Micro-Irradiation Techniques Using Synchrotron-Produced Ultrasoft X-Rays: Varying the Intracellular Dose Deposition to Study Radiobiological Mechanisms

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X-rays of energy less than 5 keV, often called ultrasoft x-rays, are important tools in radiation biology. They deposit their energy via photoelectric interactions in well-defined volumes of nanometer dimensions; thus they make excellent probes of subcellular biological targets.

Ultrasoft x-rays undergo severe attenuation even in biological material, and previous work with these low-energy photons raised some interesting questions about how intracellular dose deposition patterns may effect the biological response. This work directly investigates this question using monochromatic synchrotron-produced ultrasoft x-rays with the following two novel techniques: 1) irradiation with the isoattenuating energies 0.273 keV and 0.860 keV, which allows the study of the biological effects of different photon energies under equivalent intracellular dose deposition conditions, and 2) irradiation of cells to equivalent total energies imparted either uniformly or in partial volumes by 1.34 keV photons, which allows us to study the biological effects of concentrating an equivalent mean cellular dose into subcellular volumes. The latter technique is made possible with micro-fabricated irradiation masks consisting of a series of opaque gold stripes, 1.85 micrometers wide separated by 1.35 micrometer spaces, plated on a thin membrane.

Our results show that, under isoattenuation conditions, decreasing the x-ray energy does not effect radiation-induced cell death, contrary to the previously held notion that the biological effectiveness increases with decreasing x-ray energy. Furthermore, the partial volume irradiation experiments suggest that the mean dose to the nucleus is the critical parameter to consider in cell killing, and therefore that the induction of lethal lesions is linear with local energy imparted. In addition, we investigate the endpoints of radiation-induced DNA single- and double-strand breakage under partial volume irradiation; both these lesions show a linear response with local energy imparted. DNA damage is measured by the comet assay using the first University of Wisconsin comet analysis system, the design and implementation of which are also presented in this thesis.