metastasis development using murine melanoma metastasis model establish from our stable cell lines.

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RhoB controls estrogen receptor target genes expression through a modulation of ER recruitment on the promoter binding sites, in MCF-7 cells

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Around two thirds of mammary tumors express estrogen receptors (ER) and hormonotherapy is then recommended. Nevertheless, resistances to these treatments systematically occur and impose the search for new pharmacological targets. Estrogens act mainly through the well-known ERα but cross-talks have been clearly demonstrated between ERα and growth factors signalling pathways. Ras family proteins, such as Rho prenylated proteins, are key elements in those cross-talks. Indeed, we and others previously showed that prenylated proteins stimulate the proliferation of MCF-7 cells (hormonodependent breast cancer cell line) and on the contrary negatively regulate transcriptional responses mediated by ER. The purpose of this study was to analyze the effect of estrogens on RhoB expression and activation, and conversely, the effect of RhoB on ER expression, on its target genes expression and on ER recruitment on the promotor of target genes (progesteron receptor and pS2). We first showed that a significant increase of the active GTP bound form of RhoB is observed after 30 minutes of estrogen stimulation with no modification of RhoB expression at this stage. To decipher the mechanisms involved in the effects of RhoB on ER-mediated activities, we abolished the expression of RhoB using two sequences of interfering RNA in MCF-7 cells. On the one hand, we demonstrated that RhoB extinction significantly decreases ER protein and mRNA expression (confirmed in RhoB-/- mice). On the other hand, RhoB extinction clearly diminishes the expression of a luciferase reporter gene controlled by the vitellogenine Estrogen Responsive Element (MELN cells). Similarly, RhoB extinction decreases the progesteron receptor and pS2 mRNA levels. To explain these effects, we analyzed ERα recruitment on the Estrogen Responsive Element or ER binding site of each of these 3 genes, and demonstrated that RhoB extinction increases ERα recruitment to the pS2 and vitellogenine genes, and on the contrary, decreases it to progesteron receptor. In brief, our results evidence RhoB participation in the balance of recruitment of ER to its various target genes, individually modulating their expression. Further investigations, especially studies on hormonoresistant breast cancer cells are now ongoing for a better understanding of hormone resistance mechanisms.

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CXCR4 expression mediates the survival and proliferation of glioma cells

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Malignant gliomas are brain neoplasms that account for more than 50% of the tumours that arise within the central nervous system. They are higly proliferative, angiogenic and locally very invasive. The mechanism by which malignant glioma grow is still not understood but some evidences suggest the involvement of chemokines.

Chemokines are a family of molecules that regulate chemotaxis of leukocytes into tissues, promote mitosis and cell survival. The family of the CXC chemokines, and in particularly the CXCL12 chemokine and its receptor CXCR4, has been associated with cell proliferation and cell survival of several tumours.

To better understand the role of CXCL12/CXCR4 in malignant glioma we studied the expression of CXCR4 in a glioma cell line, the U-118 cell line. We also determined the contribution of the CXCR4 to cell adhesion, proliferation, survival and migration. The assays were performed in the presence of CXCL12 with or without AMD3100. CXCR4 expression was evaluated by western blot and immunofluorescence. To determine whether CXCR4 was functionally active, the activation of Akt was evaluated by western blot. Cell adhesion was measured under static conditions. Cell proliferation was determined using BrdUrd incorporation. Cell survival was addressed using two stains hoescht and propidium iodide. Cell migration assays were carried out using migration chambers.

Our results showed that CXCR4 is expressed in the U-118 cell line. In the presence of CXCL12 an increased adhesiveness of cells to the collagen matrix was observed. In addition, CXCL12 significantly increases the cell proliferation and survival. The CXCR4 antagonist, AMD3100, induces a significant reduction of cell proliferation and a significant increase in the number of apoptotic cells. Furthermore, in the presence of CXCL12, activation of Akt by CXCL12, the survival kinase, was also observed. The chemotaxis assay revealed that CXCL12 was chemotactic and induced the migration of glioma cells, indicating that CXCR4 expression is required in the invasion of glioma cells.

In conclusion, our in vitro studies, using the U-118 cell line, indicate that CXCR4/CXCL12 is implicated in the modulation of glioma cell proliferation, survival and migration.

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CDK-dependent phosphorylation of Bim during Taxol-induced cell death

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The mitochondrion plays an important regulatory role during caspase-dependent and caspase-independent cell death, through the release of apoptogenic proteins such as cytochrome C, Smac/Diablo, AIF, Omi/Htra and Endonuclease G from the intermembrane space. Mitochondrial release of apoptogenic proteins is regulated by the Bcl-2 protein family that is made up of both pro-apoptotic and anti-apoptotic members. Post-translational modification of Bcl-2 protein family members, such as phosphorylation and proteolytic cleavage, plays an important part in regulating their activity.[1]

The BH3-only pro-apoptotic family member, Bim, is phosphorylated by the Erk and JNK MAP kinases. Erk phosphorylates Bim resulting in proteasomal degradation of Bim.[2] The JNK MAP kinase phosphorylates Bim directly on serine and threonine residues resulting in its release from microtubules. Furthermore, JNK induces upregulation of Bim through the activation of the transcription factor, c-jun.[3,4]

It has been previously shown that chronic myeloid leukaemia (CML) cells undergo caspase-independent cell death following disruption of the microtubule network by microtubule targeting agents including Taxol.[1 and unpublished results] In this study it has been found that Bim resides in the mitochondria of CML cells. In addition, the two Bim isoforms, Bim EL and L, undergo phosphorylation following treatment with Taxol. Phosphorylation of Bim occurs in a time- and dose-dependent manner and precedes Taxol-induced cell death in CML cells. On further examination it has been found that phosphorylation of Bim EL occurs within 8 hours treatment with Taxol, whereas phosphorylation of Bim L does not occur until 12 hours after treatment. Synchronisation of K562 CML cells by double thymidine block and treatment with Taxol, has revealed that phosphorylation of Bim correlates with the accumulation of cells in G2/M.Pre-treatment of cells with the CDK inhibitors, Flavopiridol and Roscovitine, was found to block the phosphorylation of Bim EL and L upon Taxol treatment.

These results suggest that phosphorylation of Bim at the mitochondrion occurs during mitosis, which may represent an important event that connects cell cycle arrest to the cell death machinery following microtubule disruption.

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Targeting membrane rafts inhibits protein kinase b by disrupting calcium homeostasis and attenuates malignant properties of melanoma cells

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Failure of current therapeutic modalities to treat melanoma remains a challenge in clinical and experimental oncology. The aggressive growth and apoptotic resistance of melanoma are mediated, in part, by aberrantly activated protein kinase B/Akt (PKB). In many cells, PKB signaling depends on integrity of cholesterol-enriched raft micro-domains; however, it is still unclear if rafts support PKB deregulation in melanoma cells. The ablation of rafts in murine (B16BL6-8, JB/RH1) and human (GA) melanoma lines by cholesterol-chelating methyl-beta-cyclodextrin (MCD) efficiently reduced levels of active PKB in a dose- and time-dependent manner, while reconstitution of rafts restored PKB activity. PKB was also sensitive to the membrane permeable Ca2+ chelator (BAPTA-AM) and calmodulin inhibitor

(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.

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

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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.

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

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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.

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

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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).

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

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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