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

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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.

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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

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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

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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