

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

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The exposure condition of tritium radiation to living organisms from nuclear fusion reactor can be expected as a long-term, low dose rate exposure, because of the mechanical safety systems that will be applied to the reactor. However, at present, only a little experimental data are available for the biological effects of low dose or low dose rate radiation. To prepare the experimental data for biological effects of low dose tritium, the present study focused on, i) establishment of a hypersensitive assay system, at the both cellular and animal level, ii) biological adaptive responses to low-dose radiation, and iii) the mechanism of DNA damage response.

Followings are summary of the results.

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

To clarify the biological effects of low dose (rate) radiation, hypersensitive experimental systems that enable us the direct quantitative analysis are necessary. In this project, we have established a novel experimental system that can examine the biological effects of low dose (rate) tritium radiation, for the both cellular and animal level. At the cellular level, we established a hypersensitive mutation detection system using hamster cells carrying human X chromosome. In addition, at the animal level, we confirmed the availability of transgenic mice that carries a mutation reporter gene, *gpt-delta*. Another transgenic mice that are over-expressing *Rev1*, an error prone repair gene, is also under experimentation to assess their possibility to use as a hypersensitive carcinogenesis system. We also analyzed the function of p53 in tumorigenesis in order to clarify the mechanism that suppresses any genetic instability by radiation exposure.

The human-X-carrying hamster cell system appeared 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 endogenous *Hprt* gene¹⁾. Another hyper-sensitive mutation detection system using *gpt-delta* mice is also appeared to be able to detect the effect of low level tritium radiation²⁾. The *Rev1*-transgenic mice showed the high incidence of malignancy, therefore, the *Rev1* mice are possible to use as a "mammalian Ames test" which detect any mutagenic effects of DNA damaging agents.

Using p53 (a tumor suppressor gene) knockout mice, we 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, it may protect the mice from mutagenesis by both the activation of DNA damage repair and induction of apoptosis³⁾. These hyper-sensitive detection system will be further tested to establish the experimental system to monitor the biological effects of low dose (rate) exposure to tritium radiation.

ii) Biological adaptive responses to low-dose 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 which involves protein kinase C (PKC), p38 mitogen activated protein kinase (p38/MAPK), and phospholipase C (PLC). We are doing 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

Understanding the molecular mechanism of cellular DNA damage responses is another important point of view to assess the biological risk of low dose (rate) radiation. If the mechanisms are fully clarified, we believe that one can simulate the biological responses to low dose tritium radiation. We also investigated molecular function of DNA damage repair-related proteins such as histone H2AX, ATM, and NBS1. NBS1 protein is a critical factor for regulation and activation of DNA damage response. We showed that ATM as well as NBS1 is recruited to damaged-chromatin in a phosphorylated-H2AX-dependent manner. Nuclear foci (protein granules at the damaged site) formation of phosphorylated ATM and ATM-dependent phosphorylation is repressed in H2AX-knockdown cells. We also found that the antibody for phosphorylated-H2AX co-immunoprecipitates an ATM-like protein kinase activity *in vitro* and recombinant H2AX increases *in vitro* kinase activity of ATM from un-irradiated cells. Moreover, H2AX-deficient cells exhibited a defect in ATM-dependent cell cycle checkpoints. Taken together, gamma-H2AX has important role for effective DSB-dependent activation of ATM-related damage responses via NBS1⁴⁾.

The goal of our study is prepare the clear experimental data that indicate the risk/safeness of low dose or low dose rate tritium exposure. Experiments using the newly established hypersensitive systems are in progress.

- 1) Tauchi, H. et al: J. Radiat. Res. 50 (2009) 441-448.
- 2) Uehara, Y. et al: Mutat. Res. 670 (2009) 24-31.
- 3) Umata, T. et al: Int. J. Radiat. Biol. 85 (2009) 1082-1088.
- 4) Kobayashi, J. et al: Biochem. Biophys. Res. Commun. 380 (2009) 752-757.