

Genetic instability

220 Mechanism of radiation-induced delayed mutagenesis.

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Genomic instability is induced in the progeny of radiation irradiated surviving cells. Which is manifested by the expression of various delayed phenotypes. In the present study, we examined the molecular mechanism of delayed mutagenesis in CHO-LacZ cells, which harbor the reporter plasmid of *LacZ* gene. As this CHO-LacZ cells produced beta-galactosidase, they formed blue-stained colonies (LacZ⁺) in the presence of X-gal as substrate, on the other hand, these cells formed white colonies (LacZ⁻) when this gene mutate. After X-irradiation, frequency of LacZ⁻ colonies increased depending to dose, which indicated that X-irradiation caused the *LacZ* gene mutation in CHO-LacZ cells. Delayed mutagenesis was examined 15 PDN after irradiation, and the frequency of LacZ⁻ colonies in X-ray-surviving cells was higher than that in control cells. Next, we studied mutation spectrum of LacZ⁻ clones. Using PCR, the *LacZ* gene was absent in approximately 75% in delayed LacZ⁻ clones. This result was like to mutation spectrum in the spontaneous LacZ⁻ clones (72%). These results indicate that delayed mutagenesis rise spontaneous mutation level.

221 A Role of DNA Double Strand Break Repair in the Induction of Genetic Instability

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We previously demonstrated that *scid* mouse cells were hypersensitive to genetic instability induced by radiation. To confirm this result, we established *scid* mouse cells expressing human DNA-PKcs by the introduction of a human chromosome 8. The human DNA-PKcs complemented the hypersensitivity to the induction of delayed reproductive death in *scid* mouse cells, indicating that NHEJ play a role to suppress the induction of genetic instability. To know a role of double strand break repair in the induction of genetic instability further, we investigated delayed reproductive death in chicken Rad54^{-/-} DT40 cells. The result indicated no difference in delayed reproductive death between wild-type and Rad54^{-/-} DT40 cells, suggesting that homologous recombination might play little role in the induction of delayed reproductive death.

222 X-ray-induced genomic instability in *Atm* knock-out mouse cells

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The gene responsible for ataxia telangiectasia (AT) encodes ATM protein that plays a major role in the network of a signal transduction initiated by DNA-double strand breaks. To elucidate how radiation-induced genomic instability is modulated by dysfunction of ATM protein, we examined delayed chromosomal instability in individual cell lines established from wild type *Atm*^{+/+}, heterozygote *Atm*^{+/-} and knock out *Atm*^{-/-} mouse embryos. The *Atm*^{-/-} mouse cells show elevated chromosomal and telomeric instability compared with *Atm*^{+/+} mouse cells. The telomeric instability was characterized by the abnormal telomere FISH signals including loss and gain of the signals in the end of chromosome. This suggests that *Atm* deficiency makes telomeres vulnerable to breakage. Thus, the present study shows that ATM protein plays an essential role to maintain telomere integrity and prevent chromosomes from end-to-end fusions.

223 Radiation-Induced Delayed Chromosomal Instability

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Ionizing radiation induces chromosomal instability that is transmitted over many generations after irradiation in the progeny of surviving cells. It is suggested that delayed chromosomal instability plays a role in the development of cancers. However, the trigger for inducing the instability remains unknown. To know the trigger, we constructed two types of microcell hybrids by microcell-mediated chromosome transfer where a human chromosome 11 was transferred into a mouse cell line. In one type of microcell hybrids, 4 Gy-irradiated human chromosome 11 was introduced into unirradiated mouse cells, and in the other type of microcell hybrids, unirradiated human chromosome 11 was introduced into 4 Gy-irradiated mouse cells. Chromosome aberrations were analyzed by fluorescence in situ hybridization. The results indicated that not only the irradiated chromosome per se but also the irradiated recipient cells contribute to the trigger for the induction of delayed chromosomal instability by radiation.