In Vivo Induction of Aberrant Patterns of DNA Methylation and Chromosome Instability in Hematopoietic Stem/progenitor Cells (HSPCs) by Silicon (^{28}Si) Ions

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Exposure to radiation in space is the greatest hazard to astronauts venturing beyond Earth. To protect astronauts in space environments, improvement in our knowledge of radiation-induced changes in specific target cells that may impact the health of astronauts is required. Cancer of blood cells, in particular myeloid leukemia (ML), is one of the major health concerns from exposure to space radiation. However, the predictions of risks for developing ML after exposure to space radiation are unsatisfactory. To increase the reliability of predicting risk for ML, a much improved understanding of space radiation-induced changes in the target cells, *i.e.* hematopoietic stem/progenitor cells (HSPCs), is critically important.

We gave adult male CBA/CaJ mice a whole-body exposure to various doses of 300 MeV/n ^{28}Si ions (LET = 70 keV/μm) that are within the range of NASA interest, *i.e.* 0 (sham controls), 0.1, 0.25, or 0.5 Gy. The harvest times for the serial sacrifice were at 1 wk, 1 and 6 mos post-irradiation. At each sacrifice time, 5 mice were randomly selected from each dose of ^{28}Si ions for the collection of bone marrow cells for obtaining HSPC-derived clones (myeloid lineage) by means of an *in vitro* colony forming unit assay. The patterns of DNA methylation, *i.e.* the global levels of 5-methyl cytosine (5mC) and 5-hydroxy-methyl-cytosine (5hmC), were evaluated in DNA samples isolated from the HSPC-derived clones at all three harvest times. At 6 mos post-irradiation, the evaluation of DNA methylation for each mouse was coupled with the measurement of genomic instability, assessed by the presence of delayed- or late-occurring chromosome aberrations (CAs). If genomic instability does occur, the collection of samples at 6 mos post-irradiation will allow the detection of both clonal and non-clonal CAs in descendants of cells from exposed mice. It is important to study genomic instability since it has been widely suggested that elevation of genomic instability also elevates cancer risk.

We found a significant dose-dependent increase in the levels of 5mC (*ANOVA, $p<0.01$*) at 1 wk post-irradiation. Subsequently, only a slight increase in the levels of 5mC was found in all treatment groups, as compared to the corresponding sham controls. In contrast, persistent dose-dependent decreases in the levels of 5hmC (hypo-5hmC) was found up to 6 mos post-irradiation. These findings suggested that the global level of 5hmC may be a better predictor of exposure to ^{28}Si ions that, in turn, might be more useful in risk assessment than the global level of 5mC. For the study of CAs at 6 mo post-irradiation, several types of CAs (breaks and exchanges, both chromatid- and chromosome-types) were observed in HSPCs of exposed mice. Only simple one-way chromosomal exchanges were found. Robertsonian translocations were also detected. Overall, a dose-dependent increase in the frequencies of CAs was detected in HSPCs collected at 6 mos after exposure, indicating the occurrence of genomic instability in HSPCs of exposed CBA/CaJ mice. Importantly, our data suggested for the first time a link between hypo-5hmC (not hypo-5mC) and genomic instability in HSPCs of CBA/CaJ mice exposed to 300 MeV/n ^{28}Si ions since both endpoints were evaluated in the same mouse. Both aberrant patterns of DNA methylation and chromosome instability are highly relevant for assessing cancer risks; however, they have not been applied with *in vivo* exposure to 300 MeV/n ^{28}Si ions. Hence, our results provide high-priority information for NASA. *Research funded by NASA grant #NNX11AK91G.*