ALTERATION OF GENOME STRUCTURE INDUCED BY VERY LOW DOSE-RATE IRRADIATION IN MOUSE TISSUES

Tetsuya Ono*[*1], Yoshihiko Uehara[1], Naohito Okudaira[1], Kazuo Fujikawa[2], Nao Kagawa[2], Mitsuaki Yoshida[3], Isamu Hayata[3], Takehiko Nohmi[4], Tsuneya Matsumoto[5], Yoichi Oghiso[5], Kimio Tanaka[5], Kazuaki Ichinohe[5], Shingo Nakamura[5], and Satoshi Tanaka[5]

[1]*Dept of Cell Biology, Tohoku University Graduate School of Medicine, 980-8575 Sendai, Japan*
*Email:*tono@mail.tains.tohoku.ac.jp
[2]*Faculty of Science and Technology, Kinki University, Osaka, Japan*
[3]*National Institute of Radiological Science, Chiba, Japan*
[4]*National Institute of Health Science, Tokyo, Japan*
[5]*Institute for Environmental Sciences, Aomori, Japan*

ABSTRACT

To understand the effects of low dose-rate radiation on genome structure in vivo, we examined gene mutation and chromosomal abnormality in mouse tissues. The mutation was studied on transgenes in the spleen and liver and also on the Dlb1 gene in intestinal stem cells. The mice were irradiated for 483 consecutive days at three dose-rates of 0.0323, 0.65, and 12.5 μGy/min, which resulted in total doses of 21, 414, and 8,000 mGy, respectively. Statistically significant increases were observed for all indices examined when the total dose was 8,000 mGy, whereas no significant difference was observed with 21 mGy or 414 mGy.

Keywords: Low dose-rate, Radiation, Mutation, Chromosomal abnormality, Mouse

1 INTRODUCTION

A genome contains information needed for maintenance of life of each individual, and alteration of genome structure could result in serious problems, such as cell death, cellular transformation, cancer, various diseases, and acceleration of senescence. It is now well established that the genome in each living cell is damaged routinely by many extrinsic as well as intrinsic factors, such as natural background radiation, solar UV light, reactive oxygen species, etc. The damage, however, is restored by different kinds of DNA repair pathways present in cells. Thus, the genome in living cells is maintained in a dynamic balance between damage and repair (Friedberg, Walker, Siede, Wood, Schultz, & Ellenberger, 2006). Radiation exposure induces many types of DNA damage, but the damage is repaired without any alteration in the DNA sequence in most cases. Some damage, unfortunately, is repaired inaccurately and results in DNA sequence alteration. This alteration is called mutation when it changes the biological information of the DNA and is called chromosomal abnormality when it is observed as a change in chromosome structure. The efficiencies of radiation-induced mutation and chromosomal abnormality are known to be reduced if the radiation is exposed at a low dose-rate, which is called dose-rate effect (Hall, 2000). This effect is important in evaluating radiation risk because humans can be exposed to both high dose-rate and low dose-rate radiation. Although the effects of high dose-rate radiation have been studied in many biological systems, little has been investigated for low dose-rate radiation effects, especially at the very low dose-rate that corresponds to the levels of radiation workers (20 mGy/yr) or astronauts (a few 100 mGy/yr) (Ono, 2007). One exception is the mutagenic effect of radiation on germ line cells in mice performed by Russell's group. They observed dose-rate dependency in the range of 900 – 8 mGy/min but no such effect in the range of 8 mGy/min – 7 μGy/min (Russell & Kelly, 1982a, b). They concluded that the efficiency of mutation induction becomes constant and the amount of mutations depends on the irradiated dose but not on dose-rate if the dose rate is lower than 8 mGy/min. Here, we examine mutations and chromosomal abnormalities in somatic tissues of mice exposed continuously to very low dose-rate radiation for a long period of time.

2 MATERIALS AND METHODS

2.1 Mice and irradiation

The gpt-delta mice containing lambda EG10 genomic DNA (Nohmi, Suzuki, Masumura, Yamada, Matsui, Ueda, et al. 1999) were mated to SWR mice (Shaver-Walker, Urlando, Tao, Zhang, & Heddle, 1995) to obtain F1 mice, who contained both the red·gam gene and Dlb1 gene, which can be analysed separately. A schematic illustration of a gpt-delta mouse is shown in Figure 1. F1 mice were irradiated with gamma-rays from ^{137}Cs for 483 consecutive days starting at 2 months of age using the facility in the Institute for Environmental Sciences (Figure 2). The dose rates were 0.0323 μGy/min, 0.650 μGy/min, and 12.5μGy/min.

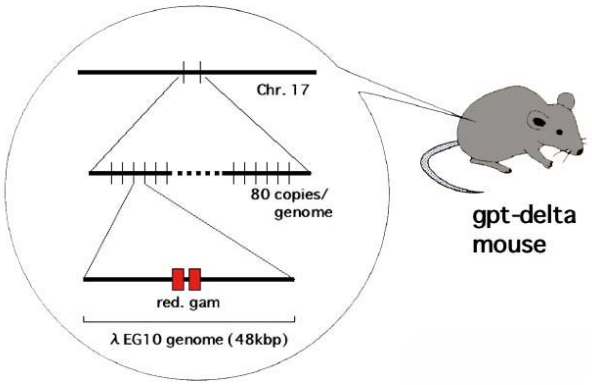

Figure 1. Schematic illustration of a gpt-delta mouse
It contains about 80 copies of lambda EG10 genomic DNA in chromosome 17 of a C57BL/6 mouse. The lambda EG10 contains red and gam genes in the middle part of the lambda genome. Spi$^-$ assay can detect mutations in the two genes.

Figure 2. Photograph of the long-term irradiation facility in the Institute for Environmental Sciences.
Mice were irradiated for 483 consecutive days under SPF conditions. A whole room was used for each radiation dose-rate.

The irradiation was stopped 2 hours every day in the morning (10:00 – 12:00 AM) for monitoring health, supplying food and water, changing cages, and cleaning of the room. After completion of irradiation, mice were kept unirradiated for one week and sampled. Unirradiated mice were treated in the same way in a separate room. The mice were maintained under SPF conditions throughout the experiment. All procedures were performed according to the Guidelines for Animal Experiments of the Institute for Environmental Sciences.

2.2 Spi⁻ assay

Genomic DNA from the spleen and liver of unirradiated and irradiated mice was extracted using phenol and treated with packaging extract, which separated lambda DNA from the mouse genome and integrated it into phage particles. Phages containing mutated red·gam genes were detected by Spi⁻ assay (Nohmi & Masumura, 2004), and the mutation frequency was calculated as the number of phages with red·gam mutation divided by the total number of phages. This system detects exclusively deletion mutation, which is the predominant type of mutation induced by radiation (Ono, Ikehata, Nakamura, Saito, Komura, Hosoi, et al., 1999; Uehara, Ikehata, Komura, Ito, Ogata, Itoh, et al., 2008).

2.3 Dlb1 mutation assay

The F1 mice contained one allele of Dlb1 gene transmitted from the SWR mice. The mutations in the Dlb1 gene in intestinal stem cells can be monitored as a streak of Dlb1-deficient cells in the villi of the small intestine after staining the Dlb1 product protein with Dolichos biflorus agglutinin-peroxidase conjugate. The fraction of streak-containing villi over the total number of villi examined was calculated as mutation frequency. The details were described previously (Shaver-Walker, Urlando, Tao, Zhang, & Heddle, 1995).

2.4 Chromosome analysis

Chromosome analysis was performed on the lymphocytes obtained from the spleen. The splenic lymphocytes were cultured for approximately 46 hours in a RPMI 1640 medium containing 20% fetal calf serum, lipopolysaccharide (10 µg/ml), and concanavalin A (3 µg/ml). In order to obtain the metaphase in the first cell cycle after stimulation by mitogens, Colcemid was added to the culture for 24 hours at a concentration of 0.02 µg/ml. The lymphocytes were treated with a 0.075M KCl hypotonic solution for 20 min at 37 °C and fixed. Air-dried preparations were stained with quinacrine mustard and Hoechst 33258 (Yoshida, Ikeuchi, & Sasaki, 1975). Dicentric chromosome frequency was used as a marker of chromosomal abnormality.

3 RESULTS AND DISCUSSION

Figure 3 indicates mutation frequencies of the red·gam gene in spleens and livers of F1 mice after 21, 414, or 8,000 mGy of low dose-rate irradiation. In both types of tissues, 8,000 mGy of radiation increased mutation frequency at statistically significant levels, whereas no difference was observed in the 21- or 414 mGy-irradiated mice.

Using the same mice, Dlb1 mutations in intestinal crypt stem cells and chromosomal abnormality in spleen cells were studied. The results are summarized in Table 1. Chromosomal abnormality after 414 mGy of irradiation was not conclusive because two different statistical analyses showed positive and negative results for the significance of difference between control and irradiated samples. From Table 1, it is obvious that chromosomal abnormality is more sensitive than gene mutation in monitoring radiation-induced genomic alterations.

Mutations on Dlb1 in intestinal crypt stem cells showed similar sensitivity to those on red·gam genes in the spleen and liver.

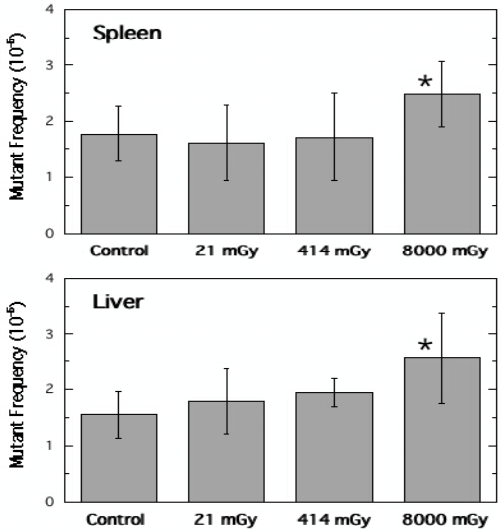

Figure 3. Mutation frequencies on the red·gam gene in the spleen and liver of gpt-delta X SWR F1 mice after 483 days of irradiation with three different dose rates; 42.6 μGy/d, 858 μGy/d, and 16.5 mGy/d. The total doses were 21 mGy, 414 mGy, and 8,000 mGy. The bars show standard deviations. Mutation frequencies after 8,000 mGy were higher than those of unirradiated control mice in both the spleen and liver at statistically significant levels. They are marked with asterisks.

Index	21 mGy	414 mGy	8000 mGy
Chromosome abnormality (Spleen)	−	±	+++
red-gam gene (spleen, liver)	−	−	+
Dlb1 gene (crypt stem cells)	−	−	+

Table 1. Comparison of radiation effects using three parameters. - indicates no change, ± indicates a slight change, + indicates a slight but statistically significant change, and +++ indicates a large increase. The total doses of 21 mGy and 414 mGy did not induce any appreciable changes in the three indices examined, whereas 8,000 mGy affected chromosome abnormality and gene mutation. The sensitivity was highest with chromosomal abnormality.

Overall, these analyses on genome structure show that the effect of 21 mGy and 414 mGy is very small, if it exists at all, whereas 8,000 mGy elicit obvious influences. Very recently, Tanaka et al. have found that the continuous irradiation of C3H mice with 1 mGy/day for 615 days increases chromosomal abnormality slightly

but at a statistically significant level (Tanaka, Kohda, Satoh, Toyokawa, Ichinohe, Ohtaki, et al., 2009). These dose responses look similar to the effects on life shortening and cancer induction elucidated before under comparable conditions in B6C3 F1 mice (Tanaka, Tanaka, Sasagawa, Ichinohe, Takabatake, Matsushita, et al., 2003; Tanaka, Tanaka, Ichinohe, Matsushita, Matsumoto, Otsu, et al., 2007). It suggests a possible association between genomic alteration and life shortening and/or cancer induction. It should be noted, however, that the studies on genomic change have been done only on limited tissues.

4 CONCLUSION

Alteration of genomic structure in somatic tissues in mice after continuous irradiation with very low dose-rate gamma-rays was detected as elevations of gene mutation and chromosomal abnormality if the total dose was 8,000 mGy at a dose rate of 16.5 mGy/d (12.5 μGy/min). Similar studies on mice irradiated with 414 mGy at a dose rate of 0.86 mGy/d (0.65 μGy/min) showed no detectable change. These characteristics correspond roughly to that observed for life-shortening (Tanaka, Tanaka, Sasagawa, Ichinohe, Takabatake, Matsushita, et al., 2003) and cancer-induction (Tanaka, Tanaka, Ichinohe, Matsushita, Matsumoto, Otsu, et al., 2007), suggesting a role of genomic alteration in radiation-induced cancer and/or life shortening.

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