Radiation-induced responses involved in intercellular signaling and *in vivo* effects.

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In the course of our studies on the effects of radiation on gene expression, we have observed a wide variety of genes whose products are involved in intercellular signaling and tissue injury responses. These studies include responses in human cells lines, peripheral blood lymphocytes irradiated *ex vivo* and *in vivo* from patients undergoing total body irradiation, as well as in studies employing mouse models. In addition to genes with roles in cell cycle, apoptosis, and DNA repair, a surprisingly large number of responsive genes were consistently found that encoded cytokines, cytokine receptors and cytokine associated proteins, cell junction and cell membrane proteins, secretory and plasma proteins, acute phase response-related proteins, tissue proteases, and inflammatory mediators. A variety of tissues from irradiated mice showed responsiveness for many of these genes. At doses of 150 to 400 cGy, strong acute (4 h) responses were observed that persisted in many cases beyond 48 h. Responses generally showed dose dependence and could be detected, albeit at lower magnitude, to 10 cGy, the lowest dose employed.

To dissect regulatory components for *in vivo* radiation responses, studies have been carried out in spleen in various mouse models. We employed p53-/- mice as well as a derivative containing a human p53 transgene. Since p38 MAP kinase has a prominent role in mediating inflammatory responses and can phosphorylate sites in p53 which are required for activation, we generated a p38α (knockin) mutant where the activating phosphorylation sites at T180 and Y182 were mutated; this mutant p38 (designated p38+/DN) functions as a dominant-negative and markedly reduced p38 signaling after stresses, such as UV radiation. p38 can be inactivated by the Wip1 phosphatase, which is known to have oncogenic properties, and Wip1 has recently been found to also dephosphorylate a key phospho-serine in ATM. We and others have observed that Wip1-/- cells and mice show heightened p38 activity. For these reasons, we included Wip1-/- mice as a potential model for increased stress responsiveness. p53-/- splenic tissue exhibited dramatically attenuated responses in that the number of genes showing significant induction was only half of that of wild type (wt), and many of the remaining responses were of lower magnitude than that of wt. The effect of p53-/- on radiation-repressed genes was not as remarkable as that for induced genes. The p38+/DN model showed reduced induction of some known p53-regulated genes such as Cyclin G, p21 (Cdkn1a), Bax and Apaf1. We observed striking ablation of the up-regulation of inflammation-related genes in p38+/DN including a variety of interleukins and other cytokines, as well as genes encoding VCAM1 and matrix metalloproteinases, and enzymes involved in leukotriene biosynthesis, such as arachidonate 5-lipoxygenase. Interestingly, the Wip1-/- model showed the opposite effect with enhanced induction of a

variety of cytokines, growth factors, and known p53-regulated genes. These results highlight the overlap between radiation and inflammatory responses, and demonstrate prominent roles for p38 signaling in radiation signaling *in vivo*.

In an effort to discern radiation-specific gene-response profiles, we have examined gene expression responses of human cell lines exposed to a diverse set of stress agents. The B-lymphoblastoid cell line TK6 (p53 wild-type) and its p53-null derivative, NH32, were treated in parallel to facilitate investigation of p53-dependent responses. Gene expression signatures were defined that discriminated between four broad general mechanisms of stress agents: Non-DNA damaging stresses [heat shock, osmotic shock and 12-*O*-tetradecanoylphorbol 13-acetate (TPA)], agents causing mainly oxidative stress (arsenite, cadmium, and H_2O_2), ionizing radiations (neutron and γ-ray exposures), and other DNA damaging agents [ultraviolet radiation, methyl methanesulfonate, adriamycin, camptothecin, and *cis*-diamminedichloroplatinum(II) (cisplatin)]. Within this dataset, non-DNA damaging stresses could be discriminated from all DNA damaging stresses, and profiles for individual agents were also defined. A 19-gene set could be used to distinguish radiation responses from other agents. Efforts are underway to extend these studies to metabolomic profiling, as well as to primary cells and *in vivo* in mice.

Considering the myriad of radiation-responsive genes that are involved in intercellular signaling and considering that many of the products of these genes, such as cytokines, can have a wide variety of effects on cellular metabolism, we are now studying the impact of radiation on the profile of metabolites. Our approach utilizes metabolomics, defined as the global analysis of low-molecular weight molecules for identifying context dependent metabolic phenotypes that vary temporally and according to physiological, developmental, or pathological state. We collected 24-hr urine samples from adult male mice before and after single exposure to 10, 300, or 600 cGy. Urines were analyzed by ultra-performance liquid chromatography-time of flight mass spectrometry (TOFMS). Data were analyzed with principal components analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) to identify markers specifically elevated in post-exposure urines compared with pre-exposure and control urines. Putative chemical formulae for candidate markers were derived based on MSTOF accurate mass measurements. Identities were verified against known standards by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Results from PCA and PLS-DA comparing urines from exposed and unexposed mice indicate that urine metabolic profiles change following radiation exposure. While changes in response to 10 cGy irradiation are subtle, we found several metabolites clearly elevated in urines from animals exposed to 3 and 6 Gy compared with pre-exposure and controls. A multi-institutional effort is underway to develop metabolic profiles for radiation biodosimetry at higher doses (1 to 10 Gy); see http://cmcr.columbia.edu/project3.html. The same approach will probably also have utility in the elucidation of metabolic changes induced at low doses of radiation.