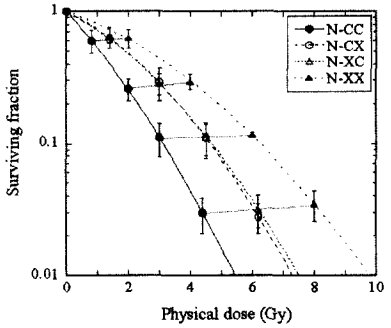
mixed beam of both (carbon-ion followed by X-ray; CX or X-ray followed by carbon-ion; XC). When utilizing mixed beam, two irradiations were done within 15 minutes. Irradiated doses were determined according to our previous assessment of biologic equivalent doses. Next, 72-hour-interval fractionated irradiation 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

O. Riesterer[1], V. Djonov[2], M. Honer[3], S. Ametamey[3], S. Bodis[1], M. Pruschy[1]. [1] *University Hospital Zurich, Radiation Oncology, Zurich, Switzerland;* [2] *University Hospital Berne, Anatomy, Berne, Switzerland;* [3] *Paul Scherrer Institute, Radiopharmaceutical Science, Villigen-PSI, Switzerland*

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

I. Skvortsova[1], T. Seppi[1], A. Haidenberger[1], A. DeVries[1], S. Skvortsov[2], H. Geiler[1], M. Saurer[1], P. Lukas[1]. [1] *University of Innsbruck, Dept. of Radiotherapy-Radiooncology, Innsbruck, Austria;* [2] *University of Innsbruck, Dept. of Internal Medicine, Innsbruck, Austria*

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

G. Morton, B. Yap, D.A. Loblaw, R. Choo, P. Cheung. *University of Toronto, Toronto-Sunnybrook Regional Cancer Centre, Toronto, Canada*

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

benign or malignant areas of the prostate. Baseline measurements were taken before the first HDR treatment, and then 7 days later before the second fraction. Control pO readings were also obtained within skeletal muscle. Seventeen patients were included in the study.

Results: At baseline, the mean hypoxic fraction was 76.5% in malignant and 63.2% in benign areas of the prostate (p=0.18, paired T test), with mean median pO values of 4.9 mm Hg and 8.7 mm Hg (p=0.40), respectively. The median pO in skeletal muscle was 29.5 mm Hg, with no values in the hypoxic range. One week following treatment, the mean HF was unchanged at 78.6% (p=0.76) in malignant areas and 66% (p=0.75) in benign areas. The mean median pO following treatment was also unchanged at 1.6 mm Hg (p=0.23) and 4.4 mm Hg (p=0.15) in malignant and benign areas, respectively. Of 27 malignant areas measured, 11 had an increase in HF, 14 had a decrease, and 2 were unchanged. Of 33 benign areas, 17 had an increase, 13 a decrease, and 3 no change.

Conclusions: The entire prostates of men with high risk prostate cancer are diffusely hypoxic. The level of hypoxia is not significantly reduced one week after receiving 10 Gy with HDR, indicating that reoxygenation had not occurred.

482 POSTER

Enhancement of radiation response by roscovitine in human breast carcinoma in vitro and in vivo xenograft model

L. Maggiorella, E. Deutsch, V. Frascogna, J. Bourhis. *Institut Gustave Roussy, laboratoire radiosensibilité des tumeurs et des ti, Villejuif, France*

Frequent deregulation of CDK activation associated with loss of cell cycle control has been found in most of human cancers. Recent development of a new class of antineoplasic agents targeting the cell cycle, has emerged as a small molecule CDK inhibitor, roscovitine which presents potential antiproliferative and antitumoral effects in human tumors. Further studies have reported that roscovitine combined with cytotoxic agents can cooperate with DNA damage to activate p53 protein. However, little is known about the biological effect of roscovitine combined with ionizing radiation in human carcinoma and no studies has been reported so far in p53 mutated carcinoma. In the breast cancer cell line MDA-MB 231 which lack a functional p53 protein, we have found a strong radiosensitization effect of *roscovitine in vitro* by clonogenic survival assay and *in vivo* in MDA-MB 231 xenograft model. Using Pulse field gel electrophoresis (PFGE), a strong impairment in DNA-DSB rejoining was observed following roscovitine + IR treatment as compared to IR alone. Cell cycle analysis has shown a G2 delay and no increase in radiation induced apoptosis in the cells treated with IR or roscovitine+IR. On the other hand, we have found a significant induction in micronuclei frequency following roscovitine +IR treatment as compared to IR alone. In MDA-MB 231 cells, the radiosensitization effect of roscovitine was associated with an inhibition of the DNA-PK activity due to a marked decrease in Ku-DNA binding when we used the electrophoretic mobility shift assay (EMSA). In conclusion, we found a novel effect on DNA repair of the CDK inhibitor roscovitine which acts as a radiosensitizer *in vitro* and *in vivo* in breast cancer cells lacking a functional p53.

483 POSTER

Erythropoietin receptor expression and the in-vitro effect of erythropoietin on the radiation-response of different cancer cell lines.

G. Shenouda, A. Belenkov, L. Souhami, M. Duclos, L. Portelance, T. Chow. *McGill University, Radiation Oncology, Montreal, Canada*

Anemia is associated with a poor outcome in patients treated with radiotherapy. Currently, Erythropoietin (EPO) is tested in phase III clinical trials to study its potential role to improve local control in patients treated with ionizing radiation. EPO is a hormone produced by the kidneys. It acts via the EPO receptor (EPOR) to stimulate growth, prevent apoptosis, and induce differentiation of red blood cells precursors. Expression of EPO and EPOR has recently been demonstrated in several nonhematopoietic tissues. This suggests a broader role for EPO in regulating cell growth and survival. It is known that autonomous EPO expression mediates autocrine growth of erythrocytic leukemia cells. This suggests that the expression of EPO and EPOR by tumors of nonhematopoietic tissues may also stimulate cancer cell proliferation.

This prompted us to study the *in-vitro* effect of EPO and the expression of its receptor on the radio-responsiveness of cultured tumor cell lines. The expression of EPO receptor (EPO-R) and its messanger (mRNA) were studied in cell lines including: MCF-7, HeLa, MDA, U87 and Colon 205, as well as a primary carcinoma cell line of the cervix (HT-100) using Reverse Transcriptase and Polymerase Chain Reaction (RT-PCR) and immuno-blot techniques. The radiation cell survival curves of all the cell lines were determined in the absence or in the presence of EPO. In all studied cell lines, there was a consistent and reproducible radiation protection in the presence of EPO. This EPO-induced radiation protection was abolished by the addition of a JAK2-kinase inhibitor, suggesting that the signal transduction pathway of EPO is functional.

Studies are underway to determine whether these *in-vitro* results are reproducible *in-vivo*. If such results are confirmed *in-vivo*, this may have implications on current ongoing clinical trials using EPO as an experimental agent to counteract the effects of hypoxia.

484 POSTER

Biological markers associated with sensitivity of tumour cells to the epidermal growth factor receptor-tyrosine kinase inhibitor ZD1839 and ionizing radiation

S. Burdak-Rothkamm[1], C.E. Ruebe[1], T.P. Nguyen[1], D. Ludwig[1], K. Feldmann[2], T. Wiegel[3], C. Ruebe[1]. *[1]Saarland University, Dept. of Radiotherapy, Homburg/Saar, Germany; [2]AstraZeneca GmbH, Wedel, Germany; [3]University Hospital Benjamin Franklin, Dept. of Radiotherapy, Berlin, Germany*

Background: ZD1839 (Iressa ®) , an orally active, selective epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor, reduces survival and augments radiation response of certain tumour cells through blockage of EGFR signaling. In this study we tested tumour cell lines for EGFR and TGF-alpha mRNA expression, cell cycle distribution and induction of apoptosis in order to identify biological markers that are associated with sensitivity to ZD1839 and that might serve as parameters in a predictive test.

Material and methods: The tumour cell lines A549, H596 (both human non-small cell lung cancer cell lines) and FaDu (human head and neck squamous cell carcinoma cell line) were subjected to ionizing irradiation, treatment with ZD1839 (1 μM, 5 μM) and combined ZD1839 / irradiation treatment. Clonogenic cell survival was determined by colony assays, EGFR and TGF-alpha expression by RT-PCR, cell cycle distribution and apoptosis by flow cytometry.

Results: Whereas in FaDu cells a considerably high amount of EGFR and TGF-alpha transcripts was detected, A549 and H596 cells both expressed moderate amounts of EGFR mRNA and very low levels of TGF-alpha mRNA. Irradiation led to early downregulation of EGFR transcripts in all three cell lines but only FaDu cells which were more radiosensitive than A549 and H596 cells showed a prolonged downregulation of EGFR mRNA expression compared to the expression level of the untreated cell line. Exposure to ZD1839 caused a decrease in EGFR mRNA expression in A549 cells whereas this effect could not be detected in the other two cell lines. Treatment with 1 μM ZD1839 showed marked inhibition of clonogenic growth in FaDu cells whereas it had little effect on clonogenic growth in A549 and H596 cells. Upon treatment with 5 μM ZD1839 survival curves revealed a radiosensitizing effect on A549 cells. A reduction of S phase cells and induction of apoptosis after treatment with 1 μM ZD1839 and combined ZD1839 / radiation treatment was most marked in FaDu cells.

Conclusions: The sensitivity of tumour cells to ZD1839 correlated with the EGFR and TGF-alpha expression level whereas a radiosensitizing effect was associated with downregulation of EGFR mRNA expression. Inhibition of cell proliferation and induction of apoptosis were correlated with a decrease in clonogenic cell survival after treatment with ZD1839.

485 POSTER

Evaluation of the effects of radiotherapy to the chiasm and optic nerve by visual psychophysical-electrophysiological tests

M. Esassolak[1], U. Karagöz[2], D. Yalman[1], S. Kose[2], Y. Anacak[1], A. Haydaroglu[1]. *[1]Ege University Faculty of Medicine, Radiation Oncology, Izmir, Turkey; [2]Ege University Faculty of Medicine, Ophthalmology, Izmir, Turkey*

Purpose: To evaluate the effects of high-dose irradiation to the chiasm and optic nerves in locally-advanced nasopharyngeal carcinoma patients by visual psychophysical- electrophysiological tests.

Materials and Methods: Series of visual tests [Visual evoked potential (VEP) latency, VEP amplitude, contrast sensitivity, visual field and visual acuity tests] were applied to 27 patients with locally-advanced (T4) nasopharyngeal carcinoma who were irradiated to high doses 6 - 74 months