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

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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

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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

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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

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

radiation with chemotherapy and/or surgery. The interaction between tumour and inflammatory cells, e.g., via S100A4 (metastasin) and the receptor for advanced glycation endproducts (RAGE), is hypothesized to play a key role in metastasis of melanoma. In this study the contribution of para- and autocrine S100A4-RAGE activation to growth, motility and migration of metastatic melanoma and inflammatory cells before and post irradiation was investigated.
Materials and Methods: Mouse melanoma cells (B16), macrophages (RAW; a model for tumour associated macrophages (TAM)) and B16/RAW cocultures (ratio 1 to 5) were exposed to single dose irradiation (5, 10, and 20 Gy, compared to sham-irradiated controls) for 0, 3 and 6 days. S100A4 and RAGE expression in these cells was quantified via real-time RT-PCR, Western-blot analysis and immunochemistry. Cell growth and cellular viability was detected by MTT assay. Migration assays of non- and irradiated cells were performed with and without chemoattractants (supernatants of irradiated cocultures after 6 days). Additionally, the actin cross-linker L-plastin was investigated as a migratory marker.
Results: Post irradiation, S100A4 and RAGE mRNA expression was significantly increased in B16 and RAW cells but not in cocultivated cells. S100A4 protein expression was only detected in irradiated B16 cells whereas RAW cells always showed high levels in non- and irradiated cells. Interestingly, cocultures showed only minor S100A4 expression levels with a further reduction of S100A4 after irradiation. In contrast, RAGE protein showed only slight differences. A significant reduction of cell viability was observed after irradiation as supposed and the remaining cells seem to be in an exceptionally aggressive shape. On the other hand, migratory activity was significantly increased in B16 and cocultures after irradiation whereas RAW cells showed a significant decrease. Furthermore, chemoattractants significantly induced the migration in non-irradiated B16 cells.
Conclusion: Irradiation of both melanoma cells and macrophages alters their migratory and invasive activity. Under conditions of cocultivation these effects were more pronounced. We suppose an involvement of para- and autocrine regulation of extracellular S100A4 and its receptor RAGE in melanoma cells and TAM, thereby changing functional properties of melanoma cells towards a promigratory phenotype.

841 PARP-1 dependent DNA damage repair and formation of reactive oxygen species in response to ionizing radiation

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Background: Poly(ADP-ribose) polymerase (PARP) is an enzyme responsible for synthesis of poly(ADP-ribose) polymers in cell nuclei in response to DNA strand interruptions. Due to PARP's ability to recognize DNA strand breaks, poly (ADP-ribose) plays an important role in repair of DNA. Modulation of its activity by inhibition is considered as a potentially important strategy in clinical practice, especially to sensitize tumour cells to chemo- and radio-therapy. This study examines the influence of PARP inhibition on DNA repair and formation of reactive oxygen species (ROS) in cells exposed to ionizing radiation (IR), and also tries to answer the question whether the addition of a PARP inhibitor to irradiated cells is reflected in an altered level of apoptosis and necrosis.
Materials and Methods: Human K562 (myelogenous leukaemia) cells pre-treated or not with the PARP inhibitor Nu1025 (100 mM) were exposed to 4 Gy of ionizing radiation. The kinetics of DNA strand break rejoining were measured by alkaline single cell gel electrophoresis (Comet) assays. Intracellular reactive oxygen species were assayed using the probe 2,7-dichlorofluorescein diacetate (DCF-DA) with detection by flow cytometry. To measure PARP activity, its product poly(ADP-ribose) was detected immunocytochemically. Apoptotic and necrotic cells were quantitated using AnnexinV-PI staining and measured by flow cytometry.
Results: The data showed a ~100-fold increase in poly(ADP-ribose) formation during the first 10 min of recovery from IR, followed by a gradual decrease up to 30 min. Addition of the PARP inhibitor almost completely stops the production of poly(ADP-ribose) and significantly decreases the rate of DNA single strand rejoining. Three hours after IR we did not observe any changes in the amount of DNA single strand breaks (ssb) between groups but ROS concentration continued to grow up to 10 h. In this time point PARP inhibitor treated cells showed 250% higher ROS level than control and irradiated cells. We didn't find any significant changes in the apoptosis and necrosis level after 48 and 72 h.
Conclusions: Poly(ADP-ribosylation) and inhibition of PARP show a critical influence on DNA repair, and appear to be responsible for keeping the concentration of ROS at a high level after exposure of cells to genotoxic stress. Significant increase of ROS level in PARP inhibited cells seems not to have any influence on apotosis and necrosis. To determine the role of PARP in formation of reactive oxygen species, further studies have to be done.
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842 Transcripts from genes located in different isochores are differently regulated in cells exposed to ionizing radiation

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Background: Studies on eukaryotic genome organization have revealed the existence of long (~300 kb average) chromosomal regions that differ significantly in GC base pair content. Vertebrate genomes contain five distinct families of DNA regions termed isochores L1, L2, H1, H2 and H3, with increasing GC content (Constantini et al., 2006). Here we compared the change of level of transcripts from genes lying in different isochores in the human genome induced by ionizing radiation. The presence of repetitive and transposon-like sequences in up- and down-regulated transcripts was also analyzed.
Materials and Methods: Transcript levels were measured with Affymetrix HG-U133A microarrays after exposing K562, Me45 and HCT116 p53+/− cells to 4 Gy of ionizing radiation. The average values of hybridization signals for multiple microarray probes corresponding to the same transcript were calculated, and sequence analysis of up- and down-regulated transcripts was performed after extraction of sequences from the EMBL Reference Sequence transcript database using mostly our custom-made computer applications.
Results: Genes whose transcripts were up-regulated after irradiation differed significantly in nucleotide composition from those down-regulated. The largest differences were observed in Me45 melanoma cells where the median GC content was 58.8% in up-regulated genes and 42% in those down-regulated. These differences were seen in the GC content of both the 3′ untranslated region and the coding sequences, which also revealed a very high codon bias. The nucleotide compositional differences between up- and down-regulated transcripts were highly correlated with the isochore location of their genes; about 78% of genes from the down-regulated group were in the GC-rich H2 and H3 isochores compared to less than 17% of up-regulated genes. The largest differences in regulation of genes lying in different isochores were observed 1 h after irradiation, with a decrease over the next 12−24 h. Up- and down-regulated transcripts also showed cell type-specific differences in the distribution of repetitive transposon sequences.
Conclusions: These observations suggest that responses of the transcriptome to radiation are related to the isochore organization of the genome.
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843 Rapamycin sensitizes glioblastoma cells to radiation by inhibiting survivin

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Background: Rapamycin, a specific inhibitor of mTOR, has a cytotoxic effect and sensitizes to radiation therapy in various types of cancer. Survivin is elevated and attributes to radiation resistance in many tumours. We investigated the radiosensitizing mechanism of rapamycin in terms of mTOR signalling, survivin and cell cycle change using glioblastoma cells which is one of the most radioresistant.
Material and Methods: Three different glioblatoma cell lines, A172, T87 and U87 were treated with rapamycin and/or radiation. We did Westernblot for the study of protein expression and analyzed the cell cycle change using flowcytometry.
Results: Rapamycin resulted in a significant reduction in the phosphorylation (ser 473) of AKT and mTOR (Ser 2448 and Ser 2481). Rapamycin also reduced XIAP and survivin. Rapamycin along with radiation more significantly reduced phosphorylation of AKT and mTOR, and suvivin level than rapamycin alone. The cell cycle assay showed that cotreatment of rapamycin and radiation increased sub-G1 population, compared with rapamycin and radiation alone. Rapamycin enhanced radiation induced $p21^{cip1}$ and $p27^{kip1}$ expression and downregulated cyclin D1 and D3. In clonogenic assay, rapamycin sensitized glioblastoma cells to radiation.
Conclusions: Rapamycin enhances apoptosis by inhibiting the pro-survival protein survivin in glioblastoma cells. Rapamycin in combination with radiation may be efficacious in the treatment of glioblastoma.