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.

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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.