689 POSTER DISCUSSION

Cooperative *in vivo* cytotoxicity of ionizing radiation and the protein kinase C-inhibitor PKC412 in p53-deficient, radioresistant tumor cells

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Purpose: To test the radiosensitizer PKC412 (N-Benzoyl-staurospofine) *in vivo*, using different treatment schedules and analysing tumor histology.

Methods: p53−/−, E1A/ras transformed mouse embryo fibroblasts (2–4 × 10^6) were injected subcutaneously into the back of nude mice. Treatment with different regimens during 4 and 10 consecutive days (3 Gy/day locoregional, 100 mg/kg PKC412/day (p.o.) or combined) was started when tumors reached a minimal size of 165 mm^3 ± 10%. Tumor size was measured daily and tumor response to combined treatment was analysed histologically with HE and TUNEL staining.

Results: Combined treatment with PKC412 (100 mg/kg daily) and locoregional irradiation (3 Gy daily) on 4 or 10 consecutive days conferred a strong tumor growth control during treatment and follow-up-period in p53-deficient tumors. Daily treatment of tumors with PKC412 or IR alone resulted only in partial tumor growth delay. In contrast to p53+/+ tumors, histological analysis of p53−/− tumors after combined treatment only showed a minimal amount of apopotic cell death.

Conclusions: These data show that PKC412 is a promising radiosensitizer in vivo. The histological analysis of the treated tumors indicates that the mechanism of induced cell death is different in p53+/+ and p53−/− tumor cells. No signs of apoptosis could be observed in p53−/− tumor cells after combined treatment. Thus, the radiosensitizing effect of PKC412 observed in the p53−/−, radioresistant tumor cells points towards a novel approach how to overcome treatment resistance.

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Changes in tumour microvessels and microcirculation during fractionated radiotherapy of mouse AT17 tumours

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Purpose: Radiation dose-dependent changes in tumour blood flow and vascular structures were analysed.

Methods: Fractionated radiotherapy (single 3 Gy doses to totals of 0, 42, 78 Gy) of mouse AT17 mammary adenocarcinomas, then dynamic contrast-enhanced MRI (Magnevist, Schering) to quantify tumour perfusion, then mapping of tumour vascular structures by intravital lectin perfusion. Pathohistology with HE.

Results: In the control group (0 Gy) radially coursing microvessels gave rise to numerous fine branches supplying nests of tumour cells. Tumour perfusion was characterised by Gd-DTPA concentration-time curves with relatively broad peaks. In the 42 Gray group tumour cell densities were attenuated and the microvascular supply consisted predominantly of large-calibre radial vessels lacking fine branches. The Gd-DTPA curves showed smaller half-peak values. In the 78 Gray group sparsely distributed small clusters of tumour cells remained. The topographical organisation of the blood vessels was altered, with fine-calibre radial microvessels and irregular arborisations of wide-calibre vessels centrally. Gd-DTPA concentration-time curves tended to revert to broader half-peak values.

Conclusions: Characteristic topographical changes in microvessel architecture during fractionated radiotherapy were reflected in changes of the time-course of Gd-DTPA concentration in the tumour tissue. This has considerable impact on the interpretation of contrast enhanced MR studies.

691 POSTER DISCUSSION

Changes of tumor oxygenation during radiotherapy

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Purpose: Hypoxia is one of the most important reasons for radioresistance. Recent studies in animals showed fractionation (hyperfractionated versus conventional fractionated) and dose dependent changes in tumor oxygenation during radiotherapy. We recently reported a decrease of the tumor oxygenation after 30 Gy conventional fractionated radiochemotherapy (Radiother Oncol 48: 157–164, 1998) whereas Lartigau et al. (Eur J Cancer 34: 6, 856–861, 1998) found an increase after hyperfractionated radiotherapy. Now we evaluated in which way the changes of the hypoxic fraction corresponded to the changes of the hypoxic subvolume (HSV) of the tumor.

Methods: We investigated 33 patients with locally advanced head and neck cancer pretherapy and after 30 Gy conventional fractionated radio- or radiochemotherapy (5 FU + Mitomycin C) by using the Eppendorf-histograph. In 23 of these patients we determined ultrasonographically the change of volume during this time interval.

Results: We observed a significant increase of the hypoxic fraction (below 5 mm Hg) after 30 Gy ($p < 0.05$). In addition we found a significant volume reduction during therapy ($p < 0.001$). Moreover the calculated "hypoxic subvolume" (hypoxic fraction (%) × tumor volume (ccm)) decreased statistically significant during the treatment ($p < 0.01$).

Conclusion: The significant decrease of the HSV during a radioncological, treatment shows that the hypoxic areas of the tumor can shrink in spite of increasing proportion of hypoxic tumor sites.

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Radiation and a p53-stabilizing agent cooperate in wild type (wt) p53-mediated growth inhibition of human lung cancer cell lines

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2-Methoxyestradiol (2-ME), a metabolic byproduct of estradiol is known to induce wt p53, and inhibit growth of human lung cancer cell lines. We evaluated whether radiation and 2-ME cooperate in inducing apoptosis in wt p53-containing H460 and A549 cells as well as mutated p53-containing H322 cells.

Cells were radiated and/or treated with 2-ME. All 3 treatments inhibited the growth of wt p53-containing cells, with the strongest growth inhibition after combination treatment. H322 cells were less sensitive to either treatment and showed no significant additional growth inhibition with the combination. Wt p53 and p21 protein expression in H460 and A549 cells was higher following radiation and 2-ME than after either single treatment, while p53 and p21 levels remained virtually unchanged in H322 cells. The proportion of apoptotic cells in H460 (43%) and A549 (31%) after combination treatment was above any of the other treatment groups.

In a nu/nu mice model employing H460 cells injected subcutaneously at the hind leg, irradiation and 2-ME as single treatments were barely effecitve. Combination therapy however, led to significant tumor growth suppression.

Our data suggest that irradiation and 2-ME cooperate in stabilizing wt p53, with possible therapeutic implications for the future.

693 POSTER DISCUSSION

Endothelial cell injury and cell kinetics in normal rectum after neoadjuvant hyperfractionated radiochemotherapy

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Purpose: This prospective study assessed pathogenic cellular and molecular mechanisms of radiation enteropathy: endothelial cell function, proliferative activity and apoptosis-regulating factors in irradiated and nonirradiated normal rectum of patients with rectal cancer. The results were correlated with histopathologic injury and also clinical symptoms according to the CTC.

Methods: Irradiated and nonirradiated normal rectal specimens from patients with rectal cancer were excised intraoperatively, six cm proximal to the tumor, and immediately snap-frozen and also fixed in formaldehyde. Specimens were analyzed by immunohistochemistry using antibodies against markers of endothelial cell function (thrombomodulin, TM), proliferative activity (Ki67), and apoptosis regulating proteins (bcl2, bax protein). DNA-sequencing of the p53 gene (exon 5–11) was also performed.

Results: Irradiated rectum showed mucosal denudation, ulceration, and microvascular injury, and exhibited significant less proportions of TM-pos-

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

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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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Non invasive measurement of oxygen in irradiated and unirradiated tumours

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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

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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

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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

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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.