Inflammatory Responses and Aberrant Patterns of DNA Methylation in the Liver of Mice Exposed Whole-Body to Titanium (^{48}Ti) Ions

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Liver is one of the target organs at risk for cancer induction from exposure to heavy and high energy (HZE) particles found in space. However, little is known about the early and late-occurring biological consequences in the liver induced by HZE particles that may be associated with increased cancer risk. Consequently, we initiated a study series to evaluate the biological effects, at the molecular levels, of ^{48}Ti ions (one of the important HZE particles found in space) on the liver tissue of exposed CBA/CaJ mice. These mice were part of a larger study series conducted to examine the genetic and epigenetic changes relevant to radiation exposure in hematopoietic stem/progenitor cells.

We gave adult male CBA/CaJ mice a whole-body exposure to various doses of 1 GeV/n ^{48}Ti ions (LET = 107 keV/μm) that are within the range of NASA interest, *i.e.* 0, 0.1, 0.25, or 0.5 Gy. Mice exposed to 0 Gy served as sham controls. After irradiation, the liver was collected from each mouse of each treatment group at 1 wk, 1 mo and 6 mos post-irradiation. There were five mice per treatment group at each harvest time. In each mouse, we prepared lysates from the liver for further analyses. Two biological endpoints known to be linked to cancer induction were used for evaluating the detrimental effects of ^{48}Ti ions on the liver of the same exposed mice. These included:

- Inflammatory responses, determined by the levels of activated nuclear factor kappa B (NF-κB) and the selected NF-κB regulated cytokines known to be involved in inflammatory responses, *i.e.* tumor-necrosis factor-alpha (TNF-α), interleukin 1-beta (IL-1β), and IL-6.
- DNA methylation patterns, assessed by the global (total) levels of 5-methyl cytosine (5mC) and 5-hydroxy-methyl-cytosine (5hmC) in DNA isolated from liver lysates.

Our data clearly demonstrated significant dose-dependent increases in the levels of activated NF-κB, TNF-α, IL-1β, and IL-6 in the liver of CBA/CaJ mice from 1 wk up to 6 mos post-irradiation (*ANOVA, $p<0.05$*). Such findings are indicative of chronic inflammation. For the study of aberrant DNA methylation, there were significant dose-dependent increases in the levels of 5mC in the liver of exposed mice collected at 1 wk and 1 mo (*ANOVA, $p<0.05$*). At 6 mos post-irradiation, however, a significant increase in the level of 5mC was found only in the liver of mice exposed to the highest dose of ^{48}Ti ions (0.5 Gy), in relation to that of sham control mice. In contrast, there were significant dose-dependent decreases in the levels of 5hmC in the liver of exposed mice (*ANOVA, $p<0.05$*) at all harvest time-points. Overall, the resulting data demonstrated that exposure to ^{48}Ti ions can induce chronic inflammation and a persistent aberrant pattern of DNA methylation (hypo-5hmC) in the liver of exposed mice. Further, since chronic inflammation and hypo-5hmC were found in the same mice at the late time-point (6 mos post-irradiation), our data suggest for the first time an association between hypo-5hmC (not hypo-5mC) and chronic inflammation. Importantly, these two biological endpoints are known to be linked to cancer induction. Hence, our findings suggest that exposure to ^{48}Ti ions during space flights may pose health risk to astronauts. Further investigation of the potential induction of ^{48}Ti-ion-induced liver cancer should be conducted to improve our understanding and mitigate risks.

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