MECHANISTIC STUDY OF LOW DOSE RADIATION INDUCED PROTEOLYTIC CASCADES IN HUMAN MAMMARY TISSUE MODELS

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We are investigating the mechanisms whereby radiation stimulates signaling cascades in tissue culture models. Human tissue models provide the opportunity to elucidate fundamental cellular mechanisms occurring following exposure to 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 is the three dimensional (3D) mammary tissue model where 184A1 human mammary epithelial cells are grown on an extracellular matrix material (Matrigel). Cells organize into tissue-like structures which recapitulate the intact human mammary gland morphology (Figure 1) enabling molecular level study of how normal tissues respond following exposure to ionizing radiation. Using this 3D model, we have demonstrated that low dose low-linear energy transfer (LET) radiation exposure leads to increased cellular invasiveness into the surrounding matrix and we propose this is due to the activation of proteolytic cascades.

Of the approximately 250 proteins released by 184A1 mammary epithelial cells, over 5% of them are proteases. These cells are found to express molecules important for ECM remodeling and for the control of protease cascades, including TIMPs 1, 2 and 4, MMP 2 and MT1-MMP. Matrix metalloproteases (MMPs) have been repeatedly implicated as having a role in matrix invasion is the. This family of proteinases degrades the basement membrane and has been implicated in promoting tumor metastasis.

To facilitate the study radiation induced signaling in these complex culture systems, we have developed and assembled a fully automated high speed confocal microscope designed to simultaneously capture the output from two intensified CCD cameras [1],[2]. Using fluorescently tagged antibodies, we have examined the level of MMP expression following exposure to low dose radiation (Figure 2). For MMP 1 and 2 we see a dramatic increase in fluorescent intensity following 10 cGy exposure. Although the level of MMP expression was reduced over time, it remained elevated at 24 hours post exposure (relative to controls). We are currently combining imaging with fluorescence activated cell sorting (FACS) and rtPCR to obtain quantitative information on radiation dose responses in 3D culture.

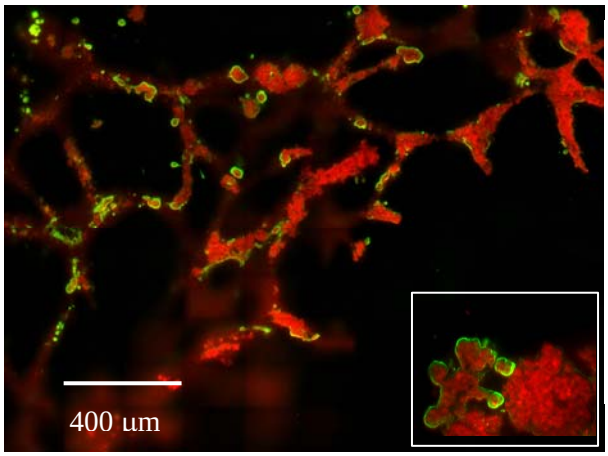

Figure 1: Confocal 10 x 10 montage of 2-day old mammary structure showing polarization. 184A1 cells were co-cultured with HB2 cells 50:50. Green = α_6 integrin, Red = Syto64. Inset: enlargement of individual frame emphasizing polarization of structure (60X).

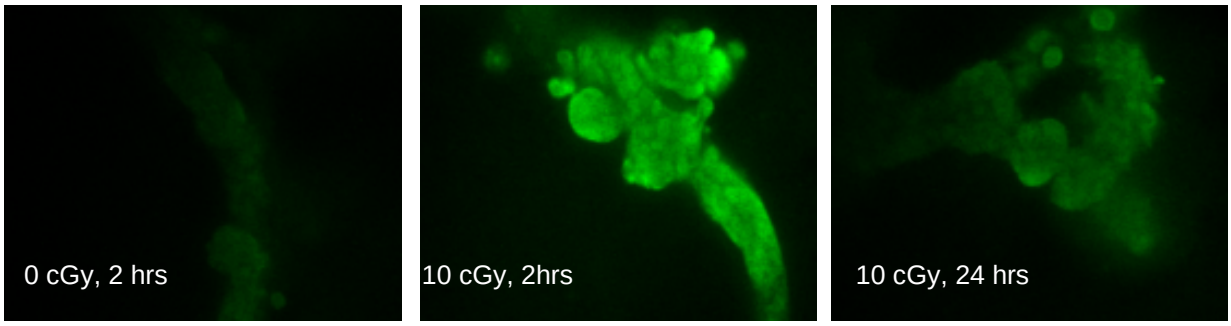

Figure 2: Time dependence of MMP 2 induction in 2-day old 3D mammary epithelial structure following 10 cGy x-ray exposurel. Control data for 24 hours did not look significantly different than data shown.

REFERENCES

[1] Perrine, K., Hopkins, D., LaMarche, B., Budge, S., and Sowa, M., (2005) *Scientific Computing & Instrumentation*, 16-20.

[2] Perrine, K. A., LaMarche, B. L., Hopkins, D. F., Budge, S. E., Opresko, L. K., Wiley, H. S., and Sowa, M. B., (2007) *Microscopy Research and Technique*. 70, 382-389.

This work was sponsored by the Biological and Environmental Research Program (BER) U.S. Department of Energy; Grant num
ber DE-AC06-76RLO.