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.

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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.

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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 μm 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