

224 Development of An Assay System to Detect Genomic Instability in Human Cells Bearing Partially Duplicated HPRT Gene

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Radiation exposure causes genetic instability in mammalian cells while the underlying mechanisms are not well understood. Although most of the animal studies used repeat sequences as the target for detection of the instability (e.g., minisatellites and pink eye-unstable locus), no pertinent assay systems are available in cultured cells. We therefore thought it useful to create cultured cells bearing partial, tandem duplication of a gene. We have chosen HPRT gene as a model since the duplication makes the cells HPRT(-) and hence selectable in the presence of 6TG, and HPRT(+) revertants due to intragenic recombination of the duplicated sequences in HAT medium. We constructed a Neo-tagged vector carrying 8.4 kb genomic DNA that covers HPRT exons 2-3. Transfection of human fibrosarcoma cells (HT1080) with the vector yielded Neo-resistant and 6TG-resistant clones. These cells reverted spontaneously from HPRT(-) to HPRT(+) with relatively high frequencies as expected [i.e., 10(-5) to 10(-4)]. Preliminary results will be presented on the relation between the reversion and forward mutation frequencies at the HPRT gene of some clones.

225 Influence of Reactive Oxygen Species in the Induction of Genetic Instability by Radiation

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Radiation generates reactive oxygen species (ROS) that may contribute to the induction of genetic instability. We examined the suppressive effect of a radical scavenger, ascorbic acid phosphate magnesium salt (APM), on the induction of delayed reproductive cell death by radiation. The delayed cell death was determined by two successive colony formation periods. The treatment with APM was applied either primary and secondary colony formation. The result indicated that the APM treatment during primary colony formation, but not secondary colony formation, suppressed the delayed reproductive cell death. We also demonstrated that the rapid increase and then decrease of the amount of hydrogen peroxide (ROS) in X-ray-irradiated cells by 5 hr postirradiation and then the level of hydrogen peroxide gradually decrease to a base line within two weeks. The APM treatment kept the hydrogen peroxide production in a lower level than an untreated control. These results suggest that the cause of genetic instability might be fixed by partly ROS during 2 weeks postirradiation.

226 Radiation-induced delayed chromosomal instability caused by large deletion

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In this study, we examined whether X-ray-induced gene deletion at the HPRT locus induces delayed instability in X-chromosome. SV40-immortalized normal human fibroblasts, GM638, were irradiated with 3 Gy of X-rays, and the HPRT mutants were isolated in the medium containing 60 µM of 6-thioguanine. The molecular structure of the HPRT mutations was determined by multiplex polymerase chain reaction of the eight exons of the HPRT gene. The size of deletions in total HPRT deletion mutants were determined by PCR amplification using sequence tagged site (STS) primers. Delayed chromosomal instability in X-chromosome was analyzed by whole human X-chromosome paint FISH. GM638 and spontaneous mutants, which showed no detectable change in the HPRT gene, did not induce X-chromosomal rearrangements, however, 3 Gy-induced mutants with large deletion showed delayed instability involving X-chromosome. These results suggest that ionizing radiation-induced large deletion is involved in initiation and perpetuation of radiation-induced genomic instability.

227 The dependency of radiation-induced genetic instability on the stage of spermatogenesis

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We previously reported that the genomic instability was observed on F1 mice born with irradiated sperm. In this report, we examined whether this phenomenon had the dependency of irradiation stage or not. It was only observed as spermatozoa stage irradiation, but not spermatid, -cyte and -gonia stage. Moreover, in two-next generation (F2) mice, the mutation frequency does not increase. These findings suggest that the genomic instability which we estimated especially occurs at spermatozoa stage.

228 Sensitivity to ionizing radiation in Recql4 -/- cells

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Mutations in the Recql helicase domain cause several genetic disorders in humans, including Werner syndrome, Rothmund-Thomson syndrome and Bloom syndrome. Common phenotypes of these diseases include premature aging and shortened lifespan. Genomic instability is also observed in the cells of these patients. In order to understand the role of RECQL4, which is the product of the gene responsible for the Rothmund-Thomson syndrome, we prepared two independent +/- ES cell lines and