A Systems Genetics Approach to Evaluate Serum Cytokine Expression Profiles in 10 cGy-Irradiated Mice: Possible Connection to Susceptibility/Resistance to Cancer

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Goal: To use a systems-genetics approach to evaluate serum cytokine expression profiles in 10 cGy-whole-body-irradiated mice (BALB/c and Spret/EiJ parental strains, and their F1 offspring backcrossed (F1Bx) to female BALB/c).

Background and Significance: Even low doses of ionizing radiation (~10 cGy) can alter the composition of the tissue microenvironment by rapidly affecting cytokine production and activities, extracellular matrix (ECM) composition, and the expression of receptors that mediate cell-to-cell interactions (1). The stroma in mammary glands is constantly changing through cycles of hormone action, differentiation and turnover of molecules and ECM, and by recruitment of migrating hematopoietic cells, particularly macrophages and eosinophils that can contribute to potentiating normal mammary gland development and tumor progression (2,3).

Approach: We selected two murine strains that have different susceptibility to tumors (BALB/c and Spret/EiJ). These strains show differences also in the levels of individual blood cell components. BALB/c have higher numbers of lymphocytes and platelets, and fewer neutrophils than the Spret/EiJ. We hypothesize that the genetic background plays a role in regulating the linkage of the cytokine-centric gene network- and ECM-component profiles. We are testing the additional hypothesis that these profiles differentially regulate targeted and untargeted stress responses, and that the Spret/EiJ mouse strain is more resistant to low-dose-induced oxidative stress than the BALB/c strain. We are asking whether the F1Bx serum cytokine expression profile 6 hours after 10 cGy shows a genotype-dependent expression that can be correlated with the later analysis of susceptibility to mammary tumor development compared to sham-treated animals. Data have been collected as a function of time from blood samples of 20 BALB/c and 10 Spret/EiJ parental mice, and 352 F1Bx mice exposed to 10 cGy or sham-treated, in order to characterize changes in cytokines using the Luminex 200 and mouse cytokine Milliplex^{R} Map Kit from Millipore run in duplicate. The microarray data are analyzed by a 2-sided, non-parametric analysis (Mann-Whitney test). The genotype association is analyzed by R/QTL linkage analysis.

Results, Conclusion and Future Plans: Nine out of 32 cytokines in multiple cytokine analyses show baseline differences between the untreated control BALB/c and Spret/EiJ lines. A 10 cGy low dose exposure to the parental lines causes small changes in serum cytokines in an early time course of hours to days after exposure. Most notably, eotaxin (CCL11) is significantly increased by 2-fold at 72 hours after exposure in the Spret/EiJ compared to the untreated controls. The F1Bx animals show a dramatic diversity of cytokine expression 6 hours after 10 cGy that is currently being correlated to their specific genotype. Further analysis is expected to reveal significant genotype-dependent differences in cytokine

expression after exposure to low dose radiation. Additional studies are in progress to evaluate the persistence of low-dose impacted cytokine expression in the F1Bx. The resultant data will be compiled with the tumor incidences from these animals to investigate whether a single low 10 cGy dose of Xrays can induce changes in cytokine levels in a genetic background, and how this can be translated to altered risk in susceptibility or resistance to mammary cancer.

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