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Editorial: June 2006

In this issue, we are pleased to announce the winner of the 2006 Pezcoller Foundation-AACR International Award for Cancer Research, Tadatsugu Taniguchi, Ph.D. Professor and Chair of the Department of Immunology, Graduate School of Medicine and Faculty of Medicine, University of Tokyo.

The Selection Committee met in Philadelphia in December 2005 and was made up of the following: Eric J. Stanbridge, Ph.D. as Chairperson and Bruno Amati, Ph.D. Anton J.M. Berns Ph.D. Elizabeth H. Blackburn, Ph.D. Suzanne A. Eccles Ph.D. Manel Esteller, Ph.D. Napoleone Ferrara, M.D. Eileen P.White, Ph.D.

2006 Pezcoller Foundation - AACR International Award for Cancer Research
Dr. Bernardi and Dr. Taniguchi

The motivation of the Award: Dr. Taniguchi was selected for a series of groundbreaking discoveries that have had a major impact on cancer research and molecular immunology. These include the isolation and characterization of the first cytokine genes (interferon-β and interleukin-2), the discovery of the IRF family of transcription factors, and the elucidation of their functions.

Dr Taniguchi's seminal studies have contributed enormously to our understanding and treatment of cancer. His groundbreaking studies on interferons and cytokines, particularly IL-2, have not only advanced our understanding of the role these factors play in cancer and its control, but have also provided important therapeutic opportunities. The same is true of his elucidation of the functions of the IRF family of proteins which have had an important impact on the study of the immune response to cancers and their role in cell cycle control and genomic stability.

Taniguchi gave the Pezcoller Lecture in Washington at the AACR Meeting on 2nd April 2006 and also the inaugural Korsmeyer Lecture at the VIMM in Padua before reaching Trento for the official Award Ceremony in the Buonconsiglio Castle on May 5.

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Prof. Taniguchi also received a Laurea Honoris Causa in Medicine at the University of Verona.
The Korsmeyer Lecture was given to honour the memory of the late Stanley Korsmeyer who received our Award two years ago.
We are also proud to celebrate the twentieth anniversary of the Pezcoller Foundation which was established in 1986.
In this issue we are presenting the speakers' abstract and the posters of the 18th Pezcoller Symposium on "Tumor Microenvironment: Heterotypic Interactions".
At the back we have also inserted the call for the 2007 Pezcoller Foundation-AACR International Award for Cancer Research.

Gios Bernardi M.D.
The Pezcoller Foundation President
and Editor of the Journal

18th Pezcoller Symposium
TUMOR MICROENVIRONMENT: HETEROTYPIC INTERACTIONS
June 27-29, 2006 - Trento, Italy

ABSTRACTS OF ORAL PRESENTATIONS

KEYNOTE

Mechanisms of Malignant Progression

Jing Yang, Sendurai Mani, Kimberly Hartwell, Piyush Gupta and ***Robert A. Weinberg,*** *Whitehead Institute for Biomedical Research, Department of Biology, MIT, Cambridge MA 02142 USA*

The mechanisms whereby carcinoma cells acquire the ability to invade and metastasize are complex and remain poorly understood. Carcinoma cells within primary tumors are generally poorly invasive and migratory, and thus lack many of the attributes that are required in order to launch the invasion-metastasis cascade. This cascade is quite complex, and its completion requires that cancer cells become locally invasive, intravasate, translocate to distant tissue sites, extravasate, form a micrometastasis, and eventually colonize their new tissue microenvironment by spawning a macroscopic metastasis. These biological steps are as complex as the preceding ones that lead initially to the formation of a primary tumor, raising the question of whether a commensurate number of mutational alterations are required in order to complete this cascade.
Work in our laboratory has studied four transcription factors – Twist, FOXC2, Slug, and Goosecoid – that are expressed in various steps of early embryogenesis and enable the completion of key morphogenetic steps, including gastrulation and emigration from the neural crest. When ectopically expressed in epithelial cells, each of these transcription factors is able to induce a mesenchymal phenotype in these cells, which results in the downregulation of epithelial markers, such as cytokeratins and E-cadherin, and the acquisition of mesenchymal markers, such as vimentin, N-cadherin, fibronectin and even α-smooth muscle actin. This shift in cell phenotype, often termed the epithelial-mesenchymal transition (EMT), thereby enables carcinoma cells to acquire the phenotypes that are associated with high-grade malignancy, including the launching of metastatic dissemination. Interestingly, expression of these transcription factors is often induced by contextual signals, such as TGF-β, that are known to be able to elicit

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the EMT in epithelial cancer cells. Indeed, it becomes plausible that the actions of these transcription factors, either singly or in concert, can empower primary cancer cells to complete all the steps of the invasion-metastasis cascade up to and including the formation of micrometastases. We suspect that the acquisition of colonizing ability requires disseminated cancer cells to adapt to their newfound microenvironment by developing cell-biological capabilities that are distinct from those programmed by these transcription factors.

Expression of various of these transcription factors can be associated with various types of malignancies, suggesting that different types of human tumors, depending on their tissue of origin, opportunistically upregulate expression of different subsets of these long-dormant transcription factors, thereby acquiring the ability to execute the invasion-metastasis cascade. This activation appears to depend often on the receipt of heterotypic signals that are released by the activated inflammatory stroma of the primary tumor, which thereby acts together with the mutant genotype of the primary carcinoma cells to induce expression of these key regulators. It is plausible that once carcinoma cells have translocated to a site of distant dissemination, their microenvironment may fail to release the types of heterotypic signals that led previously to the induction of the EMT in the context of the primary tumor. Under such conditions, the disseminated carcinoma cells may revert, via a mesenchymal-epithelial transition (MET) to the phenotype of their progenitors that resided in the heart of the primary tumor, illustrating the reversibility of the EMT and its dependence on non-genetic changes in the cell.

Relevant publications:

1. *Yang, J., Mani, S.A., Liu Donaher, J., Richardson, A., Ramaswamy, S., Gitelman, I., and Weinberg, R.A. (2004) Twist, a master regulator of morphogenesis plays an essential role in tumor metastasis. Cell, 117:927-939.*
2. *Gupta, P.B., Kuperwasser, C., Brunet, J-P., Ramaswamy, S., Kuo, W-L., Gray, J.W., Naber, S.P., and Weinberg, R.A. (2005) The melanocyte differentiation program predisposes to metastasis after neoplastic transformation. Nature Genetics 37, 1047-1054.*

Stromal and Epithelial TGF-beta Signaling in Carcer.

***Harold L. Moses**, Vanderbilt-Ingram Cancer Center and Department of Cancer Biology, Vanderbilt University Medical Center, Nashville, Tennessee USA*

*In order to examine the roles of epithelial cell autonomous TGF-β signaling in cancer, we have generated mice with **loxP** sites flanking exon 2 of the type II receptor gene, **Tgfbr2**, and crossed them with mice expressing Cre driven by different epithelial specific promoters. Loss of TGF-β signaling in six different epithelial cells gave a minimal phenotype. However, when challenged with oncogene expression or tumor suppressor gene impairment, there was rapid development of invasive and metastatic carcinomas supporting the hypothesis that epithelial cell autonomous TGF-β signaling is tumor suppressive in both early and late stages of carcinogenesis [1].*

The importance of stromal-epithelial interactions in mammary gland development and tumorigenesis is well established. These interactions likely involve autocrine and paracrine action of multiple growth factors, including members of the TGF-beta family, which are expressed in both stroma and epithelium. Again, to accomplish complete knockout of the type II TGF-beta receptor gene in mammary stromal cells FSP1-Cre and Tgfbr2flox/flox mice were crossed to attain Tgfbr2fspKO mice [2]. The loss of TGF-beta responsiveness in fibroblasts resulted in intraepithelial neoplasia in prostate and invasive squamous cell carcinoma of the forestomach with high penetrance by six weeks of age. Both epithelial lesions were associated with an increased abundance of stromal cells. Activation of paracrine hepatocyte growth factor (HGF) signaling was identified as one mechanism for stimulation of epithelial proliferation. Tgfbr2fspKO mice also exhibit defective mammary ductal development, characterized in part by increased ductal epithelial cell turnover associated with an increase in stromal fibroblast abundance. Tgfbr2fspKO mammary fibroblasts transplanted with mammary

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carcinoma cells promote growth and invasion, which is associated with increased activating phosphorylation of the receptors: erbB1, erbB2, RON, and c-Met [3]. Furthermore, the increased receptor phosphorylation correlates with increased secretion of the cognate ligands by Tgfbr2fspKO fibroblasts. Treatment of tumor cells with fibroblast conditioned medium leads to increased tumor cell proliferation and motility, which are blocked by addition of pharmacologic inhibitors of TGF-alpha signaling or neutralizing antibodies to MSP, HGF, or c-Met. A small molecule inhibitor of Met signaling inhibited tumor growth, invasion and metastasis in vivo. These studies demonstrate a significant role for stromal TGF-beta signaling in mammary tissue homeostasis and mammary tumor progression via regulation of TGF-alpha, MSP, and HGF signaling pathways.

Thus, TGF-beta signaling in fibroblasts modulates the growth and oncogenic potential of adjacent epithelia in selected tissues. The Tgfbr2fspKO mouse model illustrates that a signaling pathway known to suppress cell-cycle progression when activated in epithelial cells can also have an indirect inhibitory effect on epithelial proliferation when activated in adjacent stromal fibroblasts ***in vivo****. Loss of this inhibitory effect can result in increased epithelial proliferation and may even progress to invasive carcinoma in some tissues.*

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3. *Cheng N, Bhowmick NA, Chytil A, Gorksa AE, Brown KA, Muraoka R, Arteaga CL, Neilson EG, Hayward SW, Moses HL: Loss of TGF-beta type II receptor in fibroblasts promotes mammary carcinoma growth and invasion through upregulation of TGF-alpha-, MSP- and HGF-mediated signaling networks. Oncogene 2005;24:5053-5068.*

The urokinase receptor: cell migration, cell proliferation and cancer.

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The urokinase receptor (uPAR) is a GPI-anchored protein that facilitates extracellular proteolysis by binding the urokinase-plasminogen activator (uPA), and stimulates cell signaling via the interaction with extracellular proteins like vitronectin and trans-membrane proteins, including tyrosine kinase receptors and integrins. The importance of uPAR in human tumors is underlined by the stringent correlation between the level of uPAR (in the tumor, blood or urine) and relapse-free survival. Indeed, in colon cancers with low uPAR expression, the rate of death is indistinguishable with that of non-cancer individuals of the same age.
The availability of viable mice deleted in the uPAR gene ($uPAR^{-/-}$) allows the analysis of the role of uPAR in physiologic and pathologic processes. In particular we have analyzed the role of the uPAR genotype in vivo in the mobilization of hematopoietic stem cells, in skin wound healing and skin carcinogenesis. The effect of the uPAR genotype in vitro has been analyzed on the growth properties of non-transformed and RasV12-E1a-transformed mouse embryo fibroblasts, in osteoblasts and keratinocytes.
The results overall show a direct, yet complex, intervention of uPAR in both cell proliferation, migration and carcinogenesis, and indicate that the effect of uPAR may affect or be affected by oncosuppressor or oncogenic proteins.

Matrix Metalloproteinases as mediators of host:tumor communication

Lynn M. Matrisian, *and Lisa J. McCawley, Dept. Cancer Biology, Vanderbilt University, Nashville TN USA*

Matrix Metalloproteinases (MMPs) have long been considered to be major contributors to tumor invasion and progression by virtue of their ability to degrade the ba-

sement membrane and extracellular matrix components. In recent years, it has been realized that MMPs function as regulatory molecules in addition to their role in matrix barrier destruction. This function results in either the inactivation or the activation and solubilization of biologically active factors that mediate the communication between the tumor and its microenvironment.

The two-stage model of skin carcinogenesis is a classic mouse model that allows the molecular dissection of the processes of tumor initiation, promotion, and progression. The MMP family member MMP3, also known as stromelysin-1, is expressed at low levels in the stromal component of benign papillomas and malignant squamous cell carcinomas generated in this model (1). With continued treatment with a complete carcinogen, the tumors convert to spindle cell carcinomas with a high probability of metastasis. At this stage, MMP3 mRNA is expressed in both the stroma and the malignant keratinocytes (2). This expression pattern, combined with the assumption that MMP3 contributes to tumor invasion and metastasis by virtue of its ability to degrade basement membrane IV collagen, laminin, and proteoglycans, led to the hypothesis that MMP3 promotes tumor progression and metastasis.

To test this hypothesis, MMP3 levels were manipulated genetically in mouse skin and the mice challenged with either DMBA followed by repeated treatments with the tumor promoter TPA, or repeated treatments with the full carcinogen MNNG. The results of these experiments disproved the hypothesis. Although there was no difference in the number of papillomas/mouse, these tumors grew much faster in MMP3-null mice treated with either DMBA/TPA or MMNG than in control mice (3). In addition, MMP3-null mice had a high percentage of more advanced spindle cell carcinomas than wildtype mice, indicating faster disease progression in the absence of MMP3. Mice with keratinocyte-targeted overexpression of MMP3 had reduced tumor multiplicity vs controls, suggesting a reduction in tumor establishment with increased MMP3 levels. Cultured squamous cell carcinoma cells transfected with MMP3 had reduced tumor take, further confirming a protective effect of MMP3 on tumor development.

To determine the cellular mechanism responsible for the protective effect of MMP3 on skin tumor progression, the inflammatory cell infiltrate in chemically-induced tumors in MMP3-null mice were compared to controls. Tumors from MMP3-null mice demonstrated severely reduced levels of macrophages and a statistically significant reduction in the number of neutrophils in tumors at all stages (3). To analyze the earliest stages of neutrophil infiltration, transgenic mice overexpressing MMP3 in the skin were wounded and neutrophil infiltration measured both immunohistochemically and by cell sorting. Neutrophil counts were significantly higher in K5/6-MMP3 mouse skin compared to wildtype controls at day 1, yet were significantly reduced by 2-3 fold at day 4. The classic pattern of immune infiltration is therefore modulated by MMP3 so that neutrophil infiltration is accelerated, presumably aiding in the acute protective response of the innate immune system. We propose that in a chronic inflammatory state (eg tumorigenesis), MMP3 stimulates recruitment of innate immune cells throughout squamous cell carcinoma progression that acts in an anti-tumorigenic, wound healing function. Modulation of the infiltration of innate immune cells therefore provides a mechanism by which MMP3 levels influence both early and late stages of tumor progression in the murine model of skin carcinogenesis.

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High molecular weight-melanoma associated antigen-mediated interactions of melanoma cells with extracellular matrix. Inhibition by antibodies.

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The human high molecular weight-melanoma associated antigen (HMW-MAA) is a highly glycosylated integral membrane chondroitin sulfate which has conserved its structural and functional characteristics through phylogenetic evolution. It consists of an N-linked 280 kDa glycoprotein component and a 450 kDa chondroitin sulfate proteoglycan component. The two components share the same core protein. This antigen has a restricted distribution in normal tissues, but is expressed at high level in a high percentage of melanoma and glioma lesions. Recent studies have shown that this antigen is also expressed by putative breast carcinoma stem cells. Evidence in animal model systems and in patients with acral lentiginous melanoma is compatible with a role of HMW-MAA in the interaction of melanoma cells with extra cellular matrix and in their metastatic potential. Experimental and clinical evidence suggests that the function of HMW-MAA can be inhibited by antibodies which are administered passively or are induced by HMW-MAA mimics, i. e. anti-idiotypic (anti-id) monoclonal antibodies (mAb). Crystallization studies have defined the structural basis of the mimicry of HMW-MAA by the anti-id mAb MK2-23 which has been used as an immunogen to implement active specific immunotherapy in patients with melanoma. Furthermore these studies have shown that a 15 amino acid long peptide derived from the heavy chain of the anti-id mAb MK2-23 plays a major role in the HMW-MAA mimicry. Characterization at the atomic level of the interactions of the mAb MK2-23 derived peptide with HMW-MAA-specific antibodies has identified the residues crucial for these interactions. This information is being used to modulate the extent of HMW-MAA mimicry by anti-id mAb MK2-23 and to investigate the relationship between extent of HMW-MAA mimicry by anti-id mAb MK2-23 and its immunogenicity, as defined by its ability to elicit HMW-MAA-specific antibodies in hosts with constitutive HMW-MAA expression.

Inflammation and Cancer: The TNF- connection

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Tumour necrosis factor alpha, TNF-α, is a member of the TNF/TNFR cytokine superfamily. In common with other family members, TNF-α is involved in maintenance and homeostasis of the immune system, inflammation and host defence, with critical and non-redundant roles in innate and adaptive immunity (1). TNF-α is released pre-formed or induced de novo in response to a range of pathogenic stimuli, in the vanguard of the inflammatory cytokine cascade. However, there is a 'dark side' to this powerful cytokine; it is now clear that, especially in middle and old age, TNF-α is involved in pathological processes such as chronic inflammation, autoimmunity and, in apparent contradiction to its name, malignant disease. In recent years there has been a resurgence of interest in the parallels between chronic inflammation and cancer, and it is no surprise that TNF-α has become a focus of this research. This presentation will discuss the involvement of TNF-α in the inflammatory network that contributes the malignant process, and consider the possibility that TNF-α may be a target for cancer therapy.

There are five major areas of evidence that link cancer and inflammation: many chronic inflammatory diseases are associated with an increased risk of cancer; cancers arise at sites of chronic inflammation; many of the cells associated with chronic inflammatory processes are found at sites of inflammation; chemical mediators of inflammation are found in many cancers; deletion of the cellular or chemical mediators of inflammation has inhibits cancer development and spread, and long term use of non-steroidal anti-inflammatory agents reduces

risk of mortality from some cancers (2-4). One of the key chemical mediators implicated in these inflammation-associated cancers is TNF-α.

True to its name, high doses of loco-regional TNF-α can cause haemorrhagic necrosis via selective destruction of tumour blood vessels and generation of specific T cell anti-tumour immunity in both mouse models and human cancers (5). However, when produced in the cancer microenvironment, TNF-α can act as an endogenous tumour promoter (6). There is now substantial evidence that cancer - and stromal cell - derived TNF-α is involved in promotion and progression of experimental and human cancers, with pathways leading to activation of the NF-κB and AP-1 transcription factor complexes being key intracellular links, and general or cell selective deletion/inhibition of TNF-α reducing the incidence of experimental cancers (4, 7-10). TNF-α is a key mediator of tumour cell:macrophage communication in vitro and in vivo (11, 12) and is associated with cancer progression and metastases in a range of syngeneic and transplantable cancer models (13).

TNF-α is also frequently detected in biopsies from human cancer, produced either by epithelial tumour cells, as for instance in ovarian and renal cancer; or stromal cells, as in breast cancer (reviewed in (6, 13)). TNF-α production by tumours has been associated with a poor prognosis, loss of hormone responsiveness, and cachexia/asthenia. TNF-α is not normally detected in plasma or serum of healthy individuals but can be detected in some cancer patients, invariably those with advanced disease and poor prognosis. In addition, many cancer cell lines secrete low pg amounts of TNF-α in vitro, along with other cytokines and chemokines such as IL-1, IL-6, IL-8, M-CSF, CCL2 and CXCL12. Abnormal secretion of these inflammatory cytokines is thought to be one of the major causes of constitutive NF-κB activity in cancer cells (14). TNF-α produced chronically at low picogram levels in the tumour microenvironment, whether by tumour or stromal cells (or most likely by both) may also cause direct DNA damage, may have anti-apoptotic or mitogenic activity, may mediate tumour:stromal cell interactions and induce a range of MMPs as well as cytokines and chemokines. Moreover TNF-α can synergise with growth factors such as TGF-β and EGF. All of these actions may promote tumour development. However, the mechanism by which tumour cells produce these low pg quantities of TNF-α in vitro and in vivo in the absence of obvious inflammatory stimuli, is not clear. In summary, the evidence to date suggests that cancer cell or stromal cell production of TNF-α is involved in the development of a range of experimental tumours, is partially responsible for NF-κB activation in initiated tumour cells and for the cytokine network in found in human cancer.

Cytokine antagonist therapies are effective treatments in some inflammatory diseases. Inhibitors of TNF-α, in particular, have been used successfully in over half a million patients worldwide (15). Although there is good evidence from experimental cancer studies that TNF-α can act as an endogenous tumour promoter at an early stage of disease, increased susceptibility to opportunistic infections such as M. Tuberculosis in patients taking TNF-α antagonists (16), would preclude wide scale trial of these drugs as preventative agents against sporadic cancer. However, there is a 'natural' experiment in progress to will test the hypothesis that TNF-α antagonists may have cancer preventative actions. Large cohorts of patients receiving TNF-α antagonist therapy for a variety of chronic inflammatory conditions are being carefully monitored to see if this therapy has an impact on cancer risk.

In clinical studies in rheumatoid arthritis patients, TNF-α antagonists inhibited inflammatory cytokines, MMPs, angiogenic activity and leucocyte trafficking to sites of inflammation (15), all actions that could be useful in biological treatments for cancer. On the basis of these results and data described above, a number of clinical trials of TNF-α antagonists alone, and in combination with other therapies, are currently underway in patients with solid and haematological malignancies and some preliminary results will be presented. In patients with advanced solid cancer TNF-α antagonists are well tolerated with evidence of biological activity and possible disease stabilisation.

There is still much to be learnt about the role of TNF-α in early and advanced cancer, but a combination of good cancer models and careful clinical investigation should yield information that may lead to patient benefit.

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Immune Cells as Targets for Cancer Prevention

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During the early development of cancer, many physiological processes occur in the vicinity of 'young tumor cells' that are similar to processes that occur during embryonic development and to healing of wounds in adult tissue, e.g., inflammation, angiogenesis and tissue remodeling (Coussens and Werb, 2002). During wound healing, inflammatory cells are recruited to sites of injury to eliminate potential bacterial infection as well as to facilitate healing by providing growth factors and proteases that are essential to the process. In so doing, a new blood supply is also formed that further helps the tissue heal. When 'healing' is complete, inflammation resolves and the tissue returns to its former state. Several of these parameters are conserved during tumor development; however, instead of initiating a 'healing' response, immune cells that infiltrate premalignant tissue provide growth-promoting factors and proteolytic enzymes that promote tumor development (Balkwill et al., 2005; Coussens and Werb, 2002). These observations are significant in light of the fact that individuals suffering from chronic inflammatory diseases harbor

a greatly increased risk for cancer development in tissues infiltrated by activated leukocytes (de Visser et al., 2006), and indicate that by identifying molecular mediators regulating onset, activation and maintenance of inflammation in the neoplastic microenvioronement, we will reveal regulatory events/molecules that can be effectively targeted with anti-cancer therapeutics.

In trying to understand how chronic inflammation in premalignant tissues potentiates cancer development, we have genetically manipulated the host immune response and/or presence of various leukocyte-derived proteases (Coussens et al., 2000) in a transgenic mouse model of epithelial carcinogenesis, e.g. K14-HPV16 mice (Coussens et al., 1996). In so doing, we have found that inflammatory mast cells and granulocytes contribute in a dominant manner to the activation of neoplasia-associated tissue remodeling, angiogenesis and epithelial hyperproliferation and overall cancer incidence via their release of proteolytic enzymes of the cysteine, serine and metallo classes. Moreover, we have identified a critical regulatory pathway underlying persistent recruitment of innate immune cells toward developing neoplasms. We found that genetic elimination of mature T and B lymphocytes limited neoplastic progression to development of benign epithelial hyperplasias that failed to recruit innate immune cells into premalignant tissue (de Visser et al., 2005). Adoptive transfer of B lymphocytes or serum from K14-HPV16 mice into T and B cell-deficient/HPV16 mice was sufficient to restore innate immune cell infiltration into premalignant tissue and to reinstate necessary parameters for full malignancy, e.g., chronic inflammation, angiogenic vasculature and hyperproliferaive epidermis. These findings support a model in which B lymphocytes and/or activation of humoral immune responses in peripheral tissues is required for establishing chronic inflammatory states that promote de novo carcinogenesis, and support the concept that oncogene expression in 'initiated' cells alone is not sufficient for full malignant progression (Bissell and Radisky, 2001). Instead, additional signals provided by adaptive and innate immune cells are required for elaboration of the malignant state. Our results, in combination with results from other investigators, suggest that pharmacological interventions targeting activation and/or recruitment of innate immune cells towards premalignant tissue represent viable cancer chemopreventative strategies. To be presented, will be recent results evaluating immuogobulin-activation of innate immune cells in K14-HPV16 transgenic mice and identification of the lysosomal cysteine protease, e.g., cathepsin C, as a critical mediator of inflammation and angiogenesis associated with epithelial cancer development.

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Smouldering and polarized inflammation in tumor progression

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Macrophages are key orchestrators of chronic inflammation. They respond to microenvironmental signals with polarized genetic and functional programmes. M1 macrophages which are classically activated by

microbial products and interferon- , are potent effector cells which kill microorganisms and tumors . In contrast, M2 cells, tune inflammation and adaptive immunity; promote cell proliferation by producing growth factors and products of the arginase pathway (ornithine and polyamines); scavenge debris by expressing scavenger receptors; promote angiogenesis, tissue remodeling and repair. M1 and M2 cells represent simplified extremes of a continuum of functional states. Available information suggests that TAM are a prototypic M2 population. M2 polarization of phagocytes sets these cells in a tissue remodeling and repair mode and orchestrate the smouldering and polarized chronic inflammation associated to established neoplasia. Recent studies have begun to address the central issue of the relationship between genetic events causing cancer and activation of protumor inflammatory reactions. Rearrangement of the RET oncogene (RET/PTC) is a frequent, causative and sufficient event in papillary carcinoma of the thyroid. It was recently observed that RET/PTC activates a proinflammatory genetic programme in primary human thyrocytes, including in particular chemokines and chemokine receptors. These molecules are also expressed in vivo and more so in metastatic tumors. These results highlight a direct connection between an early, causative and sufficient oncogene rearrangement and activation of a proinflammatory programme in a human tumor.

Therapeutic targeting of cancer promoting inflammatory reactions is in its infancy, and its development is crucially dependent on defining the underlying cellular and molecular mechanisms in relevant systems. Chemokines are prime targets for interfering with tumor promotion by inflammatory reactions. Ongoing efforts along this line are encouraging.

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Hypoxia Signaling From Survival to Tumour Cell death.

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The function of the Hypoxia-Inducible Factor-1 (HIF-1), the key transcription factor involved in cellular adaptation to hypoxia is restricted to low oxygen tension (pO2) 1, 2. As such, this transcription factor is central in modulating the tumor microenvironment, sensing nutrient availability and controlling anaerobic glycolysis, intracellular pH, and cell survival.

Degradation and inhibition of the limiting HIF-1α subunit are intimately connected in normoxia. Hydroxylation of two proline residues by Prolyl Hydroxylase Domain protein 2 (PHD2) earmarks the protein for degradation while hydroxylation of an asparagine residue by Factor Inhibiting HIF-1 (FIH-1) reduces its transcriptional activity 3, 4. Indeed, silencing in normoxia, of either PHD2 or FIH-1, partially induced hypoxic genes, whereas combined PHD2/FIH-1 silencing generated a full hypoxic gene response 5, 6 . Given the fact that HIF-1α possesses two transactivation domains (N- and C-TAD), we hypothesized on a possible bi-functional activity of HIF-1α that could be discriminated by FIH-1, a modifier of the C-TAD. In human cell lines, engineered to overexpress or silence FIH-1, in response to tetracyclin, we demonstrate by quantitative RT-PCR that a set of hypoxic genes (CAIX, PHD3, PGK1 and BNIP3) respond differently towards FIH-1 expression. This finding, extended to 30 hypoxia-induced genes, indicates differential gene expression by the N- and C-TAD in response to the hypoxic gradient 7.

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We propose that the oxygen-sensitive attenuator FIH-1, together with 2 distinct TADs are central in setting the gene expression repertoire dictated by the cell pO_2.
In the tumor microenvironment context, we are intrigued by the duality of HIF-1 that can induce either cell survival or cell death. We will discuss our unpublished work on the physiological roles played by the HIF-induced pro-apoptotic gene product BNIP3, autophagy and necrotic cell death.
Ways to exploit this basic knowledge to magnify tumor regression will be presented [8].

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EMT and cancer progression: Key players identified by expression profiling

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Epithelial-mesenchymal transition (EMT), a developmental switch from a polarized, epithelial to a fibroblastoid or mesenchymal, highly motile cellular phenotype, is increasingly recognized as a central process during embryonic development, chronic inflammation and fibrosis, as well as cancer progression. In the context of cancer progression, EMT is now intensely studied in tissue culture models of epithelial cells, transgenic mouse tumor models and human cancer (1, 2). EMT can require cooperation of endogenous transforming growth factor (TGF) beta signaling with oncogenic Ras or receptor tyrosine kinases, both inducing hyperactive ERK/MAPK signaling. In epithelial cell models and transgenic mice, sustained TGF beta receptor signaling (e.g via an autocrine TGF beta loop) can be required for EMT and metastasis (3).
The term EMT comprises a wide spectrum of epithelial plasticity changes, ranging from loss of polarity and gain of a migratory phenotype without sustained gene expression changes ("scattering) to "complete EMT". The latter, defined as the development of polarized epithelial cells into a metastable, fibroblastoid phenotype plus transcriptional loss of epithelial markers (e.g. E-cadherin) and de-novo expression of mesenchymal markers (e.g. vimentin), turned to be a faithful in vitro correlate of local invasion and metastasis (1, 2).
Recently, we employed polysome-bound mRNA expression profiling to identify genes selectively up- or downregulated during complete EMT. Among ≈ 35 EMT-specific genes, we selected multiple, co-activated genes of the platelet-derived growth factor receptor (PDGFR) signaling pathway and a novel, secreted protein with no homology in the database (interleukin-like EMT inducer, ILEI) for detailed biological characterization (4).
We showed that metastatic potential of oncogenic mammary epithelial cells requires an autocrine PDGF/PDGFR loop, established as a consequence of TGF beta-induced epithelial mesenchymal transition (EMT). The cooperation of autocrine PDGFR signalling with oncogenic Ras hyper-activates PI3K and is required for survival during EMT. Autocrine PDGFR signaling also contributes to maintenance of EMT, possibly through

activation of STAT1/3 and other distinct pathways. Inhibition of PDGFR signalling interfered with EMT and caused apoptosis in murine and human mammary carcinoma cell-lines. Consequently, over-expression of a dominant-negative PDGFR or application of the established cancer drug STI571 interferes with experimental metastasis in mice. Similarly, in MMTV-neu transgenic mice TGF beta enhances metastasis of mammary tumors, induces EMT and elevates PDGFR signalling. Finally, expression of PDGFR alpha and beta correlated with invasive behavior in human mammary carcinomas. Thus, autocrine PDGFR signaling plays an essential role during cancer progression, suggesting a novel application of STI571 to therapeutically interfere with metastasis (5).

Expression profiling for EMT-specific genes also identified a novel, secreted, interleukin-related protein (ILEI), upregulated exclusively at the translational level. Stable overexpression of ILEI in normal and Ras-transformed mammary epithelial cells (EpH4; EpRas) cells caused EMT, tumor growth and late steps in metastasis, independent of TGFβ-R signaling and enhanced by Bcl2. RNAi-mediated knock-down of ILEI in EpRas cells before and after EMT (EpRasXT) prevented and reverted TGFβ-dependent EMT, also abrogating metastasis formation. Discovery of ILEI thus adds a novel key player to the growing list of mechanisms governing metastasis. Abnormal ILEI expression (cytoplasmic overexpression instead of vesicular localization) was clearly associated with EMT in human colon cancer and predictive for metastasis formation and poor prognosis in human breast cancer, rendering ILEI a promising target for therapeutic intervention by humanized monoclonal antibodies (6)

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Macrophages are a cellular toolbox used by tumors to promote progression and metastasis

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Dramatic advances have been made in defined positively acting oncogenes and negatively acting tumor suppressor genes that cause oncogenic transformation of epithelial cells. The gain or loss of function of these genes leads to loss of proliferative control, resistance to apoptosis, acquisition of a migratory phenotype, limitless replicative potential and loss of sensitivity to external growth signals (Hanahan and Weinberg, 2000). However, recently it has become apparent that for these malignant phenotypes to be fully manifested these must be an appropriate supporting stroma.

Leukocytic cells form a major component of this stromal microenvironment and of these, macrophages are particularly abundant (Pollard, 2004). Originally these tumor-associated macrophages (TAMs) were thought to be tumor rejecting through their capacity to kill cells and

to present tumor antigens. However, experimental and clinical evidence strongly suggests that they promote tumor progression and metastasis (Pollard, 2004). For example, clinical studies have correlated the abundance of TAMs with poor prognosis in over 80% of the cases with only 10% of these studies showing a correlation with good prognosis (Lewis and Pollard, 2006).

To experimentally test the role of TAMs in breast cancer, we reduced their abundance in mouse mammary tumors induced by the epithelial specific expression of the Polyoma Middle T oncoprotein, by genetic ablation of the major macrophage growth factor, colony stimulating factor-1 (CSF-1). This resulted in delayed tumor progression and a dramatic reduction in metastasis. Restoration of macrophages to the mammary tumors by the mammary-specific expression of CSF-1 restored these phenotypes in mutant mice and over-expression of CSF-1 enhanced tumor progression and doubled the rate of metastasis in wild type mice (Lin et al., 2001). Similar results have been attained through antisense or neutralizing antibody approaches against CSF-1 on the CSF-1 Receptor in xenotransplantation models of human cancer (Paulus et al., 2006). Furthermore, inhibition NF B signaling in macrophages resulted in reduced incidences of liver cancer and skin cancer (Karin and Greten, 2005). These data together with others, strongly suggest that the phenotype of TAMs is directed away from a tumor rejecting to a tumor-promoting one by education within the tumor microenvironment.

Studies on the role of macrophages in the PyMT model of breast cancer has shown that they regulate many critical processes associated with the progression to malignancy (Condeelis and Pollard, 2006). Using intravital imaging of fluorescently labeled cells together with a novel micro-capillary invasion assay, we have shown an obligate interaction between macrophages and tumor cells that is required for tumor cell migration, invasion and intravasation (Wyckoff et al., 2004). This relies on a paracrine loop of tumor-produced CSF-1 that signals to macrophages to synthesize EGF that in turn stimulates the EGFR bearing tumor cells to migrate. Inhibition of either of these receptor signaling pathways blocks tumor cell migration and intravasation (Wyckoff et al, submitted for publication). Macrophage depletion also regulates the angiogenic switch that is associated with the transition to malignancy. Thus in the absence of macrophages even tumors that have advanced to late carcinomas have ~50% reduced vascular density (Lin et al, Submitted for publication). Together these data show that in tumors a high density of macrophages confers a double whammy, not only do they enhance tumor cell invasion and intravasation but they increase the number of target vessels through which the tumor cells escape into the vasculature. This at least in part, explains the dramatic inhibition of metastasis in this PyMT model in the absence of macrophages (Lin et al., 2001). Notably, clinical studies also show that over expression of CSF-1 in tumor cells and EGF in macrophages is correlated with poor prognosis (Leek and Harris, 2002; Lin et al., 2002). Together these experimental and clinical data suggest that inhibition of specific macrophage functions should have significant clinical benefit.

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Genetic analysis of lymph/angiogenesis and the neurovascular link: therapeutic implications

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The growth of blood vessels (a process known as angiogenesis) is essential for organ growth and repair. An imbalance in this process contributes to numerous malignant, inflammatory, ischaemic, infectious and immune disorders.

Gene targeting studies in mice have elucidated a functional role of placental growth factor (PlGF), a homologue of VEGF, in pathological angiogenesis [1]. Loss of PlGF, while not causing any vascular defects during embryonic development, reproduction or normal adult life, impaired angiogenesis in pathological conditions, such as cancer [2,3]. We generated a neutralizing anti-mPlGF monoclonal antibody that inhibits PlGF binding to its receptor Flt1. This antibody efficiently blocks tumor growth and angiogenesis in several orthotopic and subcutaneous tumor models. It also inhibits lymphatic metastasis in part by inhibiting lymphangiogenesis. Interestingly, treatment with an anti-VEGFR-2 antibody upregulates levels of PlGF, and anti-PlGF treatment amplifies the effect of an anti-VEGFR-2 antibody. The antibody also has chemo-sensitizing activity, and amplifies the cytostatic effect of chemotherapy in various tumor models. Moreover, toxicology studies revealed a significantly safer profile of anti-PlGF antibody treatment regarding fertility and reproduction capacity, skin wound healing and arterial hypertension, when compared to blocking VEGF/Flk1 signaling pathway using an anti-mFlk1 monoclonal antibody. These findings support the conclusion that blocking PlGF effectively inhibits in vivo tumor growth by suppressing tumor-induced neovascularization and demonstrate the potential for therapeutic application of anti-PlGF antibodies in the treatment of cancer diseases.

Another domain of great medical importance is the lymphangiogenesis (or the formation of lymphatic vessels), as lymphatic vessels have been implicated in fluid homeostasis, immunity, metastatisis of cancer cells. Unraveling the molecular basis of lymphangiogenesis has, however, been hampered by the lack of an available animal model, allowing rapid genetic manipulation. We recently characterized the Xenopus tadpoles as a novel small animal model to study the genetic basis of lymphangiogenesis [4,5]. Tadpoles develop lymphatic vessels within the first days of development. Knockdown by morpholino oligomers of Prox-1, the master gene of lymphatic cell fate differentiation, impaired lymphangiogenesis. This model allowed us to study not only single gene functions but also genetic interactions, as illustrated by our recent findings on the cooperative regulation of lymphangiogenesis by VEGF-C, VEGF-D and Prox1 using combined morpholino knockdown. Furthermore, using inhibitor compounds such as PTK787/ZK, MAZ51, a novel multi-FGFR antagonist and other inhibitors known to block receptor tyrosine kinases involved in (lymph)angiogenesis (VEGFR-2, VEGFR-3, FGFR3, etc), normal development of lymphatics and blood vessels was impaired respectively in tadpoles and zebrafish embryos. These findings thus show that tadpoles offer unprecedented potential to study, via genetic manipulation, the molecular basis of lymphangiogenesis. The understanding of how these vessels develop offers novel therapeutic opportunities to treat cancer by blocking lymphangiogenesis.

VEGF is widely recognized as the most critical player in angiogenesis. However, we recently discovered an

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unexpected link between VEGF and ALS: reduced levels of VEGF in mice (VEGFd/d mice) caused adult-onset motoneuron degeneration reminiscent to ALS, while VEGF gene transfer and intracerebroventricular delivery prolonged survival in rodent models of ALS[6-9]. One mechanism through which VEGF is implicated in ALS is by exerting direct neurotrophic effects on motoneurons. This is perhaps not so surprising, as VEGF and its receptors first appeared in evolution in the central nervous system (CNS) of species, such as the worm, which lack a vascular system. In addition, VEGF also regulates motoneuron survival via vascular effects, as systemic delivery of various VEGF inhibitors, currently being used or tested for their anti-angiogenic activity in (pre)-clinical conditions, aggravated motoneuron degeneration. Another most exciting novel research avenue is the recent realization that vessels often use similar guidance signals as axons to navigate to their target [10,11].

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Contribution of CXCR4+ hematopoietic cells to neo-angiogenesis

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Our group and others have shown that pro-angiogenic hematopoietic and vascular cells can be mobilized from the bone marrow or organ-specific vascular niches to contribute to neo-angiogenesis during exponential phase of tumor growth or acute vascular injury. Co-recruitment of marrow-derived VEGFR2+ vascular progenitors with VEGFR1+ hematopoietic progenitors play an essential role in the regulation of post-natal neo-angiogenic processes(1-3). Marrow-derived progenitors contribute to neo-angiogenesis during wound healing (4-9), myocardial (10-13), and limb ischemia (14, 15), endothelialization of vascular grafts (16-18), retinal neovascularization (19, 20), neonatal growth (21) and tumor growth (1, 5, 22-26).

In terms of contribution of endothelial progenitors to human neo-angiogenesis, three recent reports suggest that human marrow-derived endothelial precursors incorporate, although in low numbers, into regenerating organs (27) and tumor neo-vessels (28). Fleming WH group has shown that marrow-derived cells can incorporate in intestines and certain regenerating organs (27). In another study, patients transplanted with sex-mismatched marrow for the treatment for leukemias were followed for years (28). Some of these patients with

chimeric marrow, who were cured of their leukemia later developed solid tumors. Remarkably, immunohistochemical analysis showed that between 2 to 12% of tumor endothelial cells were donor derived cells (28). The patients with lymphomas had the highest incorporation of the marrow-derived cells to the tumor vessels. Another study has shown that CD133+ endothelial progenitors also incorporate into human lung carcinomas (29). Many of these CD133+ neo-vessels also co-expressed VEGFR2.

The variability in the incorporation of marrow-derived cells depends on the time-points, aggressive nature of tumor or the degree of vascular trauma. Overall luminal incorporation of the endothelial progenitors is highest in the early phases of rapidly growing tumors or acute vascular injury. Although in all of these reports, it has been demonstrated that pro-angiogenic hematopoietic cells could contribute to neo-vessel assembly, the contribution of endothelial progenitor cells have been less consistent. This discrepancy in the contribution of the endothelial progenitors may be in part due to the difficulty in identifying or effectively transducing the true repopulating clonogenic endothelial progenitor cells with lentiviral or retroviral vectors.

In addition, one major problem in transplantation studies to evaluate the role of true repopulating endothelial progenitors is the low number of these cells present in the marrow. In many studies only few millions of marrow cells, which contain at maximum only 1000 endothelial progenitor cells (0.1%), were transplanted into the recipient lethally irradiated host. Therefore, lack of luminal incorporation of these cells, for example, GFP+Tie2+ cells (30), could be in part explained by the fact that minimal numbers of repopulating endothelial progenitors were transplanted. In studies where 27 million LacZ+ marrow cells were transplanted, there were significantly more engraftment of endothelial progenitors into the marrow and incorporation into tumor neo-vessels (1, 31). Therefore, in these studies(30), only the contribution of the mature endothelial cells have been evaluated and as such the contribution of true repopulating endothelial progenitors with the capacity to form high proliferative potential endothelial colonies (HPP-EC, CFU-EC) was not assessed(32, 33).

In addition, GFP by itself could alter differentiation of endothelial progenitors (34), while β-galactosidase is less toxic to the endothelial progenitor cells. Emerging evidence also shows that contribution of circulating endothelial cells is highest during early phases of transformation of dormant to rapidly growing tumors. These data suggest that under permissive conditions, circulating endothelial cells do contribute luminally to the generation of neovessels of specific tumors that undergo rapid tumor growth and these findings are corroborated in human sex-mismatched transplantation studies.

Severe vascular injury, such as during acute ischemia or mechanical injury, provide the optimal permissive conditions for the incorporation of the marrow derived cells luminally into regenerating neo-vessels. In one recent study, Murry C. lab (35) has shown that on average 24% (range 17 to 35%) of the luminal endothelial cells within the myocardium of patients with sex mismatched heart transplants were derived from circulating endothelial cells. Remarkably, the majority of circulating endothelial cells were incorporated into the luminal capillaries of small to medium sized capillaries. These data suggest that the magnitude of recruitment of circulating endothelial cells may not only be organ-specific, but also dependent on the extent of vascular injury and remodeling.

The absolute number of circulating of endothelial progenitors has also been shown to correlate with the degree of neo-angiogenic processes (36, 37). Remarkably, genetic background of mice also determines the extent of the mobilization of the endothelial progenitors and incorporation into the neo-vessels. These studies set forth the possibility whereby the quantification of circulating endothelial cells may be used as biological surrogate markers to evaluate response to anti-angiogenic and chemotherapeutic agents.

Collectively, these data suggest that under permissive conditions endothelial progenitors cells have the capacity to contribute to neo-angiogenesis. However, co-recruitment of specific subsets of the hematopoietic cells

may be essential for the proper incorporation or circulating and locally derived endothelial cells. Compelling evidence suggest that unique subsets of pro-angiogenic hematopoietic cells support angiogenesis both during embryonic development and post-natally by delivering bio-available angiogenic factors, including VEGFs, MMPs, and angiopoietins to the neo-vessels (38-44).

We have shown that VEGFR1+ progenitors co-expressing CXCR4 contribute to tumor angiogenesis and metastasis (1). In addition, Gr1+CD11b+ cells have been shown to contribute to tumor neo-angiogenesis by releasing MMP-9 (45). Immune dendritic-like cells can incorporate into the vessel wall and contribute to tumor neo-angiogenesis (46). These data suggest that co-recruitment of VEGFR1+CXCR4+ hematopoietic cells convey signals that support incorporation and differentiation of VEGFR2+ endothelial cells into functional neo-vessels. Therefore, mobilization of pro-angiogenic hematopoietic and endothelial cells from the marrow may be the key event regulating the mobilization of or pro-angiogenic cells from the marrow.

The pathways involved in the mobilization of the pro-angiogenic progenitors from the marrow are orchestrated by sequential activation of proteases and release of stem cell active cytokines We have shown that mobilization of pro-angiogenic cells is mediated in part by metalloproteinase-9 (MMP-9) mediated release of soluble kit-ligand from membrane kit-ligand (47-49). This results in increased motility of hemangiogenic cells facilitating their migration towards the marrow's vascular niche. Subsequently, stromal derived factor-1 (SDF-1) provides cellular road maps for the localization of these cells to the vascular niche and subsequent mobilization to the circulation.

Activation of SDF-1/CXCR4 pathway is also critical for MMP-9 mediated mobilization of hemangiogenic cells. There is a severe defect in ischemic revascularization in MMP9-/- mice, which can be reversed by introduction of soluble kit-ligand or SDF-1. Inhibition of CXCR4 blocks ischemic revascularization, while luminal incorporation of endothelial cells was significantly enhanced by SDF-1. These data indicate that collaboration of CXCR4 and VEGF-A receptor signaling facilitates incorporation of marrow cells into the neo-vessels. The number of engrafted SDF-1 recruited endothelial progenitors and magnitude of demand for neo-angiogenesis determines the extent of luminal incorporation of endothelial cells. Co-recruitment of VEGFR1 - and CXCR4+ hematopoietic cells assist in functional neo-vessels assembly or tumor metastasis. We have also shown that arrival of the VEGFR1+ hematopoietic progenitors to the specific organs may initiate tumor metastasis.

In summary, quantification of the number of functionally engrafted bona fide hemangiogenic progenitors, including true repopulating endothelial progenitors and their hematopoietic counterparts, after marrow transplantation is absolutely necessary to make any meaningful conclusions as to whether endothelial progenitors cells contribute to neo-angiogenesis. Most transplantation studies published to date have used either very few endothelial progenitors or their lentiviral/retroviral and promoter driven tracking have failed to detect repopulating endothelial progenitor cells. Most of so called endothelial specific promoters, i.e. Tie2, Scl, seem to track mature endothelial progenitors rather than repopulating endothelial progenitors. As such, determining the number of engrafted viable and functionally intact clonogenic GFP+ or LacZ+ endothelial progenitors (CFU-EC, HPP-EC) is essential to formally determine the contribution of these cells to tumor neo-angiogenesis or ischemic revascularization. Nonetheless, the extent of mobilization of hemangiogenic progenitors is a reliable and validated surrogate biomarker to evaluate the extent of neo-angiogenesis. In addition, activation of CXCR4 and VEGF-A receptors may promote ischemic revascularization, while inhibition of CXCR4 in conjunction with VEGF-A receptors may provide an effective means to block growth of hemangiogenesis dependent tumors.

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Normalization of tumor vasculature and microenvironment by antiangiogenic therapies: From the bench to bedside and back

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Solid tumors require blood vessels for growth, and many new cancer therapies are targeted against the tumor vasculature. The widely held view is that these antiangiogenic therapies destroy the tumor vasculature, thereby depriving the tumor of oxygen and nutrients. However, emerging preclinical and clinical evidence support an alternative hypothesis – that judicious application of direct (e.g., Avastin) and indirect (e.g., Herceptin) antiangiogenic agents can also transiently "normalize" the abnormal structure and function of tumor vasculature to make it more efficient for oxygen and drug delivery. Drugs that induce vascular normalization can also normalize the tumor microenvironment – alleviate hypoxia and interstitial hypertension – and thus increase the efficacy of many conventional therapies if both are carefully scheduled (1-6).

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Neutrophil and Macrophage Dynamics and their Pro-angiogenic Roles in Mouse Models of Cancer

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Studies in genetically engineered mouse models of pancreatic islet, skin, and cervical cancer have revealed that matrix degrading enzymes are important for angiogenic switching and the persistence of cargiogenesis during tumorigenesis (1-8). MMP-9 in particular is associated with the angiogenic stages in all three organs (as well as in other cancer types). A key role of MMP-9, delinea-

ted by gene knockout and pharmacological inhibition (2,3,6), involves release of latent/sequestered VEGF from the ECM, enabling its association with VEGF receptors on the endothelium to signal angiogenic responses (2, 6). Notably, the primary source of MMP-9 in all three models is infiltrating cells of the innate immune system (1-3, 6), which are therefore defined as tumor-promoting and pro-angiogenic, as opposed to tumor antagonizing. Recent efforts, to be discussed, have sought to clarify the immune cell types that supply MMP-9 in the course of angiogenic switching during pancreatic islet and cervical carcinogenesis, and to assess candidate signaling circuits that might mediate infiltration of the pro-angiogenic leucocytes.

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Vascular Tumor Targeting

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One avenue towards the development of more selective, better anti-cancer drugs consists in the targeted delivery of bioactive molecules (drugs, cytokines, procoagulant factors, photosensitizers, radionuclides, etc.) to the tumor environment by means of binding molecules (e.g., human antibodies) specific for tumor-associated markers.

Angiogenesis, i.e., the proliferation of new blood vessels from pre-existing ones, is an underlying process in many human diseases, including cancer, blinding ocular disorders and rheumatoid arthritis.

The ability to selectively target and occlude neovasculature will be potentially useful in diagnosis and treatment of angiogenesis-related diseases. In collaboration with Philogen SpA and with the lab of Luciano Zardi (Genova), my lab has developed human monoclonal antibodies, capable of selective targeting of neo-vascular structures in solid tumors and in a number of angiogenesis-related diseases. Three of these antibody derivatives are currently in clinical development programs.

One of the major challenges for the development of innovative biomedical approaches aimed at targeting neo-vascular structures in vivo consists of the identification of suitable antigens, which are specifically and abundantly expressed at sites of disease, and which are accessible to intravenously injected ligands.

We have developed a novel methodology for the discovery of marker proteins accessible from the bloodstream. This technology is based on the terminal perfusion of tumor-bearing rodents or the ex vivovascular perfusion of human surgical specimens from cancer patients with a reactive ester derivative of biotin (e.g., sulfo-NHS-LC-biotin; Pierce), which enables the covalent modification of proteins which are readily accessible from the

bloodstream. The procedure eliminates from circulation by washing both blood cells and proteins, which could compete with the biotinylation reaction. As a result, accessible proteins (e.g., membrane proteins, extracellular matrix components) carrying primary amino groups (e.g., unprotected exposed N-termini or lysine side-chains) are covalently modified with biotin. Biotinylated proteins from total organ extracts can be efficiently purified on streptavidin resin in the presence of strong detergents and submitted to a comparative proteomic analysis. Biotinylated proteins are digested on-resin with trypsin and the resulting proteolytic peptides are analyzed by mass spectrometry. We routinely use LC-MALDI-TOF/TOF for peptide analysis, which leads to the identification of several hundred vascular proteins per organ.
More recently, we have extended our methodology to the comparative analysis of vascular structures in metastases and in the host organ, and (in collaboration with Vince Castronovo and David Waltregny, Liege) to the ex vivo perfusion of surgically-resected organs bearing solid tumors.

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Role of VEGF-A in Pathological Angiogenesis

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Vascular endothelial growth factor (VEGF)-A is an endothelial cell-specific mitogen in vitro and an angiogenic inducer in vivo. The tyrosine kinases Flt-1 (VEGFR-1) and Flk-1/KDR (VEGFR-2) are VEGF receptors. Loss of a single VEGF-A allele results in defective vascularization and embryonic lethality. High expression of VEGF-A mRNA has been described in many human tumors. Anti-VEGF-A monoclonal antibodies or other VEGF inhibitors block growth and neovascularization in tumor models. Several VEGF inhibitors are undergoing clinical development. We developed a humanized anti-VEGF-A monoclonal antibody (bevacizumab). Bevacizumab is being tested in multiple types of cancer patients. A phase III study in patients with previously untreated metastatic colorectal cancer demonstrated that addition of bevacizumab to chemotherapy results in a significant increase in survival compared to chemotherapy alone. Preliminary results suggest that adding bevacizumab to standard chemotherapy results in a clinical benefit also in breast and lung cancer. Furthermore, VEGF-A is implicated in intraocular neovascularization associated with active proliferative retinopathies and age-related macular degeneration (AMD). A humanized anti –VEGF-A Fab (ranibizumab) is presently in phase III for the treatment of the neovascular form of AMD and initial results indicate that the treatment maintains or even improves vision.

Tumor Microenvironment in Cancer Progression and Metastasis

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Tumors are unorganized organs that contain many different cell types that communicate with cancer cells. The central goal of our laboratory is to evaluate the role of these non-cancer cells in cancer progression and metastasis. Cancer progression significantly depends on the influence of host cells on genetically unstable cancer cells. Whether such host responses are recruited to control cancer progression or further aid in the growth of tumors (or both) is still unclear. This lecture will highlight the role of angiogenesis (as a contribution of endothelial cells), fibroblast recruitment and innate immunity in cancer progression and metastasis.

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ABSTRACTS OF POSTERS

High TIMP-1 Levels in the Host Promote Liver Metastasis *Via* HGF-Signaling

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Tissue inhibitor of metalloproteinases-1 (TIMP-1) is a natural broad-spectrum inhibitor of matrix metalloproteinases (MMPs) some of which are clearly associated with tumor progression. The role of TIMP-1 in cancer progression has long been paradoxical as the overexpression of TIMP-1 prevents cancer cell metastasis in a number of animal models whereas elevated levels of TIMP-1 in cancerous tissues often associate with poor clinical outcome of patients. While TIMP-1 can be pro-proliferative, anti-apoptotic, and can have both pro- as well as anti-angiogenic effects, its possible role in promoting invasion and dissemination of tumor cells has not yet been assessed. In this study, we mimicked elevated host TIMP-1 levels, as found in patients with bad prognosis, by adenoviral gene transfer of TIMP-1 or N-TIMP-1 lacking the C-terminal domain. In two independent experimental metastasis models, lacZ-tagged murine lymphoma or human fibrosarcoma cells, we found a significant reduction of large metastatic foci in the liver or lung, respectively. Notably, in both models a significant induction of secondary scattered metastasis was found in livers of mice with elevated TIMP-1 levels. Overexpression of TIMP-1 was associated with induction of hepatocyte growth factor-signaling and upregulation of several metastasis-promoting genes in livers, even in absence of tumor cells. This represents a host-microenvironment with increased susceptibility to secondary scattered metastasis. Reduction of initial macrometastasis may be due to high serum levels in the blood preventing initial MMP-dependent extravasation. This work provides experimental evidence that overall promotion of metastasis to the liver can result from altered signaling and gene expression profile by broad spectrum MMP inhibition in the liver by TIMP-1. This can explain the paradoxical findings of TIMP-1 in the clinic, and may lead to the design of novel treatment-strategies for cancer patients with increased TIMP-1 levels.

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Characterization of the signalling pathways involved in the escape of leukaemia cells from tumor dormancy induced by a transient angiogenic switch into the tumor microenvironment

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We recently reported that MOLT-3 cells, derived from an adult T cell acute leukaemia (T-ALL), were poorly angiogenic and remained dormant when injected subcutaneously into immunodeficient mice.[1] However, progressive growth of lymphoid tumors was invariably recorded when irradiated angiogenic feeder cells from Kaposi's sarcoma or purified angiogenic factors, including VEGF and bFGF, were locally co-injected with MOLT-3 cells. In any case, the persistence of the angiogenic switch was short-term, as evaluated by flow cytometry and real-time PCR analysis. In this study, we investigated the cellular and molecular differences between dormant and aggressive tumors. Dormant tumors were characterized by reduced vascularization, increased expression of HIF-1α and high levels of apoptosis, compared with progressively growing tumors. Intriguingly, phosphorylated p38 (pp38) stress-activated protein kinase (SAPK) was found in the majority of the dormant tumors (15 out of 19 analyzed), along with low-level expression of phosphorylated extracellular signal-regulating kinases (ERKs). This up-modulation in the pp38/p38 levels correlated with reduced expression of the mitogen-activated protein kinase phosphatase-1 (MKP-1) in the dormant tumors, as well as with increased expression of downstream mediators of apoptosis, including caspase-3 and cleaved PARP, which could account for the increased levels of apoptosis in the dormant tumors. These findings suggest that the balance between pp38/p38 and expression of MKP-1 is a critical feature in regulating the transition between indolent and aggressive tumors in this model, and its possible relevance to the clinical behaviour of T-ALL is currently being addressed.

P190-B RhoGTPase activating protein is a critical regulator of stromal-epithelial interactions in the developing mammary gland

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Mammary gland ductal morphogenesis and maintenance of tissue homeostasis is critically dependent on stromal-epithelial interactions. It is now clear that deregulation within the extracellular environment facilitates breast cancer progression. Understanding the normal role of the microenvironment in mammary gland development is, therefore, a critical first step in elucidating how its disruption promotes malignant progression. Rho GTP binding proteins function to integrate extracellular signals to affect a wide variety of cellular processes. Importantly, the Rho signaling pathway has been implicated in breast cancer progression. The molecular mechanisms by which Rho signaling mediates normal mammary gland morphogenesis and how this signaling becomes deregulated during breast cancer progression remains unclear.
An inhibitor of the Rho family, p190-B Rho GTPase activating protein (GAP), is preferentially expressed in terminal end buds (TEBs) within the developing mammary gland. Loss of p190-B function completely inhibits postnatal ductal morphogenesis. Overexpression studies using tetracycline (tet)-regulatable p190-B transgenic mice reveal that p190-B regulates TEB architecture and the adjacent microenvironment. P190-B overexpressing TEBs display pronounced alterations in morphology, aberrant branching off the neck region, and discontinuity in the myoepithelial cell layer. Furthermore, the stroma associated with

the abnormal TEBs shows increased extracellular matrix (ECM) deposition, accumulation of collagen, and an extensive infiltration of macrophages. The pronounced changes in the TEB architecture and adjacent microenvironment lead to disorganization of the ductal tree, increased side branching, and delayed ductal elongation. P190-B overexpression during pregnancy results in the formation of hyperplastic lesions that persist after post-lactational involution. Taken together, these loss and gain of function studies demonstrate that p190-B plays an essential role in regulating stromal-epithelial interactions that contribute to mammary gland ductal morphogenesis and tissue homeostasis.

Hypoxia unravels a dichotomic functional response to CXCR4 receptor triggering in B lymphoma cells: receptor uncoupling as escape mechanism?

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The chemokine receptor CXCR4 plays a central role in organ-specific homing and tumor spreading and is induced by hypoxia in several cell types. Since B lymphocytes are exposed to low oxygen tensions as they develop and acquire effector functions, and the influence of hypoxia on their physiology and pathology is poorly understood, we addressed its role on CXCR4 expression and function in a B cell lymphoma setting. Acute and chronic hypoxia were associated with dramatic up-regulation of CXCR4 expression in human B lymphoma cells, through both transcriptional and post-translational mechanisms. Notwithstanding, a dichotomic functional response to CXCR4 triggering was observed in B lymphoma cells of different origin; following hypoxic exposure, increased responses were seen in lymphomas arising from mature B cells, whereas lymphomas originated from germinal center B cells showed virtual CXCR4 receptor desensitization. This phenomenon was associated with differential modulation of key signal-transducing molecules such as MAPK phosphatase-1 and regulator of G protein signalling molecule-1. A similar dichotomic behavior was also observed in the normal counterpart of the lymphoma cells. We propose that hypoxia in germinal center B cells may contribute to their unresponsiveness to CXCR4 triggering; thus, hypoxia may favour spreading of aggressive B cell lymphomas from lymphoid organs expressing CXCL12 through CXCR4 uncoupling.

Stroma cell derived S100A4(mts1) in mouse tumors.

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The S100A4(mts1) protein stimulates metastatic spread of tumor cells. An elevated expression of S100A4(mts1) is associated with poor prognosis in many human cancers. Our results indicate that the S100A4(mts1) protein stimulates metastasis as a stroma cell derived factor. Using S100A4-null mouse model we demonstrated that host-derived tumor stroma cells expressing S100A4 actively participate in stimulation of tumor progression and metastasis. Dynamics of tumor development was studied in S100A4-deficient mice using grafts of highly metastatic mammary carcinoma cells. A significant delay in tumor uptake and decreased tumor incidences were observed in the S100A4(mts1)-/- mice compared to the wild type controls. Moreover, tumor developed in the S100A4(mts1)-/- mice never metastasize. Co-injection of the tumor cells

with immortalized S100A4(mts1)+/+ fibroblasts partially restored the dynamics of tumor development and the ability to form metastasis. These fibroblasts were characterized by an enhanced motility and invasiveness in comparison with the S100A4 (-/-) fibroblasts, as well as by the ability to release S100A4 into the tumor environment.
Late stages of metastasis formation were studied in the S100A4(mts1)-null mice by means of experimental metastasis assay. Experimental metastasis formation was also impaired in S100A4-deficient mice. This ability was restored when tumor cells were co-injected with S100A4(mts1)+/+ fibroblasts. Taken together, these results indicate that S100A4(mts1) expression in stroma is needed throughout all the metastatic cascade.
Taken together, our results point to a determinative role of stroma cells derived S100A4 in tumor progression and metastasis.

Immense up-regulation of metastasis-promoting S100A4 (Mts1) protein in chronic inflammatory conditions: putative role in the crossroads of cancer and inflammation.

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Recently we demonstrated an important function of S100A4(Mts1), a member of the S100 family of calcium-binding proteins, in pro-metastatic activity of tumor microenvironment. We found that the active forms of S100A4 could be released by both tumor and stroma cells and in auto- and paracrine mode target cells in the tumor body. Extracellular S100A4 is able to modify actively tumor cells and various stroma cells (activation of MMPs, modulation of transcription factors, p53 and NF- B, remodeling of cytoskeleton, etc).

The aim of this study was to examine the involvement of S100A4 in inflammation that displays several facets similar to neoplasia and is recognized as a pre-cancerous condition.
We studied the involvement of S100A4 in the most severe chronic inflammatory disease Rheumatoid Arthritis (RA), where high up-regulation of cytokines, MMPs and other "tumor-promoting" factors is documented.
*Here we demonstrate a strong up-regulation of S100A4 in cells populating the synovial tissue (**ST**) of patients with RA but not OA (Osteoarthritis) and healthy persons. Most cell types identified by cell-specific markers (fibroblasts, phagocytes, granulocytes, mast cells, a fraction of T-lymphocytes, pericytes and endothelial cells) contribute to S100A4 production in RA ST. The pattern of S100A4 expression differs significantly from the expression of the pro-inflammatory proteins, S100A9 and S100A12, which are strongly restricted to phagocytes and granulocytes. The up-regulation of S100A4 production is correlated with the appearance of the protein in high concentration in plasma of RA patients (up to> 2500ng/ml) while the plasma of OA patients and healthy persons contained in average only 200 ng/ml and 80 ng/ml respectively. Moreover, we found that S100A4 in RA plasma exists in bioactive multimeric conformation whereas in OA the major S100A4 fraction is represented by less active dimeric form. Consistent with our previous observations in tumor models, extracellular S100A4 stabilizes tumor suppressor p53 in RA synovial fibroblasts (**SF**) as well, and influences its target gene regulation as we illustrated by monitoring Bcl2, p21/WAF, and HDM2 expression. These data agreed well with the observation of S100A4-mediated transcriptional activation of MMPs in RA –SFs.*
Moreover, we demonstrated that tumor cells mixed with RA-SFs but not with OA-SFs brought about more progressive tumor development in mice.
In conclusion, we speculate that S100A4 as a tumor-inflammation crossroad-factor, might have an important role in pathogenesis of RA and moreover its up-regulation might explain higher neoplasia incidences (lymphoma and others) among RA patients.

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Influence of the constitutive expression of Egr-2 and Egr-3 on the interactions of melanoma cells with their microenvironment.

Joerg Kumbrink *and Judith P. Johnson from the Institute for Immunology, University of Munich, Germany*

Melanoma cells interact with their microenvironment through the secretion of growth factors and cytokines and by direct cell-cell contact. Autocrine growth factors like bFGF and paracrine growth factors such as PDGF, TGFβ and VEGF are involved in melanoma progression by controling tumor growth and stroma formation. These growth factors induce the expression of several genes including the transcription factors of the Egr family. Egr-1 in turn induces the expression of the same growth factors, thereby establishing a positive feedback loop. To prevent a damaging over reaction by permanent secretion of growth factors in response to environmental signals, Egr-1 activates the transcription of its own repressor Nab2. The Egr-Nab2-system is shown to be altered in several tumors, including melanomas where Nab2 is constitutively expressed. In order to gain further insight into the Egr-Nab2-system in melanomas we investigated the expression pattern of the Egr`s and their influence on Nab2 expression. Transfection of Nab2 promoter luciferase reporter constructs together with the Egr`s show that Egr-1, Egr-2 and Egr-3 can activate the Nab2 promoter in an additive way. Interestingly the activation of the Nab2 promoter by Egr-1 and especially by Egr-3 is much higher in the melanoma cell line Mel888 (Egr-1 and Egr-3 8-fold) than in the colon carcinoma cell line Colo320DM (Egr-1 4-fold, Egr-3 3-fold). In addition, we identified elements in the Nab2 promoter important for further activation of the Nab2 promoter only in the melanoma cell line. Egr-1 and another member of the Egr family bind simultaneously to a cluster of overlapping Egr/Sp1 binding sites in the Nab2 promoter after stimulation with phorbol esters, while binding of Sp1 remained unaffected. In silico analysis of the 5`regions of bFGF, PDGF, TGFβ, and VEGF reveal comparable putative overlapping Egr/Sp1 binding motifs. These findings and the fact that the Egr`s share a highly conserved DNA binding domain suggests that not only Egr-1 but also Egr-2 and Egr-3 are involved in the regulation of the expression of these growth factors. Analysis of the mRNA expression of the Egr`s indicate a melanoma association of Egr-2 and Egr-3. In 77% (14/18) of the melanoma cell lines Egr-2 and Egr-3 are detectable, while Egr-2 was found only in 21% (3/14) and Egr-3 in none of the non-melanoma cell lines. In addition Egr-2 is expressed in all 9 melanoma cell lines derived from metastasis and only in 57% (4/7) melanoma cell lines derived from primary tumors, which suggests a melanoma progression association of Egr-2. This is currently being examined using frozen tissue sections of melanocytic lesions. The constitutive expression of Egr-2 and Egr-3 might contribute to the high malignancy of melanomas by maintaining the expression of growth factors that stimulate proliferation (bFGF), stroma formation (PDGF, VEGF, and TGFβ), and angiogenesis (VEGF).

Cytokines and redox potential of the microenvironment: role in tumour progression

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A number of proteins, including the two oxide-reductases thioredoxin (TRX) and MIF and the nuclear protein HMGB1, in addition to their intracellular function play an extracellular role as danger factors/pro-inflammatory proteins. These proteins lack a secretory signal sequence, accumulate in the free cytosol and are secreted by inflammatory cells through non classical pathways of export. Due to their high biological activity, their secretion is tightly regulated at different levels to ensure a positive outcome of inflammation. However, their cytosolic location allows their easy release by

dying or damaged cells: in this case, a control of their bioactivity is still maintained as these proteins are active only in a reduced state, and hence rapidly inactivated in the oxidizing extracellular milieu. TRX, MIF and HMGB1 have been found overexpressed in some tumors and associated with bad prognosis. We have analyzed several specimen from human lung, colon and kidney carcinoma and found by immunohistochemistry a strong overexpression of these proteins, both in tumor cells (in nestles and groups) and in inflammatory infiltrates. Interestingly, the same tumor samples displayed a dramatic increase in extracellular non protein thiols with respect to their normal counterparts, revealed by staining with the free thiol dye Mercury Orange.

We also studied a number of tumor cell lines in vitro and found that many of them overexpress TRX, MIF and HMGB1, and release spontaneously reduced cysteine. Several clones displaying different levels of expression of TRX and MIF were obtained from the lung carcinoma cell line SK-MES. The low-expressing clones exhibited low release of cysteine and showed a retarded growth rate both in vitro and in vivo in SCID mice. To get insight into the relationship between oxide-reductase overexpression, cysteine release and tumor progression we cultured SK-MES with low amount of reductants. Cells cultured in this reducing environment rapidly increased the rate of secretion of MIF and TRX and downregulated the expression of some adhesion molecules (α3β1, CD58, CD44), with loss of adherence to dishes coated with the relevant ligands.

Together, our results provide a molecular basis to the association between overexpression of HMGB1, MIF, TRX and bad prognosis in neoplasia. Unlike normal tissues, neoplastic tissues produce and release high amounts of free thiols. As HMGB1, MIF, TRX, and other leaderless pro-inflammatory cytokines such as galectin-1, IL-18, IL-1, also present in the tumor microenvironment, are oxidation-sensitive, abundant extracellular thiols maintain the reducing environment required for the function of these cytokines, either actively secreted by infiltrating inflammatory cells or released upon tumor cell necrosis. This would extend pathologically their life and bioactivity, contributing to the development of a chronic inflammation state in the tumor site and to a loss of adhesion of cancer cells which may improve tumor growth and spreading.

Role of IL-6 Trans-Signaling in Overcoming Hurdles to T Lymphocyte Trafficking in the Tumor Microenvironment

Qing Chen, Daniel T. Fisher, Lei Zhou, Kristen Clancy, Wan-Chao Wang, Elizabeth Repasky, and ***Sharon S. Evans***

Successful cancer immunotherapy based on vaccines or adoptive T cell transfer depends on the ability of cytolytic effector T lymphocytes to traffic to the tumor microenvironment. The mechanisms limiting the rate of entry of blood-borne CD8⁺ effector T cells across the vascular endothelial cell barrier at tumor sites are poorly understood. Here, we report on the role of a hallmark vascular gatekeeper, intercellular adhesion molecule-1 (ICAM-1), in governing T cell trafficking at three distinct sites: (1) specialized high endothelial venules (HEV) of secondary lymphoid organs (lymph nodes, Peyer's patches); (2) non-activated endothelium of normal extralymphoid tissues; and (3) tumor vessels of spontaneous and transplantable murine tumors. Tumor vessels and normal vessels of non-inflamed extralymphoid organs expressed low levels of ICAM-1, consistent with observations that these vessels did not efficiently support CD8⁺ T cell interactions or extravasation. Conversely, ICAM-1 dependent firm adhesion and extravasation of T cells occurred at high frequency in HEV which are the major portals for entry of naive and central memory lymphocytes during routine immune surveillance. Comparative analysis of vascular responses to systemic thermal therapy (39.5-40°C for 2-6 h) revealed that selected tissue microenvironments predispose vessels to respond to stress cues. In this regard, systemic thermal therapy strongly upregulated the intravascular density of ICAM-1 on tumor vessels and HEV, causing a 5-fold and 2-fold increase in ICAM-1

dependent homing of CD8⁺ T cells at these respective vascular sites. In sharp contrast, normal vessels of extralymphoid organs (heart, kidney, liver, pancreas) were not responsive to thermal therapy with respect to ICAM-1 induction or improved CD8⁺ T cell trafficking. Our studies further identified a non-redundant role for the inflammatory cytokine, IL-6, in promoting ICAM-1-dependent trafficking of CD8⁺ T cells in response to thermal stress. Regionalized control of vascular ICAM-1 expression involved an IL-6 trans-signaling mechanism whereby IL-6 and a soluble form of the IL-6 receptor engaged membrane-anchored gp130 transduction molecules. These findings provide the first evidence for an IL-6 trans-signaling mechanism that improves ICAM-1 dependent trafficking of CD8⁺ T cells through vascular checkpoints in tumor tissues or lymphoid organs. The results further demonstrate the feasibility of therapeutically exploiting the tumor microenvironment to mobilize CD8⁺ T recruitment to malignant lesions. This work was supported by NIH grants CA79765 and CA094045 and DOD grant W81XWH-04-1-0354.

Stroma content of colon carcinomas is affected by loss of 9q34 and 1p36

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Background:
Clonal expansion of neoplastic cells results from accumulation of somatic alterations in oncogenes and tumor suppressor genes. Progression of colon adenomas into carcinomas is accompanied by specific chromosomal aberrations, in particular loss of 8p, 15q, 17p, 18q, and gain of 8q, 13q, and 20q (Hermsen et al., Gastroenterology 2002). In addition, tumor development is influenced by interactions between neoplastic cells and their neighbouring stromal cells like fibroblasts, macrophages, and endothelial cells, thereby affecting processes like immune surveillance, angiogenesis, and metastasis. Hence, during adenoma-to-carcinoma progression also the tumor stroma composition is undergoing significant changes.
Aim Of This Study:
To identify chromosomal aberrations that affect stroma content of colon carcinomas.
Methods:
A panel of 23 human colon carcinomas was used to evaluate genome-wide gains and losses of chromosomal segments by BAC-array Comparative Genome Hybridization (array-CGH). Stroma content of these tumors (% non-tumor cells within carcinoma) was determined by quantitative measurements of hematoxylin-eosin stained sections, using QProdit.
Results and discussion:
Quantitative measurements revealed that stroma content of colon carcinomas varied from 18% to 64%. Array-CGH analysis indicated a total of 39 chromosomal segments that exhibited gains or losses in more than 20% of colon carcinomas. Gain of 20q was the most frequently observed genomic alteration, in 18 out of 23 carcinomas (78%). Upon statistical evaluation (ANOVA) and correction of P-values for multiple comparisons (Bonferroni) two regions were identified that affected stroma content significantly, i.e. loss of 9q34 (P=0.002) and loss of 1p36 (P=0.02). Loss of 9q34 was accompanied by a two-fold increase in the total amount of stroma (from 25% to 48%), while loss of 1p36 had the opposite effect (from 44% to 28%). Considering the importance of 20q gain for adenoma-to-carcinoma progression, we repeated this analysis using the subset of colon carcinomas with 20q gain. Loss of 9q34 and 1p36 still affected stroma content significantly (P=0.001 and P=0.01, respectively). In addition, gain of 8q was now also significantly associated with increased stroma content (from 31% to 44%; P=0.04). Collectively, these data indicate that certain somatic mutations affect tumor stroma composition, implying that they modulate functional characteristics of the tumor microenvironment.
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Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence

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Proliferation of primary diploid fibroblasts is limited by p53 activity. Activation of p53 by various cellular stresses can lead to a state of growth arrest named replicative senescence, a tumour suppressive mechanism that protects against oncogenic transformation. Although many of the molecular pathways leading to p53 activation are quite well known, little is known concerning the downstream target genes of p53 in this growth-limiting response. We have found that suppression of the p53 target gene encoding plasminogen activator inhibitor 1 (PAI-1) by RNA interference leads to escape from replicative senescence in both primary mouse embryo fibroblasts and in primary human BJ fibroblasts. PAI-1 knockdown causes activation of the PI3K-PKB-GSK3β pathway and leads to nuclear retention of cyclin D1, consistent with a role for PAI-1 in growth factor related signalling. Furthermore, we find this signalling route also to be causally involved in the senescence response. Conversely, ectopic expression of PAI-1 in proliferating p53-deficient murine or human fibroblasts induces a phenotype displaying all the hallmarks of cellular senescence. Our data indicate that PAI-1 is not merely a marker of senescence, but both necessary and sufficient for the induction of replicative senescence downstream of p53.

Activin A and matrix metalloproteinase -2 and -9 blood levels as gauges of bone metastatic spread

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Background:
Matrix metalloproteinase-2 (MMP-2) and -9 (MMP-9) have been suggested to facilitate bone metastasis formation. In fact, several investigations highlighted that tumor cells may secrete and/or induce osteoclasts to release in the bone microenvironment MMP-2 and MMP-9 which, in concert with other proteolytic enzymes, may degrade bone matrix proteins thus cleaving a number of growth factors preicorporated in the bone matrix. These factors, in turn, stimulate tumor cells to release other soluble growth factors, cytokines and hormones which further promote, cell proliferation, migration and invasion of bone tissue setting up the so called "vicious cycle". Among the growth factors released during these processes, a component of the transforming growth factor β (TGF- β) superfamily, namely, Activin A, seems to modulate the expression of MMP-2 and MMP-9 in osteoclasts and/or in malignant cells during bone matrix turnover associated to bone metastasis formation. On the basis of these findings we have assessed the clinical significance of Activin A, MMP-2 and MMP-9 circulating levels in patients with breast cancer (BC) or prostate cancer (PC) with (M1) or without (M0) bone metastases.
Methods:
Activin A, MMP-2, MMP-9 blood levels were determined by commercially available two-step sandwich enzyme-linked immunosorbent assay (ELISA) kits in 72 cancer patients and in 48 healthy subjects (HS). The diagnostic performance of these molecules to discriminate between M1 and M0 patients was evaluated by the receiver operating characteristic curve (ROC) and compared to that of tumor markers CA15.3 or prostate specific antigen (PSA).
Results.
Activin A, MMP-2 and MMP-9 blood levels were significantly increased in cancer patients as compared to HS ($P \leq 0.0001$). In breast cancer, Activin A, MMP-2, CA15.3 but not MMP-9 levels were significantly more elevated in M1 patients compared to M0 ones ($p=0.05$, $p=0.009$

p=0.0001 and p=0.52 respectively). Furthermore, in these patients a positive correlation was observed between MMP-2 and Activin A (p=0.026) or CA15.3 (p=0.012). ROC analysis showed, a good diagnostic performance of MMP-2 (p=0.006) and CA15.3 (p<0.0001), a fair diagnostic efficacy of Activin A (p=0.03) or a poor ability of MMP-9 (p=0.52) to discriminate between M0 and M1. On the other hand, in prostate cancer, Activin A, PSA and MMP-9 but non MMP-2 levels were more elevated in M1 patients compared to M0 patients (p=0.016, p<0.0001, p=0.084 and p=0.40 respectively). In these patients, a significant correlation was observed only between PSA and Activin A (p=0.042) or MMP-9 (p=0.007). ROC curve analysis showed a good diagnostic accuracy of Activin A, MMP-9 and PSA and a poor diagnostic efficiency of MMP-2 to detect M1 patients (p=0.008,p=0.05, p<0.0001 and p=0.39 respectively) ***Conclusions:*** *These results indicated that Activin A, MMP-2 and MMP-9 may play a role in bone metastasis formation. Therefore, these molecules may be potentially useful as additional gauges of bone metastatic spread and may be considered novel potential therapeutic targets for the treatment of metastatic bone disease.*

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Stroma irradiation promotes tumor invasion and metastasis by suppressing angiogenesis

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Radiotherapy is successfully used to treat a large variety of cancers. However, recurrence after radiotherapy is associated with increased local invasion, metastatic spreading and poor prognosis. While it is generally assumed that the increased aggressiveness of relapsing tumors is due to the selection of tumor cells resistant to radiation-induced apoptosis, other mechanisms may be involved. Recently it was reported that the anti-tumor effect of radiotherapy involves induction of endothelial cell death and disruption of angiogenic tumor-associated vessels. To address the question whether the tumor stroma may be involved in promoting the increased aggressiveness of tumors relapsing after radiotherapy, we established a model in which we characterized the long term effects of radiotherapy on angiogenesis and analyzed their consequences on tumor growth, invasion and metastasis.

We report here that ionizing radiation of the prospective tumor stroma results in a sustained impairment of growth factor-driven (i.e. Matrigel plug assay) and tumor angiogenesis. Tumors growing within a previously irradiated stroma have reduced growth while they display increased hypoxia, necrosis, local invasion and distant (lung) metastasis formation. Tumor cells recovered from tumors grown within an irradiated stroma retain a stable invasive phenotype under normoxic conditions in vitro and an increased metastatic capacity in vivo. Importantly, cells with increased in vitro invasiveness and in vivo metastatic capacity are also obtained in vitro through repeated cycles of culture under hypoxic (0.1% pO2) and normoxic (21% pO2) conditions. Gene expression profiling and functional experiments demonstrate that the Cyr61-αVβ5 pathway is critically involved in this process. In particular the αVβ3/αVβ5 integrin inhibitor EMD121974 (Cilengitide) fully suppresses enhanced in vitro invasion and prevents lung metastasis formation of tumors growing within an irradiated microenvironment.

Based on these results we propose that radiotherapy promotes tumor invasion and metastasis of relapsing tumors through the sustained impairment of angiogenesis and subsequent hypoxia-driven selection of aggressive tumor cells. Our data point to a critical role of the tumor stroma in promoting the aggressive progression of tumor recurrences after radiotherapy and identify αVβ5/αVβ3 integrins as potential therapeutic targets to improve outcome in patients with post-radiation recurrences. More generally, these results anticipate possible long-term side effects of anti-angiogenic drugs in cancer therapies.

The melanoma cell adhesion molecule MCAM/CD146 directly promotes angiogenesis

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The endothelial cell adhesion molecule MCAM/MUC18/CD146 is ectopically expressed by human malignant melanomas. MCAM expression enhances growth and metastasis formation by melanoma cells in immune-compromised mice suggesting that it may play a role in human tumors. It has been shown that other endothelial adhesion molecules such as E-selectin and VCAM-1, can function as an angiogenic factor raising the possibility that MCAM itself, could function as an angiogenic factor and thereby promote tumor progression. The influence of MCAM expression on angiogenesis was evaluated using stable MCAM- and neo-cDNA transfectants in 293 kidney epithelial cells in two different angiogenesis assays, the in vivo chicken chorioallantoic membrane assay (CAM-Assay) and the in vitro endothelial cell tube-formation-assay. In the CAM-Assay independently generated MCAM expressing transfectants induced from 1.5 to greater than 3 fold more vessel branching and vessel growth towards the inoculum compared to the MCAM negative cells. Using recombinant Fc-fusion proteins, the MCAM extracellular region induced an angiogenic reaction similar in magnitude to that induced by CD44, a known angiogenesis inducer. These results were confirmed in the in vitro endothelial cell tube-formation-assay. Here human umbelical vein endothelial cells were co-cultured on matrigel with either stable MCAM expressing transfectants, the human recombinant MCAM-Fc-fusion protein or their controls. Increased numbers of capillary like structures could be detected in co-cultures with MCAM expressing cells and as well in the presence of the MCAM-Fc-fusion protein. These studies show that MCAM expressing cells can enhance angiogenesis and the MCAM extracellular domain itself is sufficient to act as an angiogenic inducer. MCAM-expression of melanoma cells may therefore contribute to tumor progression not soley through adhesive interactions but also by promoting tumor angiogenesis.

Identification of new markers expressed by tumor endothelial cells

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Angiogenesis and the maintenance of the blood vessel functions are complex processes that rely on the coordination of many different activities in several cell types such as cancer and stromal cells, including endothelium and pericytes. Increasing number of evidence suggested that tumor vasculature, subjected to continuous stimulation from the surrounding microenvironment, expresses unique markers that distinguish it from normal vasculature. Our efforts have been aimed at the molecular characterization of tumor associated endothelial cells and the identification of novel markers.
Endothelial cells (EC) were isolated from tumor and normal tissue specimens and gene expression profiles analyzed by a high-throughput technique (Affyme-

trix GeneChip® Human Genome U95A Arrays). Microarray results sustained the endothelial origin of the cells, as shown by the robust expression of as many as 41 transcripts defined "typical endothelial" (by way of SOURCE batch search). Remarkably, results indicated that EC isolated from carcinoma specimens present characteristic transcriptomic features with respect to normal tissue derived EC. Specifically, one hundred fifty-nine genes, involved in a variety of biological functions including matrix degradation and remodeling, proliferation and differentiation, cell-cell and cell-matrix interaction, were more expressed by tumour derived-EC. Along with molecules known to be expressed on tumor endothelium, we have identified transcripts that might represent novel markers. RealTime RT-PCR analyses confirmed the higher expression of selected genes in newly purified tumor derived EC with respect to normal tissue EC. Tissue distribution studies showed a restricted expression, limited to a subset of adult and fetal normal tissues. Further investigation established that they could be expressed at different levels by stromal cells such as smooth muscle cells and fibroblasts, in addition to EC. Initial in situ hybridization studies are revealing that their expression is localized on blood vessel walls in neoplastic tissue specimens.

Future investigations will be required to clarify the roles of these molecules in tumor progression and vascularization.

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Stromal-derived sparc regulates tumor microenvironment toward metastasis.

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Normal tissue architecture and microenvironment change along tumor transformation. Extracellular matrix proteins take part to the complex interaction between the tumor and its stroma. Several gene profiling analysis point to SPARC as major indicator of prognosis and resistance to therapy. It remains unclear whether SPARC produced by tumor cells and stromal cell is regulated in different way and has different functions. Recently we have demonstrated that only SPARC provided by host leukocytes determines the assembly and function of tumor-associated stroma through the organization of collagen type IV. We have now evidence that SPARC produced by stromal cells is necessary for their interactions with tumor cells toward in vivo lung metastases of 4T1 mammary carcinoma. 4T1 cells injected into BALB/c but not in SPARC KO mice produce lung metastases despite the primary tumor grow without difference. Since 4T1 cells produce SPARC directly, we hypothesized that the absence of SPARC from tumor infiltrating stromal cells (mostly macrophages and fibroblasts), hampers the metastatic process. To prove such hypothesis we have injected 4T1 cells in chimeric mice expressing or not SPARC in donor bone-marrow (BM). Chimeric mice expressing SPARC only in BM–derived cells (wt> KO) show number of metastatic colonies of control chimeras (wt > wt), whereas chimeric mice lacking SPARC in their BM- derived cells (KO >wt) show a dramatic decrease in number of lung colonies. Thus host- rather than tumor-produced SPARC is needed in the metastatic process he hypothesis we are testing is that stromal SPARC favor 4T1 cell migration most likely through the induction of an intermediate state of adhesion, which is compatible with cell migration. To this end SPARC may interact with β1 and β5 integrin and competing with their normal substrates. 4T1 cell adhere to collagen type I and vitronectin through α2β and αvβ integrins respectively. Since in SPARC KO mice collagen type I is reduced, tumor β5 integrin becomes necessary for cell anchorage to substrate. Indeed there is no take of 4T1 cells silenced for β5 integrin in SPARC KO mice. Taken together, these results suggest that stroma-derived SPARC has functional role in primary tumor take

and invasion, regulating first collagen deposition and then inducing loss of focal adhesion and migration. BM from transgenic mice carrying a suicide fusion protein under csf-1 promoter will directly prove the role of macrophages as SPARC source through their specific ablation upon drug treatment.

Platelets cd40l, an environmental factor promoting mammary carcinogenesis

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Inflammatory cells are part of tumor environment and functionally can either promote or inhibit tumor growth. While cells of the innate response have been involved in such mechanisms, little is known on whether molecules of the adaptive response have direct involvement in tumor development. We studied whether CD40, key molecule for adaptive immune response, has any role in mammary carcinogenesis of BALB/NeuT transgenic tumor-prone mice. To this aim we transferred the HER2/neu oncogene into CD40-null background to obtain the CD40-KO/NeuT strain.
Unexpectedly, CD40-KO/NeuT mice showed delayed tumor onset and reduced tumor multiplicity. Bone marrow (BM) transplantation experiments excluded a role of CD40 expressed by BM-derived immune cells in the reduced tumorigenicity observed. Rather, CD40 expressed by host-derived cells, most likely tumor-associated endothelial cells, seems to be critical for CD40-KO/NeuT milder phenotype. Accordingly, BALB/NeuT tumors are characterized by large vessels, well organized around the tumor lobular structures, while tiny numerous vessels with scarce extracellular matrix, are dispersed in the parenchyma of poorly organized CD40-KO/NeuT tumors.
Among the possible sources of CD40L, activated platelets interact and activate endothelial cells, and may participate to tumor growth by contributing to the metastatic process and regulating tumor cell invasion and angiogenesis.
Their finding within tumor vessels prompted the idea of treating BALB/NeuT and CD40-KO/NeuT mice chronically with the anti-platelet drug clopidogrel, known to inhibit platelet CD40L expression. Treatment of BALB/NeuT mice reduced their tumor growth to a level similar to CD40-deficient mice, whereas CD40-KO/NeuT treated and untreated mice do not differ significantly from each other, indicating that activated platelets are likely the source of CD40L in this model.
Our results point to a role of CD40/CD40L in the angiogenic process associated to mammary carcinogenesis of BALB/NeuT mice and highlight CD40/CD40L interaction as an additional mechanisms that platelets utilize to promote tumor neo-angiogenesis, sustaining the hypothesis of using anti-coagulants in cancer therapy to prevent platelets interaction with tumor vasculature.

Tumour cell dependent effect of nami-a on cell malignant behaviour

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The progression of a benign tumour into a metastatic phenotype is the major cause of poor clinical outcome in cancer patients. Metastasis formation is a highly complex multistep process: tumour cells must modulate their

adhesion ability, degrade the surrounding extracellular matrix (ECM), migrate and proliferate to a secondary site. NAMI-A is a ruthenium compound endowed with a peculiar anti-metastatic activity in vivo and it is devoid of cytotoxic effects on primary tumour. To study NAMI-A selectivity towards metastatic tumour cells, three cell lines with different metastatic and tumorigenic ability are used: HBL-100, a non tumorigenic mammary epithelial cell line, MCF-7 poorly-invasive breast cancer cell line and MDA-MB-231 a highly-invasive breast cancer cell line, in which a pro-invasive cytokine, TGF-β, induces epithelial to mesenchymal transdifferentiation. In vitro experiments that could mime the steps of metastatic process are done: for adhesion and resistance to detachment assay, a number of ECM substrates are used. To evaluate cell motility, migration and invasion assays are done. Morphological cell changes are detected by immunofluorescence: cells are stained with Alexa Fluor-488 conjugated phalloidin to detect actin filaments and with anti-paxillin antibody to detect focal adhesion complexes and then they are observed at confocal microscope. To evaluate gelatinase activity, zymography of cell supernatants is performed. RhoA activity are measured by the affinity-precipitation assay on the basis of the specific interaction of activated RhoA with Rhotekin: protein expression and activation status are detected by electrophoresis and western blot techniques. NAMI-A interferes with every step of metastasis formation: it increases adhesion force of tumour cells to the growth substrates; it interferes with tumour cell adhesion to several substrates and to endothelial cells; NAMI-A reduces significantly cell invasion and migration, abolishing TGF-β1 pro-invasive effect. NAMI-A's anti-migratory effect is stronger in haptotaxis than in chemotaxis, suggesting that NAMI-A activity involves tumour cell contacts with molecules of extracellular matrix; and finally it inhibits gelatinase production, without affecting TGF-β1 pro-gelatinolytic effect, suggesting that NAMI-A's anti-metastatic effect is unrelated to TGF-β machinery modulation. These effects are stronger on highly-invasive tumour cells than on poorly-invasive tumour cells or on not tumourigenic cells. On the basis of recent data describing NAMI-A-induced β1-integrin activation associated to actin remodelling, we also analysed the status of an important downstream molecule, RhoA, on the metastatic cells MDA-MB-231. NAMI-A increases RhoA activation on cells still adhered to the substrate, followed by the increase of actin polymerization and by a significant modulation of cell morphology. Instead NAMI-A decreases RhoA activation on cells in suspension. These data suggest that probably NAMI-A has a differential activity on activated or inactivated integrins, leading to cell adhesion inhibition, when cells are in circulation, or blocking their movement if they are already attached to the substrate.

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Nemosis: a novel type of fibroblast activation in inflammation and cancer

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We have shown that clustering of fibroblasts induces a massive proinflammatory, proteolytic (plasminogen activation, MMP-1, MMP-10, MT1-MMP) and growth factor response. This novel type of fibroblast activation, designated nemosis, which terminates in programmed necrosis, can be induced by tumor cell-derived soluble mediators promoting fibroblast clustering. A hallmark of this cell activation is the induction of cyclooxygenase-2 expression (60-fold compared to monolayers), frequently associated with inflammation and tumor progression. Several chemokines (e.g. IL-8, MIP-1alpha, RANTES) are massively induced promoting leukocyte migration. Nemotic fibroblasts produced

large amounts of HGF/SF (>200-fold) promoting outgrowth and invasiveness into collagen lattice of tumor cells provided c-Met is properly processed and phosphorylated. Using gene silencing (conditionally knocked-out fibronectin-/- and RGD/RGE-mutated cell lines and siRNA) and function-blocking antibodies we find that nemosis is initiated by interaction of cellular fibronectin with its integrin receptors alpha-5 and -v in conjunction with beta1 subunits. Genome-wide microarrays showed nemosis signaling to induce a specific gene expression fingerprint (e.g. >100-fold induction of 30 genes). We can modulate nemosis/HGF production by e.g. COX-2 inhibitors. We intend to use nemotic fibroblasts therapeutically to promote tissue repair processes and to use "anti-nemotic factors", which we have detected, to control tumor progression and unwanted inflammation. - Supported by grants from Finnish Cancer Societies, Academy of Finland and Magnus Ehrnrooth Foundation.

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ALK5 promotes tumor angiogenesis by up-regulating matrix metalloproteinase-9 in tumor cells

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Spreading of cancer to distant sites (metastasis) is the main cause of mortality of cancer patients. Prevention of development and treatment of metastatic cancers remain important tasks for breast cancer medicine. Metastasis is a complex process involving tumor invasion, tumor angiogenesis and cell survival at distant sites. Transforming growth factor beta 1 (TGF-β1) is a potent tumor suppressor but, paradoxically, in advanced cancers TGF-β1 enhances growth and metastasis. Given a dual function of TGF-β1 in cancer progression, the development of therapies targeting TGF-β requires identification of specific TGF-β pathway components that contribute to development of metastases. This study investigated the role of TGF-β type I receptor, ALK5, and three mitogen-activated protein kinases (MAPK) signaling cascades (JNK, p38 MAPK, and ERK1/2) in metastasis using orthotopic xenografts of breast cancer MDA-MB-231 cells in SCID mice. We found that disruption of autocrine TGF-β-ALK5/TβRI signaling in carcinoma cells significantly reduced tumor angiogenesis in both orthotopic and subcutaneous (sc) xenograft models. Expression of dominant-negative ALK5 (DN-ALK5) blocked up-regulation of matrix metalloproteinase MMP-9/gelatinase-B by TGF-β1 signaling. Suppression of MMP-9 by RNA interference (RNAi) reduced tumor invasion and delayed tumor growth. Importantly, RNAi-MMP-9 reduced tumor neovasculature and increased tumor cell death. The analysis of the TGF-β signaling cascades showed that induction of MMP-9 requires MEK-ERK but not Smad, JNK or p38 MAPK. Dominant-negative MEK blocked and constitutively active MEK1 enhanced expression of MMP-9, whereas blockade of p38MAPK by inhibitors, DN-p38alpha, or RNAi-p38alpha had no effect. Together, these findings suggested that the TGF-β-ALK5-MAPK-MMP-9 pathway promotes tumor angiogenesis by enhancing recruitment of vascular cells (endothelial cells, pericytes, myofibroblasts) from stromal compartments. To test this hypothesis, tumor cells were placed subcutaneously alone or in combination with non-tumorigenic stromal cells. The analysis showed that the presence of stromal cells restored tumor growth and tumor angiogenesis in the DN-ALK5 xenografts to

the levels of control xenografts. MRI studies of tumor xenografts confirmed improved blood flow in tumors containing stromal cells.
Collectively, these findings demonstrate that the TGF-β1-ALK5-MAPK-MMP-9 pathway in carcinoma cells promotes tumor growth and metastasis by enhancing tumor angiogenesis via recruitment of vasculature-forming cells from stromal compartments.
The study was supported by PHS grant R01 CA95263 and USAMRMC grant DAMD17-02-01-0602 (to A.V.B.).

Cytoskeleton stability affects leukaemia cell migration via FLT-1, involving RhoA and Rac1 activation and lipid raft/caveolae formation.

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VEGF binds to tyrosine kinase receptors FLT-1 and FLK/KDR, regulating the survival, proliferation and migration of distinct cell types, such as endothelium and also leukemia cells. We have previously demonstrated crucial roles exerted by FLK/KDR in acute myeloid leukemia, where its blockage affects leukemia survival and proliferation (Constantino, S. and Dias, S., 2004, 103; 3883-3889). In the present study we focused on FLT-1 function and started to study the molecular mechanisms whereby it modulates leukemia cell migration in response to VEGF/PlGF stimulation. We first observed the formation of cellular protrusions on AML/ALL cells after PlGF/VEGF stimulation, with evidence for polimerized actin and FLT-1 co-localization (as assessed by phalloidin immuno-staining and confocal microscopy). Cytoskeleton destabilization by taxol and cytochalasin D both represses AML migration and affects FLT-1 distribution at cellular membrane. In vitro studies showed that FLT-1 interacts both with β-actin and β-tubulin, structural components of AML cytoskeleton and with the molecular chaperone Hsp90. Afterwards we investigated the mechanisms whereby FLT-1 and actin co-localize at cell protusions, after ligand stimulation. By Western blot analysis we observed that PlGF stimulation increases the protein level of RhoA and Rac-1 GTPases and the protein level of caveolin-1, the major component of caveolae vesicles. Next we used nystatin, an agent that impairs lipid raft formation and the formation of cholesterol/sphingolipid membrane domains and in these conditions leukemia cell migration is blocked (Fragoso, et al, 2006; BLOOD, 107; 1608-1616) with concomitant decrease in FLT-1 co-localization with actin filaments. Also, FLT-1 co-precipitates with Caveolin-1. This in vitro association is decreased when microtubules and actin filaments are disturbed and also by cell treatment with nystatin. These results demonstrate that in leukaemia cells FLT-1 and Cav-1 form a complex. Taken together, we hypothesize that FLT-1 localization in lipid-raft/caveolae membrane domains allows its interaction with actin filaments and downstream effectors resulting in cell migration.

Melanoma progression and microenvironment influence

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Malignant cutaneous melanoma is a very aggressive form of skin cancer. It generates in melanocytes, cells derived from the neural crest, that make the skin pigment called melanin. Although melanoma accounts for only about 4% of all skin cancer cases, it causes most skin cancer-related deaths.
Braf gene is involved in two thirds of malignant melanoma cases and it is a molecular element of the MAPK pathway. Activating mutations of Braf have been characterized in melanoma and in other cancers. V599E

(exon 15) is the most frequent alteration; this mutation mimes the necessary event of phosphorilation for the activation of Braf determining its constitutive activation. In our study we analyzed 55 biopsies from common nevi (n= 22), primary radial growth phase malignant melanoma (n = 15), primary vertical growth phase malignant melanoma (n= 13) and melanoma metastasis (n= 5). Global gene expression profiling of the tissues was performed using whole genome oligo- microarrays with a dye-swap duplication scheme; moreover, some samples were also sequenced to look for BRAF mutations.
Tumor microenvironment is an important factor for cancer development. Infact Braf mutations can have different effect on cells behaviour in a microenvironment-dependent way. We analyzed the gene expression profiling of BRAF mutated samples at different melanoma progression stages, in order to find any alternative activated way in mutated samples deriving from different site/microenvironment.
We also looked specific class signatures and found that melanoma metastasis clearly show a distinct expression pattern, typical of an anchor-independent growth both at local and distant sites.

Tumor-stromal cell interaction regulates CSF-1-mediated colon cancer progression

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In many solid tumor types, macrophages are recruited through the local expression of chemoattractants such as colony stimulating factor 1 (CSF-1). CSF-1 is produced by a variety of cells and represents the primary regulator of tissue macrophage production mediated through interaction with the CSF-1R receptor which is the product of the c-fms proto-oncoprotein. Importantly, over-expression of CSF-1 is correlated with poor prognosis in a variety of tumors.
We have previously shown that inhibition of host CSF-1 in human embryonic, colon and breast cancer xenografts in mice suppresses tumor growth and increases survival. Associated with this suppression, we have observed decreased tumor vascularity, reduced expression of angiogenic factors and metalloproteinases (MMPs) and decreased macrophage recruitment to tumors. These results, in combination with the recently recognized role of macrophages as vascular endothelial growth factor (VEGF) secreting cells, suggest that cancer cells can produce cytokines that upregulate host CSF-1 leading to macrophage recruitment and macrophage modification of the ECM facilitating both angiogenesis and tumor development.
The mechanisms by which colon cancer cells upregulate CSF-1 produced by macrophages that results in the promotion of tumor progression are not clearly understood. This study addressed this issue by exploring the interplay between CSF-1-negative SW620 colon cancer cell derived cytokines on cellular proliferation, migration and gene expression regulation in vitro and in an established colon cancer xenograft model.
Profiling of tumor cell cytokine expression in SW620 tumor xenografts in nude mice showed increasing human tumor necrosis factor-α (TNF-α) mRNA expression with tumor growth. Treatment of macrophages with small interfering (si)RNAs directed against TNF-α or with TNF-α-depleted conditioned medium of SW620 cells suppressed mouse macrophage proliferation and migration as well as the expression of CSF-1, VEGF-A and MMP-2. Consistent with these results, human TNF-α gene silencing decreased mouse macrophage TNF-α, CSF-1, MMP-2 and VEGF-A mRNA expression in macrophages co-cultured with human cancer cells. In addition, siRNA inhibition of human TNF-α or mouse CSF-1 expression reduced tumor growth in SW620 tumor xenografts in mice. These results suggest that colon cancer cell-derived TNF-α stimulates TNF-α and CSF-1 production by macrophages and that CSF-1 in turn, induces macrophage VEGF-A and MMP-2 in an autocrine manner. The data suggest that the proinflammatory cytokine TNF-α is linked to tumor progression

by acting as an autocrine growth factor for colon cancer cells and by upregulation of CSF-1-dependent gene expression in macrophages in a paracrine manner. Thus, interrupting tumor cell-macrophage communication by targeting TNF-α may provide an alternative therapeutic approach for the treatment of colon cancer.

Extracellular matrix molecules and their receptors undergo cell- and type-specific turnover during bone marrow recovery following irradiation: relevance for organ homeostasis and in malignancy.

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Extracellular matrix (ECM) molecules are expressed and accumulate following particular environmental and/or genetic developmental/physiological programs, and thus their presence within a certain tissue/organ is certainly essential for tissue integrity and, ultimately, organ function in normalcy and in disease. With the aim of determine the role of ECM molecules in bone marrow (BM) homeostasis, we submited mice to a sub-lethal dose of irradiation (300 rad), in order to promote cell turnover and BM recovery. Then, we analyzed the ECM and integrins expression patterns by imunofluorescence staining, RT-PCR and RQ-PCR, during BM recovery (3, 5, 10, 15 and 30 days post-irradiation). In non-irradiated BM (control), laminins and collagen IV are present in basement membranes around blood vessel and adipocytes, while fibronectin is present all over the tissue, probably associated with stromal cells. Although the number of BM cells decreases drastically in the first days following irradiation (3-10 days), ECM molecules are still present. Fibronectin seems to fill the now empty spaces and exhibits a strong association with megakaryocytes. A less intense staining is observed for collagen IV at day 5, probably due to blood vessel reconstruction. By RT-PCR we confirmed that all the ECM molecules analysed and several of their integrin receptors (α3, α4, α6 and α7-containing integrins) are also present during BM recovery at the mRNA level. However, RQ-PCR demonstrated that several of these molecules (like fibronectin, α4 and α5 integrin chains) undergo quantitative variations during BM turnover, indicating that ECM-BM cells interactions are important for the regulation of this process. Moreover, after cell isolation of non-irradiated BM extracts, we conclude that, differently from previously thought, not only stromal cells produce ECM molecules, but also the hematopoietic cells. In fact, stromal cells seem to produce only fibronectin. Also interestingly, endothelial precursor cells seem to produce small amounts of laminins and collagen IV, ECM molecules that will be part of the basement membrane around mature blood vessels. Our data indicate that in the BM microenvironment ECM molecules undergo a slow turnover, suggesting that following irradiation the remaining cells (megakaryocytes, stem cells) maintain tissue integrity. Our ongoing studies are focusing on the manipulation of ECM molecules on a cell-specific basis and the net importance of ECM production in BM recovery and for leukemia engraftment.

Characterization of Extracellular Matrix Composition in Breast Carcinoma

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Background:
A different view of the tumor as a functional tissue interconnected with the microenvironment has been described (1). Numerous recent reports have indicated that growth

and progression of breast cancer cells as well as other tumour cells, depends not only on their malignant potential, but also on stroma components present in the surrounding microenvironment (2). Recent gene expression profiling studies on breast cancer showed that molecular classification of tumours based on the gene expression patterns can identify clinically different subtypes of cancer with different prognosis or disease outcome. (3-4). Since tumours are functional tissues which are dynamically interconnected with the microenvironment molecular profiling of both stroma and tumor cells will give more information of the tumor phenotype.

Material and Methods:

Immunohistochemical staining was carried out for several extracellular matrix component (ECM) molecules like fibronectin, fibulin1, and laminin in a cohort of 29 formalin-fixed, paraffin-embedded primary breast tumours. Furthermore, evaluation of haematoxylin and eosin (H&E) sections was performed to classify the surrounding stroma in categories of loose, dense or mixed, respectively.

Gene expression analyses of the fresh frozen and grossly dissected breast tumors from the same patients were performed using 22K 60-mer Human 1A Oligonucleotide (G4110A) provided by Agilent Technology. An ECM gene list of 282 unique ECM related genes was created by querying the Agilent web site for specific keywords, and used to interrogate the breast cancer transcriptional profiles.

Results:

We defined a set of 282 ECM related genes whose expression separated the tumours in three main groups. We compared the ECM groups, defined by gene expression profile to the IHC staining results and to stroma categories. Significant correlation between stroma tumor types and ECM gene expression profile was retrieved. Using these 3 groups as variables in a multiclass SAM analyses we were able to enrich for genes that significantly classified the samples.

To validate the enriched ECM geneset of 2235 genes identified by SAM, able to distinguish the 29 tumors into 3 subclasses and to correlate the groups to clinical outcome we retrieved these genes in an independent new dataset of 77 samples, from patients who received adjuvant therapy treatment and where long term follow up information was available. The new analysis allowed us to identify the same three clusters, indicating the robustness of the ECM classification. Survival analyses showed significantly different outcomes for the patients belonging to the three ECM groups. Patients belonging to ECM1 cluster, presenting a dense-type of stroma and no over expression of collagen types, laminin chains, and matrix-associated proteins, showed the worst outcome. Samples were also classified based on breast cancer subtypes published by Sørlie et al using a select list of 534 genes, known to discriminate tumours into distinct subclasses with clinical implication. In our study, basal-like tumours showed a strong propensity to cluster to ECM group1 in both datasets, while the other subtypes were clustering across the three ECM groups.

Conclusion:

Gene expression profile of breast carcinomas allows the identification of 3 subgroups of tumors according to the ECM-associated gene expression. We show that both overall survival and distant metastasis-free survival are markedly diminished in patients whose tumors expressed a specific pattern of ECM signature. These findings provide new information on breast carcinoma biology and new parameters that may have an impact in prognosis and prediction to therapy response, arising the hypothesis that clinical outcome is strongly related to the stroma characteristic.

Tumor Endothelial Marker 8 (TEM8) Gene Expression in Proangiogenic and Cell Migratory Programs

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Tumor endothelial marker 8 (TEM8) gene was uncovered as a gene selectively up regulated in tumor versus normal blood vessels, and its product identified as a putative endothelial integrin. However, comparison of tissues-specific TEM8 expression profiles from general database and bioinformatics analysis, suggest that TEM8 gene could be actively transcribed by different cell types involved in extra cellular matrix-remodeling and migration processes, as those observed in inflammatory reactions and tumor progression.
Tumor cell migration and metastases share many similarities with leukocyte trafficking, which is critically regulated by chemokines / chemockine receptors interactions in a co-ordinate fashion with cell-surface integrins. Here we report that highly metastatic (CXCR4+) MDA-MB-231 breast cancer cells, which release potent pro-inflammatory and angiogenic mediators such as Prostaglandin E2 (PEG2) and vascular endothelial growth factor (VEGF), accumulate high levels of TEM8 transcripts (as judged by Quantitative Real-Time PCR and Northern blotting analysis). Likewise, we find that migratory (CCR7+) and proangiogenic (VEGF-releasing) monocyte-derived dentritic cells (MoDCs) matured in the presence of PGE2, markedly enhanced TEM8 expression. Neither VEGF production nor accumulation of TEM8 transcripts was observed in nonmetastatic ZR 75-1 breast cancer cell or in no migratory (CCR7-) MoDCs matured with Poly-I: C. We speculate that TEM8 may have a role in cellular mechanisms that foster tumor inflammatory angiogenesis and tumor metastases.

Norepinephrine differentially regulates the extravasation of neutrophil granulocytes and tumor cells as analyzed in vitro under flow conditions

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Extravasation of leukocytes is an important physiological process during the immune response of an organism for the defense of pathogens. On the pathological side, extravasation of tumor cells is a crucial step during the haematogenic metastasis formation. Therefore it is essential to understand the precise mechanisms by which leukocytes and tumor cells adhere and penetrate the endothelial cells to be able to prevent tumor metastasis without hindering normal immune function. We have shown previously that various neurotransmitters such as norepinephrine are strong regulators for the migration of tumor cells and leukocytes. In this work, we have investigated the influence of norepinephrine on the extravasation of different tumor cell lines and neutrophil granulocytes through human pulmonary arterial endothelial cells. Therefore we have developed a stable and sensitive in vitro extravasation assay, which enables studies under physiological flow-conditions. In this assay a suspension of tumor cells or granulocytes was applied with a constant physiological shear rate under conditions of laminar flow over a pre-activated endothelial cell monolayer. Subsequently, the adhesion process was recorded by live videomicroscopy for 30 minutes. For analysis, the number of adherent and rolling cells as well as the rolling velocity was determined. In this extravasation assay, the adhesion of neutrophil granulocytes was significantly increased after stimulation with fMLP. Additional treatment of these cells with norepinephrine decreased the number of adherent cells by 20%. In contrast, stimulation of e.g. MDA MB435 breast carcinoma cells with norepinephrine resulted in a fourfold increase of cells adhering to the endothelial monolayer. Interestingly, the observed effects of norepinephrine could be completely inhibited by treatment of the cells with the beta-adenoreceptor blocker propanol. Comparing these results with our data from in vitro migration assays, we have observed that norepinephrine acts in a cell type specific manner with distinct effects on the migratory behaviour of the cells and their interaction with the endothelial cells. Whereas in neutrophils norepinephrine decreased

both the migration and the number of adherent cells, in tumor cells the norepinephrine-induced migratory phenotype was not necessarily coupled to the development of an extravasive phenotype and vice versa.
This work was supported by the Fritz Bender Foundation, Munich, Germany.

Evasion of tumor-derived endothelial cells (tec) from the antiangiogenic activity of the interferon-inducible gene ifi16.

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The human interferon (IFN)-inducible gene IFI16 is an member of the HIN-200 gene family, implicated in the regulation of cell proliferation, angiogenesis, and modulation of the immune response by IFNs. Immunohistochemical analysis revealed that IFI16 is highly expressed in endothelial cells and squamous stratified epithelia, in addition to hematopoietic tissues. We demonstrated that overexpression of the IFI16 protein in human umbilical vein endothelial cells (HUVEC) by an adenoviral-derived vector (AdV-IFI16) suppressed tube morphogenesis in vitro, cell cycle progression and cell growth due to induction of apoptosis. These activities appear to be mediated by interaction of IFI16 with both p53 and NF-kappaB complexes, as demonstrated by transfection and co-precipitation experiments. Based on this premise, the aim of this study was to assess the capability of IFI16 to suppress angiogenesis of tumor-derived endothelial cells (TEC) compared to HUVEC. To this purpose, TEC lines derived from highly-vascularized human renal carcinoma (Eck25, Eck28), head/neck solid tumor (HN4), and breast cell carcinoma (BTEC), have been employed. These cells, which are characterized by a constant expression of endothelial activation and angiogenesis, showed enhanced survival and angiogenic properties both in vitro and in vivo experiment. To verify whether IFI16 exerted its antiangiogenic activity in TEC as well, both HUVEC and TEC were infected with AdV-IFI16 and assessed for cell growth, cell-cycle progression, tube morphogenesis and sensitivity to apoptosis. The results obtained showed that TEC, upon IFI16 overexpression, maintained their capability to proliferate, generate capillary-like structures when plated on Matrigel, progress into the cell cycle and escape apoptosis. Activation of caspase-2 and -3 by IFI16 is indeed suppressed in TEC lines compared to HUVEC. Altogether, these results demonstrate that TEC become resistant to the antiangiogenic activity of the IFI16 gene and offer new insights into the strategies adopted by tumors to escape IFN antiangiogenic activity.

Responsiveness to breast carcinoma therapy and the extracellular matrix protein fibulin-1.

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To gain insight into the biological role of the extracellular matrix (ECM) associated fibulin-1 (fbln-1) protein in response to chemotherapy, we investigated its expression and functional behaviour both in vitro and in vivo models. We firstly found by RT-PCR, FACS and ELISA analyses that fbln-1 was up-modulated in several human breast cancer cell lines and their corresponding culture supernatants upon treatment with IC_{30} doses of doxorubicin (DXR) for 72 hours. In keeping, xenotransplants from the human breast cancer cell line MDA-MB-361 grown in athymic

mice treated once with DXR showed higher fbln-1 transcript and protein levels compared to those found in tumors arisen in untreated mice and mass spectrometry confirmed that it was exclusively of human origin. To evaluate the direct involvement of fbln-1 in drug susceptibility, the breast carcinoma cell line MCF-7 was silenced for fbln-1 expression by RNAi and, following its knockdown, fbln-1-silenced tumor cells displayed a ten-fold increase in DXR-sensitivity compared to controls. An enlarged analysis of the ECM molecules involved in in vivo drug susceptibility indicated that xenografts from DXR-treated animals also overexpressed fibronectin and laminin-1 compared to controls. MDA-MB-361 cells injected in athymic mice in the presence of matrigel formed tumors significantly (p=0.01) more DXR-resistant compared to those arisen in mice injected without matrigel, strongly indicating that ECM exerts a protective effect on DXR-mediated cytotoxicity. Tunel analysis of tumor biopsies from treated and untreated animals demonstrated that matrigel protects tumor cells from drug-induced apoptosis. In keeping with these findings, a consistent number of human breast tumor cell lines grown in vitro on matrigel and incubated for 72 hours with DXR were found significantly protected from drug-induced apoptosis compared to cells incubated in absence of matrigel. In addition, a pool of MAbs directed to fbln-1 significantly reverted the matrigel-dependent drug resistance. Globally, our findings strongly indicate that the ECM milieu of breast tumor, and in particular fbln-1, strongly impacts the responsiveness of tumor cells to drugs. (Partially supported by AIRC).

Manipulation of the tumor-associated vasculature with TNF-based therapy is mediated by MMP.

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Introduction:
Manipulation of endothelial cell lining in the tumor-associated vasculature by tumor necrosis factor alpha (TNF) results in an increased permeability of these vessels. The improved permeability leads to a higher accumulation of chemotherapeutic drug inside the tumor and enhanced tumor response compared to tumors treated without the addition of TNF. We investigate the potential role of MMPs in these permeability changes.
Methods:
To investigate the production of MMPs and their effect towards endothelial cells, we stimulated human umbilical vein endothelial cells (HUVEC) with TNF, interferon-gamma (IFN) and peripheral blood mononuclear cells (PBMC) and made observations towards MMP-9 and 2 production. Tumor sections of animals treated with or without TNF were stained for localisation of MMP-9 and 2.
Results:
Endothelial cells stimulated with TNF, IFN showed a reduction in cell growth, cells began to elongate and contact between cells was breached leading to increase in permeability. Addition of PBMC even further enhanced these effects and an increase in MMP-9 production by the endothelial cells was observed. The active from of MMP-2 was found in cells stimulated with TNF with our without co-administration of IFN and PBMC and when MMP production was inhibited cell death was prevented and morphological changes were not as extreme. We also found that endogenous produced interleukin-1β by TNF-stimulated PBMC was responsible for these effects. Preliminary results in tumor section after TNF treatment showed some destruction of the vessels and re-localisation of MMP-9 positive cells around the vessels compared to sham treated tumors. Also in areas with increased permeability of chemotherapeutic drug, only observed when TNF was added, an increase in MMP-9 positive cells was found. **Discussion**: *Manipulation of the endothelial vessels in the tumor with the use of TNF, thereby increasing uptake of chemotherapeutic drug is an effective tool for tumor therapy. Endothelial cells and PBMC stimulated with TNF produce, among other*

things, MMPs that can be responsible for the destruction of the tumor-associated vasculature, leading to shrinkage of the tumor. So we conclude that in this specific TNF-based therapy MMPs are essential for a good tumor response.

Epidermal growth factor receptor transactivation is required for endothelin-1-induced activation of the -catenin pathway in human ovarian cancer cells.

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The endothelin-1 (ET-1)/endothelin A receptor (ETAR) autocrine pathway is overexpressed in primary and metastastic ovarian carcinoma. In this tumor, ET-1/ETAR contributes to cancer progression by inducing proliferation, survival, neoangiogenesis, loss of intercellular communication, and invasion. Recently, we demonstrated that the autocrine ET-1/ETAR pathway in ovarian cancer cells induces epithelial to mesenchymal transition (EMT), a key event in cancer metastasis. Because ETAR-driven signaling is in part mediated by epidermal growth factor receptor (EGFR) transactivation, we examined whether the cross-communication between ETAR and EGFR was critical to regulate the integrity and function of adhesion complex, representing an alternative mechanism for activating β-catenin signaling in human ovarian cancer cells. In this study we provide evidence that, in HEY and OVCA 433 ovarian carcinoma cells, ET-1 elicited time- and dose-dependent β-catenin tyrosine phosphorylation that was strongly prevented by both ETAR antagonists and specific small-interfiring RNA (siRNA) targeting ETAR, thus validating ETA as the receptor involved in these effects. Using cell fractionation technique we demonstrated that increased β-catenin tyrosine phosphorylation was associated with cytosolic and nuclear accumulation of β-catenin. Moreover, we observed in ET-1-treated cells a reduction in the phosphorylation of β-catenin on serine/threonine residues and high levels of both activated Akt and phosphorylated glicogen synthase kinase-3, underlying that impaired degradation of β-catenin is at least partially involved in the accumulation and activation of β-catenin. To test the hypothesis that EGFR activation via Src mediates β-catenin phosphorylation, we analyzed the phosphorylation status of β-catenin induced by ET-1 in the presence of selective inhibitors. Interestingly, the inhibitory activities of AG1478, a specific EGFR inhibitor, and PP2, a specific Src inhibitor, on ET-1-induced effects implicated that Src-mediated EGFR transactivation occurred downstream of ET-1/ETAR activation to induce β-catenin tyrosine phosphorylation and loss of β-catenin/E-cadherin association. In addition, we found that ET-1-dependent EGFR transactivation is involved in β-catenin nuclear function by promoting its binding to the T-cell-specific transcriptional factor (TCF)/lymphoid enhancer factor-1 (LEF) transcription factor and its transactivating ability. These effects were seemingly specific, because we observed the ET-1 upregulated the expression of β-catenin target genes that are implicated in invasiveness and aggressive behavior of cancers such as cyclin D1, c-Myc, uPA and MMP-7, demonstrating that the cross-talk between cell surface receptors expands the cellular communication network leading to β-catenin signaling.

Finally, we demonstrated that inhibition of EGFR activation reduced ET-1-induced matrix metalloproteinase activation as well as cell migration and invasion, indicating that the cross-signaling between the EGFR/ETAR may also account for the enhanced aggressive phenotype by ET-1. The cross-signaling between the EGFR/ETAR pathways along with the emerging role of ET-1 axis in ovarian tumorigenesis

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and progression provide a rationale for targeting ETAR and EGFR supporting the use of ETAR specific antagonists in developing new therapeutic approaches to ovarian cancer.
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Endothelin-1 and -3 induce proangiogenic and invasive behaviour via hypoxia-inducible factor-1 in human melanoma cell lines

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Endothelin B receptor (ET_BR) is overexpressed in most human cutaneous melanomas and its activation by endothelin-1 (ET-1) triggers melanoma progression by promoting cell proliferation, adhesion, migration and invasion. We investigated the relationship between endothelin axis and vascular endothelial growth factor (VEGF) in primary and metastatic human melanoma cell lines. We found that ET-1 and ET-3 increase VEGF, cyclooxygenase (COX-1), COX-2 expression and promoter activity, and do so to a greater extent under hypoxic environment. Moreover, both COX-1/2 inhibitors reduce ET-3-induced prostaglandin E_2 (PGE_2) and VEGF secretion, tumor matrix metalloproteinase-2 and -9 activation, and cell invasion indicating that both enzymes participate to these events. We next show that ET-1 and ET-3-induced VEGF expression is associated with hypoxia-inducible factor (HIF)-1α protein stimulatation under normoxic conditions and that this effect is amplified by hypoxia. The ET_BR selective antagonist, BQ788, and the small interfering (si)RNAET_BR reduce the expression of ETs-induced VEGF and PGE_2production, COX-2 promoter activity, COX-1/2 and HIF-1α protein expression under both normoxic and hypoxic conditions. Furthermore downregulation of HIF-1α expression by siRNA in melanoma cells desensitizes COX-1/-2 expression and promoter activity in response to ET-3, indicating that ETs induce COX activity via HIF-1α . These results provide a new mechanism whereby ET-1,-3 acting selectively through ET_BR can promote and interact with the HIF-1α dependent machinery to amplify the proangiogenic and invasive phenotype of melanoma cells also in normoxia. New therapeutic strategies using specific ET_BR antagonist could provide an improved approach to the treatment of melanoma by inhibiting tumor growth and progression.

"GAR-force" Dynamics in Melanoma Metastasis *(GAR = Growth-saturation- and ALCAM-associated Responses)*

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Biological development is essentially a paradigm of complex information rocessing. Exceeding preset thresholds of growth saturation in cancer could generate a homeostatic selection condition, which would ineluctably drive neoplastic progression and eventually cause metastasis. Focusing on cell-to-cell interactions in melanoma, we have shown that cell-clustering promoted by the cell-to-cell adhesion molecule ALCAM/CD166/MEMD, is rate limiting for tissue invasion, an initial step in metastasis. As a proof of concept for growth coordination by ALCAM, progressive accumulation of melanoma cells triggers metastasis-associated proteolytic cascades and a cluster of genes sensitive to ALCAM functionality and cell-confluence. Primary responding genes include Wnt-5a. Wnt-5a "best determines invasiveness" in a large-scale, NIH micro-array study of melanoma
lesions and cell lines. Since ALCAM is broadly involved in vertebrate development, our observations constitute the "GAR-force" hypothesis: <ALCAM,

a cell-surface sensor and checkpoint for growth saturation, coordinates dynamics of local and invasive cell-growth in developmental (proto-)oncoprogams>. Our experiments unveil the underlying concept of the "GAR-force" hypothesis that (tumor) tissue growth and development may be coordinated by a supra-cellular progression circuit with main molecular checkpoints and conjugated with the cell cycle. Characterizing the connections and main molecular switches of the overall circuitry is a mandatory step forward to understand neoplastic progression in general and will directly pinpoint crucial targets for innovative cancer and metastasis therapies.

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2007 Pezcoller Foundation-AACR International Award for Cancer Research

The Pezcoller Foundation-AACR International Award for Cancer Research is given annually to a scientist anywhere in the world who has made a major scientific discovery in the field of cancer, who continues to be active in the field, and whose ongoing work holds promise for future substantive contributions to cancer research. The Award recognizes extraordinary basic or translational cancer research. The Award will be presented to a single investigator for his or her highly original work. In extraordinary circumstances, two individuals may be selected to share the award when their investigations are clearly related and have resulted in prizeworthy work. The Awardee will be selected by an International Committee of AACR members appointed by the AACR President with the agreement of the Council of the Pezcoller Foundation. The selection will be made solely on the basis of the Awardee's scientific accomplishments without regard to race, gender, nationality, geographic location, or religious or political views.

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. Over the past decade the Pezcoller Foundation, in collaboration with the ESO-European School of Oncology, gave a major biennial award for outstanding contributions to cancer and cancer-related biomedical science.

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 22,000 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 word.

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 Foundation and the AACR to scientific excellence in cancer research, these organizations are now collaborating annually on the presentation of this 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 (April 14 – 18, 2007 in Los Angeles) and will receive the award in a ceremony at the Foundation's headquarters in Trento, Italy, after the AACR annual meeting (May 4, 2007).

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

In addition the winner will give the Korsmeyer Lecture at the Venetian Institute of Molecular Medicine (VIMM) in Padova (Italy).

Nomination Deadline: Friday, September 15, 2006

Questions about the nomination process – www.aacr.org - should be directed to the AACR Office (Philadelphia) via Fax at (215) 440 9372- Telephone (215) 440 9300 - or E-mail awards@aacr.org

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