(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