Genetic, cell-type, and tissue variation in low-dose and adaptive radiation damage responses as a means to understanding human cancer risks

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Goal: Our goals are to understand the low-dose and adaptive response mechanisms of the mammary gland in order to build a mechanistic model for predicting an individual's risk for breast cancer after exposure to low doses of ionizing radiation.

Approach: Our research plan utilizes a parallelogram strategy of comparing mouse *in vivo* genetic models to mouse and human *in vitro* cell culture models in order to best predict the mechanisms of susceptibility to low-dose breast cancer risks in human beings. This strategy involves obtaining low-dose data from mammary glands of genetically divergent mice exposed to a variety of low-dose exposure regimens, and from *in vitro* studies of radiation response in epithelial cells and fibroblasts from human and mouse mammary gland tissues to understand the roles of targeted and non-targeted effects of low-dose radiation and adaptive response within the mammary gland.

Results: Comparative analyses of low-dose gene expression responses in mammary gland tissue of inbred strains of mice that differ in their sensitivity to mammary gland cancer after low-dose ionizing radiation have identified a series of tissue damage response functions associated with cancer susceptibility. Our findings demonstrate that (a) low-dose ionizing radiation triggers complex damage response mechanisms in the mammary gland that vary between radiation sensitive and resistant mouse genotypes, and that are not induced after high doses, (b) expression of certain early response genes appears to be non-linear with evidence for strain differences in low-dose thresholds of induction, (c) genes with persistent changes in resistant and sensitive strains show overlaps with human genes associated with breast cancer risk and point to groups of mitotic and stromal genes that may be indicative of genetic susceptibility for breast cancer. Our analyses of blood cells from these radiation-sensitive and -resistant mouse strains has found higher pre-exposure levels of chromosomal damage in the radiation-sensitive strain than in the resistant strain, and evidence of persistent genomic instability in the blood of the sensitive strain after radiation exposure, which was not seen in the resistant strain.

Low-dose studies of human cells in culture show that the adaptive response appears to be dependent on cell type and culture conditions. Comparative analyses of adaptive protection against chromosomal damage in human fibroblasts and human mammary epithelial cells (HMECs) thus far suggest that fibroblasts are more likely to undergo adaptation than HMECs. Our findings also demonstrate that culture conditions (oxygen

tension; culture media composition) are important determinants of adaptive protection in fibroblasts. Mechanistic insights are provided by the observation that the adaptive response in fibroblasts is p53-dependent.

Conclusions and Future Plans Ongoing research continues to implement the human-mouse parallelogram strategy to understand the DNA damage response pathways involved in the low dose and adaptive response, to extend the demonstration of adaptive response to human 3D-cultures of epithelial cells and fibroblasts, and to contrast the human responses with those of murine mammary fibroblasts and epithelial cells from sensitive and resistant murine strains. We are extending our mouse *in vivo* studies of the low-dose MG risk model to investigate molecular responses in the mammary gland of animals exposed to various low-dose fractionation regimens that differ in their ability to induce MG cancer in radiation-sensitive mice, and plan to extend this work to cells from human subjects exposed to low-doses of radiation (or their cells exposed *ex vivo)*. Our findings contribute to understanding the molecular mechanisms and genetic determinants of low dose and adaptive responses in mammary gland tissues. Our studies also serve as a foundation for developing molecular strategies to identify individuals among nuclear workers and other circumstances of low-dose human exposures who might be of increased susceptibility to low-dose radiation-induced breast cancer.

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