Morphological changes in a 3D Mammary Model Following Exposure to Low Dose, Low-LET Radiation

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Human tissue models provide the opportunity to elucidate fundamental cellular mechanisms occurring following exposure to low levels of ionizing radiation. However to be truly useful the model must be capable of recapitulating the fundamental tissue architecture observed *in vivo*. One such model system that fulfills this requirement is the 3D mammary tissue model we have developed through our current DOE funding. This model uses 184A1 human mammary epithelial cells grown on a mixture of extracellular matrix material (Matrigel). In Figure 1, structures have been fixed and labeled with a fluorescent antibody for a_6 integrin and demonstrate polarity and a branching morphology similar to what is observed in situ.

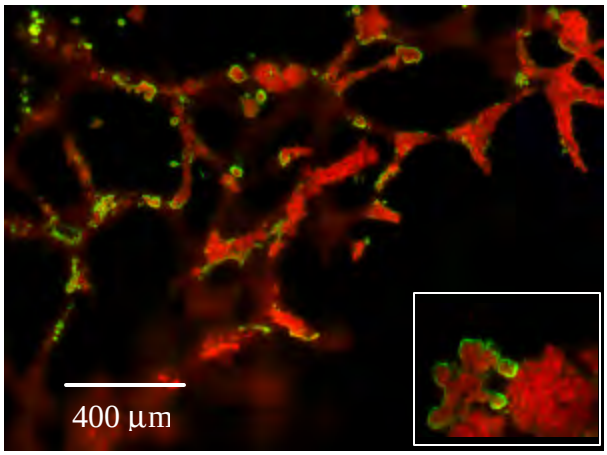

Figure 1: Confocal 10 x 10 montage of 2-day old mammary structure showing polarization. 184A1 cells were co-cultured with HB2 cells 50:50. Green = a_6 integrin, Red = Syto64. Inset: enlargement of individual frame emphasizing polarization of structure (60X).

Following exposure to low doses of low-LET radiation, we find reproducible morphological changes occurring in irradiated structures at delayed times after irradiation. Figure 2 presents some of the morphological changes observed at different radiation doses in 184A1 mammary epithelial structures seven days post irradiation. Three distinct morphological changes are observed 1) a loss of "end-bud" number and complexity with increasing dose, 2) an increase in "stalk-like" undifferentiated out growths, and 3) an overall loss of branching morphology. Since

the ECM is a complex mixture of support molecules and growth factors, extensive controls were performed to ensure that the observed effects are not due to irradiating the matrix. No morphological changes were observed following irradiation of the Matrigel without cells. The morphological dose dependence is biphasic with the above characteristics appearing most dramatically at 10 and 500 cGy but not appearing at 100 cGy.

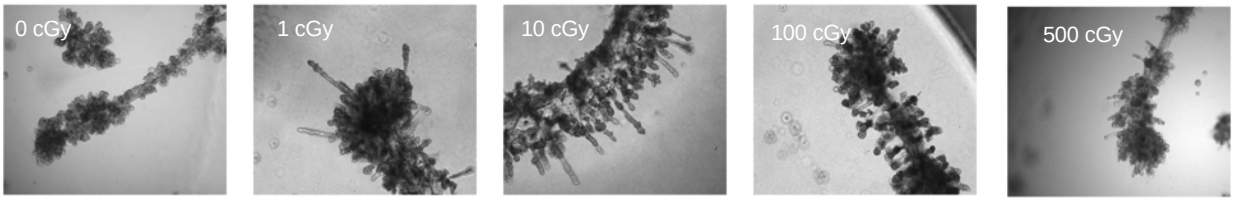

Figure 2: Morphological changes occurring in irradiated A1 mammary epithelial structures 7 days post irradiation. We see a loss of "end-bud" number and complexity, an increase in "stalk-like" undifferentiated out growths, and an overall loss of branching morphology (not shown). 4x magnification. Each structure is comprised of approximately 200,000 – 300,000 cells

We have recently examined the survival of the mammary epithelial cell line 184A1 following exposure to X-ray radiation in both 2D and 3D monocultures. We have found that cell survival is increased ~2 fold following doses of 2.5 and 5 Gy when cells are in 3D structures and that this protection is not directly attributable to the presence of ECM. Thus cells in a 3D growth environment show fundamentally different radiation responses than the same cells in 2D monolayers. *Although these results are obtained at high dose they indicate that understanding radiation responses in a realistic 3D culture system will be critical to understanding the risk of low-dose radiation.*

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