Using a Low-LET Electron Microbeam to Investigate Non-Targeted Effects of Low Dose Radiation.

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We have developed a low-LET electron microbeam that generates energetic electrons to mimic radiation damage from gamma and x-ray sources. It has been designed such that high-energy electrons deposit energy in a pre-selected subset of cells leaving neighboring cells unirradiated. In this way it is possible to examine non-targeted effects associated with low dose radiation exposure including induced genomic instability and bystander effects.

Using the microbeam in a broad beam configuration, we have measured cell survival for the gap junction null RKO36 human colon carcinoma cell line following exposure to 50 keV electrons (Figure 1). Comparing this data to the dose response for x-rays, we found that more cells survive exposure to 50 keV electrons relative to the same dose of x-rays.

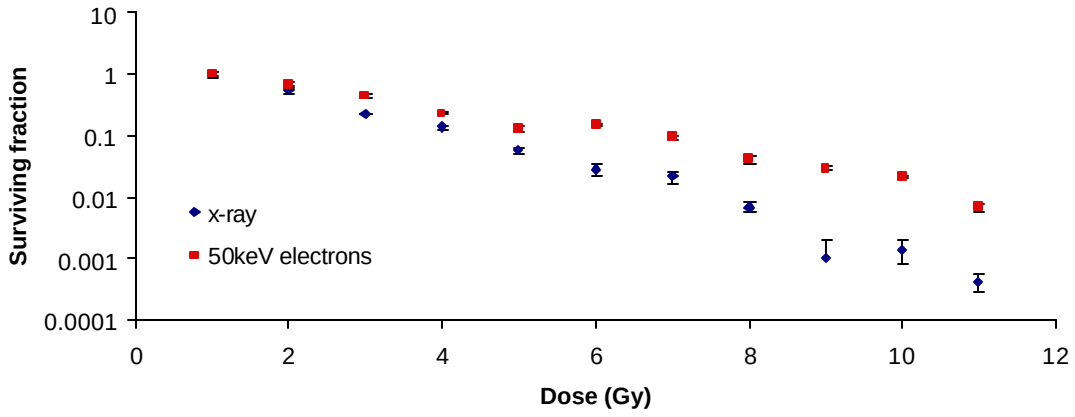

Figure 1. X-ray and 50 keV electron survival curves for RKO36 cells

We will also present data on bystander effect experiments using x-rays and electrons. In the electron microbeam bystander studies, 100, 10, or 1% of each cell population was irradiated with a lethal dose of 50 Gy. Following irradiation,

cells were left in the irradiation medium for various times to allow diffusion of any soluble bystander factors. Cells were then trypsinized, re-plated and scored for clonogenic survival (Figure 2). Since the directly irradiated cells were lethally irradiated, all cells scored were bystanders or their progeny. Samples were monitored for both direct effects of being bystanders in a radiation environment and indirect effects where medium transfer recipient cells were never exposed to a radiation environment. Based on evaluation of cell survival in these direct irradiation or medium transfer experiments, we observe no evidence of a low-LET irradiation induced bystander effect in the gap junction null RKO36 cell line.

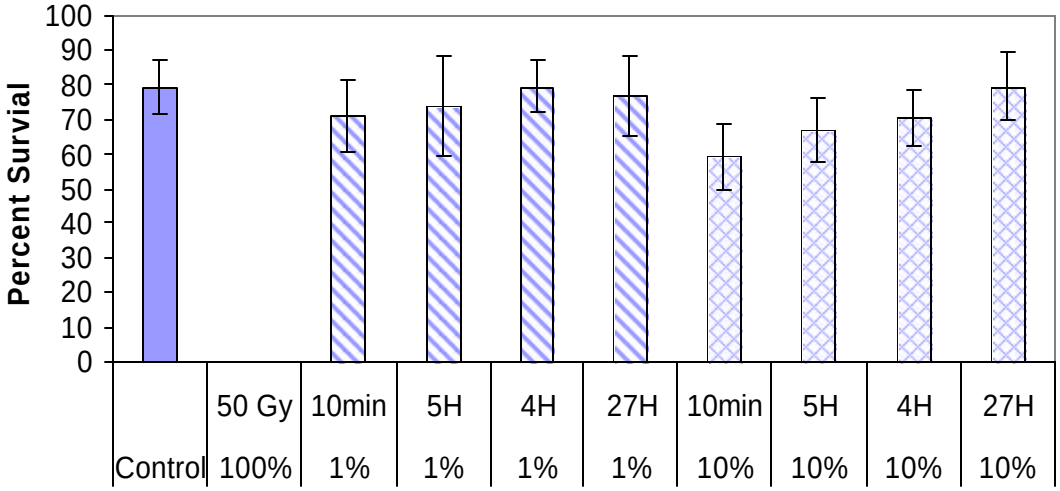

Figure 2. Percent survival versus percent of cells directly exposed to electron radiation (50 Gy, 50keV). Medium is left on cells for the times indicated before subculture.

The same samples were also scored for induced genomic instability using the GFP reporter assay previously developed. This assay provides a robust, reliable, highly sensitive assay for detecting delayed hyper-recombination/deletion events occurring in cells exposed to low doses of ionizing radiation.

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