136 Involvement of Long-Lived Radicals Induced by Radiation in the Induction of Mutation

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It is widely accepted that most of biological effects by ionizing radiation is caused by free radicals such as hydroxyl or hydrogen radicals. However, we formerly demonstrated that ionizing radiation produces organic radicals having a long lifespan whose halflife is nearly 20 h. In the present study, we studied the effect of (–)-epigallocatechin-3-*O*-gallate (EGCG), which scavenges the long-lived radicals, on X-ray-induced gene mutation. To know a role of long-lived radicals on radiation mutagenesis, the cells were synchronized, irradiated with 3 Gy of X-rays with combined treatment of 200 microM EGCG for 2 h before and after DNA synthesis, and examined for mutation frequency at the *HPRT* locus. The result indicated that the treatment with EGCG before, but not after, DNA synthesis postirradiation reduced mutation frequency. This result implies that the presence of long-lived radicals during DNA synthesis plays a role on the induction of gene mutation by ionizing radiation.

137 Induction of Gene Mutation and Cancer by Extra-DNA Bystander Effect of Long-lived Protein Radicals in Gamma Ray Irradiated Mammalian Cells.

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Though it is generally proposed that radiation mutagenesis is due to the damage of DNA by active oxygen species, the direct evidence has never been obtained. Previously we observed directly free radicals with high sensitive ESR measurement. The long-lived radicals with lifetime of several hours, produced by gamma-irradiation of cells, induce the gene mutation. The radical species, however, has not been identified yet.

In this study we discovered a new kind of long-lived radicals that disappear within 15 minutes. We have succeeded the identification of the species of the two kinds of long-lived radicals by computer simulation and the radical yields. The long-lived radicals whose lifetime is within 15 minutes are phenylalanine radicals, and those with lifetime of several hours are S-center radicals in protein.

Since the long-lived protein radicals induce gene mutation, the radicals are the plausible species responsible for extra-DNA bystander effects in cells.

138 Bystander mutagenic effect of alpha particle in human-hamster hybrid cells

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We constructed a novel hyper-sensitive assay system (Hx cell) in order to detect the mutagenic effect of radiation and also various modification factors, such as over gravity, and low dose rate. This system uses Hprt deficient hamster cells that carry a normal human X-chromosome. Although HPRT gene on the human X-chromosome is necessary for growth in HAT (Hypoxanthin, aminoputerin, thymidine) medium, any mutation or deletion of this human HPRT will not affect any viability of hybrid cells. When cells were exposed to gamma-rays, this system has been found to be 100-fold more sensitive for detecting mutations than that of the conventional system based on endogenous Hprt gene. By using an alpha particle microbeam, we show here that the mutation frequency of Hx cell cultures of which 20% were randomly irradiated with 20 alpha particles, is significantly higher than that of non-irradiated cultures. This result suggests that Hx cell is useful for analysis the bystander mutagenic effect in Hx cells.