RESEARCH OBJECTIVE

This project is part of the DOE research program on the biological effects of low dose and dose rate ionizing radiation. This DOE program is designed to support and conduct science that can impact the subsequent development of health risk policy for low dose radiation exposures in the US. The overall, long-term goal of this project is to increase understanding of the responses of cells to the low doses of ionizing radiation typically encountered in environmental level exposures. To achieve this objective, we couple use of a unique focused soft X-ray facility for low dose irradiation of individual cells or irradiation of specific subcellular regions of cells with studies of the effects of reactive oxygen species (ROS) produced in cells. The project includes seven specific goals: (1) Determine the response of individual cells to low doses of ionizing radiation from a focused soft X-ray beam with a 250 nm diameter beam spot. (2) Determine the response of cells to ROS generated by chemical agents in a fashion that mimics the endogenous cellular generation of ROS. (3) Study the interaction between cellular oxidative processes and ionizing radiation. (4) Determine the importance of the subcellular distribution of ROS from focused soft X-rays on cellular response. (5) Determine whether damage deposited in individual cells by focused soft X-rays or by chemically-generated ROS can elicit a response in other, surrounding, untreated cells, a "bystander" effect. (6) Quantify the low dose response and the targets involved in the genomic instability phenotype in cells exposed to low LET radiation and the relationship with the bystander response. (7) Develop tissue explant systems for the measurement of low dose effects in multicellular systems.

RESEARCH PROGRESS AND IMPLICATIONS

This report summarizes work after 32 months of a three-year project. It has been shown that hypersensitivity to low doses of radiation occurs in a range of animal and human tumor cell lines. However, little is known about the response of primary human cells. Here, primary human fibroblasts (AGO1522) were exposed to low doses of conventional X-rays or focused soft X-rays. Our results show that at doses of 0.2 Gy and below of conventional X-rays hypersensitivity with respect to cell clonogenicity was observed. Furthermore, a similar hypersensitive response to the same doses of conventional X-rays was found when the production of micronuclei was measured. When individual cells were irradiated through the nucleus with a focused carbon-K soft X-ray microprobe, cells were more radiosensitive compared to conventional X-rays as measured by both the clonogenic survival and micronucleus formation assays at doses greater than 0.2 Gy. However, no hypersensitivity to low doses of focused soft X-rays was observed. To test whether induction of intracellular reactive oxygen species and oxidant-antioxidant balance are involved in the mechanism of hypersensitivity to conventional X-rays dimethyl sulfoxide, a hydroxyl radical scavenger, and buthionine sulfoximine, a suppressor of intracellular glutathione production were used. Dimethyl sulfoxide had no protective effect on the hypersensitive response of cells to conventional X-ray irradiation. However, pretreatment of cells with buthionine sulfoximine before irradiation had a radiosensitizing effect with respect to cell survival at all doses, and the non-linearity of the dose-effect relationship at 0.2 Gy and below was not observed.

Our studies in V79 cells have been comparing the effectiveness of focused carbon-K X-rays (278 eV) at cell killing under conditions where every cell is targeted or only a single cell has been selected. Cells are seeded 3 hours prior to irradiation on specially constructed Mylar-based dishes. Cells are located after staining with Hoechst 33258 and positions automatically recorded. Typically around 100 - 150 single cells are present on each dish (10 x 10 mm area) at the time of irradiation. For measurements of direct cell killing, each of these cells is selected and the required dose of soft X-rays delivered through the center of the nucleus. For bystander induced cell killing a single cell is selected at random near the center of the dish and irradiated. For control bystander experiments, the same soft X-ray dose is delivered to a location in the center of the dish where no cells are present. For all dishes incubation is continued for an additional 3 days. After this time cells are re-stained with Hoechst and each of the original cell locations is revisited to determine whether colony formation has occurred. In some experiments, after targeting a single cell, dishes were uniformly exposed to low doses of conventional X-rays (240 kV).

Previously we had shown in studies where a single cell only, was targeted through the center of the nucleus a significant bystander response was observed. This increased from the lowest dose studied (50 mGy) to a maximum at 200 mGy where the response saturated at a level of 10% killing at doses up to 2 Gy. The initial slope of the induction of a bystander responses in a single cell was only slightly less than the initial slope from the situation where every cell had been targeted, illustrating the potential of the bystander effect to amplify low-dose responses. These studies suggest that every cell within a population has the ability to induce a bystander response in its neighbors and that at low doses, bystander responses may dominate the overall effect. We have also compared the effect of targeting cells with a highly focused beam to the situation where the beam is defocused to 10μm in size and the whole nucleus is irradiated. Under conditions where every cell is targeted, little difference is observed between the two situations suggesting that the response of the nucleus is relatively uniform under these conditions. This year we have extended these studies to consider the effect of irradiating more than one cell in every population. When 1, 2, 5 or all cells within the population were targeted and cell survival plotted versus dose to the targeted cell, no significant differences were observed between the situations where only 1, 2 or 5 cells were irradiated with a similar level of bystander response observed. Plotting this data versus total number of photons delivered to each dish gives a different picture. The most effective situation is where only a single cell within the dish is irradiated with the least effective when every cell is irradiated. This suggests that it is dose to the targeted cell which is the critical parameter rather than the number of cells targeted under these conditions. We have also considered the role of cell cycle phase on the degree of bystander killing observed. Using our image analysis system on the microbeam, it is possible to determine the cell cycle position of cells at the time of irradiation, even when these are asynchronous cultures using analysis of cell size and fluorescence staining from the Hoechst signal. Pilot studies have indicated that G2 cells are at increased risk of bystander killing when compared with G1 cells (see figure 1). This may have implications for likely mechanisms of action and further studies are underway to address this.

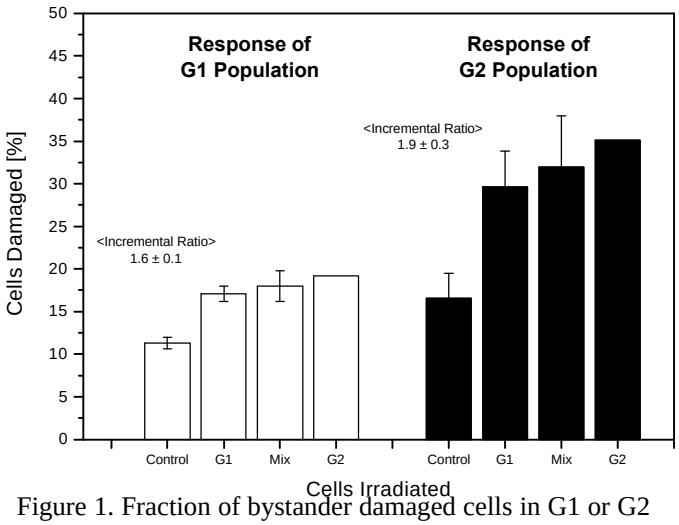

Figure 1. Fraction of bystander damaged cells in G1 or G2 of the cell cycle responding when either a single G1 or G2 cell is irradiated or multiple cells (mix).

Pilot studies have also been performed using a porcine ureter tissue model to start to address the role of cell-cell interactions in the *in vivo* situation. To date studies have only been performed using charged particles. However it is clear that bystander effects are readily observed in this model and may be more frequent than those observed in isolated cell culture systems.

INFORMATION ACCESS

Publications

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