

246 Mechanism of radiation-induced delayed mutagenesis

Hiroko YAMAJI¹, Keiji SUZUKI¹, Seiji KODAMA¹, Masami WATANABE¹, ¹Div. Radiat. Biol., Dep. Radiat. Biol., Grad. Sch. Biomed. Sci., Nagasaki Univ.

It has been shown that radiation-induced genomic instability, induced in the progeny of surviving cells, express various delayed phenotypes. In the present study, we examined mechanism of delayed mutagenesis. We established CHO cells containing the bacterial *LacZ* gene. The *LacZ*-positive cells formed colonies, which were stained blue in the presence of X-gal. We found that X-irradiation increased the frequency of white colonies, which have the mutated *LacZ* gene, in a dose dependent manner. Delayed mutagenesis was examined 15PDN after irradiation, and the frequency of *LacZ*-negative cells was higher in X-ray-surviving cells than the control cells. The *LacZ* gene was amplified using PCR, and more than half of the spontaneous *LacZ*-negative cells showed the presence of the *LacZ* gene. Although approximately 20% of *LacZ*-negative cells isolated immediately after irradiation contained the *LacZ* gene, none of the delayed mutants have the *LacZ* gene. These results indicate that delayed mutagenesis is caused by recombination-mediated gene deletion.

247 Construction of Chicken DT40 Knockout Strains of EWS, FUS/TLS and RBP56/TAF15 Genes (2)

Fumiko MOROHOSHI¹, Misao OHKI¹, ¹Cancer Genomics Div. Natl. Cancer Center Res. Inst.

Human EWS, FUS/TLS and RBP56/hTAFII68/TAF15 comprise a sub-family of RNA binding proteins. Rearrangements of these genes were found in several types of malignant tumors. Fusion proteins consisting of N-terminal half of these proteins and transcriptional regulatory proteins play important roles in pathogenesis of tumors. To study the function of these proteins in normal cells, we planned to construct disruptants of these genes using chicken DT40 cells in which the expression of these genes was observed. Library screening and RACE analysis showed that chicken FUS cDNA encoded a protein of 504 amino acids with 80 % similarity to human FUS protein and chicken RBP56 cDNA encoded a protein of 472 amino acids with 63 % similarity to human RBP56 protein. We generated disruptants of RBP56 gene using targeting vectors in which exons encoding the RNA binding domain were replaced by drug resistant cassettes.

248 Radiation Sensitivity of Breast Cancer Patients as Determined with a Micronucleus Assay.

Hitomi SUDO¹, Sadayuki BAN¹, Masashi SAGARA¹, Yoshihumi MATSUI¹, Syuhei NODA¹, Mayumi IWAKAWA¹, Yoshinobu HARADA¹, John Cologne², Takashi IMAI¹, ¹Frontier Res. Ctr. NIRS ²Statiscs, RERF,

The micronucleus assay has been extensively used to evaluate the radiation sensitivity of human individuals. Use of peripheral blood lymphocytes has become popular for quantifying human radiosensitivity, because of the ease of sample preparation. Micronucleus data obtained from 45 normal females and 112 breast cancer patients were analyzed. Both the spontaneous- and the X-ray-induced-micronucleus frequencies in patients blood were significantly higher than those in normal individuals. Because it is widely believed that one key mechanism which leads to spontaneous micronucleus formation involves an imbalance of chromosomal segregation, chromosomal instability in patients blood might be greater than that in normal individuals. Recently, Kuschel et al (2002) demonstrated that ratios in two SNPs on XRCC3 were significantly different between cancer patients and normal females. Then, we can suppose that radiation-related genes with low penetrance may be involved in mammary tumorigenesis and also, in patients radiation susceptibility.