829 POSTER

EFFECT OF HYPOXIC CELL SENSITIZATION ON GLUCOSE METABOLISM OF SQUAMOUS-CELL CARCINOMA

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Overcoming hypoxic radioresistance with chemical sensitizers has potential to improve therapeutic outcome of head and neck cancer. Squamous-cell carcinoma cell line from a patient with lingual cancer (UT-SCC-5) was exposed to moderate (1.5% pO_2) and severe (0% pO_2) hypoxia, known to increase *in vitro* uptake of 2-[^{3}H]fluorodeoxyglucose (FDG) by neoplastic cells. [^{3}H]FDG uptake in well oxygenated (20% pO_2) UT-SCC-5[2] -cells incubated with 5 mM of misonidazole (MISO) or nimorazole (NIMO) increased by 101 ± 41% and 84 ± 23%, respectively. Incubation of UT-SCC-5 in 1.5% pO_2 resulted in a decrease of [^{3}H]FDG uptake by 6 ± 4% in the presence of 5 mM of MISO while it increased by 43 ± 14% with 5 mM of NIMO. In 0% pO_2 both drugs showed a decrease of 58 ± 6% (MISO) and 6 ± 18% (NIMO), respectively. These *in vitro* studies indicate differential modification of glucose metabolism by two well known radiosensitizers in air and hypoxia. Based on these observations, we suggest that imaging of head and neck cancer with 2-[^{18}F]FDG and positron emission tomography (PET) may assist in evaluation of *in vivo* effects of hypoxic cell sensitizers.

830 POSTER

THE ROLE OF SUPEROXIDE DISMUTASE IN PROTECTION AGAINST RADIATION-INDUCED DNA DAMAGE

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Heritable variation in cellular radiosensitivity is believed to be an important determinant of normal tissue responses to radiation. There is increasing evidence that the initial level of DNA damage induced determines this cellular radiosensitivity. The superoxide dismutase genes have been shown to influence cellular radiosensitivity through their effects on free-radical damage. In this study we have examined the effect of inherited mutations in the Cu/Zn superoxide dismutase on DNA damage induction following ionizing radiation treatment.

We have examined lymphoblastoid cell lines from normal individuals and patients with Familial Amyotrophic Lateral Sclerosis (FALS)—recently identified as having Cu/Zn SOD gene mutations. Radiation-induced DNA damage has been measured in these cell lines using pulsed-field gel electrophoresis (PFGE).

A two-fold range of DNA damage induction was found between cell lines but this was unrelated to the mutations in the SOD1 gene. These data suggest that the SOD1 gene does not influence DNA damage induction but its influence on other aspects of cellular radiosensitivity require further evaluation. Despite the lack of correlation with Cu/Zn SOD mutations the wide range in DNA damage induction shown here has not been previously reported in lymphoblastoid cell lines and the implications of this on radiotherapy need to be considered.

831 POSTER

EFFECTS OF IONIZING IRRADIATION AND ADRENERGIC STIMULATION ON GENE EXPRESSION PATTERN IN RAT SUBMANDIBULAR GLANDS

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Radiotherapy administrated to patients with head and neck malignancies often results in severe xerostomia. We evaluated the expression of early response proto-oncogenes (c-fos and jun-B), tissue specific genes (proline rich protein (PRP) and kallikrein), and proteolysis linked ubiquitin gene following exposure to 15 Gy irradiation and/or adrenergic stimulation of the rat submandibular gland. We observed that the expression of the genes whose regulation is associated with DNA damage (i.e. jun-B and c-fos) was enhanced by irradiation or the combination of irradiation and isoproterenol administration. In contrast, the expression of genes associated with the routine functional integrity of the cell (i.e. kallikrein, ubiquitin and PRP) was uneffected. These findings, in addition to delayed gland dysfunction, leaves us to believe that irradiation induced injury to the submandibular glands is to be attributed to reproductive stem cell death which may be obliterated in some part in a clinical setting.

832 POSTER

ACUTE RADIATION-INDUCED THYROIDITIS—A PROSPECTIVE STUDY

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Purpose: We prospectively studied acute radiation-induced thyroid dysfunction by performing thyroid function tests.

Materials and methods: The subjects were 28 patients who underwent neck irradiation incidentally including the thyroid for various malignancies. The total dose absorbed by the thyroid was 4500–5000 cGy. Thyroid function tests included serum thyroid stimulating hormone (TSH), free triiodothyronine (T3), and free thyroxine (T4). These data were obtained before irradiation, at 40 Gy irradiation, and 3 and 6 months thereafter.

Results: Mean TSH levels were 1.57, 0.66, 1.34, and 6.23 μU/ml at preirradiation, 40 Gy irradiation, and 3 and 6 months after irradiation, respectively. The decrease in TSH levels at 40 Gy was significant (P = 0.0001, Wilcoxon signed-rank test). TSH levels significantly increased thereafter until 6 months (40 Gy vs 3 months:P = 0.001, 3 months vs 6 months:P = 0.028). Mean T4 levels increased from 1.13 to 1.21 ng/ml during 40 Gy irradiation (P = 0.02).

Conclusion: The elevation of T4 and decrease in TSH observed at the time of irradiation was attributed to acute thyroid follicular cell destruction by irradiation.

833 POSTER

NEUTRON CAPTURE POTENTIATION: HOW TO GET SOME SELECTIVITY IN A FAST NEUTRON BEAM?

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Neutron captures on ^{10}B may be a neat way to provide some selectivity in a fast neutron beam: when the neutrons are thermalized in the tissue, they produce nuclear reaction with ^{10}B nuclei, releasing energetic α particles and Li ions. Provided that a sufficient amount of ^{10}B may be carried with a specific molecule inside tumoral cells, a very efficient component of irradiation can be added.

Tumors like glioblastomas, soft tissues sarcomas or melanomas, which are highly radioresistant, may benefit of this potentiation to obtain better local control, while sparing more the normal tissues.

Results of tumor targeting with boronated compounds, of dosimetry and radiobiology in our fast neutron beam will be presented.

834 POSTER

THE INFLUENCE OF CELL DENSITY DEPENDENT PLATING EFFICIENCY ON CLONOGENIC ASSAYS

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To measure cell survival after ionizing radiation, clonogenic assays are considered the standard. Survival is expressed in plating efficiency reduction. However for some cell lines we discovered that plating efficiency was strongly dependent on the numbers of cells seeded; with increasing numbers of cells the plating efficiency decreases. Data were taken from human melanoma cell lines transfected with oncogenes c-myc and N-ras and normal human fibroblasts, that had been tested for *in vitro* radio sensitivity. For each dose a range of cell numbers was taken and survival was calculated based on (a) the total data available, (b) data from lowest number of colonies per dish, where a linear relationship between cell numbers and plating efficiency exists and (c) the highest number of colonies per dish, as can be statistically more interesting.

It will be shown that if plating efficiency is not correctly determined, that the interpretation of the data can lead to wrong estimation of survival. It is concluded:

1. Plating efficiency depends on the total number of surviving cells after treatment.

2. Linearity of plating efficiency should be tested first within a range before drawing conclusions from clonogenic assays.

835 POSTER

MODIFICATION OF THE SPARING EFFECT OF USING LOW DOSE RATE TOTAL BODY IRRADIATION ON MURINE PULMONARY TOXICITY BY CYCLOPHOSPHAMIDE

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We have thus tested the effect of radiation dose rate and combining cyclophosphamide (CTX) with single fraction TBI on lung damage in a mouse model for BMT. Total body irradiation (TBI) was given as a single fraction at high dose rate (HDR, 0.71 Gy/min) or at low dose rate (LDR, 0.08 Gy/min). CTX (250 mg/kg) was given 24 hours before TBI. Bone marrow transplantation (BMT) was performed 4–6 h after the last treatment. Lung damage was assessed using ventilation rate (VR) and lethality between 28 and 180 days ($LD_{50/28-180}$). The LD_{50} for lung damage increased from 12.0 Gy (± 0.2) using single fraction HDR to 15.8 Gy (±0.6) using LDR. The LD_{50} values for the combined treatment were 5.3 Gy (±0.2) and 3.5 Gy (±0.2) for HDR and LDR, respectively. This indicates that the combined effect of LDR and CTX was more toxic than that of combined CTX and HDR. Lung damage evaluated by VR demonstrated two waves of VR increase within the first 180 days after treatment. We conclude that lung damage following TBI could be spared using LDR, however, CTX markedly enhance TBI-induced lung damage. The combination of CTX and LDR is more toxic to the lungs than combining CTX and HDR.

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836 POSTER

P16 AND P53 LEVELS AFTER DIFFERENT TREATMENTS IN HUMAN TUMOR CELLS

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It has been suggested that the product of p53 gene inhibits cellular growth by stimulating the production of p16 protein. We have examined by ELISA the protein levels of pl6 (ranged 0.80–3.44 O.D. unit per 10^6 cells) and p53 (ranged 2.20–4.65 O.D. unit per 10^6 cells) in human tumour cell lines. We have not found a quantitative relationship between these protein levels: neither in standard growth conditions nor after 6 Gy of radiation. The p53 product function has been surveyed by flow cytometry studying G0/G1 cell cycle arrest after irradiation of the cells at 6 Gy. Taking into account that concept we have divided our cell lines in two groups (A) cells with functional p53 protein and (B) cells with functional inactivation of the p53 gene product. Higher constitutive levels of p16 product were found in group A cells. Intracellular p16 levels change after 6 Gy but not a defined time course profile has been found. We have identified that p16 levels change markedly with growth conditions, ie, age of culture, growth rate modified by use of differents serum levels or after hormonal synchronization of human breast cancer cell lines. The implications of this for the radiation response and cellular proliferation of human tumour cell lines remains to be determined.

837 POSTER

COMPARISON OF RADIATION-INDUCED TRANSLOCATIONS IN EARLY AND LATE PASSAGE TUMOR CELLS BY FISH

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Chromosomes No 1, 2, 3, 4, 9 and 12 in early (1–3rd) and late (25–30th) passage cells derived from a *squamous cell carcinoma of the gingival mucosa* were analyzed. Translocations in unirradiated as well as in irradiated (D = 4 Gy) cells were determined by painting whole chromosomes with fluorescent hybridization probes (FISH). A radiation-induced polyploidization of all chromosomes analyzed was observed with the only exception of chromosome No. 4 in late passage cells. The frequency of radiation-induced translocations as well as the clonogenic cell survival was similar in early and late passage cells but translocation frequencies were not always proportional to the length of the corresponding chromosomes. The rate of spontaneous translocations was different for individual chromosomes and was not correlated with their radio-sensitivity.

When compared with the more radioresistant fibroblasts HSF-2 and the more radiosensitive breast cancer cells MCF-7, the investigated tumor cells showed a medium radiosensitivity with respect to both, translocation frequencies and clonogenic cell survival.

838 POSTER

S PHASE DURATION IN RELATION TO S PHASE FRACTION AS SIMULATED BY A COMPUTERIZED MODEL

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For several tumour types it has been found that there is a correlation between clinical outcome and the relative number of tumour cells in S phase as measured with DNA flow cytometry (FCM). From data showing that a high S phase fraction is associated with poor prognosis it has been concluded that these tumours have a high proliferative activity and therefore grow faster. Moreover, the size of the S phase fraction has also been used as an equivalent of labelling index to determine tumour growth fraction in experimental cell kinetic studies. In the present study the relationship between DNA distribution and the duration of cell cycle phases in tumours was investigated with the aid of a computerized mathematical model. In our study we found poor correlation between the size of the S phase fraction and the proliferative activity when tumour growth fraction was taken into account. A shortening of the duration of the S phase ie, increased cell production per time unit, led to a decrease in the relative number of S phase cells as measured by FCM.

839 PUBLICATION

CLINICAL RADIOBIOLOGY OF HDR CF-252 BRACHYTHERAPY FOR CERVIX UTERINE CARCINOMA

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45 pts (I) with cervix uterine carcinoma received combined radiation therapy; external Co-60 gamma therapy (37.3 Gy) for pelvis and HDR Cf-252 brachytherapy (point A—35, Gy-eq). The treatment results were compared with historical similar group—64 pts (II) treated by external Co-60 gamma therapy (39.7 Gy) and HDR Co-60 brachytherapy (point A—47.4 Gy).

There were no significant difference in 4 yr survival: 73.3% (I) vs 79.7% (II). Local failure was observed in 22.2% (I) and 10.9% (II) cases. The rate of late radiation complications was similar—4.4% (I) vs 1.6% (II). Acute reactions were brachytherapy dose dependent with ED50:80, 1 Gy-eq and 74.5 Gy in I and II groups, respectively.

Radiobiology analysis of obtained data show some possibilities to improve treatment results in HDR Cf-252 brachytherapy group.

840 PUBLICATION

A RADIORESISTANT LINE OF RAT GUERIN'S CARCINOMA: ITS OBTENTION, PROPERTIES AND A REGIMEN OF RADIOTHERMOTHERAPY

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We obtained a radioresistant line of rat Guerin's carcinoma through repeated courses radiotherapy (5 sessions of 10 Gy each). We followed 14 subsequent passages of the line, revealed a complex of signs testifying to radioresistance of tumor cells: an increase in their morphologic heterogeneity, nuclear and cellular area, number and size of nucleolus and number of binuclear cells. We proved alterations in the subpopulation composition of Guerin's carcinoma cells with an increase in the proportion of slowly proliferating but more radioresistant cells. A radioresistant line also features increased antioxidative activity of tumor cells, levels of non-protein thyol groups, induction of stress proteins with 140–150 kDa, whose levels are the same between the courses of radiotherapy, as well. It is experimentally shown that hyperthermia before radiotherapy effective