§29. Study on Biological Effects of Tritium at an Animal Level

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At present, only a little experimental data are available for the biological effects of low dose or low dose rate radiation. To prepare the data for biological effects of low dose tritium, the present study focused on, i) analysis of biological effects of tritium by using genetically engineered animals, ii) analysis of the mechanism of DNA damage response, and iii) analysis of the biological effects of tritium at the cellular level.
Followings are summary of the results.

i) Analysis of biological effects of tritium by using genetically engineered animals
In the former project, we have established a novel experimental system that can examine the biological effects of low dose (rate) tritium radiation at an animal level. We used two strains of transgenic mice, the gpt-delta mice[1)] and Rev1 mice. The gpt-delta mice carry a mutation reporter gene, and Rev1 mice are over-expressing Rev1. Rev1 is a gene for translesion synthesis, an error prone repair pathway, which acts on DNA damage such as DNA base modification induced by UV or ionizing radiation. We also used the *p53* (a tumor suppressor gene) knockout mice[2)], in order to clarify the importance of the regulation of DNA damage checkpoint in prevention of teratogenic effects of low dose tritium.
The gpt-delta mouse is a novel system that can be successfully applied to experiments that assess the biological effects of low dose radiation. Tritiated water was administrated to the gpt-delta mice at low dose rate, and the mutation frequency at several tissues were analyzed. We found that the mutation induced by 6Gy of tritium were only deletion type mutation.
Using the Rev1 transgenic mice, we assessed the frequency of tumor induction by alkylating agent, azoxymethane. the experimental results suggested that the Rev1 mices are hypersensitive to tumor induction by any DNA damaging agent.
The frequency of teratogenic effects did not increase in *p53* knockout mice exposed to low dose tritium compared to wild mice. As the mice used were few, it is thought that the difference of the frequency of teratogenic effects between wild and *p53*-deficient mice were not able to be detected. It will be necessary to increase the number of mice to detect a significant difference statistically in the future.

ii) 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.
Activation of the DNA-dependent protein kinase (DNA-PK) and AKT signaling pathway was found in cellular response to X-ray exposure[3)]. Therefore, we tested whether the pathway is also activated by HTO exposure. The result suggested that the activation of AKT signaling pathway by HTO exposure might be regulated by the receptor of epidermal growth factor (EGFR).
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 NBS1[4)].

iii) Analysis of the biological effects of tritium at the cellular level
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* gene[4)]. We started the experiments by using the system, to test the mutation induction by low dose rate exposure at G1/G0 stage of cell cycle.
Radio-adaptive response is a biological defense mechanism in which low-dose ionizing irradiation elicits cellular resistance to the genotoxic effects of subsequent irradiation. 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. In addition, we found that an adaptive response was induced by pre-treatment of the cells with low concentration of tritium compound such as ^{3}H-thymidine.

1) Uehara, Y. et al: Radiat. Res. 174 (2010) 611-617.
2) Umata, T. et al: Int. J. Radiat. Biol. 85 (2009) 1082-1088.
3) Shimura, T. et al: Oncogene 29 (2010) 4826-4837.
4) Tauchi, H. et al: J. Radiat. Res. 50(2009)441-448.