THE ROLE OF THE BONE MARROW MICROENVIRONMENT IN SPACE RADIATION-INDUCED LEUKEMOGENESIS

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We examined the effects of simulated SPE/GCR exposures on primitive human hematopoietic stem/progenitor cells (HSC), testing the hypothesis that the quality and quantity of cells being produced can be altered not only from exposure of HSC themselves, but also via indirect effects arising from exposure of microenvironmental mesenchymal stromal cells (MSC). Human HSC and MSC were isolated by immunomagnetic sorting with antibodies to CD34 and Stro-1 (n=3), and exposed to: A) sham irradiation (to control for air transportation effects); B) 1 Gy of ^{137}Cs gamma irradiation; C) 1 Gy of 50 MeV protons (primary SPE energy); or D) 20 cGy of 1 GeV/n ^{56}Fe ions (model GCR radiation) at the NSRL Immediately following irradiation, aliquots of HSC and MSC were cultured for chromosomal aberration (CA) analyses and DNA/RNA isolations, and the remainder of cells were placed in an MSC-based HSC maintenance/expansion culture system we previously reported, testing 4 different combinations: 1) unirradiated HSC plated over unirradiated MSC; 2) unirradiated HSC plated over irradiated MSC; 3) irradiated HSC plated over unirradiated MSC; and 4) irradiated HSC plated over irradiated MSC. Hematopoietic cells were then collected after 7 days, and methylcellulose assays were used to assess the impact of irradiation of the HSC, the MSC, or both cell types on the hematopietic colony-forming potential. 1 Gy gamma radiation of HSC resulted in 20% reduction in CFU-GM potential compared to unirradiated HSC, but exerted little effect on more primitive CFU-Mix output. In contrast, gamma irradiation of MSC and subsequent use of these cells as feeder layers revealed that gamma irradiation negatively affected the MSC's ability to support the maintenance of more primitive HSC, as evidence by a 60% reduction in the output of CFU-Mix, supporting our hypothesis that the types and quantity of hematopoietic cells being produced can be altered not only as a result of HSC themselves being exposed, but also via indirect effects arising from exposure of the microenvironment. Irradiating both HSC and MSC had an additive (negative) effect, further reducing the CFU-Mix output by nearly 70%. In contrast to what was seen with gamma irradiation, exposure of MSC to 1 Gy of 50 MeV protons had no deleterious effect on their ability to support early (CFU-Mix) or more mature (CFU-GM) hematopoietic cells. To the contrary, our results to-date suggests proton irradiation of MSC may actually enhance their ability to support the maintenance/expansion of early HSC (CFU-Mix). In marked contrast, treatment of HSC with proton radiation had a pronounced deleterious effect on the functionality of these cells, reducing their CFU-GM output by 60% and CFU-Mix output by 90%. While exposure of MSC alone to protons did not appear to affect their ability to support hematopoiesis, exposure of both HSC and MSC to protons resulted in further reduction in CFU-GM colony output when compared to irradiating HSC alone with protons. Effects of exposing MSC and HSC to 20 cGy of 1 GeV/n ^{56}Fe ions had the complete opposite effect compared with gamma or proton radiation. While exposing MSC to this dose of ^{56}Fe ions had little or no effect on their ability to support HSC, exposure of HSC to this same dose of ^{56}Fe irradiation resulted in up to a 3.5-fold increase in CFU-GM output, suggesting enhanced HSC proliferative potential following low dose ^{56}Fe ion irradiation. CA analyses are currently being conducted on aliquots of all MSC and HSC to examine immediate CA induction for the three radiation types among the different donors. In addition, supernatants and cell pellets were collected from MSC and from the hematopoietic colonies from each treatment group, and we are currently performing cytokine arrays, microarray, RNASeq, and whole exome sequencing to define key changes induced by each radiation type at the protein, transcriptional, and genomic levels, respectively, within these two cell populations and further define the potential risk of leukemogenesis. Planned future experiments include modeled 1972 SPE irradiations, with some followed by low dose ^{56}Fe ions, and use of additional donors to assess potential inter-individual variation in these responses.