A Systems Genetics Approach to Analyze the Metabolic Responses to Low Dose Radiation

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Goal: American nuclear regulatory agencies consider that risk is proportional to dose of ionizing radiation and thus any doses of radiation are harmful. This is referred to as the linear no threshold model (LNT) and it assumes that the biological response to DNA damage is also linear. Such model has been questioned by the radiation biology community and is the subject of an ongoing controversy. An understanding of the genetic basis of individual variation in responses to the deleterious effects of radiation is a prerequisite for identification of high risk individuals, as well as for the development of novel strategies for prevention of tissue damage during tumor therapy. As a part of Project 2 of the Berkeley Lab SFA we are investigating the contribution of non-targeted effects to the low- dose radiation response using female murine strains with variable radioresponse (BALB/c and Spret/EiJ), and an F1-backcross (F1Bx) with the BALB/c female.

Using a systems genetics approach, our goal is to investigate the parental strains, and the individual F1Bx phenotypes in a time-course after exposure to understand the genetic, signalling and metaboloic response to a 10 cGy whole-body X-ray exposure compared to sham-treated controls. In addition, our work will contribute to the evaluation of the risk of radiation-induced tumorigenesis, since these animals are apart of a continuing study investigating how many tumors arise in unirradiated murine mammary epithelial cells implanted in their cleared mammary fat pads. Our work can investigate the parameters of the irradiated microenvironment in which the tumors develop.

Background and Significance: Mass spectrometry has been used for decades to measure metabolite levels in clinical settings, due to its ability to provide quantification and structural information (from MS/MS fragmentation patterns), as well as its high sensitivity. Recent developments in mass spectrometry now allow for 'metabolomic' profiling of complex biological samples, providing insights into metabolic networks and enzymatic activities. While these approaches maximize the number of metabolites detected, solvent extraction methods result in a loss of spatial information, averaging of metabolite concentrations. This is complicated by the fact that these are likely cell specific processes that depend critically on the cells in the tissue microenvironment and the genetic background. Given the inherent heterogeneity within tissues, the 'grind-and-find' approach averages out locally elevated and clinically-relevant metabolite levels and typical tissue culture experiments disregard tissue type variance, immunological, and paracrine effects. The alternative approach of directly imaging metabolites within tissues has the potential to overcome these limitations by measuring metabolite levels within tissues.

Approach: Parental and F1Bx mice were irradiated with 10 cGy X-rays or sham and then harvested serum in a time-course 6 hours after exposure. A second time point 15 weeks after irradiation is also in progress. Serum from a total of 351 mice was individually extracted using 700 µl of a pre-cooled methanol-isopropanol-water (3:3:2) mixture. Samples were dried and 1 µl of this RI mixture was added to the dried extracts and derivatized. A Gerstel automatic liner exchange system with multipurpose sample MPS2 dual rail was used to inject 0.5 µl sample to a Gerstel CIS cold injection system. The injector was operated in splitless mode, opening the split vent after 25 s and being by to 250°C. Analytes injected were separated using an Agilent 6890 gas chromatograph, equipped with a 30 m long, 0.25 mm i.d. Rtx5Sil-MS column (0.25 µm 5% diphenyl film and additional 10 m integrated guard column). Mass spectrometry was performed by a Leco Pegasus IV time of flight mass spectrometer with 280°C transfer line temperature, electron ionization at −70eV and an ion source temperature of 250°C. Mass spectra were acquisitioned from m/z 85 to 500 at 17 spectra s−1 and 1850 V detector voltage. Result files were preprocessed directly after data acquisition and stored as ChromaTOF-specific and analyzed using BinBase software.

As discussed above the inherent heterogeneity of biological systems, this 'grind-and-find' likely ignores much of the complexity within the systems and ionization approaches generates additional non-biological complexity. Therefore we are complementing these profiling methods with small molecule imaging and computation approaches to understand metabolism within the tissues and cells[1]. Towards this goal we have developed a surface based mass spectrometry approach that is well suited for metabolite imaging from frozen tissue sections (Nanostructure-Initiator Mass Spectrometry[2], NIMS) with a unique combination of high lateral resolution (10-75 µm), sensitivity and lack of matrix.

Conclusion and future plans: Our results indicate that there are strain-dependent differences in serum metabolomic profiles in untreated mice with BALB/c showing relatively higher expression than Spretus. We also find statistically significant low-dose specific metabolic profiles 6 hours after 10cGy. In the F1Bx mice we see a diverse array of outcomes depending on the individual genotypes. We will utilize genotype information to define genetic controls of the low-dose metabolic responses and cytokine profiles to investigate non-targeted effects, and as tumors become avalable, we will use NIMS imaging to analyze the regional variation in low-dose associated metabolites identified using metabolite profiling. These data are being extended to the mammary gland of mice as well as human breast cells in 3D cutlures. Together, this comprehensive systems biology approach is being used to identify specific genes or pathways that are differentially controlled between mouse strains, and contribute to variation in susceptibility to radiation-induced carcinogenesis.

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References:

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