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Development of a functional genomics approach to use radiation-induced changes in gene expression to monitor for low dose and low dose-rate exposures.

As part of this ongoing project, we are exploring the utility of gene induction as a means to monitor for exposure to ionizing radiation. In this study, we employed molecular biology approaches including cDNA microarray technology, to study cellular responses to radiation injury. In experiments using a human myeloid tumor cell line (ML-1), we demonstrated induction of mRNA expression of several stress responsive genes by doses of gamma rays as low as 2 cGy (Amundson et al., 1999). For instance, the dose-response for induction of CIP1/WAF1 and GADD45 appeared to be linear over the range of 2 - 50 cGy, and showed no evidence of a threshold for induction.

We have also used fluorescent cDNA microarray hybridization to investigate transcriptional stress responses following low doses of gamma-rays. Using this functional genomics approach, the responses of a substantial portion of a cell's expressed genes can be monitored. We previously demonstrated the utility of this approach using ML-1 cells exposed to high doses of ionizing radiation to identify sequences not previously reported to be radiation-responsive (Amundson et al., 1999). We found good concordance between the induction ratios determined by microarray and those determined from single probe hybridizations. We are now using data generated in ongoing experiments using different radiation doses and cell lines to identify additional radiation-responsive genes for inclusion on a stress-specific microarray under development.

Investigation was also made into the effects of protracting delivery of low dose ionizing exposures. While the results indicate a generally protective effect of low dose rate exposures on gene induction, some exceptions were also noted. The differential responses of groups of genes to reduced dose-rate exposure may indicate important differences in the regulatory mechanisms governing their expression.

As a first step to in vivo studies in humans, the responses of peripheral blood lymphocytes (PBL) irradiated ex vivo were characterized. These experiments used freshly collected blood from donors at the NIH blood bank, from which we separated the lymphocytes using a standard density gradient centrifugation. We used microarray analysis to identify radiation-regulated genes that could potentially serve as informative biomarkers of radiation exposure. Our initial studies identified several genes significantly up-regulated in human peripheral blood between 24 and 72 hours after ex vivo irradiation. Three of these genes, DDB2, CIP1/WAF1 and XPC, were induced in a linear fashion between 0.2 and 2 Gy at 24 and 48 hours after treatment, with less linearity at earlier or later times (Amundson et al., 2000). We are currently extending these studies with murine microarrays to examine the in vivo response to whole body irradiation of mice. Doses from 0.1 to 2 Gy result in the induction of a large number of genes in liver, spleen and thymus 24 and 48 hours after exposure, with the results in the two hematopoietic tissues, spleen and thymus, being the most similar to each other, and also yielding the largest magnitude responses. Studies with a number of collaborators to

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apply microarray analysis to RNA from PBL samples taken from patients undergoing radiation therapy are also ongoing. Preliminary results with a patient undergoing total body irradiation show robust induction of many genes after the first two 1.5 Gy fractions. Future studies will extend this work to lower doses from patients receiving smaller field local irradiation and low dose diagnostic procedures. Although the similarity of even the high dose responses in different individuals has yet to be determined, this is the first microarray analysis of in vivo human radiation response, and holds great promise for future endeavors.

Publications supported by this project:

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