1. Title

2. Research Term
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3. Research Fields
Biomedical Science

4. Research Categories
Phase IB Research

5. Research Theme
Mutation induction by long-lived radicals produced by radiation and its protection by antioxidant materials

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8. Summary of Research
8.1 Objective/ Significance
One of the most important biological effects by radiation in space environment is carcinogenesis. There is accumulated evidence to show that multiple genetic changes are involved in the development of cancers. However, the molecular mechanism for induction of gene mutation by radiation still remains obscure. We have found that ionizing radiation produces organic radicals of which half life is more than several hours at room temperature in the irradiated mammalian cells. We have also demonstrated that ascorbic acid efficiently scavenges the long-lived radicals (LLRs) and suppresses radiation-induced mutation frequency.

In the present study, we challenge the following three issues on molecular mechanism for radiation-mutagenesis.
(1) A role of LLRs for the induction of gene mutation
(2) An effective protection against LLRs-induced mutation by antioxidant materials in view of space utilization
(3) Assignment of radiation-induced LLRs

On the basis of the data that we have obtained, we propose a new model for radiation-mutagenesis that the LLRs induce gene mutation by reducing the fidelity of DNA replication, but not inducing DNA strand breaks, possibly by affecting the DNA replication machinery. Also, we examine the assignment of LLRs by analyzing ESR spectora.

8.2 Methods.
(1) Measurement of amount of long-lived radicals (LLRs)
Amount of long-lived radicals (LLRs) in γ-irradiated or UV-irradiated mammalian cells was directly measured by electron spin resonance spectrometry (ESR) at 77 K.
(2) Cell killing and micronuclei formation by radiation
Cell killing effect by radiation was determined by colony formation assay. Radiation-induced micronuclei were determined by scoring the number of micronuclei per binucleated cell induced by cytochalasin B treatment.
(3) Mutation frequency induced by radiation
Mutation at hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene was detected in human embryo cells.
Synchronized human embryo cells were irradiated with 3 Gy of X-rays at G1 phase and then treated with AsA or EGCG for 2 hrs at 6 hrs, and 48 hrs post-irradiation. At 50 hrs post-irradiation, the cells were collected, inoculated into medium containing 40 µM 6-thioguanine (6-TG) and incubated for two weeks.

(4) Analysis for deletion mutation by multiplex PCR
DNA was isolated from 6-TG resistant mutant cells, and deletion mutation was analyzed by multiplex PCR targeted to exons 2 to 9 of the HPRT gene.

8.3 Results and Potential for Space Experiment
(1) A role of long-lived radicals for the induction of gene mutation
In the present study, we demonstrated the following evidence about a role of long-lived radicals (LLRs) for the induction of gene mutation. First, LLRs induce gene mutation without induction of DNA double strand breaks because they do not affect cell killing effect and induction of micronuclei by radiation. Second, removal of LLRs by AsA or EGCG prior to DNA replication suppresses the induction of gene mutation, suggesting that the existence of LLRs during DNA replication is critical for inducing gene mutation. Lastly, LLRs predominantly induce point mutation rather than deletion mutation. These results indicate that LLRs may not directly interact with the DNA molecules; however, they may induce gene mutation by reducing the fidelity of DNA replication.

(2) An effective protection against LLRs-induced mutation by antioxidant materials in view of space utilization
We demonstrated the following evidence about an effective protection against LLRs-induced mutation. First, the induction of gene mutation is suppressed by treatment with AsA or EGCG not only prior to but also posterior to irradiation. Second, removal of LLRs by AsA or EGCG before DNA replication is very effective to reduce radiation-induced gene mutation. These results indicate that antioxidant materials such as AsA and EGCG can prevent radiation-induced gene mutation effectively.

(3) Assignment of radiation-induced LLRs
We demonstrated the following evidence about an assignment of radiation-induced LLRs. First, LLRs yields of 99.8% are produced in proteins and those of 0.2% are in DNA. Second, a major protein radical of LLRs is assigned to sulfinyl radicals. These results imply that radicals in proteins induced by radiation play a key role in biological effect of ionizing radiation.

9. Publication List
9.1.1 Article

9.1.2 Proceeding of International Symposium

10. URL : Not available