Microbeam Studies of Low Dose X-ray Bystander Effects On Epithelial Cells and Fibroblasts Using Synchrotron Radiation

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ABSTRACT

Evidence is emerging that radiation exposure can change communication between cells of the same type, as well as between cells of different cell compartments within tissues. We are using the X-ray Microprobe Beamline (10.3.1) at the Advanced Light Source (ALS) to investigate bystander effects of low doses in well-characterized human mammary epithelial cells (HMEC) and human skin fibroblasts (HSF). The discovery of "bystander" effects on unirradiated cells from radiation-exposed neighbors has confounded and challenged radiation researchers. It has been difficult to understand how unirradiated cells could be affected. The ALS facility is capable of producing a beam of 12.5 keV X-rays with a focused spot size of 2 μm^2 and a wide range of doses and dose-rates. Unlike normal X-ray sources, this beam has a very low background of both low and high-energy X-rays. In initial studies, cultures grown in microwell slide chambers have been irradiated with precise stripes of dose up to 100 μm wide. To evaluate the spatial dependence of intercellular communications, we varied the distance between dose stripes from 100 μm and 900 μm. We are using computer-controlled quantitative fluorescence microscopy to evaluate several classes of radiation-induced soluble signals, how these signals are communicated across cell compartments, and how radiation changes cell signaling both acutely and chronically. In particular, we measure the radiation induction of $p21^{Cip1}$ (CDKN1a), and phosphorylation of H2AX and p53 serine-15. Our preliminary results indicate that there is a dose- and cell-type-dependent expression of p53 serine-15P within 10 minutes after exposure in a 100μm wide stripe of dose. Immunohistochemistry of p53-serine-15P-positive cells traversed by the beam illuminates the path of the X-ray microbeam, with epithelial cells responding more rapidly and with greater intensity than fibroblasts. The number of p53-serine-15P-positive cells in the unirradiated cell populations between two dose stripes has been counted as a measure of the bystander effect, and compared to appropriate controls. In the dose stripe the intensity of the immunofluorescence scales with the dose. Cellular responses to doses of 400 cGy down to 2 cGy were examined in a time course from 10 min to 12 hours after exposure.

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