

## **Oxidative stress and autophagy responses of osteocytes exposed to spaceflight-like radiation**

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Weightlessness and radiation, two of the unique elements of the space environment, causes a profound decrement in bone mass that mimics aging. This bone loss is thought to result from increased activity of bone-resorbing osteoclasts and functional changes in bone-forming osteoblasts, cells that give rise to mature osteocytes. Our current understanding of the signaling factors and mechanisms underlying bone loss is incomplete. However, it is known that oxidative stress, characterized by the excess production of free radicals, is elevated during radiation exposure. The goals of this study is to examine the response of osteocytes to spaceflight-like radiation and to identify signaling processes that may be targeted to mitigate bone loss in scenarios of space exploration, earth-based radiotherapy and accidental radiation exposure. We hypothesize that (1) oxidative stress, as induced by radiation, decreases osteocyte survival and increases pro-osteoclastogenic signals and that (2) autophagy is one of the key cellular defenses against oxidative stress. Autophagy is the process by which cellular components including organelles and proteins are broken down and recycled. To test our hypothesis, we exposed the osteocyte-like cell line, MLO-Y4, to 0.5, 1, and 2 Gy of simulated space radiation (<sup>56</sup>Fe radiation at 600 MeV/n) and assessed cell numbers, cell growth-associated molecules as well as markers of autophagy and oxidative stress at various time points post-irradiation. We observed a reduction in cell numbers in the groups exposed to 1 and 2 Gy of <sup>56</sup>Fe radiation. Collectively, flow cytometry and gene expression analysis revealed that radiation caused a shift in cell cycle distribution consistent with growth arrest. Compared to sham-treatment, 2 Gy of <sup>56</sup>Fe increased FoxO3, SOD1, and RANKL gene expression yet unexpectedly decreased LC3B-II protein levels at 4 and 24 hours post-IR. Taken together, these findings suggest that simulated space radiation invoke antioxidant, pro-osteoclastogenic, and growth arrest responses in osteocytes. The implications of reduced autophagy flux at the time points examined remain to be elucidated.

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