1. Title

2. Research Term
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3. Research Fields
Biomedical Sciences

4. Research Categories
Germinating Research

5. Research Theme
Effect of gravity on the fidelity of DNA damage repair

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8. Summary of Research
Objectives:
Space ionizing radiation causes human cells to induce DNA double strand breaks (DSBs). If the DSB is not properly rejoined, that possibly causes cell death, somatic mutations, chromosomal aberrations, or carcinogenesis. In this aspect, fidelity of DSB rejoining is one of the most critical factors that determine quality of genetic stability. The quality of genetic stability might be an issue for long term life under the space environment because one might be exposed to higher doses of cosmic radiation. It is known that the most of DSBs are properly repaired in the terrestrial environments, however, it is not clear whether those are repaired at the same efficiency even under the space environment. There are at least two pathways which can repair DSBs: homologous recombination (HR) and non-homologous end joining or micro-homology mediated recombination. Although NHEJ pathway causes a frequent deletion or insertion at the site of rejoining, HR is the error free and it contributes to maintain the genetic stability of living organisms. Therefore, the efficiency of HR might be a good indicator for assessment of the genetic stability of living organisms under the space environment. We assess the effect of gravity change on the fidelity of DNA repair because the gravity is the most significant factor which differs between terrestrial and space environment. To achieve the research purpose, we have established a cell line for site-specific DNA repair assay.
Methods:

A modified SCneo reporter construct was used in this study. In this SCneo construct, a site specific DSB can be induced by transient expression of a rare cutter restriction enzyme, I-SceI. When the induced DSB is repaired by HR, an active neo-resistant gene will be generated. Therefore, one can assess the HR frequency by counting the number of neo-resistants after DSB induction. An I-SceI expression vector was transfected to exponentially growing cells for transient expression of I-SceI endonuclease. Then the cells were incubated at 37 °C under hyper-gravity at 20 G from time 18 h to time 30 h (total of 12 h) during 48 hs’ incubation to allow the cells to repair DSBs. This hyper-gravity treatment did not cause any changes in plating efficiency (survival) of the cells.

Results and Potential for space experiments:

When the cells were treated with hyper-gravity at 20 G for 12 h during repair time after DSB induction, detected number of G418-resistant was slightly increased. The increase in HR frequency by hyper-gravity treatment could not be seen for background samples, which were assayed by transfection of empty vectors. Therefore, we suppose that the difference in HR frequency between hyper-gravity treated and untreated cells might be caused by difference in DSB repair efficiency. Our results suggest that the gravity change might affect on the fidelity of DNA damage repair pathway although further analysis to confirm the gravity effect on HR frequency is necessary. We believe that this molecular biological approach using SCneo system will provide us important information to maintain our individual health and to maintain the genetic stability of living organisms in space.

Publication List


URL