212 Development of a new experimental system to detect biological effect of Radon using mouse cells.

Radon exposure contributes to nearly half of the environmental radiation, however, its molecular nature of the biological effects has not been elucidated. We, therefore, have started the development of the experimental procedure for detecting the biological effects, especially genetic one, caused by radon. In the control experiments, mouse FM3A cells were grown on soft-agar plates, and irradiated by up to 10 Gy of X-ray. DNA sample was extracted from the cells, and analyzed by Southern blot hybridization technique using hypervariable repeat Pc-1 DNA as a probe. Dynamic mutation at Pc-1 locus was not observed in the cells exposed by up to 10 Gy of X-ray. Also the cells growing on soft-agar plates were exposed to 100K-1MBq/cubic-meter of Radon, and the Pc-1 locus was analyzed. The experiments are still on the half way, and we will describe the results and the reliability of this new experimental procedure.

213 Development of an automatic analyzer of DNA break, based on atomic force microscopy
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We have developed a novel automated system for the quantitative evaluation of DNA damage based on an atomic force microscopy (AFM). By visualizing DNA molecules and measuring their lengths directly and automatically, we succeeded to detect the increase in DNA damage caused by several hundred Gy-irradiation with the statistical significance (p < 0.05). The result demonstrates the improvement of sensitivity by one order of magnitude compared to previously reported AFM-based systems. We found AFM more suitable for the quantitative detection of DNA break than electron microscopy, because it is easy to operate, and can be used in high throughput analysis. These results suggest that our automatic analyzer is powerful tool for the quantitative evaluation of DNA break.

214 The complementation of abnormal phenotypes of Werner syndrome cells by expressing WRN gene

Werner syndrome (WS) is autosomal recessive disorder exhibits features of premature aging. The precise role of werner protein (WRN) remain to be determined. In order to reveal the causes of abnormalities, We introduced human normal chromosome 8 codes WRN gene into the ws cell immortalized by htert. Ws cell has previously been shown to have hypersensitivity to 4-nitroquinolinol-1-oxide (4NQO), and cis-platinum (II) dichloride (CDDP), hydroxyurea (HU) and have increased chromosomal instability after X-ray irradiated. So we tested these agents and treatment, the results show the hyper sensitivity to 4nqo and cddp, chromosomal instability were complemented. And hu was partially complemented. These findings suggest WRN protein has functions concerning recombinational DNA repair. Further more, these complementation have not seen in the ws cell has human normal chromosome 8 immortalized by SV40. This may be the result of occurring further mutation caused by deficient of WRN protein.

215 Analysis of nucleosome positioning by the use of ionizing radiation and psoralen

We analyzed the nucleosome positioning in the promoter of the human c-FOS gene, using ionizing radiation and 4,5',8-trimethylpsoralen as in vivo nucleosome footprinting agents. The distribution of the lesions induced by these agents was visualized by ligation-mediated PCR or terminal transferase-dependent PCR. We firstly studied the influence of the transcriptional activation of the c-FOS gene on a nucleosome positioned in the promoter of this gene. When human fibroblasts were serum-induced, we could not detect any change in the position of this nucleosome, although concurrent chemical modifications of nucleosomes in the c-FOS region upon induction have been reported. Then we studied cell cycle-dependent changes in the positioning of this nucleosome. In mitotic HeLa cells, the positioning of the nucleosome appeared to be lost. This result suggests random distribution of nucleosomes in this promoter in mitotic chromosomes.