Results: The sensitizing effect of IFN-beta was associated with an increase of the alpha-component of the survival curves. IFN pretreatment increased the apoptotic index in ZMK-1-, MCF-7- but not in A549-cells. The sensitizing effect of IFN-beta was more pronounced in proliferating cells compared to resting cells. There was no increase in initial DSBs and no alteration of DNA rejoining after IFN-beta treatment. The radiosensitizing effect was enhanced in LDR experiments compared to HDR experiments. Treatment with IFN-beta reduced the delayed plating effect in tumour cells.

Conclusions: Our observations are suggesting an influence of IFN-beta on repair mechanisms. Further studies should be aiming at identifying the subcellular pathways of the IFN-beta interaction with radiation repair.

699 POSTER

Influence of ERK1/ERK2 inhibition on radiation induced apoptosis and cell death in human squamous cell carcinoma cell lines

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Introduction: The constitutive activation of the Ras GTPase or the kinase cRaf-1 with subsequent activation of MEK1 and Erk1/Erk2 is frequently found in human carcinoma and mediates anti-apoptotic survival signals. We analyzed the impact of an specific MEK-1 inhibitor (PD98059) on radiation induced cell death in human carcinoma cell with high constitutive activity of Erk1/Erk2.

Material and Methods: Activation of Erk1/Erk2 was determined employing an antibody directed against active, phosphorylated Erk1/Erk2. PD98059 was used as specific inhibitor. Apoptosis induction was analyzed by activation of caspase-3 and parallel Hoechst staining. Clonogen cell survival was determined by standard colony formation assays.

Results: Active Erk1/Erk2 was detectable in all tested squamous cell carcinoma lines. PD98059 inhibited Erk1/Erk2 almost completely. Apoptosis induction as determined by morphology and caspase-3 activation was not influenced. In parallel, no influence of PD98059 on clonogen cell kill was detectable.

Conclusion: Inhibition of Erk1/Erk2 using PD98059 is not associated with increased radiosensitivity or apoptosis.

700 POSTER

Activation of caspase-8 by ionizing radiation is associated with high radiation sensitivity

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Introduction: The activation of caspases is a key step during apoptosis induction in response to different stimuli. We analyzed the involvement of caspase-8 which is the key caspase for CD95 induced cell death in radiation induced cell death in 8 different human lymphoma cell lines.

Material and Methods: Activation of caspase-8 was determined by western blotting directed against the p18 subunit of caspase-8. Apoptosis was analyzed by FACS and in parallel by Hoechst stain.

Results: Activation of caspase-8 in response to CD95 was detectable in 3 lines (CEM, Jurkat and Molt-17). In parallel, these lines were highly sensitive to CD95 induced apoptosis. CEM, Jurkat and Molt-17 also reacted with apoptosis and caspase-8 activation in response to ionizing radiation. 698, EHEB and K422 cell were resistant upon stimulation with both triggers reflected by no activation of caspase-8. K1 and DOHH cells only responded to ionizing radiation. In parallel, caspase-8 activation was only detectable in response to radiation.

Conclusion: Caspase-8 activation is detectable in all cell lines responding to ionizing radiation. Since there were two lines responding with caspase-8 activation in response to XRT but not to CD95 disparate pathways for activation of caspase-8 are likely to exist.

701 POSTER

Short- and long-term histopathological changes in the canine liver following single high dose intraoperative radiation therapy (IORT)

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Purpose: The histopathological changes in the canine liver following single high dose intraoperative radiation therapy (IORT) were investigated, in order to establish the tolerance of liver tissue to IORT thus providing a framework for clinical IORT treatment of patients with metastatic disease to the liver.

Materials and Methods: Following partial resection of the liver, IORT in doses of 10, 20, 25 or 30 Gy was applied to the resection plane and a non-surgically manipulated part of the liver of 25 Beagles.

Results: There were no postoperative complications, and no morbidity or mortality during a maximal follow-up of 5 years. Elective sacrifice was performed 3 months, and 1, 2, 3, and 5 years following IORT. Light microscopic examination revealed capsular thickening, severe parenchymal fibrosis, liver cell atrophy, and bile duct proliferation at the irradiated area 1 to 2 years following IORT. At 3 and 5 years however, only mild parenchymal changes were found that consisted out of slight periportal fibrosis, an incidental portal-central fibrous septum and vascular changes with endothelial proliferation and focal arteriolar hyalinosis.

Conclusions: This study demonstrated that following partial hepatic resection IORT to the liver in the canine model can be safely applied, without short- or long-term treatment morbidity. Although doses up to 30 Gy result in severe local tissue damage 1–2 years following IORT, these changes are largely reversible due to hepatic regeneration.

702 POSTER

Effect of genomic instability on radiation response of leukemic cells

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Data on radiation response derived from immortalized cell lines is sometimes regarded as little meaningful due to their genomic instability. It has been proposed that this genomic instability might have an effect on the radiosensitivity, proliferation and apoptotic index of cell cultures after radiation exposure.

To investigate this correlation, we looked at four genomic instable leukemia cell lines (HL-60, K562, ML-1, Raji I). Prior to irradiation, we prepared metaphase spreads to encounter the genomic instability of the cell lines. Therefore, we counted the chromosomes per metaphase, performed conventional cytogenetic analysis and three color FISH to detect numerical and structural chromosomal variability.

After irradiating each cell line with single doses of 0–4 Gy we looked at the following biological parameters: Radiosensitivity by the colony formation test, proliferation kinetics by flow cytometry and frequency of apoptosis by flow cytometry (Annexin V) and microscopy.

In all four cell lines, we detected varying numbers of chromosomes as well as different translocation chromosomes within the cell populations, proving their genomic heterogeneity and indicating genomic instability. Even though the cell lines showed genomic instability to a certain degree, parameters of cellular radiation response like proliferation kinetics, apoptosis and radiosensitivity always remained constant.

We conclude that genomic instability has no obvious effect on our measured radiation response parameters. Therefore, immortalized cell lines can serve as suitable model systems for measuring proliferation kinetics, apoptotic frequency and radiosensitivity but might not be suitable for investigations aiming for induced genomic instability.

703 POSTER

Different position of caspase-8 and bid activation within CD95 or radiation-induced apoptotic cascades

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Introduction: Activation of caspase-8 is crucial for apoptosis in response