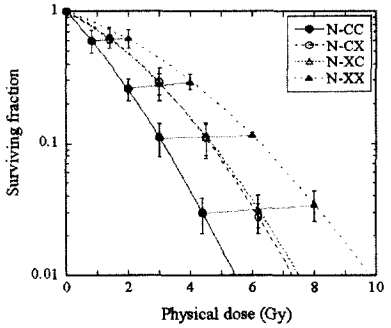
mixed beam of both (carbon-ion followed by X-ray; CX or X-ray followed by carbon-ion; XC). When utilizing mixed beam, two irradiations were done within 15 minutes. Irradiated doses were determined according to our previous assessment of biologic equivalent doses. Next, 72-hour-interval fractionated irradiation was performed with cells cultured under standard condition (Interval) to observe the difference of sublethal damage repair. Cell survival was assessed with the usual colony formation assay and survival curves were fitted by linear-quadratic model.

Results: In all experiments, the survival curves for cells irradiated with carbon-ion showed the steepest curves with the smallest shoulders, X-ray-irradiated cells showed the gentlest curves with the largest shoulders, and mixed beam irradiation showed intermediate curves. The difference of cell survival in the irradiation sequence of carbon-ion and X-ray (CX or XC) was not significant. In Hypoxia, Synchronized, and Interval conditions, surviving fractions were generally higher than in Normal condition, but not statistically significant. In mathematical analyses, mixed beam irradiation of carbon-ion and X-ray had no synergistic effect, and its cell-killing effect could theoretically be estimated from survival curves of carbon-ion and X-ray by using geometric internal dividing point method. These findings were observed in Hypoxia, Synchronized, and Interval conditions as well as in Normal condition.



Conclusions: The therapeutic effect of mixed beam irradiation of carbon-ion and X-ray is intermediate between carbon-ion only and X-ray only, and can be estimated without any complicated calculations. This provides very important information for the clinical use of mixed beam irradiation.

479 POSTER

Inhibition of angiogenesis and ionizing radiation: treatment-dependent influence on the tumor microenvironment

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Background: The combined treatment approach using inhibitors of angiogenesis (IoA) and ionizing radiation (IR) is a promising strategy against solid tumors. Several preclinical studies demonstrated that IoA enhance radiation-induced tumor growth control but so far the mechanism of combined treatment effect *in vivo* is far from clear. Here we investigate the effect of different treatment modalities on the tumor angiogenic system and on tumor hypoxia with innovative imaging techniques.

Material and methods: *In vivo* growth control experiments (IR (4x3Gy), PTK787 alone and in combination) were performed with tumor allografts derived from the murine c-neu (erbB2) over-expressing breast cancer cell line NF9006. The effect of different treatment modalities on the three-dimensional tumor vessel morphology was assessed by mercox casting followed by electron microscope scanning. Analysis of tumor hypoxia was assessed by 18F-fluoromisonidazole ([18F] FMISO) PET. Expression of distinct angiogenesis and microenvironment parameters was analyzed by immunohistochemistry.

Results: The combined treatment regimen exerted an at least additive growth control effect in NF9006 tumor allografts. Analysis of the different microvessel structures revealed that a distinct angiogenic phenotype resulted in a treatment-dependent way. Whereas in control tumors the morphological pattern of sprouting angiogenesis predominated, treatment with PTK787 (and to a certain extent) with IR alone drastically changed the pattern to intussusceptive microvessel growth. Furthermore combined treatment with PTK787 and IR markedly damaged and shrinked tumor vessels with dramatically reduced microvessel density and total vessel volume. Analysis of tumor hypoxia indicated that treatment with PTK787 alone increased tumor hypoxia to a higher extent than combined treatment or IR alone.

Conclusions: Treatment with PTK787 and/or IR changes the intratumoral angiogenic system as investigated on the morphology and oxygenation level. The treatment-induced angiogenic switch from sprouting to intussusceptive angiogenesis might be part of a treatment-induced tumor environmental stress response.

480 POSTER

Comparison of in vitro growth-inhibitory activity of paclitaxel and docetaxel on squamous cell carcinoma under normoxic and hypoxic conditions during irradiation

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Background: Hypoxia may influence tumor biology and physiology reducing cytotoxic effects of anticancer therapy. Our privious studies showed that docetaxel is more potent to kill hypoxic cancer cells *in vitro*. It is generally believed that both of taxanes, paclitaxel and docetaxel, are promoters of apoptosis in cancer cells. However, the apoptotic mechanisms of paclitaxel and docetaxel in cancer cells under the low concentration of oxygen are still not enough clear.

Materials and methods: Human squamous cell carcinoma cell line, A 431, was treated with paclitaxel, docetaxel and gamma-ray irradiation under normoxic and hypoxic conditions. Growth inhibition and induction of apoptosis were studied by SRB assay, flow cytometric analysis and M30-Apoptosense ELISA. Expression of p53, bax, bcl-2, bcl-XL, HIF-1α was investigated by Western blotting.

Results: Continious paclitaxel and docetaxel exposure over 96 h resulted in a dose-dependend decrease in the survival of A431 tumor cells incubated under normoxia. In the lower concentration range from 0,5 nM to 50 nM docetaxel was 1,3-fold more potent in average than paclitaxel. At concentrations above 500 nM both agents exhibited similar cytotoxic activity. Hypoxic treatment conditions significantly affected paclitaxel cytotoxicity in the lower concentration range. Under hypoxic conditions docetaxel (viability of 31,1% ± 1,3) was 2,0-fold more effective than paclitaxel (63,1% ± 6,4) at concentration 5 nM. Paclitaxel and docetaxel showed a synergistic effect with irradiation under normoxia even at the low concentrations. Hypoxic conditions affected synergism of paclitaxel and irradiation. Docetaxel completely maintained its toxicity despite the changed atmospheric incubation conditions. In the analysis of paclitaxel and docetaxel-induced expression of apoptosis-regulating molecules, the most significant changes were observed for HIF-1α, p53, and bcl-2 family members.

Conclusion: Docetaxel is more potent agent to show cytotoxicity in human squamous cell carcinoma under hypoxia, than paclitaxel. The key elements of the high potency of docetaxel are increased expressions of p53 and apoptosis-regulatory genes.

481 POSTER

An investigation of reoxygenation in high risk prostate cancer following high dose-rate (HDR) brachytherapy

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Background: Reoxygenation of hypoxic tumours is believed to occur during a course of radiotherapy, and is one of the basic principles on which fractionated treatment is based. The primary objective of this study was to directly measure changes in prostate oxygenation following a single 10 Gy fraction of high dose-rate (HDR) brachytherapy in men with prostate cancer.

Materials and Methods: The study was approved by the institutional ethics review board. Eligible patients had high risk localised prostate cancer (stage T3, Gleason 8-10, or PSA >20 ng/ml) with no previous cancer therapy (hormones or radiotherapy). Treatment consisted of two separate HDR brachytherapy treatments of 10 Gy, one week apart prior to external beam radiotherapy. Prostate oxygenation was measured using a 20 cm custom made polarographic needle electrode (Eppendorf), with the patient in the dorsal lithotomy position under spinal anaesthesia. The needle electrode was advanced through the perineum using a brachytherapy template under ultrasound guidance in 0.7 mm pilgrim steps. At least four tracks, one in each quadrant, were made (median of 32 pO readings per track). Median pO, and the hypoxic fraction (HF) considered as the percentage of values < 2.5 mm Hg, were obtained for each quadrant. Clinical, imaging and biopsy data were used to determine if the measurements were being made in