

- 133 Somatic Mutation Caused by Low Dose Rate Tritium Radiation: Studies Using A Hyper-sensitive Detection System

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In some types of high LET radiation, the reversed dose rate effects are observed at a very low dose rate. To investigate whether the effect could be seen with low dose rate tritium radiation, a novel hyper-sensitive detection system was developed to detect Hprt-deficient mutations. This system uses Hprt deficient hamster fibroblast cells which carry a normal human X-chromosome, and appears to be able to detect a wide spectrum of mutations, even mutations affecting the expression of important genes in neighborhood of the *Hprt* gene. Exponentially growing cells were grown in medium containing different amounts of tritiated water (HTO) for appropriate periods. The frequency of Hprt-deficient mutations was then assayed by colony formation in medium containing 6-thioguanine. Differences in mutation frequencies induced by 1 Gy of tritium radiation were not observed within the range of dose rates used, suggesting that if a reverse dose-rate effect exists, it may not be observable with tritium radiation at dose rates over 0.04Gy/h. Molecular analysis of the Hprt locus in 6-TG resistant mutants induced by tritium showed that deletion sizes observed in the hamster cell's human X-chromosome under these conditions is much smaller in cells exposed at 0.04Gy/h than in cells exposed at 0.9Gy/h.

- 134 Dose-dependent Increase of Spi⁻ Mutations in the Spleen and Liver of *Atm*-disrupted Mice Following X-irradiation

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We have studied X-ray-induced mutations in *Atm* homozygously disrupted(-/-)mice. The *gpt*-delta transgenic mice with *Atm* deficiency were generated for *in vivo* mutation assay. After X-irradiation of the transgenic mouse, the genomic DNA was isolated from the liver and spleen, and subjected to Spi⁻ selection as described previously. The spontaneous mutant frequency of the spleen in *Atm* homozygously disrupted(-/-) mice was the same for that in *Atm* wild-type(+/+) mice. A linear increase in mutant frequency was observed as the dose of X-rays increases in *Atm*(-/-)mice and *Atm*(+/+) mice. The induced mutant frequency in the spleen of *Atm*(-/-)mice was significantly higher than that in the spleen of *Atm*(+/+) mice. We previously reported that *Atm* gene gave no appreciable influence on mutant induction in mouse liver with Spi⁻ assay. These results indicate that effects of *Atm* deficiency on mutation induction in the spleen are different from those in the liver. More detail information will be obtained through PCR analysis of the Spi⁻ mutants isolated from irradiated mice.

- 135 Scavenging of long-lived radicals by (-)-epigallocatechin-3-O-gallate and simultaneous suppression of mutation in irradiated mammalian cells

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Our previous studies indicate that mutation or transformation in the irradiated mammalian cells are induced by long-lived radicals(LLR) which exist in the cells for several hours after the irradiation. In this study, we chose (-)-epigallocatechin-3-O-gallate (EGCg) as an antioxidant of LLR to confirm the relationship between the induction of mutation by LLR. When EGCg is added to the gamma-ray irradiated GHE cells 2 h after the irradiation, EGCg reacts with LLR in irradiated GHE cells to reduce the radicals. The rate constants of the reaction between LLR and EGCg in GHE is $1.1 \times 10^{-3} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. When the EGCg is treated to HE17 cells after the X-ray irradiation, the mutation frequency is decreased drastically to that of the irradiated cells which are not treated with EGCg. These results strongly indicate that LLR in irradiated mammalian cells induce mutation. Our study suggests that the LLR located in proteins in the irradiated cells probably induce mutation. This is a new kind of extra-DNA bystander effect on mutation.