Investigation of non-targeted effects of low dose ionizing radiation on the mammary gland utilizing three-dimensional culture models of mammary cells derived from mouse strains that differ in susceptibility to tumorigenesis

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Goal: Within the Lawrence Berkeley National Laboratory's SFA, Project 2, our studies focus on utilizing three dimensional (3D) cell culture models as surrogates for *in vivo* studies to determine how low doses of ionizing radiation influence mammary gland tissue architecture and how this may relate both to tumor progression and/or adaptive response.

Background and Significance: Responses to ionizing radiation include both targeted and non-targeted effects to particular cells within tissues or organisms. Although the mammary gland is known to be susceptible to radiation-induced cancer, the mechanisms that coordinate interaction of epithelial and stromal cells with the microenvironment allowing radiation-induced tumors to occur are not well understood. Using a radiation chimera model, the Barcellos-Hoff laboratory showed previously that irradiation of the stromal component of a cleared fat pad prior to transplantation of mammary epithelium significantly accelerated tumor formation and increased tumor size (1). These pivotal experiments revealed that non-targeted effects of ionizing radiation can indeed contribute to mammary carcinogenesis. Thus, targeted and non-targeted responses of tissue must be considered in order to identify mechanisms of radiation-induced tumor progression.

Approach: We are utilizing 3D cell culture models of mouse mammary epithelial and fibroblast cells to investigate the mechanisms that lead to radiation induce mammary cancer. As tissue architecture is an early hallmark of tumor progression, these culture models that morphologically mimic tissue structure and function provide a simpler system to decipher responses of the mammary gland to low doses of radiation as compared to *in vivo* models. Furthermore, if these mouse culture models faithfully recapitulate the *in vivo* situation, these systems could be applied to model human cell responses. In our mouse model system, we are using two stains of mice (BALB/c and SPRET/EiJ) that differ in susceptibility to cancer. The SPRET/EiJ inbred strain is more resistant to tumor formation than the BALB/c strain; however, mechanisms behind this resistance are unclear. Comparison of mammary fibroblasts of these two strains revealed that levels of cytokine secretion were quite different. Moreover, we observed that the SPRET/EiJ fibroblasts were more resistant to ionizing radiation induced death as compared to BALB/c.

To determine whether radio-resistance of the stromal cells could be in part a reason for resistance of SPRET/EiJ mice to carcinogenesis, a 3D co-culture model was developed. In this model, fibroblasts from BALB/c or SPRET/EiJ mice are embedded in a collagen matrix and subjected to ionizing radiation. Immediately following radiation exposure, the fibroblasts are co-cultured with EpH4 cells embedded as single cells within laminin rich extracellular matrix (lrECM) and plated on top of the collagen. EpH4 cells are a mammary epithelial cell line derived from BALB/c mice. These cells have retained normal p53 status and the ability to undergo mammary differentiation programs, including the ability to form polarized acinar colonies in 3D lrECM and to actually secrete milk proteins into the lumen of the colonies (2). Thus, the EpH4 colony structure constitutes a functional readout of signals received from the collagen embedded

fibroblasts. Our initial results from these co-culture experiments are quite encouraging and suggest that irradiated BALB/c fibroblasts disrupt the epithelial architecture to a greater extent than do irradiated SPRET/EiJ fibroblasts. This implies that the SPRET/EiJ fibroblasts may be able to more successfully process radiation damage resulting in less disruption to the tissue microenvironment and retention of normal tissue homeostasis that would be less likely to develop into a microenvironment permissive to tumor formation.

Conclusions and Future Directions: Although the ultimate test of genetic attributes that confer resistance or susceptibility to tumor formation will require *in vivo* studies, our 3D culture studies will provide excellent surrogates for mechanistic information in relation to cell-cell, cell-ECM and stromal/epithelial signaling that contribute to resistance or susceptibility of radiation-induced mammary tumors. We have begun to identify signaling mechanisms by proteomics and metabolomics and will validate the responsible signaling pathways that may provide targets for radiation-induced tumor progression. In the future, we will be extending these studies to interrogate the influence of fractionated low dose- and adapting- regimes on mammary gland tissue architecture and extending these 3D culture models to examine the effects of low dose radiation on human mammary cells.

References:

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Supported by the DOE Low Dose SFA Program at Berkeley Lab under DOE Contract No. DE-AC02-05CH11231