

The Bystander Effect in Normal Human 3-D Tissue: Experiments, Models, and Implications

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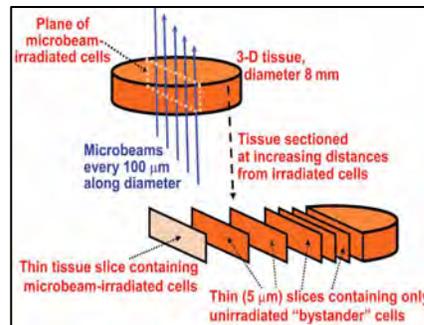
Background

The radiation-induced bystander effect, wherein non-hit cells can be damaged as a result of signals sent by neighboring hit cells, has the potential to significantly alter our understanding of the hazards of very low doses of ionizing radiation. The reason for this is that it casts serious doubt on essentially all of the various methodologies that have been proposed for extrapolating radiation risks from epidemiologically-tractable doses down to very low doses. Most of the early work on the bystander effect, starting from the pioneering work of Jack Little, was done with experimental systems based on independent cells, either confluent or well-separated. However, given that the bystander effect is clearly a cell-to-cell damage-signalling phenomenon, it is important that we use normal human 3-D tissue models, if our conclusions are to be pertinent to human low-dose risk estimation

Methods

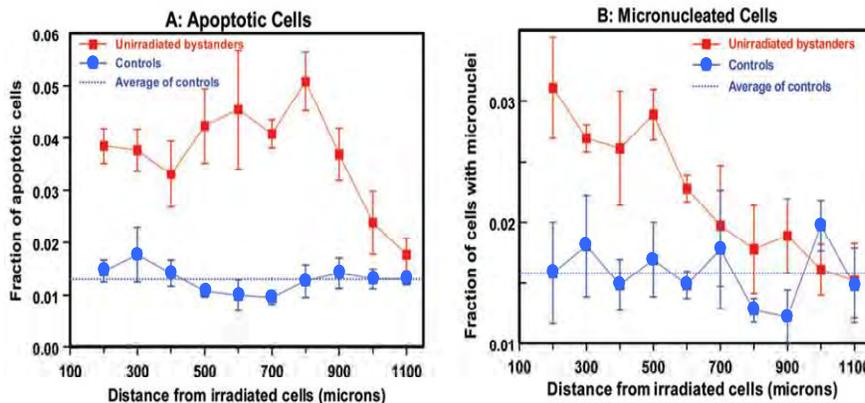
We have studied bystander responses in two types of reconstructed, normal human three-dimensional skin tissue; these systems are generated by growing differentiated keratinocyte cultures on a cellular or fibroblast-populated dermal substrates. One of the systems reconstructs the human epidermis, and the other is a "full-thickness" skin model corresponding to the epidermis and dermis of normal human skin. The model of the human epidermis consists of normal human epidermal keratinocytes that have been cultured to form a multilayered, differentiated epidermal model. It closely resembles normal human epidermal microarchitecture, with *in-vivo*-like morphological and growth characteristics that are uniform and highly reproducible. The model that we have typically used contains 8-12 cell layers and is ~75- μm thick, samples being 8 mm in diameter.

We use the Columbia University microbeam to irradiate a thin (microns) slice of cells along the diameter of the tissue, while guaranteeing that cells outside this thin slice are not directly irradiated. We use both low-LET protons and high-LET alpha particles in our experiments. After irradiation, the 3-D tissue is sectioned (with or without fixation, depending on the endpoint), and various endpoints in bystander cells are then assayed as a function of distance from the irradiated cells, time post irradiation, and LET.

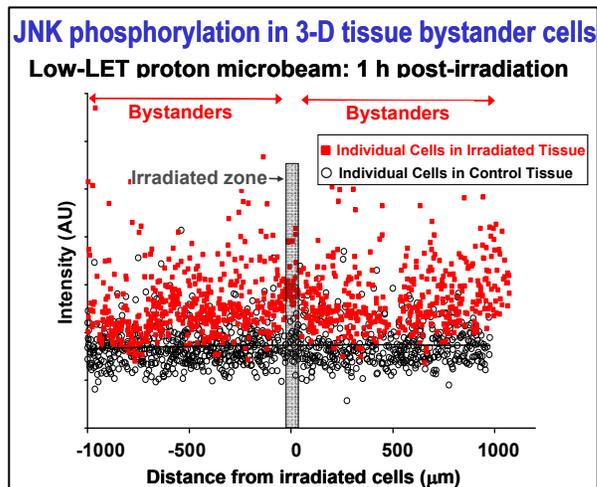


Results with 3-D Tissue Models

- 1) Our initial work used the endpoints of apoptosis and micronucleus induction, our logic being to investigate one endpoint that is potentially protective and one that is a surrogate for a potentially carcinogenic event. The results shown here (1), indicate that unirradiated cells up to 1 mm (~50 cell diameters) distant from irradiated cells showed a significant enhancement in effect over background, with an average increase in effect over background of about 1.7-fold for micronuclei and 2.8-fold for apoptosis.

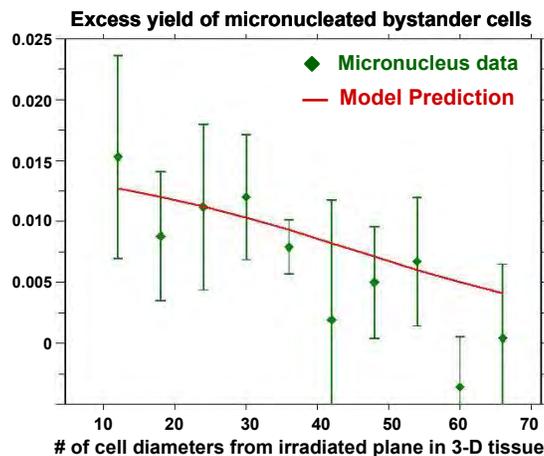


2) There is considerable evidence that MAP kinase pathways are involved in bystander responses (2), so we are systematically probing key components of these pathways using single-cell immunohistochemistry on the normal human 3-D tissue bystander samples described above – again as a function of distance from the irradiated cells, time post irradiation, and LET. As an example, shown here are measurements in human 3-D tissue of phosphorylated JNK in bystander cells which are near to cells irradiated with low-LET protons, 1 hour post irradiation. Again a range of at least 1 mm for bystander-effect signals is observed.



Modeling Bystander Spatial Effects

Although we do not know the identity of all the molecular players in the bystander effect, enough basic principles are apparent to be able to model bystander spatial patterns – which are key to understanding the significance of bystander effects at low doses. In this first approach to mechanistic modeling bystander spatial effects, we assume that bystander responses result from signaling molecules (S) that propagate from irradiated cells to neighboring cells, and can induce reactive molecules (R), which in turn can damage DNA or change the internal signaling state of the cell (e.g. induce proliferation, differentiation or apoptosis). We assume that cell-to-cell propagation of S is rapid, and that the concentration of S decreases exponentially with distance, as in ordinary diffusion or in many juxtacrine models. The R level in a bystander cell is assumed to be a function of the S level in that cell and, based on much experimental data, the shape of the R vs. S function is assumed to be non linear, with saturation. Finally, the endpoint of interest is assumed to be proportional to the R level in bystander cells. This scenario is an oversimplification, but lends itself to subsequent detailed modeling of DNA damage repair/misrepair and cell cycle effects. Our assumptions result in a minimally parameterized model, most of whose parameters are taken from the literature. A typical result, modeling our 3-D tissue data, is shown here, analyzing effects occurring up to 70 cell diameters away from the irradiated region.



Implications

The long range of bystander signals in human 3-D tissue suggests that bystander responses may well be important in extrapolating radiation risk estimates from epidemiologically-accessible doses down to very low doses where non-hit bystander cells will predominate. This conclusion could have major implications for the future of nuclear power, and for the multi-trillion dollar cost of cleaning up nuclear weapons facilities. 3-D tissue studies hold out great promise for elucidating the relevant pathways and providing the data needed to model bystander-related human risks at very low doses.

1. O. V. Belyakov, S. A. Mitchell, D. Parikh, G. Randers-Pehrson, S. Marino, S. A. Amundson, C. R. Geard, D. J. Brenner, *Biological effects in unirradiated human tissue induced by radiation damage up to 1 mm away*. Proc. Natl. Acad. Sci. U.S.A. 102, 14203-8 (2005).
2. H. Zhou, Ivanov VN, Gillespie J, Geard CR, Amundson SA, Brenner DJ, Yu Z, Lieberman HB, Hei TK. *Mechanism of radiation-induced bystander effect: role of the cyclooxygenase-2 signaling pathway*. Proc Natl Acad Sci USA. 102, 14641-6 (2005)