and supra and infratentorial in 40%. Patients presented metastases from primitive tumor of the lung (74%), the breast (9,1%), colorectal origin (9%), cutaneous origin (6,4%) head and neck origin (5,3%), other origin (7%) and unknown origin (7,3%).

**Results:** Survival of patients with CM was dependent on the type of treatment tumour, it was about 339 days [28-662], 222 days [90-390 ] and 64 days [8-678], respectively in the event of complete surgical resection, of biopsy or partial resection or exclusive radiotherapy. In addition survival was also conditioned by the type of primary tumour, it was 197 days, in case of non small cell lung cancer and 119 days for the small cell lung cancer. In case of breast cancer, colorectal cancer, cutaneous cancer, head and neck, other origin or unknown, survival was respectively 106, 90, 63, 120, 226, 174 days.

**Conclusion:** Survival was dominated by the achievement of a surgical resection and by the aggressive nature of the primary tumor. It seem possible to use different radiotherapy scheme according to primary tumor site

# Radiobiology

742 POSTER

## Role of Bcl-2 subcellular localization for radiation-induced apoptosis

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**Introduction:** The anti-apoptotic proto-oncogene Bcl-2 is expressed in membranes of mitochondria and endoplasmic reticulum and mediates resistance against a broad range of apoptotic stimuli. Although several mechanisms of Bcl-2 action have been proposed, its role in different cellular organelles remains elusive.

**Material and Methods:** We analyzed the function of Bcl-2 targeted specifically to certain subcellular compartments in Jurkat lymphoma cells. Bcl-2 expression was restricted to the outer mitochondrial membrane by replacing its membrane anchor with the mitochondrial insertion sequence of ActA (Bcl-2/MT) or the ER-specific sequence of cytochrome b5 (Bcl-2/ER). Additionally, cells expressing wildtype Bcl-2 (Bcl-2/WT) or a transmembrane domain-lacking mutant (Bcl-2/DTM) were employed. Apoptosis induced by ionizing radiation was quantified using scatter characteristics and by determination of the mitochondrial membrane potential (DYm) using FACS Calibur flow cytometer. Furthermore activation of different caspases was analysing by western blotting.

**Results:** Bcl-2/WT and Bcl-2/MT strongly inhibited radiation-induced apoptosis and caspase activation, whereas Bcl-2/DTM had completely lost its anti-apoptotic effect. Interestingly, Bcl-2/ER conferred protection against radiation-induced mitochondrial damage and apoptosis similarly to Bcl-2/MT.

**Conclusion:** Here we show for the first time that not only mitochondrial Bcl-2 but also ER-targeted Bcl-2 interfered with mitochondrial DYm breakdown and caspase-9 activation. Our finding therefore indicates the presence of a crosstalk between both organelles in radiation-induced apoptosis.

743 POSTER

## Immunohistochemical study on reoxygenation of FaDu-tumours during fractionated radiotherapy

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**Purpose:** Previous investigations indicated that reoxygenaton might be the stimulus for accelerated repopulation of FaDu-tumors during fractionated radiotherapy. In addition to these experiments immunohistochemical studies on the oxygenation status and the tumormicromilieu during radiotherapy were performed.

**Methods:** Tumorbearing mice were irradiated with 3 to 15 daily fractions (3 Gy) under normal blood flow and clamp hypoxia. Mice were sacrificed one day after end of irradiation after injection of different histological markers and tumors were stained and evaluated. Vascularization (ERMP-12), perfusion (Hoechst) and the amount of cellular hypoxia (Pimonidazol) was quantified by multiparameter image analysis.

**Results:** Vascular density in the vital tumor area was constant with increasing number of fractions (5-8%). The perfused fraction of vessels decreased considerably after irradiation with 3 and 6 fractions compared to unirradiated controls from 37% to 7% but increased after 12 to 15 fractions to values comparable to unirradiated tumors. The amount of cellular hypoxia in the vital tumor area decreased with increasing number of fractions from 17% to 2%.

**Conclusion:** From these immunohistochemical and morphometric studies we conclude that there is a high degree of hypoxia during the initial part of radiotherapy in FaDu-tumors. After 12 fractions reoxygenation occurs. These data are in good agreement with our functional studies on radiobiological hypoxia.

Supported by the Deutsche Forschungsgemeinschaft Ba/1433-2

744 POSTER

## Comparison of biodistribution of two hypoxia markers [18F]fmiso and [18F]fetnim in an experimental mammary carcinoma

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Nitroimidazole compounds labelled with positron emitting radionuclides such as fluorine-18 offer a means for non-invasive detection of tumour hypoxia with positron emission tomography (PET). A good marker for clinical use would apparently be one with a high hypoxia-specific signal-to-background ratio in target tissues. Our goal was to compare the intratumoural biodistribution of [18F]fluoromisonidazole ([18F]FMISO) with that of [18F]fluoroerythronitroimidazole ([18F]FETNIM) in carbogen treated and untreated mice, in order to compare the hypoxia-specificity of the tracers. Female CDF1 mice with a C3H mammary carcinoma grown on the backs were used. Tumours were size matched and animals breathed either normal air or carbogen gas (95% O2 + 5% CO2). The gassing procedure was started at least 5 min prior to the intravenous injection of either [18F]FMISO or [18F]FETNIM and continued throughout the experiment. A minimum of six mice were used for both gas conditions with each tracer. The hypoxia markers were allowed to distribute for 120 min. Blood, tumour, muscle, heart, lung, liver, kidney, fat, and bone tissues were immediately removed, counted for 18F-radioactivity and weighed. Tumour and muscle were frozen in dry ice/isopentane and cut with a cryomicrotome into 20 $\mu$m thick slices. The spatial distribution of 18F-radioactivity from the tissue slices was determined with digital autoradiography.

The treatment had no effect on the biodistribution of either tracer in the normal tissues, but had an effect on the tumours. Autoradiography results showed that the whole tumour-to-muscle 18F-radioactivity uptake ratios were significantly higher in untreated mice as compared to carbogen treated mice for both [18F]FMISO ($p = 0.004$) and [18F]FETNIM ($p = 0.004$). The autoradiograms showed that the 18F-activity was heterogeneously distributed within tumours showing regions with high and very low uptakes. These uptakes will be correlated to the histological status of the tumour slices.

In conclusion, our study shows that both [18F]FMISO and [18F]FETNIM uptake correlates with the oxygen status in tumours.

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## Does selection of rapidly proliferating clonogenic tumour cells contribute to accelerated repopulation during fractionated RT? A study on human squamous cell carcinoma in nude mice

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**Purpose:** FaDu hSCC exhibits a clear-cut time factor of fractionated irradiation due to accelerated repopulation of clonogenic tumour cells during treatment. The underlying mechanisms of accelerated repopulation are not fully understood. Beside other mechanisms genetically stable selection of rapidly proliferating clonogenic tumour cells may be involved in this phenomenon.

**Materials and methods:** Three FaDu tumours (R1, R2, R3) that recurred locally after fractionated RT with high doses and long overall treatment times were retransplanted s.c. into the right hind leg of NMRI nude mice. Human origin was confirmed by LDH isoenzym pattern. Six millimeter tumours were irradiated either with single dose, 18 fractions of 3 Gy within 18 days, or 18 fractions of 3 Gy within 36 days. To obtain complete dose effect curves, graded top-up doses were given after fractionated RT. All irradiations were applied to anaesthezised animals under clamp hypoxia. For data evaluation

tumour control dose 50% (TCD50) were calculated and compared with the TCD50 values of the parental FaDu line after the same irradiation schedule.

**Results:** The TCD50 values after single dose irradiation were 37 Gy [95% CI 33;42], 39 Gy [33;43], 37 Gy [35;40] and 38 Gy [35;41] for FaDu-R1, FaDu-R2, FaDu-R3 and the parental FaDu, respectively. All investigated retransplanted recurrences showed a clear-cut time factor, i.e. TCD50 values after 18 fractions within 36 days were significantly higher than after 18 fractions within 18 days. The comparison of TCD50 values after the same overall treatment time revealed no significant differences between R1, R2, R3 and the parental FaDu line indicating an identical magnitude of the time factor in the retransplanted recurrences and in the original FaDu.

**Conclusion:** A genetically stable selection of rapidly proliferating clonogenic cells does not contribute to accelerated repopulation in poorly differentiated FaDu hSCC in nude mice.

Supported by the Deutsche Forschungsgemeinschaft Ba 1433-2

746 POSTER

## Effect of recombinant human Keratinocyte Growth Factor (rhKGF) on proliferation, clonogenic capacity, and radiation response of human epithelial tumor cells in vitro

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**Purpose:** A fatal consequence of breaks in radiotherapy of head and neck cancer caused by severe mucositis may be a significant decrease in local control and cure. Amelioration of the mucosal response aiming at avoiding breaks could increase the therapeutic ratio of radiotherapy. Keratinocyte growth factor has been identified to ameliorate the acute response to radiation in animal models. The application of KGF in tumor treatment should not protect tumor cells. The purpose of this study is to investigate the in vitro effect of rhKGF on proliferation, clonogenic capacity, and radiation response of low passage human epithelial tumor cells in media containing low FCS concentration.

**Material and Methods:** Five tumor cell cultures derived from head and neck squamous cell carcinomas, three cultures derived from pleural effusions of lung carcinomas and normal nasal epithelial cells were analyzed. For experiments, cells in passage 2-6 were incubated with rhKGF (10;200 ng/ml) immediately after plating for clonal growth in serum-depleted media. To determine cellular radiosensitivity single doses of 1;8 Gy of X-rays were applied. Colony formation as well as the number of cell doublings was determined after 10;14 days of growth in rhKGF-treated and control cells. Each experiment was repeated twice, radiation survival curves were fitted by the linear-quadratic equation, and statistical comparison was preformed betweeen rhKGF-treated and non-treated cultures.

**Results:** Normal epithelial cells showed a two- to three-fold increase in the number of cell doublings due to KGF-treatment ($P < 0.0001$). In contrast, in tumor cell cultures only slight, not significant stimulation of proliferation occurred in 2 out of 8 samples ($P = 0.20$ and 0.07, respectively). This stimulation was abolished either by serum addition to the medium or in irradiated cells. In the remaining tumor cell cultures, which were not growth stimulated by KGF neither radiation-induced impairment of proliferation nor clonogenic cell survival was influenced by the addition of KGF to the medium.

**Conclusion:** A clinical pilot study indicate that KGF is well tolerated and effective in humans. In animal models, KGF has been shown to ameliorate the radiation tolerance of normal epithelia. Together with the minimum in vitro tumor cell response to KGF, compared to normal epithelial cells in this study, these results suggest a potential for selective protection of normal epithelia during clinical radiotherapy.

747 POSTER

## The cytotoxicity of Ukrain does not involve the TP53/p21/p27-signal transduction cascade

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**Purpose:** Ukrain, a Chelidonium majus L.-Alkaloid/Thio-TEPA derivative, has shown cytotoxicity in vitro and in vivo. The mechanism responsible remains to be elusive. In this study the influence of Ukrain and ionizing radiation on the TP53-p21 pathway and the cell-cycle was investigated in human wild-type (wt) TP53 lung carcinoma cells (A549), TP53-overexpressing glioblastoma cells (U138MG) and normal wt-TP53 fibroblasts (HSF1).

**Materials/methods:** Exponentially growing cell lines/cell stem were irradiated with 1x5 Gy or treated with 1.0$\mu$g/ml Ukrain for 2, 6 or 24h. Except colony formation, TP53, p21 and p27 were examined using western blot technique. Analyses of the cell-cycle were performed by flow cytometry.

**Results:** Ukrain treatment demonstrated a radiosensitizing effect in A549 and U138MG cells and a radioprotective effect in normal fibroblasts. TP53 induction/stabilization (>2-fold) and subsequent induction of p21/p27 (>10-/>8-fold) could be shown in A549 cells and HSF1 after irradiation but not after Ukrain exposure. TP53-overexpression without p21/p27 induction was detected in U138MG cells. An accumulation of cells in the G2-phase after a 24h-Ukrain treatment was detected in A549 (50%) and U138MG cells (70%) whereas the HSF1 showed no alteration of the cell-cycle.

**Conclusion:** Ukrain did not exert its cytotoxicity via the TP53-pathway. Radioprotection of wt-TP53 cells after Ukrain was TP53/p21/p27-independent and without G1-phase block. However, in tumor cells a radiosensitizing effect was demonstrated that was possibly based on blocking cells in the G2-phase. To provide more insight into Ukrain's unique molecular mechanisms optimal for radiochemotherapeutic approaches further experiments still have to be performed.

748 POSTER

## ACE-inhibition with Ramipril improves survival after thoracic irradiation in mice

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**Purpose:** The doselimiting effect of radiotherapy are in most clinical situations the 'late effects' of normal tissue tolerance. In this study we hypothesize that activation of matrix-metalloproteases may play a part in the pathogenesis of late effects. As Angiotensin Converting Enzyme (ACE) inhibitors have been demonstrated to inhibite at least some matrix-metallo-proteases, it was our hypothesis that they might protect against late irradiation morbidity. In addition we wanted to test if Ramipril had any effect on tumorgrowth.

**Methods and materials:** Single dose thoracic irradiation to the thorax in C57bl/6J mice was used as a model for late tissue tolerance. We used doses of 12, 15, 18 and 21 Gy and compared mice receiving Ramipril 30 mg/kg, continously 24 hours after irradiation in the drinking water, with mice receiving only plain water. The primary endpoint was survival, and as secondary endpoint for the 12, 15 and 18 Gy experiments we used breathrate measurements every second week for 180 days.

In a second experiment we tested the effect of Ramipril 30mg/kg in a micetumor model using the 'LPB-tumor'.

**Results:** Mice receiving Ramipril lived significantly longer than controls when the mice were irradiated with 18 or 21 Gy. For the lower doses the difference was not significant, but there was a trend in the same direction. The breathrate measurements supports these results. In Kaplan Meier survival plots with tumorarea $\geq$ 200 mmsq as endpoint we found a significant difference in' survival ' between mice receiving Ramipril and controls for both irradiated and non-irradiated mice.

Conclusions

The ACE-inhibitor Ramipril given 24 hours after single dose lethal thoracic irradiadition significantly prolongs lifetime in C57bl/6J mice. In addition Ramipril attenuates tumorgrowth in LPB tumors in mice.

749 POSTER

## Comparison of tumor control probability and normal tissue complication probability between 3D-CRT and IMRT plans in patients with prostate cancer

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**Purpose:** To compare tumor control probability (TCP) and normal tissue complication probability (NTCP) between conventional three Dimensional Conformal RadioTherapy (3D-CRT) and Intensity Modulated Radiation Therapy (IMRT) in prostate cancer patients using radiobiological response models.

**Methods:** Ten prostate cancer patients had planning CT studies at the Houston VAMC. The prostate was immobilized using an endorectal balloon inflated with 100 cc of air. The Raptor/3D and Peacock/Corvus treatment planning systems were used to generate 3D-CRT and IMRT