Targeted radiation studies in tissue explants

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The overall goal of this project is characterization of 3D tissue models that can be used for investigation of the mechanisms underlying radiation-induced bystander effects at low doses of low LET ionizing radiation. We are using a unique focused soft X-ray microprobe that provides a range of focused soft X-ray energies, some sufficient to penetrate 3D models. Our initial studies have involved further development of a porcine ureter model which allows the response of urothelial tissue to be followed after localized exposure.

Porcine ureters are isolated and sectioned into ~5x5x5 mm samples and then placed on specially designed microbeam dishes consisting of 0.9 μm thick Mylar film with the lumen cell layer in contact with the film in a small volume of medium. The samples were irradiated using the Gray Cancer Institute ultrasoft X-ray microprobe using either 278 eV carbon-K or 1.5 keV aluminium-K soft X-rays. The spot size of both of these beams are around 500 nm, although the attenuation of the carbon-K X-rays is such that only 0.2% of the dose extends beyond the first cell layer whereas for the aluminium-K ~10% extend beyond the first cell layer. The samples were either irradiated at a single location or at a series of 25 locations over a 5 x 5 μm array. Following irradiation the tissue fragment is incubated in defined medium to allow a urothelial explant to form. After 7 - 14 days, the explants are typically a few millimetres in diameter and then samples are fixed and stained for the presence of micronucleated and apoptotic cells. In a series of studies we have monitored the increase in damaged cells in response to a single location in the tissue being irradiated with doses ranging from 1 – 200cGy. A significant bystander response was observed for both carbon-K and aluminium-K which was similar to our previous reported data using charged particles (Belyakov *et al.*, 2002), although some dose dependency is observed.

The role of the adaptive response under these conditions is also important. In pilot studies, tissue fragments were pre-irradiated with 10cGy of 240 kVp X-rays prior to microbeam irradiation. The level of bystander response was reduced under these conditions suggesting significant protection. Interestingly, studies in cell models also show similar responses when bystander and adaptive treatments are combined. Baseline studies have also been carried out isolated cells to monitor the response and mechanisms underpinning bystander responses. Locations outside the nucleus in individual cells have been targeted and early markers of DNA damage signalling responses quantified under conditions where every cell or only a few cells were exposed. What is emerging is a commonality between the response of cells to non-nuclear irradiation and bystander responses.

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