Using *ex vivo* organ culture models as surrogates to investigate morphological and functional differences of mammary glands derived from mouse strains that differ in cancer susceptibility to understand the underlying mechanisms of radiation sensitivity or resistance

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Goal: Within the Lawrence Berkeley National Laboratory's Low Dose SFA, as part of Project 2, we are using a systems genetics approach to determine the contribution of non-targeted and targeted radiation effects for risk of mammary carcinogenesis. The goal of this work is to characterize the mammary gland of the parental mouse strains, and the F1 and F2 generations used in these studies with respect to tissue architecture and morphogenesis in *ex vivo* three-dimensional (3D) culture to find both faster functional end points and to be able to manipulate the microenvironment in order to understand underlying molecular mechanisms of tumor progression.

Background and Significance: The use of mouse models with different genetic backgrounds provides valuable information with regard to susceptibility to cancer and prediction of risk. BALB/c mice are known to be susceptible to mammary carcinogenesis. On the other hand, the SPRET/EiJ strain of mice is resistant to tumor formation and these mice have been used to identify genetic variants that contribute to cancers of the skin (1). We are using a similar strategy to produce a genetic backcross model of SPRET/EiJ and BALB/c mice to investigate factors that increase risk for radiation induced mammary carcinogenesis. Currently, little is known regarding the particular tissue architecture of the SPRET/EiJ mammary gland or the specific signaling pathways utilized during mammary gland morphogenesis in SPRET/EiJ mice. Furthermore, it is not clear whether the genetic background of the SPRET/EiJ strain would be dominant when crossed with BALB/c female mice. Thus, investigation and characterization of the normal mammary glands from the parental strains (BALB/c and SPRET/EiJ), the F1 generation and the F1 backcrossed with BALB/c (F2 generation) using *ex vivo* culture models will provide a faster and more robust end point than 'cancer' to identify traits and signaling mechanisms associated with resistance or susceptibility to radiation-induced mammary tumors.

Approach: Mammary gland morphogenesis involves interplay between epithelial and stromal cells and the extracellular matrix (ECM), as well as precise amplitude and duration of particular growth factor signaling pathways (2,3). Thus, in addition to classical histological techniques, we are using *ex vivo* organotypic 3D culture models, developed in our laboratory (2,3), to investigate specific mechanisms required for mammary gland morphogenesis in the SPRET/EiJ mice compared to BALB/c and the resulting F1 and F2 generations. Whole mounts of mammary gland tissue revealed that SPRET/EiJ females have less side-branching as compared to BALB/c females examined at an identical age. Additionally, SPRET/EiJ mammary epithelial cells were characterized with respect to morphogenic programs of branching morphogenesis and alveolargenesis using conditions established previously for the BALB/c mammary gland. Remarkably and surprisingly, the SPRET/EiJ mammary epithelium failed to undergo branching in collagen gels or to undergo robust alveolargenesis in a laminin-rich extracellular matrix (lrECM). This failure to undergo morphogenesis was not dependent on the particular growth factor used, because changing the growth factor from transforming growth factor-alpha to fibroblast growth factor did not allow the SPRET/EiJ mammary gland to undergo

morphogenesis in these assays. Examination of the F1 generation in the 3D *ex vivo* morphogenic assays revealed that the mammary glands of the F1 generation behaved identically to the Spret/EiJ. These results suggest that mammary organoids provide promising surrogates for a rapid functional assay. It may be that mammary cells from the SPRET/EiJ genetic background utilize unique cell-cell and cell-ECM signaling mechanisms in order to undergo mammary gland development, and that these characteristics may contribute to resistance of the SPRET/EiJ to carcinogenesis in general, and radiation-induced cancers in particular.

Conclusions and Future Directions: These data indicate that genetic background influences tissue architecture and the behavior of cells during developmental programs and these are carried through to the F1 generation. As developmental programs are often re-initiated or hijacked during tumor progression, it will be important to understand the different signaling pathways at work in the SPRET/EiJ mammary gland. Understanding how these pathways are modulated during development of the SPRET/EiJ mammary gland and how they differ from BALB/c could provide important insight into the mechanisms underlying resistance of the SPRET/EiJ mouse to cancer. We will interrogate the regulation of the signaling pathways in these mouse models and integrate these results with the genetic information from the F1 backcross generation. We will use these morphogenic 3D culture models to determine the direct effects of low-dose radiation on tissue architecture and developmental programs.

References:

1. Quigley DA, To MD, Pérez-Losada J, Pelorosso FG, Mao JH, Nagase H, Ginzinger DG, Balmain A. (2009) Genetic architecture of mouse skin inflammation and tumor susceptibility. *Nature* 458:505-8.
2. Simian M, Hirai Y, Navre M, Werb Z, Lochter A, Bissell MJ. (2001) The interplay of matrix metalloproteinases, morphogens and growth factors is necessary for branching of mammary epithelial cells. *Development* 128:3117-31.
3. Fata JE, Mori H, Ewald AJ, Zhang H, Yao E, Werb Z, Bissell MJ. (2007) The MAPK(ERK-1,2) pathway integrates distinct and antagonistic signals from TGFalpha and FGF7 in morphogenesis of mouse mammary epithelium. *Dev Biol*. 306:193-207.

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