Overall cellular radiosensitivity correlates well with the level of radiation-induced G1 arrest (r = 0.856, $P = 0.0067$), with p53 constitutive levels (r = 0.874, $P = 0.0046$) and with p53 protein fold induction (r= -0.882, $P = 0.0038$). The mechanistic basis of these correlations remains to be elucidated in these cells, but the data do suggest that both the constitutive p53 level, and the p53 protein response to radiation, may be good predictive tests for radiosensitivity in some cell types.

819 ORAL

SIGNIFICANCE OF P53 AND BCL2 LEVELS IN THE RADIO-SENSITIVITY OF HEAD AND NECK CANCER

O. Csuka, G. Németh, K. Koronczay, Z. Doleschall, É Remenár
National Institute of Oncology, Budapest, Hungary

Radiosensitivity of tumor cells is thought to be modulated by p53 and bcl2 proteins. High level of wild type p53 is required for radiation induced apoptosis. The aim of the present study is to investigate the significance of gene dosages of p53, bcl2 and c myc in radiation induced apoptosis. The base line and 30 Gy, 60 Gy gamma radiation induced values of p53 and bc12 were estimated by Western blot in 60 biopsies of head and neck cancer. Our results suggest that the radiosensitivity of head and neck cancer depends on the ratio of p53 and bcl2 gene dosages. High level of p53 is counter-balanced by high bcl2 gene dosage resulting in radioresistance. Amplification of c myc can compensate for the low p53 level and suggests radiosensitivity of tumor cells. Our studies indicated that the prediction of radiosensitivity of tumor cells could be based on the simultaneous evaluation of p53, bcl2 and c myc levels.

820 ORAL

IMMUNOHISTOCHEMICAL ANALYSIS OF P34^{CDC2} AND CYCLIN B CELL LOCALIZATION IN RECURRENT HEAD AND NECK SQUAMOUS CELL CARCINOMA AFTER IRRADIATION

E. Cohen-Jonathan[1], C. Toulas[2], P. Rochaix[3], J.F. David[3], N. Daly-Schveitzer[1], G. Favre[2]
[1]Radiotherapy Department
[2]Laboratory of Molecular Oncology
[3]Histopathologic Department-Centre Claudius Regaud, 20-24 rue du pont Saint Pierre, 31052 Toulouse, France

After exposure to ionizing radiation, eukaryotic cells undergo a G2 delay which contributes to the ability of cells to survive irradiation. For some radioresistant cell lines, this delay is prolonged. Entry of cells into mitosis is regulated by a complex of two proteins cyclin B, and the serine-threonine p34^{cdc2} kinase. When this complex is activated, it undergoes a transport from cytoplasm into the nucleus and phosphorylates proteins which lead to mitosis. P34^{cdc2} kinase is activated by binding to cyclin B and by phosphorylation/dephosphorylation of p34^{cdc2}. Since G_2 delay after irradiation has been correlated with a rapid inhibition of p34^{cdc2} activity and an enhanced tyrosine phosphorylation, we hypothesized that radioresistant tumors could have a lack in regulation of p34^{cdc2} kinase activity. In this study, we entered 32 patients treated, from 1983 through 1989 at the Claudius Regaud Center, for head and neck squamous cell carcinoma by surgery and standard post-operative doses of radiotherapy. The paraffin embedded tumor specimens had been sampled before radiotherapy for long term controlled patients (n = 7) and before and after radiotherapy for patients who had developed a recurrence in the irradiation fields (n = 25). Immunohistochemical staining was performed with monoclonal antibodies against p34^{cdc2} (sc-54) and cyclin B (sc-245). A semi-quantitative score was used. For p34^{cdc2} analysis, no difference in intensity of staining was observed between long term controlled patients and those who recurred or, when there was a recurrence before and after radiotherapy. However, there was a highly significant difference ($P < 0.001$) in p34^{cdc2} cell localization with a preferential cytoplasmic localization only for the patients who have a recurrence in the radiotherapy fields. This cytoplasmic localization was present in the primary tumor before radiotherapy and in the recurrence, too. No preferential localization was observed in long term controlled patients. For cyclin B, no difference in intensity of staining was observed anywhere and conversely to p34^{cdc2}, no difference in localization appeared in long term controlled patients nor for patients who have had a recurrence. No correlation existed between localization of p34^{cdc2} and those of cyclin B in patients who recurred. Our results suggest a probably intrinsic abnormality of p34^{cdc2} activity and a lack of association between cyclin B and p34^{cdc2} in head and neck radioresistant squamous cell carcinoma.

821 ORAL

GLUCOSE UTILIZATION AND *IN VIVO* CELL KINETICS IN RECTAL CANCER, BEFORE AND AFTER PREOPERATIVE RADIOTHERAPY

K. Haustermans, C. Schiepers, F. Penninckx, K. Geboes, J. Nuyts, L. Filez, L. Mortelmans, G. Bormans, M. De Roo, E. van der Schueren
Department of Oncology, Nuclear medicine, Abdominal surgery and Pathology, University Hospital, Leuven, Belgium

Patients with T3/T4 rectal cancers were injected i.v. with 400–555 MBq F-18 FDG. Dynamic imaging was immediately started. Maximum tumor glucose utilization was calculated. Cell kinetics were measured by flow cytometry 6–8 hrs after i.v. injection of IUdR.

Two groups of patients were studied: 1) surgery only (n = 8); and 2) preoperative radiotherapy (30 Gy/10 fractions) followed by surgery (RT, n = 6). At baseline, TuGluc for group 1 was 280 ± 141 (SD) nmol/min/ml, and for group 2:269 ± 161 (P = NS). After RT, TuGluc decreased to 109 ± 61 ($P > 0.05$). Tpot was 3.50 ± 1.21 days for group 1 and 3.23 ± 2.23 days for group 2 before RT (P = NS). A negative correlation was found between TuGluc and Tpot, suggesting increasing glucose utilization for faster dividing cells. After RT, Tpot did not change significantly (4.88 ± 3.80 days), whereas TuGluc fell significantly. *In conclusion:* these results show a direct correlation between tumor cell metabolism and tumor cell proliferation *in vivo*. RT results in a decrease in TuGluc utilization indicating cell loss due to RT, because the proliferating cells don't proliferate more slowly, and so should not have a smaller TuGluc.

822 ORAL

ASSESSMENT OF HUMAN FIBROBLAST RADIOSENSITIVITY BY THE MICRONUCLEUS AND COLONY-FORMING ASSAY

J. Johansen, S.M. Bentzen, J. Overgaard, B. Stausbøl, M. Overgaard, C. Fuhrmann, C. Streffer
Danish Cancer Society and Department of Oncology, University Hospital of Aarhus, Denmark
Institut für Medizinisches Strahlenbiologie, Essen, Germany

The relationship between radiosensitivity of normal human skin fibroblasts determined by the micronucleus (Mn) assay and the colony-forming assay was investigated. Prediction of radiation-induced normal-tissue response in individuals necessitates a rapid and reproducible *in vitro* assay that correlates well with cell survival after irradiation. The Mn-assay is a quick test that was applied to primary skin biopsies from 10 unselected breast cancer patients in whom a significant correlation previously was found between the expression of subcutaneous fibrosis after radiotherapy and the cell surviving fraction at $SF_{3.5}$ (Spearman's rho = −0.81, $P < 0.01$). Early generations of fibroblasts in exponential growth were irradiated with 250 kV X-rays at room temperature. The micronucleus frequency in Cytochalasin B-induced binucleated cells (Mn/BNC) was scored after doses of 0, 1, 2, and 3.5 Gy, and Mn/BNC after irradiation with 3.5 Gy was estimated (range 1.11–1.77). Mn/BNC at 3.5 Gy showed no correlation with $SF_{3.5}$ as determined by the colony-forming assay. A possible reason for lack of correlation include variable numbers of scorable binucleated cells after irradiation with 3.5 Gy (4%–21%).

823 ORAL

IN VIVO AND *IN VITRO* EFFECTS OF TIRAPAZAMINE (SR-4233) ALONE OR COMBINED WITH RADIATION OR CYTOTOXIC DRUGS ON HUMAN CELL LINES

E. Lartigau, M. Guichard
Laboratory of Radiobiology, Institute Gustave-Roussy, 9488 Villejuif, France

Solid human tumours contain areas with low oxygen tension (pO_2). For bioreductive drugs it is important to define the cytotoxic effect according to drug concentration and to clinically relevant pO_2. *In vitro*, the pO_2 dependence of the survival of three human cell lines (HRT 18, Na11 +, and MEWO), exposed to tirapazamine alone or combined with ionizing radiation, was studied at five different oxygen concentrations: air (20.9% O_2), 10, 2, 0.2 and 0.02% O_2 (hypoxia). Tirapazamine below a concentration of 100 μM was not cytotoxic in air or at 10% O_2. At 100 μM tirapazamine was toxic in 2% O_2, and at 50 μM in 0.2% O_2. For $pO_2 < 0.2\%$ O_2, there was a marked increase in cell killing when 10 μM tirapazamine was combined with 2 Gy, compared with either 10 μM or 2 Gy given alone ($P < 0.03$). *In vivo*, tirapazamine was combined with various drugs (5 FU, VP 16, DTIC, CDDP, BLEOMYCINE). Mice lethality, regrowth delay and excision assay were studied. In the latter, the most effective combination was tirapazamine-bleo for HRT 18 and tirapazamine-VP 16 for NA 11+. The activation of tirapazamine at a low concentration and at pO_2 found mainly in tumours could yield a very