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

to the CD95 death receptor. We aimed to determine the role of caspase-8 activation during radiation-induced apoptosis in human lymphoma cells.

Material and Methods: Activation of caspase-8, its substrate BID and apoptosis in response to CD95 stimulation or irradiation (XRT) was tested in Bcl-xl overexpressing Jurkat cells and the respective vector controls.

Results: In contrast to CD95 stimulation, apoptosis in response to XRT was abrogated completely in Bcl-xl expressing cells. CD95 induced apoptosis was delayed. In parallel, caspase-8 and BID activation by ionizing radiation was abrogated almost completely in Bcl-xl expressing cells. BID cleavage by XRT was still detectable in caspase-8 negative Jurkat cells, whereas no activation was visible after CD95 stimulation. Using the caspase-8 negative cells and a caspase8 dominant negative cell line we found that inhibition of caspase-8 activation interferes partially with radiation induced cell death.

Conclusion: Radiation induced activation of caspase-8 is secondary to a bcl-xl controlled process. BID can be activated independently of caspase-8 during radiation induced cell death. Caspase-8 is involved but not required for radiation induced cell death in human lymphoma cells.

704 POSTER

Ionizing radiation effect on transcription of estrogen receptor in human breast cancer cells

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Purpose: Potential alteration of ionizing radiation on estrogen receptor (ER) transcriptional activity was assscsssed in vitro.

Method: Transcriptional activity of ER under estrogenic and antiestrogenic stimulation was assessed by spectrophotometric measurements of luciferase concentration in MVLN cells (i.e. MCF-7 cells stably transfected with a plasmid in which expression of lucifcrasc gene (LUC) is under the control of an estrogen-response element (ERE))

Results: Ionizing radiation (3 to 12 Cy) failed to suppress the transcriptional activity of ER. Thus, after irradiation luciferase activity or residual cells still increased with 10^{-10} M E_2 and decreased with both pure (RU58668) and partial (OHTain) antiestrogens (both at 10^{-7} M). Hence, no major loss of transcriptional activity was recorded. Interestingly RU 58668-induced inhibition of luciferase was not amplified by irradiation while the inhibition produced by OHTam significantly decreased.

Conclusion: ionizing radiations may select a receptor machineD, less sensitive to antiestrogen inhibition.

705 PUBLICATION

Different radiosensitizing effects of Gemcitabine in human squamous cell carcinoma cell lines

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Purpose: Gemcitabine (dFdC) has shown promising activity in different solid tumors in vivo and in vitro. Combined with irradiation a radiosensitizing effect is observed. We investigated the influence of dFdC on the radiosensitization of different human squamous cell carcinoma cells (#4197-oropharyngeal cancer cells, Hep2-larynx cancer cells, HeLa-cervical cancer cells).

Methods: Under standardized conditions monolayer cultures of each cell line were incubated in medium with dFdC for different times (4–24 h) and in non or slightly cytotoxic concentrations (0.01 + 0.03 μmol/l). After dFdC-exposure the cells were irradiated with 0–6 Gy. Cell survival was determined by colony forming assay. Using the linear-quadratic model survival curves were fit and the radiation enhancement ratio was calculated by the means of the mean inactivation dose.

Results: Depending on concentration (0.01 + 0.03 μmol/l) and time of exposure (4–24 h) the effect of dFdC on #4197-, HeLa-, and Hep2-cells decreases survival from 1–50%, 0–50%, and 4–18%, respectively. Combined with irradiation directly after 4-h- and 24-h-exposure the enhancement ratios are 1.03–1.05 (0.01 μmol/l) and 1.39–1.67 (0.03 μmol/l) in #4197-cells, 1.07–1.14 (0.01 μmol/l) and 1.49–2.48 (0.03 μmol/l) in HeLa-cells, and 1.04–1.14 (0.01 μmol/l) and 1.07–1.16 (0.03 μmol/l) in Hep2-cells, respectively.

Conclusion: Our results demonstrate that dFdC is a potent radiation sensitizer of HeLa- and #4197-cells. The only slight effect on Hep2-cells could be caused by a reduced or lacking activity of intracellular deoxycytidine kinase which is important for phosphorylation of inactive dFdC (pro-drug) into active dFdC-triphosphate.

706 PUBLICATION

Cytogenetic disorders and changes in peripheral blood in children living in the areas polluted after the accident at the Chernobyl AES

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Dynamic investigations carried out in the population of children living in the areas of Russia radionuclide polluted as a result of the accident at the Chernobyl AES, allowed to determine the correlation of specific changes in peripheral blood with cytogenetic status changes. It is indicated by the high frequency of different blood disorders (74.2%) in children with moderate and high extent of the cytogenetic disorders aggregate (75%). Increase in dicentric number and the stable-unstable aberration sum in 13.5% of all cases combined with erythrocytosis, in the same percent of cases – with two-blast cytosis, and in 10.8% of cases With trombocytosis. In all those children more than two chronic diseases and thyroid functional disorders were found. In the most polluted regions trombocytosis was found besides lymphocytopenia and anemia. The high sensitivity of erythrocytes and platelets is also confirmed by the variation size between lower and upper limits of the control indices in the total population of inspected individuals. Those indices dispersion values appeared to be the most unstable, deviations from the permissible range being 28–30%. The stability of the diagnostic index disorders in the blood counts of 85% of children is worth mentioning as an independent fact. It also concerns the most of sideropenic anemiae The immune diseases, such as neutropenia and trombocytopenia, obtain chronic and clinically marked nature. The problems also arise with functional disorders indicating of disorders of maturation, differentiation and active processes in the mature cell, directed towards its destabilization.

707 PUBLICATION

The effect of GM-CSF on wound healing in irradiated rats

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Purpose: Preoperative radiotherapy (RT) is frequently used as an adjuvant therapy to surgery for same solid turnouts, however irradiation can complicate surgical wound healing. Granulecyte macrophage-colony stimulating factor (GM-CSF) is one of the cytokins that have important roles in wound healing. We developed an animal model to investigate the effect of GM-CSF on wound healing in irradiated rats.

Methods: Thirty male adult wistam rats were divided into the following three groups: group 1 (n = 10), control (no irradiation, no GM-CSF); group 2 (n = 10), RT (irradiation, no GM-CSF); group 3 (n = 10), RT and GM-CSF (irradiation and GM-CSF). The irradiated groups received 30 Gy to their skin. The treatment schedule was 300 cGy/fraction, one fraction in a day, five fraction in one week. Three weeks after the final dose of radiation, all irradiated and normal rats received skin flaps. The wound healing was evaluated by histological, histochemical, and immunohistochemical studies. Biopsies taken on the postoperative day 3 and 10 were analyzed according to the following criteria: presence of crust, epidermal regeneration, presence of acute inflammatory elements (AIEs), collagenization of granulation tissue, collagen fibers, and neovascularization. Chi-square test was used to compare each criteria.

Results: On the postoperative day 3, there were statistically significant differences between the groups according to the present of crust, present of AIEs, and collagenization of granulation tissue. On the postoperative day 1 O, group 3 also displayed significant difference in epidermal regeneration, neovascularization, and collagen fibrils compared to the other groups.

Conclusion: Our experiments confirm that RT impairs wound healing and that GM-CSF therapy for radiation-damaged skin improves wound healing.