itive vessels, increased numbers of p53- and bax-positive crypt cells and less bcl2- and Ki67-positive cells than unirradiated controls. Histopathologic radiation injury was associated with high grad diarrhea.

Concluson: Our data support a prominent role for endothelial dysfunction in he pathogenesis of radiation proctitis and clarify mechanisms of intestinal radiation injury and repair in the *in-vivo* situation.

694 POSTER

No effect of increased cell loss and decreased neoangiogenesis on clonogen tumor cell proliferation in human fadu scc during fractionated RT

D. Zips[1], C. Petersen[1], F. Hessel[1], M. Baumann[1]. [1]*Dept. of Radiooncology, University Hospital, Dresden, Germany*

Purpose: Preirradiation of the tumor bed causes decreased neoangiogenesis and increased necrotic cell loss in FaDu-hSCC. To investigate the impact of these factors on repopulation of clonogenic tumor cells during fractionated RT, tumor control (TCD50) experiments were performed.

Methods: FaDu hSCC was transplanted s.c. into the preirradiated hindleg of nude mice. A series of 10 TCD50 assays under clamped hypoxia was performed. 3, 6, 9, 12, 15, 18 daily fractions of 3 Gy and 3, 12, 18 fractions of 3 Gy given every second day were followed by graded top-up doses. The top-up TCD50 values after 120 days follow-up were compared with results obtained from experiments with FaDu without pre-RT of the tumor bed.

Results: With increasing number of daily fx, the top-up TCD50 decreased from 30 Gy after 3 fx to 7 Gy after 18 fx. In the group treated every second day no decrease was observed indicating a clear-cut time factor. All TCD50 values were 7 Gy lower in the preirradiated tumor bed compared with data from FaDu without pre-RT of the tumor bed.

Conclusion: Preirradiation of the tumor bed causes an increased cell loss, a decreased neoangiogenesis and a decreased number of clonogenic tumor cells per tumor but does not affect the repopulation kinetics in FaDu-hSCC. The potential benefit of inhibition of neoangiogenesis in combination with fractionated RT will be investigated further in ongoing experiments.

supported by the Deutsche Forschungsgemeinschaft Ba 1433/2-1

695 POSTER

Non invasive measurement of oxygen in irradiated and unirradiated tumours

L. Jüling-Pohlit, K. Borowsky, M. Schneider, J. Röhrborn, B. Schopohl, H.D. Böttcher. *Inst. of Radiotherapy, Hs 21D, Univ. of Frankfurt, Theodor-Stern Kai 7, 60596 Frankfurt, Germany*

Purpose: The oxygen supply of tumors has an important influence on the success of a radiation therapy. Therefore we measured Hb_{total}, HbO_2 and the oxygen saturation in tumors. These Data were corralated with the rate of mitosis, necrosis and vasculogenesis.

Methods: Solid tumors in the leg muscle of mice were irradiated with electrons. The total dose was given in one or 5 fractions with 12 hours interval. Every two days the oxygen parameters were measured non invasivly with near infared reflection spectoscopy and some of the animals were sacrefied for the determination of histological parameters.

Results: Unirradiated tumors: With increasing tumorvolume the necrosis, vasculogenesis, and all the oxygen parameters increase. Only the rate of mitosis remains constant. In the irradiated tumors the change of histological parameters as well as of the oxygen parameters depend on the total tumor dose and the fractionation scheme.

Conclusion: The oxygen supply of tumors changes with tumor volume. After irradiation with decreasing tumor volume the oxygen parameters increase, which might be an indication for reoxygenation of the tumor.

696 POSTER

Direct and transgenerational carcinogenic effect of ionizing radiation

I. Klementis, K. Lumniczky, S. Antal, E.J. Hídvégi, G. Sáfrány. [1]*National Research Institute for Radiobiology and Radiohygiene, Molecular and Tumor Radiobiology, Budapest, Hungary*

Purpose: Ionizing radiation can induce genetic instability and mutations both in somatic and germ line cells. We have investigated the genetic events leading to cancer in prenatally exposed individuals and tried to estimate the risk of transgenerational effects.

Methods: Mice were irradiated in utero with gamma radiation and the presence of point mutations as well as loss of heterozygosity (LOH) in different oncogenes and tumor suppressor genes were studied in the developed tumors. In addition, male mice were exposed to gamma and fission neutron radiation and mated with unirradiated females in different intervals after irradiation. We analyzed the litter size and followed the mutation rates at different hypervariable minisatellite DNA regions in the offspring.

Results: H-ras mutations were found in liver carcinomas, K-ras mutations in lung tumors and p53 mutations in lymphomas. LOH at the p53 and mts tumor suppressor genes was observed in all types of malignancies. Male germ cells were most sensitive to ionizing radiation at the spermatid stage. The litter size decreased in a dose dependent manner and mutation rates at minisatellite loci were increased by 4–5-fold. Irradiation of male germ cells at the spermatozoa stage hardly affected the litter size, however mutation rates were increased by 2-fold. When male germ cells were irradiated at the spermatogonium stage we have not observed alterations in litter size and in mutation rates.

Conclusion: Paternal exposure to ionizing radiation induces detectable transgenerational effects on gene level. This might increase the cancer risk in the offspring of exposed parents.

697 POSTER

Induction of TGF-β in lung tissue after thoracic irradiation

C.E. Rübe[1], Ch. Rübe[1], D. Uthe[1], K.W. Schmid[2], N. Willich[1]. [1]*Depts. of radiotherapy;* [2]*pathology; University of Münster, Germany*

Purpose: The lung is the major dose-limiting organ for radiotherapy of cancer in the thorax. The pathogenesis of radiation-induced lung injury at the molecular level is unclear. Immediate cellular damage after irradiation is hypothysed to result in cytokine-mediated multicellular interactions with induction and progression of fibrotic tissue reactions. The purpose of these experiments was to evaluate the acute and long term effects of radiation on the gene expression of TGF-β in a modell of lung injury using the fibrosis-sensitive C57BL/6 mice.

Methods: After thoracic irradiation (6/12 Gy) the mice were sacrified at times corresponding to the latent, pneumonic and fibrotic phase. The mRNA expression in the lung tissue was quantified by competitive RT-PCR; the cellular localization of the TGF-β protein was identified by immunhistochemical staining. The cytokine expression on mRNA and protein level was correlated with the histopathological alterations.

Results: Following thoracic irradiation with a single dose of 12 Gy, radiation-induced TGF-β release was appreciable already within the latent period and reached a significant increase during the pneumonic phase; at the beginning of the fibrotic phase, the TGF-β expression gradually declined. The elevated levels of TGF-β mRNA have been found to correlate with immunhistochemical staining of alveolar macrophages, type II pneumocytes and fibroblasts. Increased TGF-β expression was detected prominently in regions of histopathologic radiation injury. After exposure to a single radiation dose of 6 Gy, the lung tissue revealed no significant radiation-mediated TGF-β response.

Conclusion: This study demonstrates a dose-dependent expression of TGF-β in lung tissue following irradiation. The predominant localization of TGF-β in areas of inflammatory cell infiltrates and fibrosis suggests involvement of this cytokine in the pathogenesis of radiation-induced pulmonal fibrosis.

698 POSTER

Interaction of interferon-beta and irradiation

H. Schmidberger[1], M. Rave-Fränk[1], P. Virsik-Peuckert[2], K. Thürnau[1], A. Thole[1], C.F. Hess[1]. [1]*Univ. Göttingen, Radiotherapy, Göttingen;* [2]*Univ. Göttingen, Clinical Rad. Biol., Göttingen, Germany*

Purpose: In vitro studies on five different tumor cell lines suggested an additive or supra additive interaction of IFN-beta and radiation. We aimed at elucidating the underlying biological and biochemical characteristics of the enhancement.

Methods: The interaction of IFN-beta and radiation was tested in the following 5 cancer cell lines: A549 (lung), MCF-7 (breast), CaSki (cervix), WiDr (colon), ZMK-1 (head and neck). Cell survival was measured by a colony forming assay after incubation with IFN-beta for 24 h, and quantified by sensitizer enhancement ratios (SER) at the 37% survival level, as well as the isobologram method. Apoptosis was measured in acridine orange stained cells. DNA-DSB were determined by constant field gel electrophoresis. Low dose rate experiments (LDR), and delayed plating experiments were performed.

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

C. Belka, P. Knippers, W. Budach, P. Marini, H. Faltin, M. Bamberg.
Departments of Radiation-Oncology, University Tuebingen, Germany

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

V. Heinrich, C. Belka, W. Budach, P. Marini, H. Faltin, M. Bamberg.
Department of Radiation-Oncology, University Tuebingen, Germany

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)

H.J. Hoekstra[1], A.J.K. Grond[2], B.G. Szabó[3], C. Cromheecke[1].
[1]Groningen University Hospital, Surgical Oncology, Gronigen; [2]Groningen University Hospital, Pathology, Groningen; [3]Groningen University Hospital, Radiation Therapy, Groningen, Netherlands

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

K.M. Greulich[1], A.P. Rhein[1], K.P. Gilbertz[2], M. Brueckner[1], D. van Beuningen[2], M. Molls[1]. *[1]Techn. Univ. Munich, Dept. of Radiation Oncology, Munich; [2]Inst. of Radiobiology, Federal Armed Forces Medical Academy, Munich, Germany*

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

J. Rudner[1], W. Budach[1], P. Marini[1], E. Gulbins[3], K. Schulze-Osthoff[2], M. Bamberg[1], C. Belka[1]. *[1]Departments of Radiation-Oncology; [2]Internal Medicine; [3]Physiology, University Tuebingen, Germany*

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