In this model, primary tumour cells derived from genetically engineered mouse medulloblastomas (*Ptc*$^{+/-}$ or ND2:SmoA1) are orthotopically transplanted into wild type host mice. Hosts are monitored for early tumour development by MRI or bioluminescence imaging. To model medulloblastoma relapse, implanted tumours are established and then treated with a dose of radiation that leads to near-complete regression by MRI. Mice bearing implanted tumours survive radiotherapy, in contrast with the lethality observed with genetically engineered mouse models. While this radiation treatment provides a significant survival advantage as compared to untreated mice, the tumours eventually relapse, allowing for further analysis of medulloblastoma recurrence.
The initial tumours that form after transplant are histologically indistinguishable from spontaneously occurring medulloblastoma. In contrast to the uniform masses formed by xenografts, tumours that result from the implantation of primary tumour cells display perivascular niche architecture resembling that of spontaneous tumours. Radiation-resistant nestin-positive stem-like cells reside near blood vessels, and the tumour bulk is comprised of nestin-negative cells that are sensitive to radiation, undergoing apoptosis within 6 hours after exposure to a single dose of 2 Gy.
We are currently using tumours generated in this model to study treatment-acquired phenotypic alterations in medulloblastomas, and to identify pathways that can be targeted in relapsed medulloblastomas in order to better provide treatment for this devastating disease.

835 Pancreatic stellate cells modify tumour growth and radioresponse of pancreatic cancer

S. Lunardi[1], T. Mantoni[1], O. Al-assar[1], T.B. Brunner[1]. [1]*University of Oxford, Gray Institute for Radiation Oncology and Biology, Oxford, United Kingdom*

Background: Pancreatic ductal adenocarcinoma (PDAC) is characterised by an abundant stromal response also known as desmoplastic reaction. One of the main actors in promoting the desmoplastic reaction is a stromal cell type known as pancreatic stellate cells (PSCs). There is accumulating evidence that PSCs influence the malignant phenotype of PDAC. The aim of our study is to analyse the tumour response to radiation treatment in the presence of PSCs and to determine the stromal and tumour factors involved in this response.
Material and Methods: PSCs were used in a coculture system with Panc-1 and PSN-1 PDAC cell lines *in vitro*. Their effect on radiation survival was tested using clonogenic survival assays. Conditioned media from the monocultures and from the coculture were analysed for the expression of different factors using a proteomic approach. *In vivo*, subcutaneous and orthotopic injection of pancreatic cancer cells with or without PSCs were used for the evaluation of the tumour growth. Tumour regrowth was measured on subcutaneous tumours after irradiation. All animal experiments were carried out in accordance with U.K. Home Office regulations.
Results: PDAC cell lines showed decreased radiosensitivity when cocultured with PSCs. Coinjection of PDAC with PSCs in nude mice enhanced tumour proliferation. Furthermore, tumour regrowth experiments after irradiation showed that tumours from coinjection of PDAC with PSCs respond less to radiotherapy than tumours from PDAC only. At last, we identified three factors differentially expressed *in vitro* in the coculture supernatant compared to the monocultures.
Conclusions: These data demonstrate that PSCs promote tumour growth and decrease radiation response in PDAC. Further investigations of the mechanisms of communication between tumour and stromal cells may lead to a better understanding of pancreatic cancer biology and to new targets for multimodal therapy.

836 Effects of irradiation on viability, growth, metastatic properties and expression of Eph receptors and their ephrin ligands in human melanoma cells

B. Mosch[1], J. Pietzsch[1]. [1]*Institute of Radiopharmacy Forschungszentrum Dresden-Rossendorf, Department of Radiopharmaceutical Biology, Dresden, Germany*

Background: It is accepted that X-ray irradiation influences growth, viability and metastatic potential of tumour cells. Furthermore, it is supposed that tumour cell invasion and metastasis is regulated by Eph receptors and their ephrin ligands. The aim of our study was to investigate the influence of irradiation on cell viability, growth, and metastasis in human melanoma cells and whether this is mediated by dysregulated Eph receptor or ephrin ligand expression.
Material and Methods: Primary (Mel-Juso) and metastatic (A375, A2058) human melanoma cell lines were irradiated with 5 or 10 Gy. Up to 7 days after irradiation we examined cell viability (MTT test). At 1 day and 7 days post irradiation we further analyzed cellular growth, motility (scratch assay), adhesion to fibronectin, and migration through a porous membrane. Furthermore, the mRNA expression of 8 different Eph receptors and 6 ephrin ligands was analyzed using RT-PCR.
Results: In all cell lines a dose dependent decrease in viability and cell growth for up to 1 week after irradiation was demonstrated. Analysis of metastatic properties 1 day after X-ray showed decelerated scratch closure, slight increase in migration, and increased adhesion to fibronectin in all investigated cell lines. In contrast, 1 week after irradiation we detected faster scratch closure in irradiated primary Mel-Juso cells but unaltered motility in metastatic cell lines and, moreover, decreased migration in primary Mel-Juso cells and, by trend also in metastatic A375 cells. In addition, in Mel-Juso and A375 cells capability to adhere to fibronectin remained elevated. RT-PCR analysis revealed that Eph receptors and ephrins investigated have similar mRNA expression levels in primary and metastatic cell lines, with exception of both EphA2 and ephrinA5 showing enhanced expression in metastatic A375 cells. After irradiation changes in mRNA expression were not detected with exception of an increase in EphA2 and EphA3 in A375 cells and ephrins A1 and A5 in A375 and Mel-Juso cells 7 days after treatment.
Conclusion: Irradiation considerably influences viability and metastatic properties of melanoma cells. The different effects depending on time after irradiation observed suggest an involvement of cell-cell interaction via A-type Eph receptors and ephrins in irradiation-induced metastatic potency of melanoma cells.

837 Concomitant targeting of cyclooxygenase-2 and oxidant stress pathways for radioprotection of normal vascular tissue

J. Pietzsch[1], F.J. Pietzsch[1], M. Laube[2], R. Bergmann[1], T. Kniess[2], F. Wuest[3]. [1]*Institute of Radiopharmacy Forschungszentrum Dresden-Rossendorf, Department of Radiopharmaceutical Biology, Dresden, Germany*, [2]*Institute of Radiopharmacy Forschungszentrum Dresden-Rossendorf, Department PET Tracer, Dresden, Germany*, [3]*Cross Cancer Institute University of Alberta, Department of Oncologic Imaging, Edmonton, Canada*

Background: Radiotherapy of various cancers is closely associated with increased cardiovascular morbidity and mortality. Arachidonic acid metabolites are supposed to play a key role in radiation-induced vascular dysfunction, inflammation, and injury. This study was designed to evaluate the effects of novel selective cyclooxygenase-2 (COX-2) inhibitors on radiation-induced formation of arachidonic acid metabolites via cyclooxygenase-2 and oxidant stress pathways in endothelial cells.
Materials and Methods: Acute effects (1 d, 3 d) of X-ray radiation at moderate doses (2 to 10 Gy) without or with presence of selective COX-2 inhibitors (cyclopentene/indole/indomethacin derivatives (2 each); 1 μM, 10 μM) in human arterial (HAEC) and microvascular (HDMEC) endothelial cells compared to sham-irradiated controls were assessed. Therefore, the following parameters were measured: COX-2 induction; secretion of cytokines tumour necrosis factor-α, interleukin-6, and monocyte chemoattractant protein-1; release of prostaglandins PGE_2 and PGI_2; release of isoprostanes 8-iso-PGE_2 and 8-iso-$PGF_{2\alpha}$; and oxidative stress (lipid peroxides).
Results: Irradiation of endothelial cells without presence of COX-2 inhibitors resulted in a dose-dependent augmentation of all parameters studied. When endothelial cells were exposed to COX-2 inhibitors during and for 24 h post irradiation, indole derivatives showed highest potency to inhibit release of both prostaglandins and isoprostanes. Furthermore, when irradiated cells were treated with indole derivatives a significant decrease of lipid peroxide formation and cytokine secretion could be observed, which indicates a direct interaction with oxidant stress-pathways. By contrast, both cyclopentene and indomethacin derivatives majorily inhibited prostaglandin release, but showed only slight effects on formation of isoprostanes, lipid peroxides and cytokines. Model experiments using human low density lipoproteins oxidized by radiolytically generated oxygen radicals showed that indole derivatives differently interact with peroxidation of polyunsaturated fatty acids, than the cyclopentene/indomethacin derivatives, suggesting a physico-chemical rationale for observed anti-oxidant activity.
Conclusion: Indole-based selective COX-2 inhibitors substantially decreased radiation-induced formation of vasoactive isoprostanes 8-iso-PGE_2 and 8-iso-$PGF_{2\alpha}$ by endothelial cells. These findings may have particular importance in radiation-induced processes in which COX-2 is induced and oxidant stress occurs. The reduction of radiation-induced vascular dysfunction by antioxidative COX-2 inhibitors may widen the therapeutic window of cyclooxygenase-2 targeted treatment.

838 Withdrawn

839 Withdrawn

840 Influence of irradiation on para- and autocrine regulation of extracellular S100A4 (metastasin) and its receptor RAGE in B16 mouse melanoma cells

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

Background: Malignant melanoma is one of the most invasive and metastatic tumours. A common therapeutic approach towards metastases will combine