UNDERSTANDING RADIATION DAMAGE TO CELLS USING MICROBEAMS

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Cellular micro-irradiation using ion beams is now recognised as a highly versatile technique for understanding how ionising radiation interacts with living cells and tissues. The strength of the micro-irradiation technique lies in its ability to deliver precise doses of radiation to selected individual cells (or sub-cellular targets) *in vitro*. The application of this technique in the field of radiation biology continues to be of great interest for investigating a number of phenomena currently of concern to the radiobiological community. In particular, it is the study of so-called 'non-targeted' effects that are benefiting most from the use of microbeam approaches. Non-targeted effects are those where cells are seen to respond *indirectly* to ionizing radiation and are in conflict with the conventional view of cellular radiation damage, which assumes that that *direct* damage to the DNA helix is necessary to induce critical effects (either through direct ionization of the DNA, or through the action of reactive radical species from the ionization of water close the DNA molecule). One important non-targeted effect is the so-called 'bystander-effect' where it is observed that unirradiated cells can also respond to signals transmitted by irradiated neighbours. Clearly, the ability of a microbeam to irradiate just a single cell or selected cells within a population is well suited to studying this phenomenon. The conventional representation of radiation damage (i.e. one that does not consider non-targeted effects) is consistent with the 'linear no-threshold' model used to estimate the risk associated with exposure to occupational and environmental levels of radiation. This model is based on a linear extrapolation of known risks at higher doses to the low-dose region where reliable data from epidemiology or experiments are not currently available. However, if non-targeted effects are considered, then their effects will predominate at low doses and our confidence in the linear no-threshold is dramatically undermined. Non-targeted effects such as the bystander effect are also of potential relevance to the advancement of the treatment of cancer by radiotherapy. For example, it raises the possibility of increasing the therapeutic benefit by selectively modifying the response of either the tumour, or the healthy tissue to radiation by chemical action directed at the signalling molecules involved in the bystander effect.

The Gray Cancer Institute has developed several microbeams, using either charged-particles or focused low-energy X-rays. Low-energy X-rays are the source of choice for achieving the finest probes. This is because they interact almost entirely through the photoelectric effect and are therefore are not scattered. However, environmental exposure to particles (i.e. through exposure to radon) is important and therefore particles must also be studied. Our charged-particle microbeam has a targeting accuracy of ±2μm, achieved by using a 1μm diameter bore glass capillary to collimate protons, or $^{3}He^{2+}$ ions accelerated by a 4MV Van de Graaff. Automated systems enable up to 9000 cells per hour to be irradiated. Using our microbeam facilities, we have undertaken a number of successful studies that have identified and quantified the bystander effect in cells and tissues and demonstrated that cell survival, apoptosis, chromosome damage, genomic instability and adaptive responses can all be influenced by bystander effects. We have also shown that direct damage of the DNA is not a necessary requirement for inducing the bystander response and the nitric oxide has an important role in mediating the bystander effect.