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The relationship between normal tissue radiosensitivity and radiation induced cell-cycle delays in asynchronous populations of normal fibroblasts analysed by bivariate flow cytometry

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Introduction: It is possible to study irradiation induced cell-cycle delays in asynchronous populations with bivariate flow cytometry.

Materials: Twelve normal human fibroblast cell strains were studied, including, two genomic radio-sensitive cell strains, a radioresistant cell strain and nine strains derived from vaginal biopsies from pre-therapy patients with carcinomas of the cervix.

Methods: Confluent cells were irradiated at 0, 2 and 4 Gy, and then plated out at low cell density with the addition of bromodeoyxuridine for 72 hrs. and then lysed and double-labelled with Hoechst 33258-ethidium bromide for bivariate flow cytometry.

Results: Cell-cycle analysis showed emptying of the G_2 compartment which indicates a G_2 block for cells irradiated in G_2. This was maximal in the most radioresistant cell strains (occurring at a dose of 2 Gy), whereas in the most radiosensitive cell strains this does not occur after a dose of 4 Gy. The proportion of cells which cycle following irradiation correlates with the cell surviving fractions at 2 Gy, SF_2, $r = -0.86$, $p = 0.026$, after a dose of 2 Gy. The accumulation of cells in the first G_1 compartment following irradiation is greater in radioresistant cells than in radiosensitive cells: after 4 Gy this relationship reaches significance when correlated with SF_2, $r = 0.81$, $p = 0.05$. The percentage of cells entering the G_2 compartment in the first cycle is the same irrespective of whether the cells are irradiated or not. In cycling cells G_2/M delay measured by the ratio of the percentage of cells in G_2 in the first cycle, to the percentage of cells in G_1 in the second cycle, correlates with SF_2 $r = -0.89$, $p = 0.03$ at 2 and 4 Gy.

Conclusions: Irradiation induced cell-cycle delays correlate with normal tissue radiosensitivity.

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Fibroblast contamination in assays of tumour radio-sensitivity

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Cellular *in vitro* radiosensitivity was measured in 69 tumour biopsies from SCC of the head and neck using the modified Courtenay-Mills soft agar clonogenic assay. In the cultures of all tumour biopsies, stromal fibroblasts colonies were obtained (range 14–100%), determined by immunocytochemistry. The tumour cell and fibroblast radiosensitivities were uncorrelated. In an attempt to compare the growth and radiation characteristics of fibroblasts with different origin, skin, mucosa and stromal tumour fibroblasts were obtained from 7 of the patients, and cultured in culture flasks and in soft agar. Plating efficiencies were significantly higher for fibroblasts originating from normal mucosa ($p < 0.012$) than for the stromal tumour fibroblasts. In this small sample, the SF_2 values of the normal tissue fibroblasts did not correlate significantly with the SF_2 values of stromal tumour fibroblasts, which fell into the range of SF_2 values obtained from 36 skin biopsies from women who received postmastectomy radiotherapy in a previous study in the lab. This study illustrates once more the problem with fibroblast contamination in assays of tumour radiosensitivity. A pilot study on SCC of the uterine cervix indicates that the problem with stromal fibroblast contamination exists in this case also. The growth and radiation characteristics of the stromal tumour fibroblasts did not differ considerably from the normal tissue fibroblasts. A more definitive study investigating these characteristics would include many patient biopsies and would be very time consuming and expensive.

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Chromatin structure in human glioma cell lines with different radiosensitivity

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Purpose: To study the correlation between cellular radiosensitivity and chromatin organisation.

Methods: Two human glioma cell lines, U87 and EA14, of differing radiosensitivity were examined. Radiation induced changes in chromatin organisation were assessed on histone-depleted nuclei (nucleoids) following exposure to high-salt buffers. The ability of propidium iodide (PI) to introduce positive supercoils in nucleoids was measured as forward light-scatter by using flowcytometry analysis.

Results: Cell survival varied by a factor of 1.4: U87 (a 0.19 Gy-1; b 0.011 Gy-2) and EA14 (a 0.27 Gy-1, b 0.041 Gy-2). Both cell lines expressed similar maximum loop relaxation at comparable PI concentrations indicating no differences in DNA loop size or native supercoiling. As a result of irradiation nucleoids from EA14 displayed more forward light-scatter than those from U87.

Conclusion: These results suggest the involvement of differences in the arrangement of supercoiled nuclear DNA into loop domains anchored to the nuclear matrix in the ability of these two cell lines to express radiation-induced DNA damage.

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Effects of fractionation and dose rate in PDR-brachytherapy (PDR-BT) of B14-fibroblasts

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Purpose: The aim of our study was to evaluate the impact of different pulse doses and dose rates of pulsed brachytherapy on cell survival under clinically conditions.

Methods: Hamster fibroblasts were exposed to a radiation source at a distance of 9 mm with following RT-schemes: dose per pulse: 1/2.5/5 Gy (PDR); 2.5/5 Gy (HDR); total dose: 5–30 Gy (PDR/cLDR), 5–20 Gy (HDR); dose rate: 50 cGy/h (cLDR); 100–300 cGy/h (PDR); 1500–2000 cGy/h (HDR); pulse repetition: 1 Gy/1 h or 1 Gy/2 h (PDR); 2.5 Gy/2.5 h and 5 Gy/5 h (PDR/HDR). Cell survival was measured by dye exclusion test and clonogenic survival assay.

Results: Cell survival decreased for pulse doses of 5 Gy compared to 2.5/1 Gy (PDR/HDR) or when using HDR- brachytherapy. No differences were observed with dose rates of 100–300 cGy/h with a biological equivalence of cLDR- and PDR- BT, keeping the total exposure time constant.

Conclusion: Radiobiological effects of PDR-BT are dependent on the total dose, the dose rate, the dose per pulse and the total exposure time.

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PR-350, the least toxic 2-nitroimidazole hypoxic cell radiosensitizer for clinical use

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Purpose: PR-350 is a newly developed 2-nitroimidazole nucleoside analogue radiosensitizer which is now undergoing clinical trials. The purpose of this study was to extensively evaluate its efficacy, toxicity and suitability for clinical studies.

Methods: In vitro sensitizing activity was assessed by colony and micronucleus assays in various murine and human cancer cells. In vivo effects were assessed by tumor growth delay and in vivo-in vitro assay of SCCVII tumors. The drug was combined with a single 20 Gy dose or 5 doses of 4 Gy. The 50% lethal dose in ICR mice and the concentrations in the sciatic nerve of C3H mice were determined to evaluate toxicity. Etanidazole was used for comparison.

Results: In vitro enhancement ratio of PR-350 was 1.4–1.6 at 1 mM, which was similar to that of etanidazole. In vivo, PR-350 was as efficient as etanidazole. The 50% lethal dose in mice was ≥ 5.8 g/kg. The concentration of PR-350 in the sciatic nerve was as low as that of etanidazole.