Low-Dose Studies with Focused X-rays in Cell and Tissue Models: Mechanisms of Bystander and Genomic Instability Responses

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Research Objectives

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 100 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 five months of a three year project. Progress has been made on goals 1and 2 from the list in the previous section. For goal 1 we have targeted individual V79 cells through the center of the nucleus with focused soft X-rays such that cells were exposed to a beam with a focal spot of ~100 nm. The survival of these cells is similar to that observed with conventional soft X-ray exposures. Interestingly, our initial studies suggest that the degree of radiation-induced low dose-hypersensitivity is less than that predicted from conventional low LET exposures or studies using targeted low LET protons. We are currently determining if this is due to the distribution of radiation dose within individual cells by changing the focal spot size of the beam. Preliminary studies are also being performed with the RKO cells being utilized in aim 2 to measure both clonogenic survival and micronuclei formation. With regards to aim 2, we have been determining the pathways for induction of apoptosis in HL-60 cells exposed to oxidative stress from hydrogen peroxide and oxidizing thiols, especially dithiothreitol (DTT). Although there are some similarities in the pathways used by the two agents, e.g., apoptosis and all cell killing from either agent is blocked by overexpression of BCL-2, there are significant differences also. Hydrogen peroxide utilizes the mitochondrial/ caspase 9 pathway to activate the effector caspase 3, whereas DTT does not cause any mitochondrial changes and activates caspase 3 before any other caspases. Preliminary studies with RKO cells have shown these cells to be much less susceptible to oxidative stress-induced apoptosis than the HL-60

cells. We have also used 2'7'-dichlorofluorescine diacetate (DCFH-DA) as an intracellular probe for ROS to show that intracellular ROS can be detected in cells treated with either hydrogen peroxide or DTT.

Planned Activities

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

Information Access

None