

## 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 20 months of a three-year project. Progress has been made on goals 1, 2, 3, 4 and 5 from the list in the previous section. 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).

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

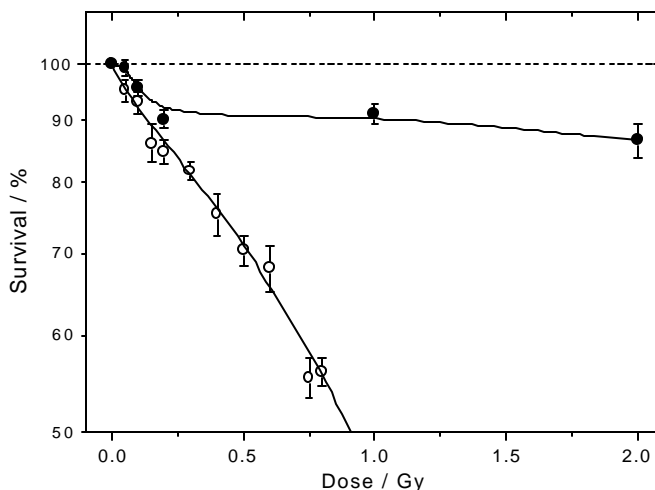


Figure 1. Survival of V79 cells after exposure to focused carbon-K X-rays (O) where every cell was targeted through the nucleus or only one cell was targeted (●).

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 10 $\mu$ m 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.

With regards to aims 2 and 3, in one set of studies, we have used HL-60 human promyelocytic leukemia cells to compare the apoptotic pathways induced by four different treatments: bolus H<sub>2</sub>O<sub>2</sub>, an oxidizing thiol compound dithiothreitol (DTT) which we have shown acts as a “slow release” source of H<sub>2</sub>O<sub>2</sub> in cells (Tartier *et al.*, 2000), high dose IR and low dose IR. Addition of H<sub>2</sub>O<sub>2</sub> to cell medium causes rapid apoptosis, evident by DNA fragmentation or appearance of a sub-G1 fraction in flow cytometry of propidium iodide-stained cells, starting within 1-2 h, depending on the concentration of H<sub>2</sub>O<sub>2</sub>. The H<sub>2</sub>O<sub>2</sub>-induced apoptosis occurs in a fashion that depends on both mitochondria and caspase 3 activation. In contrast, the “slow release ROS” agent, DTT, leads to apoptosis on a somewhat slower scale and in a fashion that requires caspase 3 activation as a relatively early event, but is independent of mitochondrial involvement and caspase 8 or 9. Different patterns still are seen by low (e.g., 3 Gy) and high (20 Gy) dose IR, with the most striking differences being the apparent lack of caspase 3 involvement in the relatively rapid apoptosis caused by high dose IR and the very long time to apoptosis after the low dose IR. The data are summarized in the table, and clearly show that not all ROS are created equal.

	ROS generation	Time to maximal apoptosis	Mitochondrial involvement	Caspase 3 activation
H <sub>2</sub> O <sub>2</sub>	<5-30 min	1-4 h	Yes	0.5-2 h
DTT	5-60 min	5 h	No	2-4 h
High dose IR	Immediate	4-5 h	2-4 h	None
Low dose IR	Immediate	72-96 h	48-72 h	48-72 h

In studies where apoptosis is not a major pathway for expression of cell damage, primary human fibroblasts (AGO1522) were used to assess the responses of cells to low doses of IR using clonogenicity and micronuclei formation as endpoints. Both conventional X-rays and the focused soft X-ray microprobe were used. With conventional X-rays, hypersensitivity was seen at  $\leq 0.2$  Gy using both clonogenicity and micronuclei formation as end points. When low doses of focused X-rays were targeted to the nucleus, the cell sensitivity (clonogenicity) was greater than that seen using conventional X-rays. The low dose hypersensitivity to conventional X-rays was increased by pre-treating cells with the thiol depletor buthionine sulfoximine (BSO). These data suggest that ROS are involved in the increased response of cells to low dose X-rays.

#### PLANNED ACTIVITIES

Remainder of Year 2 Complete goals 1-3 and continue with goals 4 and 5  
Year 3 Complete goals 4 and 5  
Work on goals 6 and 7, which were designed to be largely exploratory

#### INFORMATION ACCESS

##### Publications

Tartier L, McCarey YL, Biaglow JE, Kochevar IE, Held KD. Apoptosis induced by dithiothreitol in HL-60 cells shows early activation of caspase 3 and is independent of mitochondria. Cell Death and Differentiation 7:1002-1010 (2000).