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Project Progress

This is the final report for our study "Mechanisms of Adaptive Responses and Genomic Instability Induced by Low Dose/Low Dose Rate Radiation". The project provided support for three Ph.D. students to investigate the effect of LD/LDR X-rays on induced genomic instability and adaptive responses (ARs), and investigate the molecular mechanisms for these phenomena. Three projects were proposed based upon a rapid and reliable plasmid assay for detecting delayed radiation effects involving expression of green fluorescence protein (GFP). The assay measures delayed recombination events we believe are similar to those occurring in irradiated cells that lead to chromosomal rearrangements. This assay has advantages over the time and labor-intensive cytogenetic analysis in that surviving clones can be studied as a function of time after irradiation by automated identification of GFP+ cells. Large numbers of colonies can be rapidly screened permitting investigation of rare events like those expected after LD/LDR radiation. Three specific aims were proposed:

Specific aim 1 tested the hypothesis that LD/LDR radiation can induce genomic instability in human cells, and that the frequency of induced instability can be modified by dose rate.

Specific aim 2 tested the hypothesis that LD/LDR radiation can induce an AR that makes cells refractory to damage induced by a subsequent challenge with an acute high dose radiation exposure.

Specific aim 3 tested the hypothesis that induced genomic instability and ARs result from differential gene expression used micro array technologies to investigate the molecular mechanisms underlying induced instability and the AR.

The goals proposed in aim 1 were accomplished. We developed and characterized the GFP-based assay and demonstrated that

ionizing radiation induces genomic instability in human RKO-derived cells. Up to 10% of cells cultured after a 0.01-10 Gy dose of IR produce colonies with a small number of GFP+ cells in a background of GFP- cells or a small number of GFP- cells in a background of GFP+ cells, indicative of delayed homologous recombination or mutation/deletion. Delayed homologous recombinated was induced at 1, 2, 5, and 10 Gy doses of ionizing radiation, indicating that it is a sensitive measure of low-dose radiation effects. However, delayed homologous recombination did not show a typical dose response relationship. Consistent with prior studies, delayed chromosomal instability was induced in RKO-derived cells by relatively high doses and correlated with delayed reproductive cell death. In contrast, cells displaying delayed homologous recombination showed no evidence of delayed reproductive cell death, and there was no correlation between delayed chromosomal instability and delayed homologous recombination, indicating that these forms of genome instability arise by distinct mechanisms. Because delayed homologous recombination is induced at low doses of ionizing radiation that are not associated with reduced cell viability and there is no dose response, it may suggest that low dose irradiation is potentially more dangerous than cytotoxic doses.

In collaboration with Dr. Marianne Sowa and colleagues at the Pacific Northwest National Laboratory significant progress has been made towards fully automating colony counting and expression of fluorescence GFP. In this way we can rapidly and reliably evaluate large numbers of colonies to investigate delayed effects associated with low dose radiation exposure.

We also made significant progress on aim 2, to investigate ARs and bystander effects, both of which are associated with low dose radiation exposure. ARs refer to the phenomenon by which cells irradiated with a sub-lethal dose are less susceptible to subsequent high-dose exposure. Bystander effects are nontargeted effects observed in cells that were not irradiated but were either in contact with, or received soluble signals from, irradiated cells. These non-hit bystander cells can exhibit damage typically associated with direct radiation exposure. We found that human RKO cells do not exhibit an AR or bystander effects when cell killing is the endpoint evaluated. However, low radiation doses suppressed the induction of delayed homologous recombination by a subsequent high dose,

indicative of an AR for radiation-induced delayed homologous recombination. These results highlight the inherent variability in cellular responses to low dose radiation exposure and add to the uncertainties associated with evaluating potential hazards at these low doses.

Specific aim 3 was more complicated and frustrating. We completed the transcriptional profiling of four chromosomally unstable clones. Our strategy was to mix equal numbers of cells from two unstable clones prior to RNA extraction and array hybridization and compare these with an irradiated but chromosomally stable clone. The Genomic Solutions GeneMAP human cancer chip consisting of 1152 human cancer related cDNA elements was used to find genes associated with radiation induced genomic instability. The criteria for considering a gene differentially expressed used two methods. First, if a gene had a +/-2.0-fold change in expression in experimental versus stable control samples in at least six of eight spots for that gene it was considered further. Second, the Significance Analysis of Microarray (SAM) software package was employed to find statistical significance in expression among unstable clones and the control clone. Interestingly, 61 genes were identified and all were down-regulated in the unstable clones. We then confirmed the microarray analysis by RT-PCR, Northern and western blot analysis and where feasible functional assays. After a huge amount of work we were unable to identify a single gene or molecular pathway that was differentially down-regulated in all the chromosomally unstable clones tested. We interpreted these data to imply that there were multiple potential molecular pathways and/or events that maintain the unstable phenotype, and that no single expression pattern was linked to instability in the clones analyzed.

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