Using Three Dimensional Cell Culture and Tissue Architecture to Monitor an Adaptive Response in Mammary Epithelial Cells

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Exposure of tissues to ionizing radiation results in targeted effect on cells as well as non-targeted effects on tissues. Although, targeted effects such as the DNA damage response have been studied extensively, non-targeted effects leading to modification in tissue architecture and tumor progression have been less studied and are not well understood. The mammary gland is a tissue that has been shown to be susceptible to tumor formation and cancer progression following exposure to ionizing radiation. In conjunction with the laboratories of Mary Helen Barcellos-Hoff and Catherine Park we showed previously that in the presence of TGF-β, irradiation of non-malignant human breast cells in a three-dimensional (3D) cell culture assay leads to altered tissue architecture and disruption of cell-cell and cell-matrix interactions (Park, et al., 2003, *PNAS 100:10728-33*). We are continuing to explore the effects of low dose irradiation on tissue organization using mouse mammary epithelial cells grown in the 3D culture assay. Like the human cells, the mouse cells form organized, polarized acinar-like structures that recapitulate structures observed in vivo. Furthermore, when the mouse cells become polarized and are provided hormonal stimulation they can be induced to produce milk proteins, such as beta casein, that are secreted into the hollow lumen of the acinar structures. Thus, this 3D cell culture assay provides a powerful functional cell culture system in which to model the effects of low doses of ionizing radiation on tissues. Moreover, observations from the mouse cell culture model can be extended to mammary organoid 3D culture models and in vivo models using mouse strains with different genetic backgrounds to explore genetic components responsible for alteration in tissues following radiation exposure.

Our studies have shown that EpH4 cells, a p53 wild type mouse mammary epithelial cell line, exposed to a single dose of 5Gy form colonies with improper apical-basal polarity and impaired lumen formation. However, providing a small priming dose of as little as 5 or 10 cGy prior to the 5Gy treatment permits the cells to form acinar-like structures with proper polarity and a hollow lumen similar to sham-treated colonies. These observations suggest that the cells are undergoing an adaptive response. Interestingly, the same conditions that yielded the adaptive response in the 3D culture system were not sufficient to produce an adaptive response when the cells were assayed on a two-dimensional (2D) substratum. Both proliferation rates and colony forming ability were unaffected by providing the priming dose in the 2D cultures. These observations suggest that the adaptive response observed in these cells is highly dependent on culture conditions and context. We are currently exploring mechanisms responsible for the adaptive response observed in the 3D cultures by examining soluble factors in the conditioned medium as well as key intracellular factors such as NF-kB. Furthermore, we are expanding the characterization of the adaptive response observed in the 3D culture assay to the analysis of mammary tissue harvested from in vivo experiments. Coupling the results and observations from cell culture model systems with in vivo models will allow extensive evaluation of the response of the mammary gland to low doses of ionizing radiation.