

Proteomic and Biochemical Studies of Human Mesenchymal Stem Cells in Response to Low Dose Ionizing Radiation

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We will present data obtained during the first year of our DOE/NASA Low Dose Radiation Research program. We utilized a comprehensive approach including transcriptomics, proteomics, phosphoproteomics, and biochemistry to characterize human mesenchymal stem cells (MSCs) in response to low dose ionizing radiation.

We first determined the cell survival, proliferation, and osteogenic differentiation of MSCs in response to IR. We showed that cell survival over a period of 8 days was decreased in a dose-dependent manner with high-dose radiation (from 1 to 10 Gy). However, the cell survival didn't change significantly with low-dose radiation (from 0.01 to 0.1Gy). We next determined the effect of irradiation on MSC proliferation 24 hr after IR using flow cytometry. We showed that IR regulated cell cycles in a dose-dependent manner. Finally, we discovered that the osteogenic differentiation of MSCs was modulated in response to IR, particularly at high doses. We also observed that p53 was activated in MSC after IR and the dose-dependence was consistent with the DNA double-strand break as measured by rH2AX foci.

To examine the global change of gene expression in response to high and low dose of irradiation, we subjected MSCs to irradiation at 0.1 and 1 Gy and analyzed the transcriptome using an Affymetrix platform. The effects of irradiation were revealed clearly in the gene expression profile after 24 hr. For all 22,000 genes probed, 176 and 175 genes changed more than 2 folds in response to 1 and 0.1 Gy respectively. The genes with changes included cell cycle-related genes, signaling molecules, matrix proteins, and metabolic proteins etc. Many genes related to different aspects of cell cycle were decreased by irradiation. The number of cell cycle-related genes that decreased in 1 Gy-treated cells was larger than that in 0.1 Gy-treated cells, suggesting that mRNA profiling can distinguish the quantitative difference of irradiation effects. To confirm the results from DNA microarray, we verified selected genes using qRT-PCR.

To characterize the proteome and phosphoproteome changes of MSCs after irradiation, we combined 2D gel electrophoresis and LC-MS/MS strategies. We showed that expression, posttranslational modification, and truncation of annexin A2 and annexin A1 were regulated in response to IR as low as 0.1 Gy. To understand the mechanisms underlying radiobiological responses of MSCs, we carried out experiments to define ROS signaling pathways in MSCs. We currently focus on MEK pathways to delineate the effects of H₂O₂ treatments. Dose-dependent and time-course studies have already been carried out. Ongoing efforts will continue to elucidate the mechanisms and pathways involved in the radiobiological responses of MSCs.