(W7) implying the contribution of Ca2+ signaling to PKB deregulation in melanoma cells. Indeed, highly malignant and apoptosis-resistant clone of B16BL6 melanoma (B16BL6-8) displayed significantly higher [Ca2+]i and store-operated Ca2+ influx relative to non-malignant apoptosis-sensitive B16BL6 clone (Kb30) expressing barely detectable basal levels of active PKB. MCD-mediated raft ablation in B16BL6-8 cells robustly inhibited store-operated Ca2+ influx and decreased [Ca2+]i to levels comparable to those detected in Kb30 cells. Exposure of cells to PKB-inhibiting doses of MCD dramatically impaired their apoptotic resistance and capacity to generate experimental tumors. Furthermore, weekly intraperitoneal injections of MCD to mice grafted with melanoma cells at doses of 300 and 800 mg/kg significantly attenuated tumor development. Our data implicating raft micro-domains in protecting melanoma.

81 Poster

Quantification of neo-angiogenesis by micro-computed tomography in xenograft mouse model of lung cancer

R. Savai[1], R.T. Schermuly[2], R. Dumitrascu[2], S.S. Pullamsetti[2], A.C. Langheinrich[3], H. Traupe[4], W.S. Rau[3], W. Seeger[2], F. Grimminger[2], G.A. Banat[1]
[1]Medical Center University of Giessen, Department of Hematology and Oncology, Giessen, Germany; [2] Medical Center University of Giessen, Internal Medicine II, Giessen, Germany; [3] Medical Center University of Giessen, Department of Radiology, Giessen, Germany; [4] Medical Center University of Giessen, Department of Neuroradiology, Giessen, Germany

Evaluation of lung tumor angiogenesis using imaging and immunohistochemical techniques has been limited by difficulties in generating reproducible quantitative data. In this study, for analysing the intrapulmonary tumors and their vascularisation we used high resolution micro computed tomography (μCT). C57/BL6 mice were inoculated with Lewis lung carcinoma cell line via intratracheal injection for lung tumor formation that was confirmed with flat panel volumetric computed tomography. Further the lung vasculature was filled with radiopaque silicone rubber (Microfil). After polymerization of the microfil, the lungs were harvested and investigated with μCT. For filling of the pulmonary vascular tree, two different methods were applied 1) via jugular vein for intravenous in vivo application of microfil and 2) via pulmonary artery for ex vivo application of microfil. Quantitative analysis of lung tumor microvessels with the analyze 7.0 software showed more vessels mainly the smaller vessels (<0.02 mm^2) were filled with in vivo (5.4%) compared to ex vivo (2.1%). In addition, lung tumor bearing mice were treated with anti-VEGF antibody bevacizumab (avastin) using osmotic minipump infusion for 14 days. Avastin treatment significantly reduced the lung tumor volume and the lung tumor angiogenesis compared to control mice as assessed by μCT. Interestingly neo-angiogenesis mainly the smaller vessels (<0.02 mm^2) were reduced following avastin treatment. This observation with μCT was nicely correlated with immunohistochemical measurement of microvessels. Therefore, μCT is a novel method investigating tumor angiogenesis and might be considered as an additional and complementary tool to quantitative histopathology.

82 Poster

SC35, a member of the Ser-Rich Arg (SR) splicing factors family, cooperates with the transcription factor E2F1 to control cellular proliferation

V. Edmond[1], G. Merdzhanova[1], A. van den Broeck[1], C. Brambilla[1], E. Brambilla[1], S. Bazzeri[1], B. Eymin[1]
[1]INSERM U823, Team 2 Molecular Basis of Lung Cancer Progression, Grenoble, France

SR proteins are a family of RNA binding proteins that play critical roles in both constitutive and alternative pre-mRNA splicing. While knockout studies have demonstrated their essential functions during animal development, less is known about the cellular signalling pathways and targets that are controlled by these proteins. The E2F1 transcription factor belongs to the E2F family and plays a crucial role in driving cell cycle progression in S phase. Using various human lung carcinoma cell lines, we previously identified the SR protein SC35 as a new transcriptional target of E2F1 and further demonstrated that both proteins are involved in apoptosis following genotoxic stresses. In this study, we postulated that SC35 could also play a role during cell cycle progression. Consistently, using cellular synchronization and immunoblotting experiments, we first show that SC35 protein level peaks during the G1 to S phase transition concomitantly with E2F1 and some of its transcriptional targets. Furthermore, using RNA interference strategy, BrdU incorporation analysis and immunoblotting experiments, we provide evidence that knockdown of SC35 in various lung carcinoma cell lines decreases the number of cells in S phase and leads to a strong downregulation of some E2F1-target genes involved in DNA replication, such as the dihydrofolate reductase, the thymidilate synthetase and the cyclin E, as well as is associated with an accumulation of the cyclin-cdk inhibitor p21WAF1. At the molecular level, using co-immunoprecipitation and GST pull-down analyses, we demonstrate that both E2F1 and SC35 proteins directly interact and identify in this setting the DNA binding domain of E2F1 as the site interacting with SC35. Finally, in cells transfected with plasmids encoding luciferase under the control of various E2F1-targeted promoters, we show that SC35 is able to stimulate the transcriptional function of E2F1 towards some of these promoters. Overall, these findings unravel a role of the splicing factor SC35 in the control of cellular proliferation through its cooperation with the transcription factor E2F1. Moreover, as we obtained preliminary results indicating that SC35 is overexpressed in neuroendocrine lung tumors as compared to normal lung tissues, these results suggest that a deregulation of SC35 could contribute to the tumorigenesis of these highly malignant cancers.

83 Poster

Irradiation-induced promigratory phenotype of melanoma cells - role of S100A4-RAGE interaction

C. Haase[1], S. Wolf[1], J. Pietzsch[1]
[1]Forschungszentrum Rossendorf e.V., Institute of Radiopharmacy Department of Radiopharmaceutical Biology, Dresden, Germany

Metastases are a devastating and debilitating complication of melanoma with a poor prognosis for the patient. The treatment of metastases would be either radiation only or surgery combined with adjunctive postoperative radiation therapy. S100A4 (metastasin) is known to play a direct role in these metastatic processes. We hypothesize that RAGE (receptor for advanced glycation endproducts) is a putative receptor for S100A4. However, the role of S100A4-RAGE interaction in melanoma metastasis is still unclear. The purpose of this study was to find out how mouse B16-F10 melanoma cells restrained to irradiation. Furthermore, we examined changes in the S100A4-RAGE interaction and the ability for migration of irradiated melanoma cells in the presence of tumor associated macrophages. B16-F10 cells were exposed to single dose irradiation (5 Gy, 20 Gy) and mouse RAW 264.7 cells were used as a model for tumor-associated macrophages. S100A4 and RAGE expression in these cells was quantified via real-time RT-PCR and Western-blot analysis three and six days after irradiation. Cell migration was investigated with B16-F10 cells six days after irradiation in a 24-transwell chamber for 16 h and 24 h. Furthermore, migration was influenced by seeding RAW cells as a chemoattracant into the lower compartments and recombinant S100A4 as a stimulus to the upper compartments. After labeling the cells with Calcein-AM the migratory cells were quantified in a standard fluorescence microplate reader. The total number of vital irradiated B16-F10 cells is significantly decreased with increasing dose up to 20 Gy and up to six days, thereby altering morphological appearance. Surprisingly, in surviving B16-F10 cells expression of S100A4 and RAGE significantly increased three and six days after 20 Gy ($p<0.05$). Furthermore, irradiated B16-F10 cells showed higher migratory activity supposed due to enhanced expression of S100A4 and RAGE. In the presence of RAW cells and/or recombinant S100A4 a further increasing migration activity of irradiated cells (20 Gy) was found. Our findings suggest an association of melanoma and macrophages with alterations of their migratory and invasive activity after irradiation due to a perpetual para-/autocrine expression mechanism of extracellular S100A4 and RAGE, and thereby changing functional properties of melanoma cells towards a promigratory phenotype. This study was supported in part by the Deutsche Forschungsgemeinschaft (grant Pi 304/1-1).

84 Poster

LDH-A gene suppression affects cell growth of colon carcinoma xenografts but not in culture conditions

S. Langhammer[1], M. Najjar[1], K. Berhoerster[1], H. Hess-Stumpp[1], K.H. Thierauch[1]
[1]Bayer Schering Pharma AG, TRG-Oncology, Berlin, Germany

High serum LDH activity correlates with a bad prognosis in different tumour entities. LDH metabolises pyruvate under hypoxic conditions to lactate for NAD^+ provision. A recent study provided evidence that LDH is causally involved in tumour growth, showing that LDH-A shRNA interference led to a growth retardation of mammary epithelial tumour cells in vivo.

We generated HT29 colon carcinoma LDH-A shRNA clones and analysed for LDH-A gene product, for LDH activity and for in vitro growth and for hypoxia induced proteins. The shRNA expressing clones were implanted as xenograft tumours into mice for an evaluation of tumour growth. In addition, the influence of LDH-A deficiency on LDH-B and on hypoxia regulated genes (Hif1α, CA9, PHD2) and not directly hypoxia regulated genes like FIH was evaluated.

The LDH-A gene product and the LDH activity were significantly reduced in LDH-A shRNA generated clones compared to the controls. LDH-A

suppression did not decrease the proliferation rate in vitro,rather a slight increase was observed. However, tumour growth generated from LDH-A deficient clones was significantly reduced. LDH-B was not increased by shRNA interference for LDH-A in a compensatory mode, while Hif1α expression was increased and PHD2 and CA9 expression were significantly decreased in the LDH-A deficient clones.

We show that LDH-A is critical for the growth of colon carcinoma cells in vivo but not in vitro. The LDH-A deficiency seems to induce cellular stress resulting in an increased Hif1α expression and a decreased expression of its regulator, PHD2. A reduced expression of CA9 in those cells may depend on an abrogated lactic acid production. The generation of mouse melanoma (B16F10) and mouse lung carcinoma (Lewis Lung) LDH-A shRNA clones has been successful and the effect in HT-29 cells was reproduced with Lewis lung carcinoma cells but not with B16F10 clones.

85 Poster

Analysis of TGFBI overexpression and silencing in the proliferation, migration and chemoresistance of NSCLC cells

M. Irigoyen[1], J. Agorreta[1], M.J. Pajares[1], L. Montuenga[1], A. Rouzaut[1]
[1]Center for Applied Medical Research, Oncology, Pamplona, Spain

Background: TGFBI (transforming growth factor-β, induced protein) is an extracellular matrix protein that has been described to mediate cell adhesion to the extracellular matrix by its interaction with integrins. In spite of recent reports dealing with its expression in tumors, little is know about its role in tumor migration and adhesion. In the present work we aim at studying the role of TGFBI overexpression or silencing on cell adhesion, migration, proliferation and resistance to chemotherapy in NSCLC.

Methods: We analyzed the effects of TGFBI silencing in the NSCLC cell line A549, which expresses high levels of this molecule, and TGFBI overexpression in H1299, that shows low basal TGFBI expression. Cell viability was determined by the incorporation of the vital dye neutral red and apoptosis was measured by PARP degradation. Cell adhesion was measured by the fluorescent labelling of adhered cells while their migration was in Boyden chambers. We have also analyzed TGFBI expression in 22 NSCLC cell lines and in 80 samples derived from NSCLC relative to normal lung tissues.

Results: TGFBI silencing in non metastatic A549 cells increased their proliferation and migration, but decreased extracellular matrix cell adhesion and while recovery of TGFBI expression in H1299 metastatic cancer cells decreased their proliferation and migration and induced H1299 cell adhesion to the extracellular matrix.

We also demonstarte that TGFBI overexpression increases tumour cell sensitivity to chemotherapy whereas loss of TGFBI induced resistance.

Expression studies showed a heterogeneous TGFBI expression in the 22 NSCLC cell lines tested. Besides, we studied the correlation between TGFBI expression, tumor stage and resistance to chemotherapy in 80 NSCLC samples.

Conclusion: Loss of TGFBI is able to increase cell proliferation and migration, and to decrease sensitivity to apoptosis, and points it as a candidate tumor suppressor. The study of TGFBI expression in human lung carcinoma relative to normal lung tissues, and its correlation with several pathological and histological parameters, including tumor stage and chemotherapy response, should be explored as a useful tool in a clinical setting.

86 Poster

Epidermal growth factor receptor distinguishes between stem and transient amplifying cell fate in squamous cell carcinoma cell line

H. LeRoy[1], T. Zuliani[1], D. Vaudry[2], M. Ould-Aklouche[3], H. Obriot[1], M. Benard[2], P. Formstecher[1], R. Polakowska[1]
[1]Faculté de Médecine Université Lille 2, Centre de Recherche JP Aubert Inserm U837, Lille, France; [2] Université de Rouen, Inserm U 413, 76821 Mont-Saint-Aignan, France

[3] Faculté Dentaire Université Lille 2, Centre de Recherche JP Aubert Inserm U837, Lille, France

Cancer cells are phenotypically and functionally unequal in the tumor mass and in established cultures. This is accounted for by a small subpopulation of cancer cells which have the unique ability of stem cells to generate differentiating progeny while maintaining their own number. Regulation of this dual ability is controlled at the level of asymmetric division by mechanisms that are, as yet, not well defined. Our findings suggest that in the squamous cell carcinoma (SCC) cell line, the fate of cancer cells is linked to the expression level and subcellular distribution of epidermal growth factor receptor (EGFR). Interestingly, though essential for epithelial cell proliferation, differentiation and survival, this factor was not found on the surface of cells that satisfy criteria for stem cells, including asymmetric division, high clonogenic potential, expression of stem cell markers and migration profile. We determined that EGFR can be asymmetrically distributed during cell division and identified several cell cycle, TNF-pathway, survival, mitochondria and self-renewal controlling genes that were differentially regulated in EGFR-negative and EGFR-positive cells and whose expression differed in SCC cells and their normal counterparts. Our data suggest that EGFR might be an important cell fate determinant which switches the stem cell phenotype into transient amplifying during asymmetric division, and that the set of genes associated with this switch is different for normal and cancer stem cells.

87 Poster

Inhibitory effects of unliganded estrogen receptor alpha on breast cancer cell growth and invasion

M. Maynadier[1], J.M. Ramirez[1], A.M. Cathiard[1], N. Platet[1], D. Gras[1], M. Gleizes[1], M.S. Sheikh[2], P. Nirdé[1], M. Garcia[1]
[1]Institut de Recherche en Cancerologie de Montpellier, Inserm U896, Montpellier, France; [2] Upstate Medical University, Departement of Pharmacology, Syracuse New York, USA

Breast cancer, the most frequent malignancy in western women, is a model of hormone dependent malignancy. While estrogens are mitogenic in breast cancer cells, the presence of estrogen receptor alpha (ERα) indicates a favourable prognosis in breast carcinoma. To improve our understanding of ERα unliganded action, we used mutants deleted in ligand and/or DNA-binding domains. In previous studies, we have shown that unliganded ERα protects against invasion through matrigel via protein-protein interaction in its first zinc finger region. Recently, we demonstrated that expression of ERα mutants also inhibits cell outgrowth in three dimensional matrices as well as tumor formation in nude mice. Using GST-pull down and two-hybrid techniques, we found that ERα, via its amino acids 184-283, interacts with the cyclin-dependent kinase inhibitor $p21^{WAF1}$. The interaction between these proteins is detected in absence of estrogens or in the presence of pure antiestrogen $ICI_{182,780}$, whereas estradiol treatment disrupts the interaction. By cross-linking experiments, a large complex of ~200 kDa containing $p21^{WAF1}$, ERα and both cdk2 and cyclin E was identified. We further demonstrate that ERα expression after gene transfection significantly increases $p21^{WAF1}$, while ERα silencing by RNAi significantly reduces $p21^{WAF1}$. Moreover, the silencing of $p21^{WAF1}$ prevents the ERα –induced growth inhibition. In conclusion, our findings point to an anti-invasive and an antiproliferative function of the unliganded ERα through its physical interaction with $p21^{WAF1}$ that may explain, at least in part, the favourable prognosis associated with ERα-positive breast cancers.

88 Poster

Caveolin-1 regulates glioblastoma aggressiveness through the control of a5β1 integrin expression and modulates glioblastoma responsiveness to SJ749

S. Martin[1], J. Terrand[1], A. Maglott[1], S. Froelich[2], M. Dontenwill[1]
[1]IGL UMR7175, Pharmacologie et Physicochimie, Ilkirch, France; [2] CHU Strasbourg, Service de Neurochirurgie, Strasbourg, France

Background - Gliomas are the most common deadly brain tumors. Despite advances in neurosurgery, radiation and medical oncology, the prognosis for patient with glioblastoma did not improve in the last 30 years. A better molecular and biological knowledge of glioma will lead to advances for the management of glioma. Increased expression of caveolin-1 seems to be the norm in glioma. As caveolin-1 plays a checkpoint function in the regulation of processes altered in cancer, we investigated its role in glioblastoma carcinogenesis.

Methods - Caveolin-1 was overexpressed or knocked down in U87MG cells and proliferation, clonogenicity and invasion were examined. PCR Arrays were undertaken to determine pathways altered after caveolin-1 manipulation. The involvement of α5β1 integrins was studied by overexpressing or knocking down α5 in U87MG cells and using an antagonist (SJ749). Finally, the expression levels of both caveolin-1 and α5β1 integrin were analyzed in 24 glioma patient samples and normal brain by qPCR.

Results - The reduction of caveolin-1 levels in U87MG shifted cells towards a more aggressive phenotype (increased proliferative, clonogenic and invasive capacity) as conversely the forced expression of caveolin-1 slowed down proliferation, clonogenicity and invasion. Using PCR array strategies, we showed that only 20% of the genes studied were significantly affected by caveolin-1 modulation. The most exciting finding was that half of them belonged to the integrin family and above all that their expression was always inversely correlated to caveolin-1. Focusing on α5β1 integrin, we showed that caveolin-1 could in fact control α5β1 integrin at the transcription level and consequently alters cell sensitivity to the specific α5β1 integrin antagonist, SJ749. We also report here for the first time that the inverse correlation between caveolin-1 and α5β1 integrin had biological