103 Effect of introduction of a normal chromosome 8 on Werner syndrome cells

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Werner syndrome (WS) is a rare autosomal-recessive human disorder characterized by the premature appearance of several features of normal aging in young adults. Cells derived from WS patients demonstrate a severely limited capacity to divide in vitro. Recently, the gene responsible for WS(WRN gene), mapped on short arm of chromosome 8, has been cloned by positional cloning. However, its function is still unknown. Therefore, we examined whether introduction of a normal chromosome 8 complements the deficiency in WS cells or not. We analyzed mutations at HPRT locus in WS cells and three micro cell hybrids with an extra chromosome 8 by multplex PCR. We demonstrated that WS cells showed an usually high proportion of deletion mutations compared to control cells as reported by other investigators. In addition, we found that this unique phenotype was not corrected by the introduction of chromosome 8. The results suggest a dominant-negative nature of a mutation of the WRN gene.

104 Analysis of Mutations in the Human HPRT Gene Induced by High LET Heavy-ion Irradiation

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Not only from the viewpoint of basic research on radiation biology but also from that of the application of heavy-ions in radiation therapy, it is interesting to study the characteristics of the mutations induced by heavy-ion irradiation. We have analyzed the hprt mutations in human cultured cells, WI-L2-NS and TK6, at DNA sequence level. The WI-L2-NS has a mutation in codon 237 of p53. The survival curves were determined by limiting dilution method and mutant clones were selected as resistance to 5 μg/ml of 6-thioguanine. The difference in radiosensitivity for X-rays between these two cell lines were preserved at only low-LET (high-energy) region of C- and Ne-ions. DNA analysis for these mutant clones also showed the differences between the two cell lines but we have to confirm this result. On the other hand, we have observed a significant difference in the mutational events of TK6 cell-line between C- and Ne-ion at high LET obtained by the ions just stopping. The majority of mutational events recovered from the the high-LET C-ions was a point-mutation in contrast to the very few recovery of such event by the high-LET Ne-ion.

105 Detection of Deletion Mutants of CPD-Photolyase Gene Using Medaka by a PCR System

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We tried to detect γ-ray induced deletion mutants of CPD-photolyase (phr) gene of the medaka by a PCR system utilizing their DNA polymorphism between different populations. Males from the northern population were irradiated with 4.75Gy γ-rays and from 1day to 12 days after irradiation they were mated with females from the southern population.

We got 2182 normally hatched embryos and 313 abnormally developed embryos from γ-irradiated sperm or spermatid. We found 4 mutants out of 313 abnormal embryos. This mutation rate is close to previously reported one ditermined by a SLT system or a AP-PCR system.