. HIF is a master regulator of genes involved in acute or chronic adaptation to hypoxia including genes linked to glucose uptake, glycolysis, angiogenesis, and erythropoiesis. Deregulation of HIF, perhaps in conjunction with some of the other functions cited above, appears to play a prominent role in the development of hemangioblastomas and renal cell carcinomas. Genotype-phenotype correlations suggest that the degree of HIF deregulation correlates with hemangioblastoma and renal carcinoma risk. Moreover, suppression of HIF appears to be both necessary and sufficient for pVHL to inhibit VHL-/- renal carcinoma growth based on experiments in which HIF activity has been genetically manipulated in renal carcinoma cells prior to implantion in nude mice. In contrast, deregulation of HIF does not appear to be linked to the development of pheochromocytoma (intradrenal paraganglioma), which is a tumor of the sympathetic nervous system.

EglN1 (PHD2) is the primary regulator of HIFα turnover under normal conditions among the three EglN family members. We recently discovered that EglN2 and EglN3 play HIF-independent roles in the regulation of proliferation and apoptosis, respectively. For example, precursors of the sympathetic nervous system compete with one another for growth factors such as NGF during embryological development, with the losers undergoing c-Jun-dependent apoptosis. We recently reported that all of the genes linked to familial paraganglioma (VHL, NF1, c-Ret, SDH B, SDH C, SDH D) impinge upon this pathway and that c-Jun transcriptionally activates EglN3, which delivers a HIF-independent death signal. In collaboration with Daniel Peeper (NKI), we then screened for shRNAs that protect against EglN3-induced apoptosis in neural crest-derived tumor cells. One of the few shRNAs that scored in this screen inhibited the function of KIF1Bβ, a member of the kinesin family. KIF1Bβ maps to 1p36.2, which is frequently deleted in a variety of tumors, including neuroblastomas. KIF1Bβ acts downstream of EglN3 and we have

identified loss of function KIF1Bβ mutations in some pheochromocytomas and neuroblastomas, arguing that KIF1Bβ is one of the long sought after "1p" tumor suppressor genes. Our genetic and functional studies suggest that haploinsufficiency of KIF1Bβ might be sufficient to promote tumor growth, which could explain why 1p deletions (with retention of a wild-type allele) are much more common than KIF1Bβ missense mutations. We are currently exploring whether EglN3 agonists can be harnessed as cancer therapeutics and conducting mechanistic studies to determine how KIF1Bβ induces cell death.

Immune Checkpoint Blockade in Cancer Therapy

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Ludwig Center for Cancer Immunotherapy, Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

While there are exciting examples of successful clinical strategies to mobilize the immune system to attack cancer cells, overall the results have been disappointing. One reason for less than optimal results is that until recently insufficient attention has been paid to multiple inhibitory mechanisms that serve to shape the immune response and minimize harm to normal tissues and can frustrate generation of effective anti-tumor responses. The prototype of these inhibitory pathways is CTLA-4, which upon engaging its ligands B7-1 and B7-2 limits T cell proliferation. We have shown that blockade of CTLA-4 can greatly enhance anti-tumor responses in a number of experimental tumors in mice. Recent studies of the mechanisms involved suggest that CTLA- blockade enhances anti-tumor responses by blocking a cell intrinsic inhibitory pathway in effector T cell, thus rendering them resistant to inhibition by Treg cells.
Anti-CTLA-4 (MDX-010, Ipilimumab) is being co-developed by Medarex, Inc. and Bristol Meyers Squibb. The results of clinical trials in over 1700 patients have demonstrated objective, durable responses with manageable in a high fraction of melanoma, renal prostate, ovarian, and pancreatic cancer.
More recently we and others have shown that there are additional B7 family members that limit T cell responses at distinct stages, and that at least three of these are also expressed by tumor cells. Thus the extended B7 family offers a number of targets for immune checkpoint blockade in the treatment of cancer. Finally, recent studies have shown that tumors multiple coding mutations which should result in generation of multiple neoantigens. I will discuss the implications for this to immunologically based as well as conventional cancer therapy.

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An *in vitro* protein interaction profile of *Drosophila* p53 uncovers novel partners and potential new functions of mammalian p53 and p53-related proteins.

Andrea Lunardi, Giulio Di Minin, Giannino Del Sal and Licio Collavin
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In the genome of the fruit-fly Drosophila melanogaster there is a single member of the p53 family of tumor suppressors. Sequence analysis indicates that this protein is phylogenetically closer to vertebrate p63/p73 than to

p53, yet it displays functional features of p53: it has a marked genoprotective activity, responds to DNA damage, and triggers apoptotic responses.
This convinced us that mapping the protein interaction profile of the single p53 protein in D. melanogaster (Dmp53) might reveal conserved mechanisms underlying the function of p53, p63 and p73, thus providing information on the biology of the entire p53 family.
We employed a small-pool based approach to screen more than 8000 non-redundant cDNAs from the Drosophila Gene Collection in search of proteins that bind to baculovirus-expressed Dmp53 by in vitro pull-down.
We report the results of such screening, which led to the identification of 94 novel proteins that interact with Dmp53 in vitro.
Among these, we found some p53 interlogs (fly orthologs of mammalian proteins that are known to bind p53), confirming the validity of our approach. In addition, we identified 59 Dmp53 binding proteins that have conserved mammalian orthologs not previously reported to associate with p53 family members.
We selected 40 of these human proteins for further analysis, and assayed their ability to interact with p53, p63 and p73 in co-transfected mammalian cells. Under these conditions, 85% of the proteins tested bound to at least one of the members of the p53-family, supporting the strong evolutionary conservation of such interactions.
These data suggest that our current understanding of the protein interaction profile of p53 and p53-related proteins is probably far from being exhaustive. Analysis of the biological function of this additional group of interactors has the potential to uncover novel connections between members of the p53 family and conserved cellular pathways.

Role of VEGF and other mediators in tumor angiogenesis

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Vascular endothelial growth factor (VEGF)-A is an important angiogenic factor, involved in both physiological and pathological growth of blood vessels. Anti-VEGF-A monoclonal antibodies or other VEGF inhibitors block tumor growth and neovascularization in numerous preclinical models. We developed a humanized anti-VEGF-A monoclonal antibody (bevacizumab) to test the hypothesis that blocking VEGF-A may have a clinical benefit in tumor patients. Bevacizumab has been approved by the USA FDA for the treatment of previously untreated and relapsed metastatic colorectal cancer, non-squamous non-small-cell lung cancer and metastatic breast cancer, in combination with chemotherapy.
We have been investigating the mechanisms of refractoriness/resistance to anti-VEGF therapies. These studies indicate that tumor-infiltrating Gr1+ myeloid cells play an important role in these processes. The secreted Bv8 protein was identified as a mediator of myeloid cell-dependent tumor angiogenesis, potentially implicated in refractoriness to anti-VEGF therapy.

The NPM paradigm and the "Co-clinical trial" project

Pier Paolo Pandolfi
Cancer Genetics Program, Division of Genetics, BIDMC Cancer Center and Harvard Medical School

NPM (Nucleophosmin, B23, NO38) is a multifunctional protein directly implicated in cancer pathogenesis in view of its involvement in cancer associated chromosomal translocations and its extremely frequent mutation rate in leukemia. Here we will discuss how NPM has become a paradigmatic example for the dramatic consequence of subtle variation in gene expression level and aberrant trafficking in tumorigenesis, as determined through a direct genetic approach in vivo in the mouse.
Time permitting, we will discuss a novel methodological approach, which we refer to as the "Co-Clinical trial" project, towards the optimized and accelerated testing of novel therapeutic modalities for cancer eradication in faithful animal models of human cancer.

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ABSTRACTS OF POSTERS

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Among these, we found some p53 interlogs (fly orthologs of mammalian proteins that are known to bind p53), confirming the validity of our approach. In addition, we identified 59 Dmp53 binding proteins that have conserved mammalian orthologs not previously reported to associate with p53 family members.
We selected 40 of these human proteins for further analysis, and assayed their ability to interact with p53, p63 and p73 in co-transfected mammalian cells. Under these conditions, 85% of the proteins tested bound to at least one of the members of the p53-family, supporting the strong evolutionary conservation of such interactions.
These data suggest that our current understanding of the protein interaction profile of p53 and p53-related proteins is probably far from being exhaustive. Analysis of the biological function of this additional group of interactors has the potential to uncover novel connections between members of the p53 family and conserved cellular pathways.

Effects of L19mTNFα on inhibition of angiogenesis in pre-clinical models of oesophageal cancer

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Angiogenesis is a pre-requisite for tumors to grow and metastasize. Dependence of tumors on angiogenesis is the starting point to draw new therapeutic strategies striking tumor indirectly.

Fibronectin (FN) is an high molecular weight adhesive glycoprotein present in extracellular matrix. Processes of alternative splicing give rise to different FN isoforms, among which oncofoetal FN, containing the ED-B domain, considered a marker of tissue remodelling and angiogenesis.
Antibodies specific to ED-B and conjugated to bioactive molecules, such as chemokines or cytokines, capable of selective tumor targeting, are available. scFv(L19) is a human antibody fragment with high affinity for the ED-B domain of FN, which is specifically expressed in tumor lesions. L19mTNFα represents an example of immunoconjugate: it strengthens the selective toxicity of the drug by acting preferentially on the tumor vasculature and causing massive destruction of endothelial cells with subsequent tumor necrosis. However, the viable rim is not affected by this immunoconjugate, since it benefits from the vicinity of peri-vascular vessels.
This work focused on the development of anti-vascular strategies applied to pre-clinical models of oesophageal cancer. We investigated the short- and long-term effects of L19mTNFα on tumor volume, amount of necrosis, mean vessel density (MVD) and perfusion by immunohistochemistry and immunofluorescence techniques.
ED-B was expressed at high levels in s.c. tumors formed in SCID mice by an established oesophageal cancer cell line (Kyse-30) and also in xenografts derived from primary tumours (ES2) as well as in surgical samples of oesophageal cancer.
Kyse-30 tumors were characterized by an heterogeneous inflammatory infiltrate, including $CD45^+$, $F4/80^+$ and $CD11b^+Gr1^+$ cells. Following short-term treatment of these tumors with L19mTNFα, we observed a significant increase in the necrotic area and a reduction of the (MVD) and of perfusion. Treatment reduced the overall percent of $Gr1^+CD11b^+$ myeloid and $Gr1^+$ cells. In contrast, infiltration of leucocytes and macrophages was not appreciably modulated by the immunocytokine. Preliminary studies in ES2 tumors showed that L19mTNFα caused a decrease in perfusion, without marked changes in the MVD.
In long-term studies, we observed an increase of both hypoxic areas and perfused vessels, as well as a decrease in necrotic areas in L19mTNFα-treated tumors compared to controls.
These observations form the basis for future studies concerning the combinatorial effects of L19mTNFα with anti-antiangiogenic agents, which are expected to improve the therapeutic efficacy of the vascular disrupting agent.

Angiogenesis unmasks the tumorigenic potential of dormant cancer cells through the DLL4-NOTCH3 interaction

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***OBJECTIVES**: While recognized that an angiogenic switch can drive outgrowth of dormant tumor cells, this effect has generally been attributed to the fostering role of angiogenesis and relatively little is known regarding contact-dependent signals between endothelial cells and cancer cells that may promote tumor growth. Here, we investigated expression of the Notch ligand Dll4 and activation of Notch receptors during angiogenesis in experimental models of tumor dormancy.*
***MATERIALS**: Human T lymphoblastic acute leukaemia (T-ALL) cells were injected subcutaneously in NOD/SCID mice with or without angiogenic factors (bFGF, VEGF); moreover, non-tumorigenic and tumorigenic variants of colorectal cancer cells were also utilized. Analysis of Notch1, Notch3, Dll4 and Notch target genes (pTα, HES1) expression was performed by Western blotting and real-time PCR. Silencing of Notch3 was obtained by lentiviral vector delivery of specific shRNA. Apoptosis was evaluated by Annexin V staining and*

analysis of PARP cleavage. Tumor growth rates were monitored by live imaging of tumors.
***RESULTS**: escape from tumor dormancy was invariably associated with augmented expression of the Notch ligand Dll4 on endothelial cells (EC) and increased expression of the intracellular domain of Notch3 and the Notch3-target genes in T-ALL and colorectal cancer cells. In vitro, treatment of human and murine EC with bFGF and VEGF up-regulated Dll4, induced activation of the Notch3 pathway in co-cultivated T-ALL cells, and protected T-ALL cells from stress-induced apoptosis, thus indicating that this heterotypic interaction could trigger Notch3 signalling and deliver a survival signal in the leukaemia cells. These effects were largely blocked by a neutralizing antibody to Dll4. Silencing of Notch3 by shRNA delivery resulted in increased apoptosis in vitro in T-ALL cells and it prevented tumor growth in mice. Importantly, Dll4 and Notch3 were found expressed in human T lymphoblastic lymphoma samples, thus showing the clinical relevance of these observations.*
***CONCLUSIONS**: These findings indicate that modulation of the Notch3 activation status by an heterotypic interaction between tumor cells and EC expressing the Notch ligand Dll4 can promote tumor growth. These observations can highlight a novel mechanism of angiogenesis-regulated escape from tumor dormancy.*

Leukocyte-derived TNF-α promotes tumor growth in a spontaneous model of mammary carcinogenesis

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Solid tumors comprise tumor cells and surrounding stromal cells, among which leukocytes and blood vessel cells foster tumor development and progression. Cancer cells and infiltrating inflammatory cells communicate through a complex network of pro-inflammatory molecules, mostly unknown. Critical is the role of the transcription factor NF-kB and of the inflammatory mediator TNF-α, which, through a multifaceted interaction, ultimately promote cancer development and progression, at least in some tumor types
On this line, we are investigating the role of TNF-α in HER-2/neuT transgenic mouse model of mammary carcinogenesis, which, because of the expression of the mutated rat neu oncogene under the control of the MMTV promoter, spontaneously develops mammary carcinomas during life time.
Bone-marrow transplantation (BMT) experiments from TNF-α KO mice into NeuT significatively delay the onset and reduce mammary tumor growth, indicating that the relevant source of TNF-α fostering tumor promotion is of BM origin. Whole mount analysis of mammary glands confirms the less severe tumor phenotype of mice transplanted with TNF-α KO BM. BMT experiments performed at different time points during tumor progression (8, 15, 20 weeks of age) indicate that TNF-α is critical during early steps of mammary tumorigenesis as well as later time points when carcinomas in situ are already present. This finding differs from other models such as skin carcinogenesis in which the role of TNF-α is relevant mainly for tumor initiation/promotion.
To clearly identify which leukocyte population is the relevant source of TNF-α BMT experiments with mice deficient for TNF-α in selected cellular compartments, such as monocytes, T cells and B cells.
From these experiments we expect to uncover the role of TNF-α in the various phases of mammary transformation and progression and to identify the best time window to neutralize its activity using specific monoclonal antibody.

INTERFERON-α counteracts the angiogenic switch and reduces tumor cell proliferation in a spontaneous prostatic cancer model

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Interferon-α (IFN-α) is the prototype of anti-angiogenic cytokines with recognized therapeutic activity in transplantable and orthotopic tumors, and also in prostatic cancer models. This activity has been mainly attributed to indirect effects, such as the down-regulation of pro-angiogenic factors, or direct effects on proliferation and motility of endothelial cells. Aim of this study was to investigate the effects of IFN-α on the angiogenic switch occurring during the early phases of tumor development in the Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model in which tumorigenesis is driven by an androgen-regulated early gene (T/t antigen) of SV40. To provide sustained IFN-α production, TRAMP mice were injected i.p. with lentiviral vectors, followed by analysis of the IFN-mediated transcriptional and biologic effects. Moreover, IFN-α activity was analyzed on TRAMP tumor-derived cell lines in vitro.
In the prostate of TRAMP mice, IFN-α administration resulted in sustained up-regulation of IFN-α-regulated genes endowed with anti-angiogenic and anti-proliferative functions, including guanylate binding protein 1 (GBP-1), IFI16 protein and CXCL10-11; since the levels of these transcripts increased at early time points following treatment, they could be primary mediators of the biologic effects of IFN-α. A moderate down-regulation of the basic fibroblast growth factor (bFGF) transcript was also observed. These transcriptional changes were accompanied by effects on the tumor vasculature, including reduction of intraductal microvessel density and increased pericyte coverage, and marked reduction of tumor cell proliferation, whereas tumor cell apoptosis was unchanged. Intriguingly, a subgroup of human prostatic tumors analyzed (15 out of 31) disclosed GBP-1 protein expression, and this correlated with the expression of another IFN-regulated protein, MxA, thus hinting at endogenous IFN-α expression in a subset of prostate cancer patients. Overall, our findings demonstrate that IFN-α is able to counteract the angiogenic switch and impair tumor cell proliferation during the early phases of prostatic cancer progression. The detection of GBP-1 and MxA expression in clinical samples of prostatic cancer may identify a tumor subset with distinct biological features.

Contributions of osteopontin to prostate carcinoma progression

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Osteopontin, a non-collageneous bone matrix protein, is produced in several human tumors and it has been investigated for use as a biomarker for advanced disease and as a potential therapeutic target in the regulation of cancer progression. Osteopontin is a secreted phosphoprotein highly glycosilated and contains several binding sites for integrins. In our study we investigated the potential role of osteopontin in the progression of prostate cancer (PCa). The in vitro study using PCa cell lines demonstrated a differential response to osteopontin according to the pattern of expression of integrins. In highly aggressive PCa cell line, PC3, chemotaxis and chemoinvasion analyses revealed a dose-dependent increase in cell movement induced by osteopontin and a strict dependence of cell invasion on alphavbeta3 integrin function. The pattern of protease expression was modified by osteopontin and was characterized by an upregulation of plasminogen activators. In the LNCaP

cell model, osteopontin stimulates cell proliferation in serum-free medium and colony growth at high dilution but this effect is visible only in presence of epidermal growth factor (EGF). Proliferation induced by OPN is accompanied by a sustained activation of EGF receptor (EGFR) whose phosphorylation is detectable up to 12 hr after treatment in association with EGF. The colocalization of integrin beta1, a ligand of OPN, and of EGFR on the cellular membrane, suggests that the association of these cell surface receptors may be the principal mechanism involved in the long-term activation of the EGFR.
These results suggest a leading role of integrins in the determination of tumor cell phenotype in presence of osteopontin and may drive the development of future therapeutic approaches according to the various molecular and clinical stages of PCa progression.

Understanding the complex crosstalk between p53 and the Estrogen Receptors at a polymorphic variant of the VEGF receptor Flt-1 promoter

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Recently we established that a C>T single nucleotide polymorphism (SNP) in the Flt-1 promoter generates a functional half-site p53 response element (RE-T). We also showed that p53 is required but not sufficient for Flt-1 transactivation and that there is cooperative interaction with ligand-bound estrogen receptors (ER) via an ER half-site response element (ERE) located 225nt upstream the p53 RE-T. Disruption of the ERE in a reporter construct containing a 1kb fragment of the Flt-1 promoter resulted in loss of p53 responsiveness in HCT116 (p53 wt, weakly ERβ positive) and U2OS (p53 wt, negative for ER) cells. Surprisingly, we have now observed that disruption of the ERE has no impact on transactivation in MCF7 cells (p53 wt, ERα and ERβ positive) treated with doxorubicin (doxo) to induce p53. Searches for transcription factor binding sites revealed another putative half-site ERE in the promoter fragment located 145bp downstream the p53 RE. Using site-directed mutagenesis, we showed that while the mutation of this second site has no impact, mutation of both EREs greatly reduced transactivation. Over-expression of ERα or ERβ in HCT116 phenocopied the MCF7 results in terms of the EREs contribution. To induce p53 in MCF7 cells we also used the thymidylate synthase inhibitor 5-FluoroUracil (5FU). Although 5FU was similar to doxo in stabilizing the p53 protein and inducing the p21 target gene, there was minimal transactivation of the Flt1-T construct, suggesting that doxo might have a specific impact on the p53, ER transcriptional cooperation or might enlist additional transcription factors/cofactors that contribute to the activation of the promoter. Using HCT116 cells (p53 wt and p53-null clones), which are heterozygous for the C>T SNP, we are also examining the expression of the endogenous Flt-1 gene, using qPCR. The Flt-1 transcript undergoes alternative splicing resulting in a soluble form of the receptor. These experiments are confirming the p53-dependent regulation of the Flt-1 gene and the different impact of doxo and 5FU. Notably, we are also observing an additional layer of complexity in the regulation of the gene, as the relative abundance of the two splice variants is differentially affected by the doxo treatment. This observation is currently being followed up with the development of assay systems probing stress-dependent stability of the two Flt-1 mRNAs, which have distinct 3'UTRs, as well as relative efficiency of alternative splicing.

PRIMA-1 synergizes with Adriamycin and to induce cell death in Non Small Cell Lung Cancer Cells

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p53-dependent apoptosis has an important role for the efficacy of cancer treatment, and tumours carrying mutant p53 are often resistant to chemotherapy. Among tumor types, Non-Small Cell Lung carcinomas (NSCLC) exhibit a strong resistance to drug- and radio-therapy and clinical response to these treatments is very rare. By using PARP cleavage and FACS analysis, we first investigated apoptosis induction by adriamycin or UV-C in three lung cancer cell lines carrying different p53 proteins: A549 (p53wt), LX1 (p53R273H), SKMes1 (p53R280K). After both treatments, A549 and LX1 underwent apoptosis, while SKMes1 did not. Recently, the new molecule PRIMA-1 has been shown to induce apoptosis in human tumour cells by restoration of the transcriptional activity of mutated p53s. Thus, we investigated the apoptotic potential of PRIMA-1 in our lung cancer cells. PRIMA-1 alone did not trigger apoptosis but significantly reduced cell viability, indicating that the reported ability of PRIMA-1 to induce a massive apoptotic response in cells expressing mutated p53 can depend on cell type. However, in combination with adriamycin, PRIMA-1 strengthen the adriamycin-induced apoptosis in A549 and LX1. Interestingly, SKMes1 cells that were not able to develop an apoptotic response following adriamycin alone, showed a strong PARP cleavage when PRIMA-1 was added after a low dose adriamycin treatment.
To investigate a correlation between the apoptosis observed and a restoration of p53 transactivation activity towards some effector genes, we look at the level of p21, Mdm2 and PUMA. ChIP experiments were also performed to explore the restoration of p53 binding activity. Our results indicated that the activation of PARP cleavage, observed after adriamycin/PRIMA-1 treatment, did not correlate with p21, MDM2 and PUMA activation. Thus, in NSCLC cell lines expressing mutated p53, PRIMA-1 or the combined adriamycin/PRIMA-1 treatments did not rescue the p53-transacrivation activity. However, other p53–dependent pathways are possible and remain to be explored. We hypothesized that in NSCLC cells, PRIMA-1 may induce cell death through pathways other than apoptosis but, depending on cell types, it may enhance the apoptotic potential of drugs already used in chemotherapy.

Integrin β_3 expression is regulated by let-7a miRNA in malignant melanoma

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Although integrin β_3 is known to play an important role in melanoma progression and invasion, regulation of integrin β_3 expression in melanoma has not been analysed in detail until today. As transcriptional regulation of integrin β_3 was ruled out by our analysis we concentrated on regulation by microRNAs (miRNAs). Comparing primary melanocytes and malignant melanoma cell lines, we found that one candidate miRNA, miR-let-7a, was lost in melanoma and sequence analysis suggested an interaction with the 3'untranslated region (3'UTR) of integrin β_3 mRNA. Transfection of melanoma cells with let-7a pre-miR™ molecules resulted in downregulation of integrin β_3 mRNA and protein expression. In addition, we cloned the 3'UTR of the integrin β_3 mRNA containing the let-7a target sequence into a reporter plasmid and revealed that let-7a negatively regulates reporter gene expression. The repressed expression of integrin β_3 accompanies with reduced invasive potential of melanoma cells transfected with synthetic let-7a molecules observed in Boyden chamber assays. On the other hand, induction of expression of integrin β_3 was achieved in melanocytes by transfection with let-7a anti-miRs resulting in invasive behaviour of transfected melanocytes. In summary, we determined miRNA let-7a to be an important regulator of integrin β_3 expression and showed that loss of let-7a expression is involved in development and progression of malignant melanoma.

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Zolendronic acid converted microenvironment hampers Her-2/neu driven mammary carcinogenensis

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Zoledronic acid (ZA) is one of the most potent aminobisphosphonates clinically available. In vivo preclinical data suggest that it is effective in hampering the growth of both transplantable and autochthonous mouse tumors in cancer-prone transgenic mice. Antitumor effects are observed when 80-120 μg/kg ZA are administered at least 3 times/week. The present study was undertaken to assess the ability of fractionated low doses of ZA (100μg/kg) administered only once a week to impair the onset of neu+ mammary carcinomas. Virgin BALB/c female mice transgenic for the activated rat Her-2/neu oncogene (BALB-neuT mice) provide one of the most aggressive and consistent models of autochthonous mammary carcinogenesis. Mammary tumor penetrance is 100% and its step-wise progression closely mimics that of breast carcinoma in women. Seven-week-old BALB-neuT mice displaying atypical mammary hyperplasia received 100 μg/kg ZA i.v. once a week for four weeks. A second course was given after 3 weeks of rest. This alternation of treatment and rest was repeated throughout the life of the mice. As compared with saline-treated control mice, those receiving ZA displayed a significant reduction in the mean number of carcinomas per mouse and a reduction of their growth rate, resulting in an extension of the tumor-free and overall survival. Whole mount analysis documented that inhibition of carcinogenesis is an early event already evident after 2 courses. To assess whether it was due to direct activity on tumors, the ability of ZA to inhibit BrdU uptake of a tumor cell line established from a BALB-neuT carcinoma (TUBO cells) was evaluated. Uptake was only significantly inhibited in TUBO cells cultured in the presence of 100 and 10 μM ZA, whereas their culture with 1 μM ZA had no effect. These data rule out the possibility that inhibition of cancer progression in our in vivo experiments was due to a direct effect of ZA on tumor cell proliferation, since the plasma ZA concentrations following the dose injected are well below 10 μM, as shown by pharmacokinetic studies.
Histological and immunohistochemical analysis of stromal and immune reactive cells from the hyperplastic and tumor lesions of ZA-treated mice revealed a strong reduction in the number of CD11b+ macrophages in the tumor stroma paralleled by a significant decrease in the number of blood vessels associated with the mammary lesions. Tumor associated macrophages (TAMs) are known to play an important role in promoting tumor growth and neovascularisation through the production of growth factors and angiogenic inducers, such as VEGF. This decrease in TAM density was associated with a strong reduction in VEGF production in the mammary tumors and in the VEGF present in the sera of ZA-treated mice. Since VEGF has a key role in inducing an M2 polarization of TAMs, we evaluated whether its reduction was associated with a shift of macrophage phenotype from M2 to M1. TAMs of ZA-treated mice displayed a markedly reduced secretion of IL-10, a representative type-2 cytokine, along with a markedly enhanced secretion of IFN- γ, a representative type-1 cytokine. Moreover, peritoneal macrophages isolated from ZA-treated BALB-neuT mice displayed an increase in iNOS expression, which is considered a hallmark of M1 polarized macrophages.
As a whole, our in vivo and in vitro data show that fractionated low doses of ZA impair the onset of neu+ mammary carcinomas, mainly by acting on the tumor microenvironment rather than on tumor cells directly.

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A novel mechanism for regulation of angiogenesis in cancers: hERG1 potassium channels regulate VEGF-A secretion and in vivo angiogenesis in leukemias and epithelial cancers.

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A relevant aspect of tumour growth is represented by intratumoral angiogenesis. It has been hypothesized that cancer cells begin to promote angiogenesis early in tumorigenesis. This "angiogenic switch" is characterized by oncogene-driven tumour expression of pro-angiogenic proteins. Tumour-associated hypoxic conditions also activate the Hypoxia-Inducible Factor 1a (HIF-1a), which promotes up-regulation of several angiogenic factors. The Vascular Endothelial Growth Factor-A (VEGF-A) is the most relevant angiogenic factor and its expression and secretion are regulated by both the two types of stimuli.
The aim of the present study was to analyse the regulation of VEGF-A expression and secretion through an unconventional mechanism, i.e. the activity of K+ channels encoded by the human ether à-go-go-related gene 1 (hERG1). hERG1 channels are are over- and mis-expressed in a wide variety of human cancers and their activity is involved in the regulation of neoplastic cell growth and progression (Arcangeli A and Becchetti A. Trends Cell Biol., 16: 631-639, 2006). hERG1 activity is modulated by hypoxia (Fontana et al., Biochem. Biophys. Res. Commun., 286: 857-862, 2001) and is linked to VEGF-A secretion in astrocytomas (Masi A et al., Br. J. Cancer, 93: 781-792, 2005). We recently provided evidence that hERG1 channels mediate VEGF-receptor-1 (FLT-1)-induced cell migration and signalling in acute myeloid leukaemias (AML). In vivo experiments in NOD/SCID mice injected with AML cells showed that herg1 over-expression confers a greater malignancy, witnessed by a higher bone marrow angiogenesis, and a stronger leukemia blast exit into the peripheral blood and into extramedullary organs. What is more, herg1 expression in AML patients correlated with a higher probability of relapse and shorter survival periods (Pillozzi S et al, Blood 110:1238-1250, 2007). The treatment of NOD/SCID mice injected with human leukaemia cells with specific hERG1 blockers, as well as with a monoclonal anti-hERG1 antibody raised in our laboratory, led to a significant decrease of leukaemia cell engraftment into the bone marrow and migration into the bloodstream and peripheral organs. Moreover, treated mice showed a longer survival compared to untreated mice.
We also provide evidence that hERG1 channels are functionally linked to the VEGF-A pathway in epithelial cancers. In fact a direct link between hERG1 and VEGF-A expression was found in human gastric cancer cell lines, where hERG1 activity apparently regulates VEGF-A expression and secretion. Gastric cancer cells lines subcutaneously injected into nu/nu mice displayed a growth pattern and a degree of intratumoral angiogenesis which were related to the amount of hERG1 channel expression. The inhibition of hERG1 channels with specific drugs led to a significant decrease of in vivo tumour growth.
On the whole we can conclude that hERG1 channel expression in cancer cells can represent a mechanism for the regulation of tumour angiogenesis. hERG1 channels can therefore be envisaged as novel targets for anti-neoplastic therapy.

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The actin cytoskeleton regulator hMena is downstream to HER2 activity in breast cancer

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hMena and the epithelial specific isoform hMena^{+11a} are cytoskeleton regulatory proteins belonging to the Ena/VASP family. hMena isoforms expression is modulated during breast carcinogenesis, and may represent an early marker of transformation. hMena/hMena^{+11a} expression level is influenced by ErbB receptors activity and hMena^{+11a} undergoes phosphorylation following EGF treatment of breast cancer cell lines, suggesting that hMena/hMena^{+11a} couple tyrosine kinase receptors to the actin cytoskeleton.
The aim of this study was to determine whether a cross-talk between HER-2 activity and hMena/hMena^{+11a} occurs in breast cancer, affecting the HER2 mitogenic activity.
The analysis of a panel of human breast cancer cell lines demonstrated that hMena/hMena^{+11a} are expressed at higher levels in cell lines overexpressing HER2. MCF7 cells transfected with HER2 showed, in parallel to the receptor activation, hMena/hMena^{+11a} overexpression, and an increase in hMena^{+11a} phosphorylation. This phosphorylation is further increased by EGF and NRG1 treatments, suggesting that hMena^{+11a} is downstream to signalling pathway of both EGFR/HER2 and HER2/HER3 heterodimers. Similarly, the treatment of SKBr3 and MCF7 cells with EGF and NRG1 respectively determined an up-regulation of hMena expression at mRNA and protein levels.
The hMena/hMena^{+11a} knock-down, although not able to directly influence the constitutive HER2 expression and phosphorylation, is able to inhibit the EGF and NRG1-mediated phosphorylation of HER2 and of the downstream p42/44 MAPK and AKT. Of functional significance, the hMena/hMena^{+11a} knock down cells are not responsive to the mitogenic activity of EGF and NRG1.
In a series of 292 breast cancer the higher frequency of hMena overexpressing tumors was found in the HER2 subtype and a significant correlation between hMena, proliferation index (Ki67 high, p=0,01), and phosphorylated MAPK (p=0,0001) and AKT (p=0,0001) was found.
Collectively these data provide new insights in the cross-talk between HER2 signalling pathways and cytoskeleton and point out on the relevance of hMena and hMena^{+11a} in the proliferative signature of epithelial breast tumors.
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hMena^{+11a} Isoform Serves as a Marker of Epithelial Phenotype and Sensitivity to EGFR Inhibition in Human Pancreatic Cancer Cell Lines

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hMena, a member of the Ena/VASP proteins family, is a cytoskeletal protein involved in the regulation of cell motility and adhesion. We have shown that hMena, is overexpressed in human breast tumors, and a splice variant termed hMena^{+11a} recently isolated from a breast cancer cell line with an epithelial phenotype, is phosphorylated

following EGF treatment. Of interest, experimental data suggest that hMena isoforms couple tyrosine kinase receptor signaling to the actin cytoskeleton.
In the present study we evaluated whether hMena and hMena^{+11a}, may represent key mediators of EGFR activity in pancreatic cancer cells and their expression may correlate with sensitivity to EGFR tyrosine kinase inhibitors.
The analysis of hMena expression in the pancreatic tumor model revealed that hMena is expressed in all of the pancreatic tumor cell lines tested as well as in the majority of the human tumor samples [primary (92%) and metastatic (86%)]. On the contrary, hMena^{+11a} isoform was exclusively expressed in the pancreatic cancer cell lines E-cadherin positive and negative for expression of vimentin and N-cadherin. Notably, these epithelial cell lines also displayed constitutive phosphorylation of the EGFR pathway and significant sensitivity to erlotinib.
In the epithelial BxPC3 pancreatic tumor cells EGF treatment up-regulated whereas Erlotinib down-regulated hMena/hMena^{+11a} expression. hMena/hMena^{+11a} knock-down reduced cell proliferation and MAPK and AKT activation in BxPC3 cells and promoted the growth inhibitory effect of Erlotinib.
Collectively, our data indicate that the hMena^{+11a} isoform is associated with an epithelial phenotype and identifies EGFR dependent cell lines that are sensitive to the EGFR inhibitor erlotinib.
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The endothelin axis promotes lymphatic formation and function in cultured lymphatic endothelial cells

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The lymphatic vasculature is essential for tissue fluid homeostasis, immune functions, and cancer metastasis. Although endothelin-1 (ET-1) axis plays a crucial role in angiogenesis and tumorigenesis, the biological relevance of ET-1 in lymphangiogenesis is unknown. We analyze the functions of ET-1 axis in highly purified human lymphatic endothelial cells (LEC) isolated from lymph nodes. We demonstrate that LEC produce ET-1, ET-3, and express the endothelin B receptor (ET_BR). In LEC ET-1 promotes proliferation and phosphorylation of AKT and p42/44 mitogen-activated protein kinase through ET_BR. Furthermore ET-1 enhances LEC differentiation into cord vascular-like structures and this effect is additive in the presence of vascular endothelial growth factor (VEGF)-C. In normoxic conditions, ET-1 is also able to upregulate the expression of the major mediators of lymphangiogenesis, including VEGF-A, VEGF-C and the VEGF receptor-3, similarly to hypoxia. In these cells ET-1 increases hypoxia-inducible factor-1α (HIF-1α) expression to an extent similar to hypoxia and its silencing by siRNA desensitises VEGF-C and VEGF-A production in response to ET-1 or hypoxia, implicating HIF-1α/VEGF as downstream signalling molecules of ET-1 axis. ET_BR blockade with the specific antagonist BQ788 inhibits ET-1-induced effects indicating that ET-1 through ET_BR can directly regulate different steps of lymphangiogenesis and by interacting with the HIF-1α-dependent machinery can amplify the VEGF-mediated lymphatic vascularization. These findings provide new insights into the molecular mechanisms that control growth and differentiation of lymphatic vessels and indicate that targeting pharmacologically ET_BR and related signaling cascade in lymphatic system may be therapeutically exploited in a variety of diseases including cancer.
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Mutations detection in some molecular markers on patients with colorectal cancer

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Colorectal cancer is one of the most frequent causes of cancer deaths in the world. The chromosomal instability pathway involves the activation of oncogenes (e.g. K-ras), the inactivation of tumour suppressor genes such as APC, p53, DCC are associated with microsatellite alteration. In colorectal cancer, instability at the level of chromosomes results in losses or gains of whole chromosomes or large portions of it.

The aim of this study was to analyze mucinous and nonmucinous colorectal adenocarcinomas in order to emphasize: i) the clinicopathological characteristics; ii) allelic imbalance/loss of heterozigosity or microsatellite instability (AI/LOH or MSI) status of APC and sPLA2 and expression of these early proteins; iii) establish a possible correlation between AI on APC gene and deletion or duplication detecting by MLPA method iv) late expressed protein such us mucins and p 53.

Immunoflorescence analysis for APC, sPLA2, mucin proteins, p53 and microsatellite markers designed in order to evaluate AI status of APC and sPLA2 were used to investigate twenty-seven patients with colorectal cancer. The phenotypic expression of mucin proteins (MUC1 and MUC2) and nuclear accumulation of p53 predict the clinical outcome of patients with colorectal cancer. The AI/LOH or MSI status on chromosome 1p and 5q, where PLA2G2A and respectively APC genes are localized, have been done on ABI PRISM 310 Genetic Analyzer by using a panel of 8 microsatellites loci. The whole APC gene was also investigated for large genomic rearrangements by MLPA using the same samples and also the 3 samples of DNA isolated from blood belong a health patient (as a control for this case). Following the specific PCR amplification, the probes were also analyzed by capillary electrophoresis on ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems).

Allelic imbalance analyses revealed that the most frequent alterations appeared to the microsatellite markers D1S2644, D1S2843, D1S199, and D1S234 loci mapped in 1p35-36.2 and D5S82, D5S421 loci mapped in 5q14-q23. At 6 patients out of 27 (22,22%) LOH/ MSI appears to the PLA2G2A locus and/or in the surrounding microsatellites loci. At 5 patients out of these 6, AI appear at the D1S234 locus. At 12 patients out of 27 patients (44,44%) LOH/MSI appears at different microsatelittes loci on chromosome 5q. At 9 patients aut of these 12, AI appear at the D5S421 locus. These results are confirmed by results on APC and PLA_2 type IIA expression by immunohistochemistry.

By MLPA method, any patients do not show the modifications at the blood samples, except only one patient diagnosed with FAP. This patient show two deletions, in blood and in tumor, at the region of promoter 2 and mutation 1309, although do not have any modification at the microsatellite loci. When we analyzed the tumor sample of the same patients, we found by MLPA technique, variable deletion starting with exon 10 up to exon 15 that are or not associated also with AI. These results underline the sporadic character of colorectal cancer from Romania and the clinical data also sustain that the alimentation habitude are the majority cause of colorectal cancer.

Taking together all these results show that only 22,22% LOH/ MSI appears to the PLA2G2A locus and 44,44% of patients have AI at microsatellite loci surround APC and for approximately half of theme (18,5%) we observed an association with MLPA. The difference of percent, which underlines the mutational mechanism of APC gene, is still difficult to explain as a consequence of chromosomal rearrangements complexity.

Mucinous adenocarcinoma is a distinctive form of colorectal cancer, where MUC1 protein expression is involved in the progression from the non-metastatic to the metastasis stage is associated with expression of p53 and MUC2 that underline their roll in analyse of metastatic potential of colorectal cancer.

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Synergist Anticancer Effects between Curcumin and Adriamycin in Human Breast Cancer

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Chemotherapy plays a critical role in virtually every phase of cancer treatment and rarely uses a single drug, but usually combine two or more drugs with different mechanisms of action. The synergist effects of curcumin (diferuloyl methane), the yellow colored chemopreventive agent from turmeric, and adriamycin, one of the most efficient medicine used in mammary cancer therapy, on cytotoxicity, proliferation and apoptosis in breast cancer cells (MDA-MB-231) were investigated for their eventual use in combination therapy. The purpose of using a natural compound and a chemotherapy drug was to determine whether greater therapeutic effects could be obtained by this combination rather than those provided by adriamycin alone which is known to have high toxicity and many side effects.
Cell viability was measured by MTT test; the protein expression of proliferation marker PCNA was evaluated by Western blot; the apoptotic process was relieved with TUNEL test which shows the nuclear fragmentation induced by treatments; the caspase 3 activity was quantified with ELISA; the invasive potential of tumor cells was evaluated by a chemotaxy assay based on Boyden chamber principle; the main matrix metalloproteinase (MMP) associated with tumor progression were evaluated by gelatin zimography. Studies were pursued with different exposure times.
The simultaneous exposure of tumor cells to curcumin (25μM) and adriamycin (0.5μM) caused a significant decrease of cell viability up to 65% compared to adriamycin alone 88%. Association of adriamycin and curcumin resulted in a marked inhibition of PCNA protein expression compared to adriamycin alone. The positive staining for DNA fragmentation was observed by TUNEL assay only in the presence of curcumin. After 24h of treatment, adriamycin induced a 1.5-fold increase in caspase 3 activity compared to untreated cells, but with curcumin present this increase was 2-fold. The increasing was time dependent. The enhancement of antitumor activity of adriamycin by curcumin was showed also by inhibition of matrix metalloproteinase activity, known to be involve in metastasis process. The MMP-2 activity displayed a decrease only in curcumin presence after 24h treatment. The capacity of tumor cells to migrate through the artificial Matrigel showed o reduction of invasive potential in both treatments with curcumin or adriamycin but the combined therapy did not lead to additional decrease.
Our results demonstrated that curcumin enhances cytotoxicity of adriamycin to breast cancer cells in culture. We conclude that curcumin may be useful as a potential activator of anticancer activity for adriamycin.

Endothelin A receptor links β-Arrestin to promote β-catenin signaling and metastasis in ovarian cancer cells

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Metastatic relapses remain a major challenge in the management of ovarian cancer. In this tumor, the activation of endothelin A receptor (ET_AR) by endothelin-1 (ET-1) promotes epithelial to mesenchymal transition (EMT), a metastatic early event. In search of downstream mediators in ET-1-induced EMT, we focused on β-*arrestin,* as adaptor protein of G-protein coupled receptors*. Here, we identify a new mechanism, whereby* β-*arrestin is a novel interaction partner of ET_AR to pro-*

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mote β-catenin stabilization and transcriptional activity forming two trimeric signaling complexes. The first through the interaction with c-Src, consequent epidermal growth factor receptor (EGFR) transactivation resulting into tyrosine phosphorylation of β-catenin, the second through the direct association with axin. Z-stack analyses of HEY cells by confocal microscopy revealed that ET-1 induced the membrane translocation of β-arrestin and its dephosphorylation on serine-412, leading to the formation of an ET_AR/β-arrestin/Src signaling complex ("signalplex"). By expressing WT- or mutant β-arrestin-1, we showed that this signalplex was crucial for EGFR transactivation, that, in turn, controlled the degree of β-catenin protein stabilization by affecting its tyrosine (Y) phosphorylation. The Y-phospho β-catenin translocated to the nucleus and bound the TCF4 transcription factor, thus representing a transcriptional active pool. Concurrently, ET_AR activation leads to the association of the β-arrestin with axin, contributing to release of GSK-3β from axin-containing degradation complex and to β-catenin stabilization. At the functional level, β-arrestin siRNA inhibited β-catenin/TCF transcriptional activity and cell invasion, delineating previously unknown biological functions of β-arrestin in EMT-related signaling. ZD4054, a small molecule ET_AR antagonist, prevented the functional role of β-arrestin in the interplay between ET_AR and β-catenin pathway in invasive signaling. *In an xenograft model of ovarian i.p. metastasis, ZD4054 treatment significantly inhibited tumor burden and metastatic tumor nodules, that were maximally impaired by combination of ZD4054 and gefitinib, an EGFR inhibitor. Interestingly, HEY cells that express the S412D mutant β-arrestin 1 metastasized at a reduced rate, highlighting the importance of β-arrestin–mediated EGFR signaling in the ovarian cancer metastasis formation. Our results indicate that β-arrestin links ET-1 axis to β-catenin signaling, and hence identify new therapeutic opportunities for ovarian cancer. Supported by AIRC, Ministero della Salute, AstraZeneca.*

The role of the Prolyl-isomerase Pin1 in cancer: a fine-tuner and/or a dangerous amplifier of cell proliferation?

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Cancer results from perturbations in signaling pathways controlling cellular and tissue behavior and genome stability, which invariably produce aberrant cell proliferation. Recently, a crucial role has emerged for phosphorylation-dependent conformational changes caused by the prolyl-isomerase Pin1 on several highly interconnected substrates. Pin1 binds and catalyzes cis/trans isomerization of prolines on S/P or T/P motifs in many proteins involved in different processes. Work done in our laboratory has established that Pin1 controls the functional activation of p53 tumor suppressor in response to DNA damage and is also required to control the activity of the p53 homologue p73 and its binding to apoptotic promoters. Under physiological conditions Pin1 thus behaves as an essential fine tuner of the coordinated activation of the p53 protein family network. Pin1 is itself under control of signaling pathways that could become aberrantly activated in cancers and high levels of Pin1 have been observed in several tumors. In search for new substrates we recently identified Notch1 as a new Pin1 target. Pin1 regulates the activation of Notch signaling at several levels while Notch directly activates Pin1 at transcriptional level. Tumors over expressing Pin1 have high levels of Notch and Hes1. This indicates that Notch and Pin1 could establish a positive feed back loop to enhance cell proliferation and tumor growth. All these evidences suggest that depending on the cellular context Pin1 could behave as a fine tuner or a dangerous global amplifier of signaling pathways thus contributing to cancer progression.

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2009
Pezcoller Foundation-AACR International Award for Cancer Research

The prestigious Pezcoller Foundation–AACR International Award for Cancer Research was established in 1997 to annually recognize a scientist:

- *who has made a major scientific discovery in basic cancer research or who has made significant contributions to translational cancer research;*
- *who continues to be active in cancer research and has a record of recent, noteworthy publications; and*
- *whose ongoing work holds promise for continued substantive contributions to progress in the field of cancer.*

The Award is intended to honor an individual scientist. However, more than one scientist may be co-nominated and selected to share the Award when their investigations are closely related in subject matter and have resulted in work that is worthy of the Award. In the rare event that there are dual winners of the Award, the cash award will be shared equally between them, and the AACR Executive Committee will determine which of the two co-recipients will present the Pezcoller-AACR Award Lecture at the AACR Annual Meeting.

Candidates for the Award will be considered by a prestigious international Selection Committee of renowned cancer leaders appointed by the President of the AACR and the Council of the Pezcoller Foundation. The Committee will consider all nominations as they have been submitted; the Committee may not combine submitted nominations, add a new candidate to a submitted nomination, or otherwise make alterations to the submitted nominations. After careful deliberations by the Committee, its recommendations will be forwarded to the Executive Committee of the AACR and the Council of the Pezcoller Foundation for final consideration and determination.

Selection of the Award winner will be made on the basis of the candidate's scientific accomplishments. No regard will be given to race, gender, nationality, or religious or political view.

The Pezcoller Foundation was established in 1980 by Professor Alessio Pezcoller, a dedicated Italian surgeon who made important contributions to medicine during his career and who, through his foresight, vision and generous gift in support of the formation of the Foundation, stimulated others to make significant advances in cancer research. Previously the Pezcoller Foundation, gave a major biennial award for outstanding contributions to cancer and cancer-related biomedical science, in collaboration with the ESO-European School of Oncology.

The American Association for Cancer Research (AACR) was founded in 1907 by eleven physicians and scientists dedicated to the conquest of cancer and now has over 25,000 laboratory, translational, clinical and epidemiological scientists engaged in all areas of cancer research in the United States and in more than 60 other countries around the world.

The AACR is dedicated to its mission of preventing and curing cancer through the communication of important scientific results in a variety of forums including publications, meetings and training and educational programs. Because of the commitment of the Pezcoller Foundation and the AACR to scientific excellence in cancer research, these organizations are now collaborating annually on the presentation of the Award. This will strengthen international collaborations and will be a catalyst for advancements in cancer research internationally.

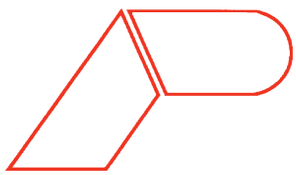
The winner of the Pezcoller Foundation-AACR International Award for Cancer Research will give an award lecture during the AACR Annual Meeting (Denver, April 18-22 2009), and the memorial Korsmeyer lecture at the VIMM in Padua and will receive the award in a ceremony at the Foundation's headquarters in Trento, Italy (May, 2009).

The award consists of a prize of € 75.000 and a commemorative plaque.

Nomination Deadline: Monday, September 15, 2008

Questions about the nomination process:
Monique P. Eversley, Staff Associate - American Association for Cancer Research, 17th Floor, 615 Chestnut Street, Philadelphia, PA 19106-4404 - Tel. +1 (267) 646-0576; E.mail: eversley@aacr.org - www.aacr.org

Notes



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