Genetic susceptibility to low-dose ionizing radiation in the mouse mammary gland as a means of understanding human risk for breast cancer

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Goal: Our goal is to develop an *in vivo* mechanistic model of genetic variation in the low-dose damage responses of mammary glands using inbred mice known to vary in their sensitivity to low-dose induced mammary gland cancer, and to develop molecular predictors for susceptibility or resistance to low-dose induced breast cancer.

Background and Significance: It is increasingly believed that individuals differ in their genetic susceptibilities to environmental insults for diseases such as cancer. This concern is especially important for the large numbers of individuals receiving low-dose exposures in the nuclear energy industry, from natural sources, and from medical procedures. The human breast is sensitive to radiation-induced cancer, but little is known of low-dose damage response pathways or how they might vary across individuals. Studies in mice have shown that genetic background is an important factor for mammary gland cancer risks after low-dose exposures. Aside from collecting epidemiological data, more needs to be done to increase our understanding of the molecular mechanisms of genetic susceptibility to radiation-induced diseases and to identify individuals who are resistant or susceptible to cancer after low-dose exposures.

Approach: We are investigating the damage response mechanisms of mammary glands (MG) of mice with differing sensitivities to low-dose induced MG cancers to identify genes and pathways that have bearing on risk predictions for human breast cancer. We have characterized the MG expression profiles of radiation-sensitive BALB/c versus radiation-resistant C57BL/6 inbred mice by sampling MG tissue before exposure, at 4 hours after exposure (early response) and 1 month after exposure (persistent response). Because of relevance to human occupational and medical exposures, we utilized a fractionation regimen consisting of 4 weekly whole body exposures to 7.5 cGy X-rays (low-dose group), 4 weekly 1.8 Gy exposures (high-dose reference group) or 4 weekly sham exposures (unexposed reference group). Differentially expressed genes were evaluated using qPCR, bioinformatics tools, and known human breast cancer biomarkers.

Results: We found significant strain differences in the unirradiated baseline expression of MG genes associated with metabolism (45% of affected genes), immune response (19%), DNA repair (2%), apoptosis (10%), extracellular matrix (10%), and vasculature (10%). For example, unirradiated BALB/c mice showed lower expression of several DNA damage response genes (Ku70/XRCC6, PARP3, RAD23A/B, GADD45G and GADD45GIP1), including some not previously reported.

The early low-dose MG responses involved ~4x as many genes in BALB/c as in C57BL/6, and were associated with inflammation, cell-to-cell signaling, hypoxia and MG developmental functions, none of which were detected in C57BL/6 mice. The early responses also showed dose non-linearities in BALB/c mice (both qualitatively and quantitatively) and strain differences in low-dose induction at 4h after exposure.

Certain persistent low-dose MG responses (i.e., at one month after the last exposure) were in opposite directions between the two strains in reference to the shams and primarily involved mitotic genes and microenvironmental functions. C57BL/6 mitotic genes were broadly down-regulated in a network with upregulated p21, whereas BALB/c mitotic genes were up-regulated within a myc-interaction network.

Comparison of the persistent low-dose MG responses in mice against >40 studies of biomarkers of human breast cancer risk (Abba et al, 2010) identified ~30 cell cycle control genes and ~5 stromal genes whose expression was in opposite directions in BALB/c and C57BL/6. The directions of responses in BALB/c were the same as those associated with increased breast cancer risk in humans. In addition, among 32 human mitotic genes whose expression was associated with poor prognosis, 17 were persistently upregulated in BALB/c whereas 29 were persistently downregulated in C57BL/6 after the low-dose regime.

Conclusions/Future directions: Our findings demonstrate that (a) low-dose ionizing radiation triggers complex MG damage response mechanisms 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 of breast cancer risk and point to groups of mitotic and stromal genes associated with genetic susceptibility for breast cancer. In mice, low dose fractionation can significantly modify MG cancer outcome, and tumor incidence appears to depend on the delivered dose per fraction (Ullrich et al, 1987). We are conducting experiments to extend our low-dose MG risk model to low-dose fractionation regimens that differ in their ability to induce MG cancer in radiation-sensitive mice and then to extend this work to human low-dose exposures. Our findings contribute to understanding the genetic risk factors for radiation sensitivity and resistance. They may serve as a foundation for identifying individuals in the nuclear energy industry and other circumstances of low-dose human exposures who might be of increased susceptibility to low-dose radiation-induced breast cancer.

References:

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