138 Detection of instability with minisatellite maker in mouse sarcoma

Kzunori Shiraishi[1], Ryou Kominami[2], Yoshiaki Takahashi[2], Ohtura Niwa[1]; 1) Radiation Biology Center, Kyoto Univ., 2) Faculty of Medicine, Niigata Unv.

Minisatellite mutation can serve as one of markers of genomic instability. In this study we report analyses of mutation at the minisatellite Pc-3 locus in mouse sarcoma cell lines.
Sarcoma cell lines were established from MCA (methylcholanthrene)-induced sarcomas of C57BL/6N×C3H/He F1 mice. Subclones were isolated for further analysis. Minisatellite Pc-3 has a repeat of AGGCAGG and spans about 1.5kb in length for C57BL/6N and C3H/He. Pc-3 marker has a superior point that the only small quantity of DNA sample is required, because it allows to detect mutation with PCR. In this experiment, we analyzed PCR products by Southern blotting.
The mutant frequency differed between sarcoma cell lines. Although we previously had reported that minisatellite Pc-1 mutation correlated with c-myc amplification, we found that Pc-3 did not. In addition, two minisatellites differed in mutant frequencies. We showed that minisatellite Pc-1 and Pc-3 markers were able to detect mutation in almost all sarcoma cell lines.
It is possible that the mutation is affected by sequences, binding proteins and chromatin structure in tumor cell. Therefore, use of various makers is required to detect mutation inflicted by various stresses.

139 Radiation-induced delayed damage in *scid* mouse cells

Seiji KODAMA[1], Mohamad MD. DESA[1], Keiji SUZUKI[1], Fumio SUZUKI[2], Masami WATANABE[1], [1]Lab. Radiat. & Life Sci., Schl. Pharm. Sci., Nagasaki Univ., Nagasaki 852-8521, [2]Dept. Regul. Radiobiol., Res. Inst. Rad. Biol. & Med., Hiroshima Univ., Hiroshima 734.

We previously showed that UV-irradiation does not induce delayed damage and that pre-irradiation of a low dose of radiation reduces the amount of delayed damage, suggesting the involvement of DNA strand breaks and its repair to produce the delayed damage by radiation. To know how the repair for DNA strand breaks is involved in the delayed damage formation, we studied delayed giant cell formation and delayed reproductive death in X-ray-irradiated *scid* mouse cells. The *scid* mouse cells are hypersensitive to radiation and defective in DNA dependent protein kinase catalytic subunit (DNA-PKcs) which plays a crucial role in the DNA strand break repair. We determined the X-ray-induced delayed damage at a dose equivalent to 10% survival in a *scid* mouse cell line (2 Gy), a heterozygous *scid* mouse cell line (6 Gy) and a wild-type mouse cell line (6 Gy). The amounts of delayed damage observed in the radiation sensitive *scid* cell line and other two cell lines with normal sensitivity were similar although the doses exposed were different. The result indicates that the amount of initial DNA strand breaks *per se* is not directly responsible for that of the delayed damage, suggesting that some radiation effect responsible for the delayed damage remains after the repair of the initial DNA damage and that the similar amount of such a radiation effect may remain in both the repair proficient cells and the repair deficient cells when they are irradiated at a dose for the same survival.

140 Genome Abnormality Appeared in X-Irradiated Scid Mouse Embryo Fibroblasts

Fumio SUZUKI[1], Kiyokazu TAKADA[2], Hirohiko YAJIMA[1], Masaaki TATSUKA[1], Osamu NIKAIDO[2], Toshiaki OGIU[3], [1]Hiroshima Univ., [2]Kanazawa Univ., and [3]Natl. Inst. Radiol. Sci.

Severe combined immunodeficient (scid) mice, which are deficient in DNA-dependent protein kinase (DNA-PK) activity, are hypersensitive to ionizing radiation because of a related defect in repair of DNA double-strand breaks. In this study, we analyzed biological response of scid cells to ionizing radiation *in vitro*. Three scid, three C.B-17 and two (C.B-17 X scid) F1 mouse cell lines were established from 16 or 17-day-old embryos. As reported previously, all scid mouse embryo cell lines were about twice as sensitive to X-rays as their C.B-17 and F1 counterparts, and showed an enhanced production of micronucleated cells after X-irradiation. Furthermore, an extensive stimulation of plasmid (pSV2*neo*) DNA transfer were observed when scid cells were irradiated with X-rays after a certain period of DNA transfection. These results suggest the presence of abnormal response to ionizing radiation in scid mouse embryo cells.