§42. Assessment Study on Biological Effects of Low-dose Radiation

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An exposure condition of tritium radiation from nuclear fusion reactor could be a long-term exposure at low dose rate. In this study, we focused on i) establishment of a hypersensitive assay system for radiation biological experiments and ii) biological responses to low-dose (rate) radiation, and iii) the mechanism of DNA damage response. Followings are summary of the results obtained in this study.

i) Establishment of hyper-sensitive assay system for radiation biological experiments.

The biological effects of low dose (rate) radiation are still unclear because any experimental system, which allows us to obtain quantitative data with low dose radiation, has not been established. Therefore, we are trying to establish a novel experimental system that can examine the biological effects of low dose (rate) tritium radiation, for the both *in vitro* and *in vivo*. In this study, we established a hypersensitive mutation detection system using hamster cells carrying human X chromosome. We also tested availability of transgenic mice that carries a mutation reporter gene, gpt-delta. Another transgenic mice line that uses Rev1, a error prone repair gene, also tested their possibility to use as a hyper-sensitive system of carcinogenesis.

In the human-X-carrying hamster cell system, any somatic mutations and gene deletions in the additional human X chromosome do not affect the cell viability when cells are cultured in normal medium. This system appears to be able to detect a wide range of mutation spectrum, even if those mutations affect the expression of important human genes for cell survival. The system showed about 100-fold sensitivity compared to the conventional system that uses *Hprt* gene locating the internal X-chromosome.

Because the Rev1-transgenic mice showed the high incidence of malignancy we are now testing the possibility to use as a "mammalian Ames test" to detect any mutagenic effects of DNA damaging agents.

Using p53 (a tumor suppressor gene) knockout mice, we also investigated the induction of chromosomal aberrations by tritium radiation. It was suggested that p53 stimulates repair system and suppress chromosomal aberrations. Because p53 induces apoptosis after low dose tritium uptake [1], it may protect the mice from mutagenesis by both the activation of DNA damage repair and induction of apoptosis. The p53-knockout mice could be useful to test the in vivo effects of low dose tritium radiation. These hyper-sensitive detection system will be further tested to establish the experimental system for low dose (rate) exposure of tritium radiation.

ii) Biological responses to low-dose (rate) radiation.

Radio-adaptive response is a biological defense mechanism in which low-dose ionizing irradiation elicits cellular resistance to the genotoxic effects of subsequent irradiation. However, its molecular mechanism remains largely unknown. We have demonstrated that the recognition of primary-dose and adaptive response could be mediated by a feedback signal pathway that involves protein kinase C (PKC), p38 mitogen activated protein kinase (p38MAPK), and phospholipase C (PLC). We have started experiments to clarify the effect of PKC knockdown by siRNA on radio-adaptive response. By the experiments, we may verify the importance of PKC pathway for expression of radio-adaptive responses.

iii) Analysis of the mechanism of DNA damage response

We are investigating molecular biology of DNA damage repair genes. For example, NBS1 is a critical protein for regulation and activation of DNA damage response [2]. We have established a reporter cell system for homologous recombination repair and studied the effect of NBS1 mutation on homologous recombination. We found that the NBS1 regulating homologous recombination through the regulation of nuclear localization and foci formation of MRE11/RAD50 protein complex. Interestingly, ATM. another critical regulator of DNA damage response, was not essential for regulation of homologous recombination. This suggests that ATM may functions on radiation-damage specific end-processing or regulation of non-homologous recombination that is known to be a major pathway for DNA damage repair in mammalian cells [3].

1) Umata, T. Rad. Biol. Res. Commun. 42, 282-292. 2007 (in Japanese)
2) Morishima K. *et al*. Biochem Biophys Res Commun. 362, 872-879, 2007.
3) Sakamoto, S. *et al*. Oncogene 26, 6002-6009, 2007.