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Adaptation of Endothelium to Microlesions Created by X-ray Microbeam Irradiation

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Beamline: X17B1

Introduction: Microbeam radiation therapy (MRT) is an experimental therapy that delivers an array of parallel, microscopically thin (<100 μm) planar slices of synchrotron-generated x-ray beams [1-4]. While effectively killing experimental tumors in rodent models [2, 4], it also exhibits an unexpectedly large tissue-sparing effect in normal tissue [1-4]. It has been conjectured that the sparing is caused, at least in part, by undamaged capillary endothelial cells that reside in the areas not directly hit by individual microbeams. Cells that are hit by microbeams can be lethally irradiated, but the unirradiated survivors may contribute to the repair of microlesions created by several mechanisms -- such as hypertrophy, spreading, migration, and cell proliferation. This hypothesis has been difficult to substantiate in intact animal tissues; therefore, studies were initiated in an endothelial cell culture model.

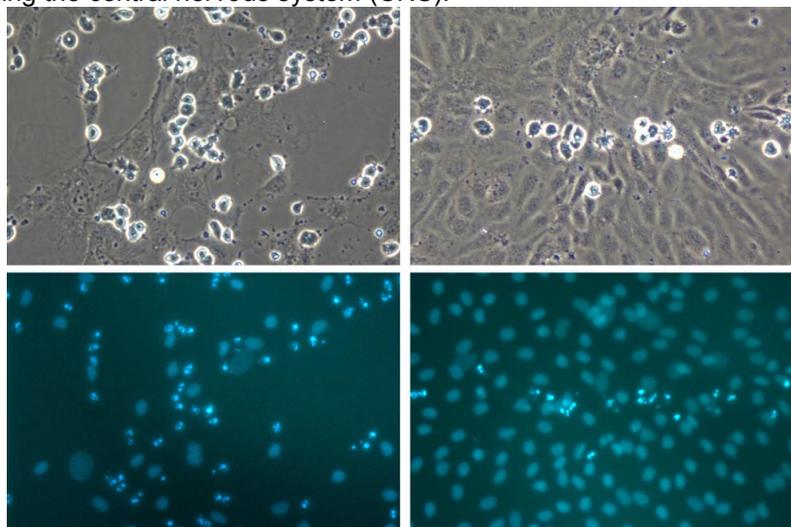
Methods and Materials: Bovine aortic endothelial cells were cultured in EGM2-MV medium (Cambrex BioWhittaker; Walkersville, MD) as a confluent monolayer on plastic microscope slides configured as 8-well Chamber Slides (Nalge Nunc; Naperville, IL). The chamber slides then were positioned vertically in front of the beam at the X17B1 beamline, and were irradiated with three parallel microplanar beams entering from their underside. The microbeams were 27 μm wide and 2 mm, positioned at 2 mm center-to-center with a ~24 Gy absorbed dose. The ring operated at 2.584 GeV, and the X17 wiggler operated at 4.7tesla. The beam was filtered with 3 mm Si and 25.4 mm Cu, providing a median spectral energy of about 160 keV, and an incident dose-rate in tissue of 0.8 Gy/s at 200 mA ring current. Therefore, the irradiation time for each microplanar beam was about 30 sec. Six hours after irradiation, cultures were fixed with paraformaldehyde, stained with bis-benzamide, and examined by phase-contrast and fluorescent microscopy for the extent of radiation-induced apoptosis (programmed cell death).

Results: The figure below shows microbeam-irradiated cultures (right panels) side-by-side with similar samples irradiated with a conventional broad-beam (left panels) at 24 Gy dose. Cells undergoing apoptotic death are evident in the microbeam-irradiated sample under phase-contrast illumination as rounded, bright cells (upper panels). In the same microscope fields under UV illumination, condensed and fragmented bis-benzamide-stained nuclei indicate apoptotic cell death (lower panels). Evidence of the path of a single horizontal microbeam is clearly visible as a straight row of apoptotic cells against an otherwise undisturbed monolayer of cells (right panels).

Conclusions: The results demonstrate that the endothelial cells neighboring the lethally injured cells rapidly occupy the space formerly held by the dying irradiated cells within 6 hours by a simple process of hypertrophy and cytoplasmic streaming. In other experiments this effect has been observed in as little as 3 hours. In the time frame and the conditions of these experiments, it is far too soon for cell proliferation to be a contributing mechanism. The same process is likely to occur in normal tissue *in vivo*, and thus may be the basis for the high tolerance of normal tissues to MRT, including the central nervous system (CNS).

References:

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