

Low Dose Radiation Exposure: Exploring Bystander Effects *In Vivo*.

¹Blyth, B.J., ¹Sykes, P.J.

¹Department of Haematology and Genetic Pathology, Flinders University and Medical Centre, Bedford Park, South Australia, 5042,

The general population is daily exposed to chronic, low doses of ionizing radiation from both natural and artificial sources. The shape of the radiation dose-response curve at these low doses is currently linearly extrapolated from data obtained after high dose exposure due to the low sensitivity of traditional biological assays after near-background exposures. At odds with this Linear No-Threshold model, are the phenomena collectively referred to as the radiation-induced bystander effect.

The bystander effect describes a collection of *in vitro* observations that suggest the presence of a soluble, transmissible factor(s) released from irradiated cells that can induce a biological response in un-irradiated cells. The induction, nature and magnitude of the bystander effects vary between cell culture systems, radiation sources and end-points measured. The contribution of bystander effects *in vivo* may significantly alter biological responses to low dose radiation exposure and could result in a cancer-protective effect by eliminating genetically compromised cells or low dose hypersensitivity.

Validation of bystander effects *in vivo* has been mired by the difficulty of selectively irradiating cells within an animal model. Using the pKZ1 *in vivo* mouse inversion assay, this research is aimed at studying bystander effects using an adoptive transfer of syngeneic splenic T cells receiving chronic low radiation doses from the internal β -particle emitter tritium (^3H) incorporated into the donor cell DNA as tritiated thymidine. The donor cells will be transplanted intravenously into recipient pKZ1 mice, where a proportion of the cells will lodge in the recipient animal's spleen and can be tracked with a intracellular-bound fluorescent dye. Using multi-colour fluorescence microscopy, the location of transplanted cells can be viewed simultaneously along with a number of biological endpoints.

By examining the modulation of chromosomal inversions (by identifying expression of the pKZ1 transgene), apoptosis and proliferation as well as candidate signalling and effector molecules (including Transforming Growth Factor- β , Interleukin-8 & nitric oxide), the presence of bystander effects *in vivo* can be evaluated. The effect of sparse, low dose-rate irradiation in the spleen can be examined by comparing mice receiving chronically irradiated cells with those receiving unirradiated control cells. Using point-pattern analysis, the spatial distribution of biological events (apoptosis, proliferation, chromosomal inversions) in relation to irradiated cells can be examined to identify clustering and localised effects. This *in vivo* model for studying bystander effects can be expanded to conduct a variety of fluorescence-based biological assays.

If a bystander effect is indeed induced *in vivo* after chronic exposure to low dose radiation, this would challenge the assumed linearity of low radiation dose effects and suggest a possible mechanism for previously observed hormetic and hypersensitive low dose responses.

This work is funded by the Low Dose Radiation Research Program, Biological and Environmental Research (BER), U.S. Department of Energy Grant No. DE-FG02-05ER64104.