Meeting Report

1st Kloster Seeon Meeting "Cellular and molecular mechanisms of tumor progression and metastasis – Seeon Monastery, Sept. 22-25, 2007

Session 1: GET OUT AND RUN

"Invasive growth: A MET-driven genetic programme for cancer and stem cells"

Paolo Comoglio introduced recent data showing that the MET proto-oncogene is a key regulator of invasive growth in cancer and stem cells. He presented data demonstrating that lentiviral transduction of liver progenitor cells with MET in a mouse model led to the progression of liver cancer preceded by a thrombo-hemorrhagic syndrome. In addition, MET-silencing experiments revealed the importance of MET for invasion and metastasis. Further examinations detected the hypoxia-dependent transcription of MET in primary tumors. Taken together, the data indicate that MET tyrosine-kinase receptor is a sensor of adverse microenvironmental conditions (such as hypoxia) and drives cell invasion and metastasis through the transcriptional activation of a set of genes that control blood coagulation.

"Distinct mechanisms of tumor cell invasion and metastasis"

Gerhard Christofori presented three distinct pathways of tumor cell invasion. Single cell invasion is accompanied by an epithelial-mesenchymal transition (EMT) that involves the activation and repression of a large number of genes that modulate the migratory and invasive behaviour of tumor cells (loss of E-cadherin and gain of N-cadherin, upregulation of NCAM). In contrast, collective cell migration requires the maintenance of cell-cell adhesion and EMT does not occur. Furthermore, it was shown in cultured human breast cancer cells and in the Rip1/Tag2 transgenic mouse model that podoplanin, a small mucin-like protein, induces a novel molecular pathway of collective tumor cell migration and invasion that does not involve the loss of E-cadherin function or EMT. Podoplanin induces rearrangements of the actin cytoskeleton and the formation of filopodia. The third type of metastatic tumor cell dissemination involves the upregulation of lymphangiogenic factors, such as VEGF-C and VEGF-D, and the subsequent induction of lymphangiogenesis. Hence lymphatic vessel density within and in the proximity of the expanding tumor increases and leads to lymph node metastasis.

"CD44v6 peptides block metastatic spreading and interfere with angiogenesis"

Véronique Orian-Rousseau gave an interesting talk about the function of CD44v6 in c-Met activation. Her group demonstrated that CD44v6 is instrumental in the metastatic process and angiogenesis by using CD44v6 peptides that interfere with the presentation of the ligand HGF to c-Met and block the activation of the receptor. *In vivo*, these peptides blocked the metastatic dissemination of rat pancreatic carcinoma cells but had no influence on primary tumor growth. In addition, CD44v6 was demonstrated to be a co-receptor for receptor tyrosine kinases in angiogenesis. This was shown by CD44v6 blocking experiments. Upon inactivation of CD44v6, endothelial cell migration, tube formation as well as the *in vitro* spouting of endothelial cells was decreased.

Session 2: GOING THROUGH THE CHANGE

"Control of sprouting and vessel quiescence by MAP-kinases and Rho-GTPase signaling"

Georgia Mavria presented data on the role of Rho-kinase in maintaining endothelial cell quiescence. Rho-kinase activity is upregulated in established tubules and its inhibition leads to a delocalization of VE-cadherin in tumor blood vessels. Delocalization of VE-cadherin promotes endothelial cell protrusions, migration and Rac-dependent sprouting. Taken together, she showed that homophilic

interactions of VE-cadherin promote Rho-kinase activity which phosphorylates myosin-light chain thus promoting its own stability at cell-cell junctions.

"The epithelial-specific integrin alpha v beta6 and its role in tumor invasion"

Ian Hart presented data showing that the integrin αvβ6 is not detectable in healthy tissue, but upregulated in tissue-remodeling events including wound-healing, inflammation and cancer. It mediates the migration of keratinocytes and is preferentially expressed at the leading edge of migrating cells and at the tumor cell-stroma interface. He showed that the 35kD protein HAX-1 binds to the β6 subunit of αvβ6. Using siRNA directed against HAX-1, he presented data showing that HAX-1 regulates αvβ6 dependent migration and invasion in tumor cells. Mechanistically, HAX-1 is involved in αvβ6-mediated migration by regulating its clathrin-dependent endocytosis.

"Role of cadherins and catenins in tumor progression"

Frans van Roy presented an overview of the role of E-cadherin in the suppression of tumor growth, invasion and metastasis. Stimulating data regarding the hypermethylation and binding partners of E-cadherin were presented, and how this is altered in 'cadherin-switching' which is sufficient to induce multiple steps in tumor progression. In addition, the intracellular roles of the cadherin family were discussed, raising the profile of p120ctn, and how this is regulated at both the transcriptional and posttranslation level. Finally, the requisite role of E-cadherin expression upon levels of the transcription factor Nanos1 was demonstrated, which when considered together highlights the signaling role of cell-cell contacts during tumor progression.

"Direct probing of tumor cell invasion by atomic force microscopy"

In his presentation, **Thomas Ludwig** introduced Atomic Force Microscopy (AFM) techniques which allow obtaining data on cell deformability, maximal unbinding forces, individual unbinding events and the total work required to remove a cell from the surface. In addition, it is possible to obtain biophysical parameters like the local plasticity of the extracellular matrix. Taken together, AFM can be used to investigate basic mechanisms of tumor cell invasion.

Session 3: TUBES

"Targeting growth factor pathways for inhibition of angiogenesis, lymphangiogenesis and metastasis"

Kari Alitalo presented data showing that VEGF-C binds to VEGFR-3, thereby promoting the formation of lymphatic vessels especially during development. VEGF-$C^{-/-}$ embryos fail to develop lymphatic vessels, whereas adenovirally expressed VEGF-C induced lymphangiogenesis. Dr. Alitalo's group could show that the lymphatic flow through the axillary are a of mice could be restored by VEGF-C /-D induced formation of lymphatic condroid vessels in this area following removal of the axillary lymph node. Similarly, adenoviral treatment with VEGF-C /-D resulted in an augmented integration of the lymph node transplant and a complete restoration of the lymphatic and sentinel function in transplantation experiments where the lymph node was removed and exchanged against a donor lymph node. Treatment with Lac-Z as control resulted in an atrophic lymph node. VEGF-C also affected the metastatic potential of tumors. Using VEGF-C / -D traps that only affect growing but not resting lymphatic vessels, lymphatic metastases could be reduced. VEGFR-3 antibodies resulted in a reduction of the primary tumor, an effect that could be intensified by Avastin treatment leading to a reduced microvessel density and increased necrosis as well as hypoxia. VEGFR-3 is also expressed by tip-cells in endothelial sprouts in tumors and could be used as a novel marker for this cell type. Blocking of the Notch pathway by Dll4 inhibition led to an upregulation of VEGFR-3 in the tip cell area and excessive angiogenesis, which could be blocked by inhibiting VEGFR-3.

"Tumor and lymph node lymphangiogenesis: Impact on cancer metastasis"

Nadia Tobler dealt with the correlation between lymphangiogenesis and metastasis. She demonstrated that primary human melanomas without lymphangiogenesis showed no metastasis to the sentinel lymph node whereas extensive lymphangiogenesis was correlated with metastatic spread. So the degree of tumor lymphangiogenesis in the primary tumor can be used as a novel marker of patient survival and presence of sentinel lymph node metastasis. Because it is known that VEGF-C is upregulated in metastasizing melanoma, Nadia Tobler performed *in vivo* experiments with VEGF-C and VEGF-A transgenic mice. She demonstrated that VEGF-C or VEGF-A overexpression in tumor cells resulted in increased lymphangiogenesis in the tumor that promotes more numerous and bigger lymph node metastases indicating the ability of tumor cells to actively induce the formation of lymphatic vessels. These mice showed induced lymphangiogenesis in the sentinel lymph node prior to primary tumor metastasis. Thus, tumor cells are able to condition the sentinel lymph node for their arrival.

In the second part of her talk, she addressed the possibility of preventing metastasis by interfering with lymphatic growth. Mcrodissection experiments were pursued towards this end in order to identify genes differentially expressed in tumor lymphatics vs. normal lymphatic vessels, embryonic bodies and the *Xenopus* model to identify new molecules interfering with lymphangiogenesis. The Adenosine A1 receptor and the voltage gated Calcium channel were identified through this approach as novel candidates for anti-lymphangiogenic treatment.

"Contribution of individual MMPs in angiogenesis and lymphangiogenesis"

Agnes Noel addressed the role of different matrix metalloproteases (MMPs) that are involved in tissue remodeling, angiogenesis and lymphangiogenesis. By analysis of different MMP deficient mice, she showed the multiple and even opposite roles of MMPs during tumor progression. MMP-19$^{-/-}$ mice display stronger vessel migration towards the tumor and showed stronger invasion indicating a protective role of MMP-19. MMP-12 and MMP-8 were also identified as negative regulators. Other MMPs including MMP-2, MMP-9, MT1-MMP, and MT4-MMP acted as positive regulators of angiogenesis. MMP-11 played a progression-dependent role as a positive regulator in the early stage of cancer and as a negative regulator during metastasis.

To analyze lymphangiogenesis *in vitro,* she established the lymphatic ring assay (lymphatic rings isolated from mice thoracic duct) by modifying the aortic ring assay. Employing these two assays, she demonstrated a function of plasminogen activation inhibitor 1 (PAI-1) specific during angiogenesis. Aortic rings of PAI-1$^{-/-}$ mice showed no sprouting whereas sprouting out of lymphatic rings from these mice was not affected. In contrast, lymphatic rings from MMP-2$^{-/-}$ mice showed reduced lymphangiogenesis although there was no lymphatic phenotype in these mice. With these examples Dr. Noel introduced the lymphatic ring assay as a new method to study lymphangiogenesis *in vitro*.

Session 4: TUMOR MEETS VESSEL

"The role of the host in organ-specific metastasis: Lung and liver"

Ruth Muschel compared the interaction of metastatic tumor cells with the lung and liver in models of colon and breast cancer metastasis. Metastases remain intravascular in the lungs, whereas tumor cells in liver metastases proliferate and destroy microvascular networks. Angiopoietin-2 (Ang-2) expression is induced by cells adjacent to the tumor vasculature in the liver. Vascular disruption did not occur in Ang-2 deficient mice, although colony growth was more extensive. Taken together, these data suggest that Ang-2 dependent co-option occurs in the liver but not in the lung.

"Crosstalk between tumor and endothelial cells involving the Notch3-Dll4 interaction marks escape from tumor dormancy"

Stefano Indraccolo's showed that endothelial Dll4 triggers Notch3 activation in neighboring tumor cells thereby promoting tumorigenesis. Notch3 and its target genes are upregulated in aggressive

compared to dormant tumors and silencing Notch3 had an anti-proliferative and pro-apoptotic effect on tumor cells *in vitro*, which was associated with the maintenance of the dormant phenotype *in vivo*.

"EphB/eprhinB interactions controlling leukocyte and tumor cell adhesion to endothelial cells"

Mélanie Héroult presented data showing that EphB4/ephrinB2 interactions play a role in the adhesion of leukocytes and tumor cells to endothelial cells. Furthermore, EphB4-expressing tumor cells preferentially home to the lungs upon intracardiac injection *in vivo*, in contrast to cytoplasmically truncated EphB4 expressing cells, suggesting that EphB4 forward signaling is necessary for *in vivo* adhesion of tumor cells to endothelial cells.

CANDLE LIGHT LECTURE

Translational oncology: Quid est?

Christof von Kalle gave a broad overview over the progress in the establishment of the National Center for Tumor Diseases (NCT) in Heidelberg since 2003. Patients benefit from the exemplary structure of the NCT with its central reference point, where comprehensive care is provided. New findings and promising approaches from fundamental research can be applied in clinical practice more swiftly. Furthermore, the German Cancer Research Center contributes two application-oriented research areas to the NCT. "Preventive oncology" is concerned with research on the causes and prevention of cancer, like performing epidemiological studies and creating a tumor database. The second research area, „experimental diagnostics and therapy", is focused on the development of cancer vaccines, therapeutic antibodies, cancer killing viruses or small molecules that block metabolic steps within tumor cells.

Session 5: TUMOR MICROENVIRONMENT

"Tumor microenvironment in cancer progression and metastasis"

Raghu Kalluri analyzed the role of fibroblasts in the interplay of tumor cells with their surrounding stroma during tumor progression. To answer the question regarding the expression pattern of tumor fibroblasts, he established immunofluorescent stainings with α-SMA, FSP-1, PDGFR-β, NG-2 and vimentin. Therewith he identified subpopulations of fibroblasts that are either α-SMA positive or FSP-1 positive. Fibroblast subpopulations expressing both proteins were not found and conversion between the two populations was not possible. FSP-1$^{-/-}$ mice were generated to analyze the function of the FSP-1-positive fibroblast subpopulation. These mice displayed impaired wound healing, angiogenesis and tumor progression as well as a disorganization of the cytoskeleton indicating impaired motility in comparison to wild type mice. Ablation of FSP-1-positive fibroblasts resulted in a decrease of metastatic spread, whereas the number of cancer cells and their proliferation status remain unaffected in the primary tumor,.

"Inflammation in epithelial skin tumors"

Margareta Müller addressed the role of inflammation, especially the recruitment of neutrophils during tumor progression. She used towards this end HaCaT cells in the matrix inserted surface transplantation model in mice. HaCaT cells can be induced by inflammatory agents to develop a benign as well as a malignant phenotype. She detected G-CSF, GM-CSF and VEGF expression in malignant HaCaT cells. The expression of these molecules was controlled in a GF-network involving IL-6 expressed by fibroblasts in the activated stroma. Two contrarily acting subtypes of monocytes play a role during tumor progression. Immune M1 macrophages have a tumor inhibitory effect, whereas tumor associated M2 macrophages promote tumor progression. The M2 phenotype could be induced by stimulation of M1 type macrophages with IL-4. Neutrophil depletion experiments were performed in order to analyze the source of IL-4 in the tumor stroma. This neutrophil depletion resulted

in a decrease of angiogenesis, invasion and MMP expression but did not affect the number of macrophages in the tumor. These results displayed the enhancing role of neutrophils during tumor progression and the balance between neurophils and macrophages in the tumor.

Session 6: UPON FIRST SIGHT

"The metastatic process: Imaging strategies and identifying molecular mechanisms"

Ann Chambers referred in her talk to the necessity for a better understanding of the metastatic process and dormancy. She pointed out that metastasis is an insufficient process because most cells behave in a non-metastatic way. The majority of cells die in early stages and also micrometastases fail to continue to grow. However, metastasis is responsible for most cancer deaths and can occur years after a successful cancer therapy. Thus, long term monitoring of the metastatic process is needed. *In vivo* videomicroscopy (IVVM) is a high-resolution light microscopy but invasive imaging method, thus limiting its ability to monitor metastatic processes over time. Dr. Chambers was able to identify steps controlling the metastasis efficacy through the coupling of IVVM with quantitative "cell fate analyses". The study of the fate of breast cancer cells metastasizing to the brain over time was possible by combining IVVM with Magnetic Resonance Imaging (MRI).

"Quantitative analysis of tumor cell migration in three dimensional matrices through experiments and simulations"

The group of **Muhammad Zaman** studies how cancer cells interact with extracellular matrices in native environments. *In vivo* tumor cell movement is modulated by cell matrix interactions. Adhesion forces, matrix stiffness and proteolysis are critical factors in this process. The group of Dr. Zaman used a combination of high resolution and high throughput imaging and novel computational tools. 3D migration assays allowed to vary concentrations of matrix, ligands and integrin activity and allowed to study the interaction of tumor cells with other cell types. For example, the effects of integrin mutants could be tested. The group could show that the migration speed of human DU-145 prostate carcinoma cells depends on integrin activity, matrix digestion and adhesion forces.

"Collective cancer cell invasion along blood and lymph vessels"

Peter Friedl investigated if tumor cell invasion occurs along pre-existing vessels or if a neovasculature is necessary. To address this question, HAT-1080 fibrosarcoma cells were injected into the dorsal skinfold chamber of nude mice and tumor cell movement was analyzed using intravital multiphoton microscopy. He could show that 80% of tumor cells invade in strands along preexisting blood vessels. The cells moved around 150 µm/day. Cells in central tumor regions did participate in active migration. Cells migrated within these strands and divided at the same time. Invasion occured prior to neoangiogenesis by elongation of pre-existing vessels.

"*In vivo* assessment of experimental melanoma metastasis by intravital microscopy reveals a critical role of platelets and glycoprotein IIb"

The group of **Christian Schulz** investigated the role of platelets and platelet fibrogen receptor glycoprotein GPIIb for melanoma metastasis. Highly metastatic B16-BL6 melanoma cells were fluorescent labelled and injected in the jugular vein of $GPIIb^{-/-}$ or wt mice. Real time intravital video fluorescence microscopy showed an arrest of multicellular aggregates in the lung of wt mice 1h after injection. The accumulation of aggregates was significantly reduced in $GPIIb^{-/-}$ mice. The size of the aggregates was consistently reduced. The number and size of aggregates increased after infusion of wt platelets in $GPIIb^{-/-}$ mice prior to tumor cell injection. Platelet-B16 cell aggregates could be detected in mouse blood by FACS analysis and by immunofluorescence staining of the lung. Only few melanoma cells adhered to endothelial monolayer in flow chamber experiment. Most tumor cells form aggregates. Pre-incubation with GPIIb antagonist abolished aggregate formation.

"Conditional mouse models for hereditary and metastatic breast cancer"

Jos Jonkers created mouse models for BRCA-associated hereditary breast cancer and E-cadherin associated metastatic breast cancer to study the role of these cancer-associated genes in tumorigenesis.

Breast cancer gene BRCA-1 was inactivated combined with p53 inactivation. $p53^{-/-}$ animals tumors grew faster in BRCA-$1^{-/-}$ mice compared to BRCA-$1^{+/+}$, $p53^{-/-}$ or BRCA-$1^{+/-}$, $p53^{-/-}$ animals. One aim of the work was to develop anti-tumor therapies. The BRCA-$1^{-/-}$ model can also be used to test the efficacy of anti-tumor drugs. Tumors develop resistance against some substances like doxorubicin. A combination of cisplatin and PARP inhibitor could increase tumor free survival.

E-$cadherin^{-/-}$, $p53^{-/-}$ mutants showed increased tumor growth, loss of ductal morphology and increased metastatic potential. Furthermore E-cadherin loss induced anoikis resistance. It could be shown that p120 knockdown reduced cell viability, tumor growth and metastasis.

Session 7: CANCER STEM CELLS

"CANCER STEM CELLS: Definitions and clinical implications"

Piero Dalerba introduced the cancer stem cell concept. The phenotypic subpopulation that is selectively endowed with tumorigenic capacity is operationally defined as the "cancer stem cell" (CSC) subset. The CSC working model is currently being extended and confirmed in several types of human solid tumors, including major epithelial cancers, such as breast and colon cancer. Human "Colorectal Cancer Stem Cells" (Co-CSC) can be robustly and reproducibly isolated using a novel combination of three independent surface markers (EpCAM, CD44, CD166). The transcriptional profile of human "Breast Cancer Stem Cells" is associated with poor clinical outcome and increased risk of metastasis independently of other known clinical and pathological risk factors.These results suggest that analysis of the CSC subsets could provide important insights in the processes of tumor relapse and metastasis and that these processes may be associated with a core set of "Cancer Stem Cell" functional properties shared across different tumor types.

"Normal mammary stem cells and tumor progression"

Jean-Paul Thiery gave a talk about the characteristics of mammary progenitor cell. His data provide evidence for the existence of basal-type mouse mammary progenitors able to participate in the morphogenic processes occurring during mammary gland development. Constitutive expression of truncated β-catenin in the basal layer of mammary epithelial cells, thus activating the Wnt/β-catenin signaling pathway, affects the entire process of mammary gland development and induces the amplification of a subpopulation of mammary progenitors that give rise to tumors. In addition, he showed that the deletion of β1 integrin specifically from the basal mammary epithelial cells abolished the ability of the mammary epithelium to reconstitute after serial transplantion. These β1 integrin-null basal cells produced luminal cells instead of renewing the basal cell layer. Dr. Thiery´s group demonstrated that β1 integrin-mediated interactions between basal mammary epithelial cells and ECM are important for the regenerating mammary epithelium.

"Malignant progression in colorectal cancer: EMT, β-catenin and cancer stem cells"

Thomas Brabletz´s talk about malignant progression in colorectal cancer highligheted the important role of nuclear β-catenin expression for tumor cell mobility and cancer stem cells. He showed that tumor cells at the invasive front of colorectal cancers accumulate β-catenin, undergo EMT and express EMT-associated transcriptional repressors, such as ZEB1. The number of these cells correlates strongly with clinical outcome and metastasis. In contrast, tumor cells in central tumor areas as well as metastases are differentiated and lack nuclear β-catenin, indicating a mesenchymal-epithelial re-transition (MET) and a regulatory role of the tumor environment during malignant tumor progression. Dr. Brabletz suggested that both primary tumors and metastases are derived from a pool

of EMT-associated “migrating cancer stem cells” at the tumor host interface and that they have a migratory as well as a stem cell phenotype.

Session 8: CHEMOKINES

“Chemokines and cancer”

Albert Zlotnik introduced the chemokine family, and discussed the evolutionary and genomic taxonomy of the family members. Compelling evidence for developmental and homeostatic functions was provided, which in part reflected their evolutionary heritage. In turn, expression of certain chemokines is reflected with higher occurences of lymph node metastasis (CCR7), of lung, liver and bone-marrow metastasis (CXCR4), whereby physiological chemokine ligand and receptor patterns are found in tumors, suggesting a mechanism how certain tumors migrate to lymph nodes. Additionally, he reported that blocking CXCR4 could specifically block lung metastasis by blocking extravasation of tumor cells into the lung tissue.

“Chemokine receptors CXCR4 and CCR7 mediate inhibition of anoikis in cancer cells”

Marina Kochetkova described a novel role of the CXCL12 and CCL19/CCL21 chemokines and their receptors CXCR4 and CCR7 in inhibiting anoikis in metastatic breast cancer cells via the phosphatidylinositol 3-kinase (PI3K) pathway. By regulating Bmf and Bcl-x the PI3K pathway may represent a mechanism used by malignant cells to survive in the circulation.

“Role of Tie2-expressing monocytes (TEMs) in tumor angiogenesis”

Michele De Palma demonstrated a subset of Tie2-expressing monocytes which accumulate in tumor tissue, are located perivascularly, and contribute to tumor angiogenesis through the release of angiogenic cytokines. These cells can be manipulated *ex vivo* to deliver suicide genes specifically into a murine glioma model, which resulted in a significant reduction in tumor growth and associated angiogenesis.

“The role of chemokine/chemokine receptor system in tumor-vessel interactions”

Bence Sipos presented data showing that the chemokines/chemokine receptors CXCL12, CCL21 and CCR10 are upregulated and CXCR4, CXCL2, CCL23 downregulated in lymphatic endothelial cells (LEC) compared to blood endothelial cells (BEC). Furthermore, the receptors for CXCL12 and CCL21, CXCR4 and CCR7 are expressed in pancreatic ductal adenocarcinoma cell lines (PDAC) at the mRNA level. CXCL12- and CCL21.mediated transmigration of these cells *in vitro*. Dr. Sipos presented data showing that the expression of CXCR4 correlates with the formation of liver metastasis and CCR7 with higher rates of lymphatic vessel invasion following subcutaneous injection of PDAC into SCID mice,.

Session 9: TUMOR SIGNALING

“Identification and functional analysis of the serine protease kallikrein 6 in tumor development and malignant progression”

Peter Angel showed that the serine protease kallikrein 6 is highly expressed in human skin tumors. Furthermore, ectopic kallikrein 6 expression leads to spindle-like morphology of the cells and increased cell proliferation, migration and invasion, which is likely due to ectodomain shedding of E-cadherin. In addition, tissue inhibitors of matrix-metalloproteinases (TIMPs) inhibited kallikrein 6-induced shedding and rescued the cell-cell adhesion defect, implying the involvement of matrix-metalloproteinases and ADAM proteolytic activity.

"High-throughput animal models: Molecular mechanisms in AIDS-malignacies and a role for the Semaphorin-Plexin signalling system in tumor-induced angiogenesis"

Silvio Gutkind reiterated the previous notion that tumors need to be considered as a physiological (yet unordered) organ, and provided evidence for a role of virally encoded GPCRs for tumor progression. One such GPCR (vGPCR) is sufficient for the development of Kaposi's sarcoma. A cascade of events including small GTP binding proteins, Semaphorins and plexins was elucidated through the use of *in vivo* and *in vitro* systems providing a mechanism for the development of highly vascularized Kaposi sarcoma. He established the role of Sema4D in the tumor compartment. Tumor Sema4D is cleaved by MT1-MMP and the soluble form binds to plexinB1 on endothelial cells inducing a pro-angiogenic response through the PI3K/PKC signalling pathway.

"Phospholipid hydroperoxide glutathione peroxidase (PHGPx) as a key enzyme governing arachidonate and linoleic metabolism"

Heike Beck introduced lipoxygenases (LOXs) and cyclooxygenases (COXs) during tumor development. Using mouse embryonic fibroblasts, endothelial progenitor cells as well as embryonic stem cells out of phospholipid hydroperoxide glutathione peroxidase (PHGPx) knock out mice, she introduced data on the role of the arachidonic and linoleic acid metabolism in tumor growth and angiogenesis. PHGPx counteracts cyclooxygenase and lipoxygenase activities, and tumors from PHGPx deficient embryonic stems cells and transformed fibroblasts grew larger than control tumors.

"Signaling via receptors for PDGF and TGF-β – possible targets in tumor treatment"

Carl-Henrik Heldin introduced the PDGF/PDGFR family that plays an important role during embryonic development, regulation of the interstitial fluid pressure and stimulation of wound healing. Activation of PDGFRβ by phosphorylation leads to activation of different signaling pathways (Ras-Erk/MAPK, Rho/Rac and Akt pathway) which induces mitogenicity, chemotaxis, and survival. He presented data showing that PDGF-BB stimulation resulted in inverse correlated regulation of MKP3 and Erk MAPK levels. PDGF-BB induced degradation of MKP3 could be inhibited by a proteosomal as well as a MEK inhibitor. In turn, MKP3 induction by PDGF-BB stimulation was also inhibited by a MEK inhibitor and siRNA-mediated knock down of MKP3. This resulted in an enhanced PDGF-BB induced Erk MAP kinase activation.

Dr. Heldin discussed the downregulation of PDGFRβ by internalization in the second part of his talk. He demonstrated that PDGF stimulation of TC-PTP$^{-/-}$ cells resulted in a PDGFRβ hyperphosphorylation and decreased PDGFRβ degradation.

In the last part of his talk, Dr .Heldin addressed the consequences of PDGF overexpression in human diseases, especially in the tumor in which PDGF affects tumor cells as well as fibroblasts and blood vessels. A B16 melanoma model with PDGFRβ expression specifically restricted to pericytes was used in order to answer how PDGF stimulated pericytes influence tumor progression and angiogenesis. PDGF-BB or -DD overexpression by B16 melanoma cells resulted in increased pericyte coverage and tumor growth thereby leading to decreased tumor cell apoptosis. Activation of PDGFRβ in normal tissues resulted in an increased interstitial fluid pressure (IFP). This mechanism prevents drug uptake by the tumor. Different PDGF antagonists were tested, resulting in a decreased IFP and an enhanced drug uptake in tumors and in combination with α-VEGFR treatment in reduced tumor growth.

Session 10: METASTASIS SIGNATURES

"The role of Delta4 Notch signalling in tumor angiogenesis and response to therapy"

Adrian Harris demonstrated the critical role for Notch signaling in regulating branching and vessel size. He showed that an expression of Dll4 in tumor cells leads to an induction of larger vessels, much less branching and a smaller number of vessels. The Dll4 tumor cells were resistance to Bevacizumab. Further characterization showed that these vessels are not covered by pericytes. He

investigated the effects of Notch activation by Dll4 on the secretion of soluble VEGFR-1 and determined a substantial increase of sVEGFR-1 secretion. This suggests that Notch signaling may be able to exert systemic effects by regulating sVEGFR-1. The studies indicate the important biological role of Dll4 signaling in mediating resistance to VEGF and radiation therapy and that modulation of Notch signaling may become an important therapeutic modality to therapeutically manipulate tumor angiogenesis

"Extracellular RNA-induced permeability changes and signal transduction in the vascular system"

Klaus Preissner reported that extracellular RNA (eRNA)-induced permeability changes in the vascular system are related to VEGFR-2-mediated signaling cascades. Based on these findings, eRNA could play an important role in humoral and cellular activities within the tumor-vessel interface. Pre-treatment with RNase delayed occlusive thrombus formation in an arterial thrombosis model. Moreover, the VEGFR-2 system was shown to be responsible for the cellular activity of eRNA. Strong binding of eRNA to VEGF and other heparin-binding growth factors was demonstrated. This indicated that RNA serves as natural hyperpermeability co-factor upstream of VEGF. Activation/phosphorylation of VEGF-receptor-2 by RNA was demonstrated and eRNA-induced permeability changes were abolished by tyrosine kinase inhibitors such as genistein. eRNA increased the binding of VEGF to Neuropilin-1 leading to an activation of protein kinase C as well as to an increase of intracellular Ca^{2+}-concentration. These results identify eRNA as a permeability factor, upstream of VEGF and RNase provides a novel protective substance for substance.

"Complex formation between EpCAM, claudin-7 and CD44 variant isoforms in tetraspanin-enriched microdomains promotes colorectal cancer progression"

Margot Zöller showed that EpCAM, claudin-7, CO-029 and CD44v6 are frequently co-expressed in rat gastrointestinal tumors and that they can form a complex suggesting a role of this complex during tumor progression. The expression of all four molecules was analyted in colorectal cancer, liver metastasis and tumor-free colon and liver tissue. Dr. Zöller concluded that the expression of the molecules by themselves cannot be considered as prognostic markers in colorectal cancer. However, the association of EpCAM with claudin-7 interferes with cell-cell adhesion, which facilitates metastasis. Recruitment of the EpCAM-claudin-7 complex into CO-029 and CD44v6 containing TEM (tetraspanin-enriched membrane microdomains) provides a survival advantage for dissociated tumor cells.

"Identification and characterisation of metastasis progression genes"

Don Nguyen introduced the biology of the metastatic cascade as a multi-step process. He suggested that metastasis is a result of genetic variability and natural selection. Using a functional approach for the selection of metastastic cells *in vivo*, he and his colleagues identified different gene sets that mediate the tissue specific dissemination of cancer cells. The human breast cancer-expressed genes [EREG (an epidermal growth factor receptor ligand), COX2, MMP1, and MMP2] facilitate the formation of new tumor blood vessels, the release of cancer cells into the bloodstream, and the penetration of tumor cells from the bloodstream into the lung.

Session 11: FROM BENCH TO BEDSIDE

"Akt/mTOR signalling in the tumor stroma"

Laura Benjamin presented her lab's work on Akt signaling during angiogenesis and tumor growth, and how this might be therapeutically manipulated. The wide-ranging roles and functions of Akt and mTor signaling in the homeostatic cardiovascular system was considered, and a detailed appraisal of the specific pathways activated by this cascade was discussed, building a rationale that this pathway may be a feasible target for tumor progression.

“Modulation of tumor angiogenesis by CEACAM1 in a mouse model for mammary carcinogenesis (WAP-T mice)”

Andrea Horst talked about the *in vivo* functions of carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) during angiogenesis. She also talked about the WAP-T mouse model which is well suited to test the effects of modulators of angiogenesis on tumor progression and metastasis. She has generated double transgenic WAP-T/CEACAM1-mice that either overexpressed or systemically lack the CEACAM1 gene within the endothelium. Analysis of these mice is ongoing. Analysis includes the morphological analysis of tumor progression in these mice by flat panel CT.

“Inhibition of the PI3K-Akt pathway sensitizes the tumor vasculature to TNF”

Leander Huyghe could show that the inhibition of the PI3K-Akt survival pathway promotes the anti-tumor activity of TNF. He treated B16-Bl6 melanoma bearing mice in combination with TNF and the PI3K inhibitors Wortmannin or Ly294002. This sensitization enables a 10-fold reduction of the effective dose of TNF. Combination of Wortmannin and TNF induces the rapid occlusion and destruction of tumor blood vessels within 6 h after injection. The inhibition of mTOR by rapamycin or the blocking of the VEGFR-2 with a specific antibody had similar effects.

“Vascular targeting antibody derivatives: from bench to the clinic”

Dario Neri delivered an overview about the development of vascular targeted anti-cancer drugs. This includes monoclonal antibodies capable of selective targeting neo-vascular structures in solid tumors and in angiogenesis-related diseases. He presented relevant preclinical findings as well as recent clinical data.

Mélanie Héroult

(with the assistance of Andrew Benest, Chris Dietz
Katja Fischer, Claudia Prahst, Melanie Rothley,
Renate Winkler, and Joyceline Wüstehube)