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# Frequencies of Chromosome Aberrations are Lower in Splenic Lymphocytes from Mice Continuously Exposed to Very Low-Dose-Rate Gamma Rays Compared with Non-Irradiated Control Mice

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**Chromosome aberrations have been one of the most sensitive and reliable biomarkers of exposure to ionizing radiation. Using the multiplex fluorescence in situ hybridization (M-FISH) technique, we compared the changes, over time, in the frequencies of translocations and of dicentric chromosomes in the splenic lymphocytes from specific pathogen-free (SPF) C3H/HeN female mice continuously exposed to 0.05 mGy/day (18.25 mGy/year) gamma rays for 125 to 700 days (total accumulated doses: 6.25–35 mGy) with age-matched non-irradiated controls. Results show that the frequencies of translocations and of dicentric chromosomes increased significantly over time in both irradiated and non-irradiated control mice, and that the frequencies were significantly lower, not higher, in the irradiated mice, which differs from our previous reports of increased chromosome aberration frequencies at higher radiation dose rates of 1 mGy/day and 20 mGy/day. These results will be useful when considering the radiation risk at very low-dose rates comparable to regulatory dose limits.** 

## INTRODUCTION

One of the major problems in radiation risk assessment is the uncertainty in the estimation of the health effects of radiation exposure at low doses and/or at low dose rates (*1, 2*). Although human epidemiological studies have been extensively used as basis for radiation risk assessment, they cannot provide sufficient data for doses below 100 mGy, and this is mainly due to the presence of confounding factors as well as statistical limitations. The frequency of chromosome aberrations has been considered to be one of the most sensitive and reliable indicators of exposure to radiation and thus suitable for complementing epidemiological studies and addressing the gap at low-dose and low-dose-rate exposures. The introduction of sensitive techniques such as fluorescence in situ hybridization (FISH) (*3*) has further increased the sensitivity and reliability of chromosome assays.

Chromosome aberration analyses have been applied to various population cohorts exposed to low dose or low-dose-rate radiation, including workers at nuclear facilities (*4, 5*), residents in contaminated areas (*6*), and residents in high-background-radiation areas (*7–10*). As studies using human populations are not exempt from confounding factors, cultured human cells and laboratory animals are often used. Most of these studies showed dose dependent increases in the frequency of chromosome aberrations (*1, 11, 12*). Dose-dependent relationships were observed at doses of more than 20 mGy in the studies involving high-dose-rate exposures. In the low-dose-rate studies, the lower limits appear to be substantially higher. Some studies (*9, 10*) on residents in high background radiation areas (1.5–35 mGy/year dose rates), however, reported no detectable increases in the frequency of chromosome aberrations.

We have been analyzing the frequency of chromosome aberrations, using sensitive techniques including multiplex fluorescence in situ hybridization (M-FISH), in the lymphocytes collected from the spleen of the laboratory mice kept under environmentally controlled, specific pathogen free (SPF) conditions for entire lifespans and exposed to $^{137}$Cs gamma rays at various dose rates from 0.05 mGy/day to 800 mGy/min, in the unique animal irradiation facilities of the Institute of Environmental Sciences (*13*).

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**TABLE 1**
**Frequencies of Translocations and Dicentric Chromosomes in Splenic Lymphocytes from Female C3H Mice Exposed to 0.05 mGy/Day of Gamma Rays Compared to Age-Matched Non-Irradiated Controls**

| Sacrifice day (Age in days) | Experiment Group | Total dose (mGy) | Number of mice analyzed (n) | Translocations/cell (95% CI) | Dicentric chromosomes/cell (95% CI) |
|---|---|---|---|---|---|
| 0 (53–56) | non-irradiated | 0 | 10 | 0.0026 (0.0020–0.0035) | 0.0004 (0.0002-0.0009) |
| 125 (178) | non-irradiated | 0 | 7 | 0.0026 (0.0019–0.0036) | 0.0011 (0.0007–0.0019) |
| | 0.05 mGy/day | 6.25 | 7 | 0.0032 (0.0024–0.0043) | 0.0005 (0.0003–0.0011) |
| 200 (255–256) | non-irradiated | 0 | 10 | 0.0046 (0.0037–0.0057) | 0.0010 (0.0006–0.0016) |
| | 0.05 mGy/day | 10 | 10 | 0.0025 (0.0019–0.0032) | 0.0007 (0.0004–0.0011) |
| 300 (353–360) | non-irradiated | 0 | 10 | 0.0024 (0.0018–0.0033) | 0.0015 (0.0010–0.0022) |
| | 0.05 mGy/day | 15 | 10 | 0.0038 (0.0030–0.0049) | 0.0010 (0.0006–0.0017) |
| 400 (457–460) | non-irradiated | 0 | 10 | 0.0050 (0.0041–0.0061) | 0.0012 (0.0008–0.0018) |
| | 0.05 mGy/day | 20 | 10 | 0.0036 (0.0029–0.0046) | 0.0008 (0.0005–0.0014) |
| 500 (556–565) | non-irradiated | 0 | 10 | 0.0089 (0.0075–0.0106) | 0.0024 (0.0017–0.0033) |
| | 0.05 mGy/day | 25 | 10 | 0.0049 (0.0039–0.0061) | 0.0014 (0.0009–0.0021) |
| 600 (656–670) | non-irradiated | 0 | 10 | 0.0092 (0.0078–0.0108) | 0.0025 (0.0019–0.0035) |
| | 0.05 mGy/day | 30 | 10 | 0.0067 (0.0055–0.0082) | 0.0014 (0.0009–0.0021) |
| 700 (761–774) | non-irradiated | 0 | 10 | 0.0125 (0.0108–0.0144) | 0.0029 (0.0021–0.0039) |
| | 0.05 mGy/day | 35 | 10 | 0.0076 (0.0063–0.0092) | 0.0017 (0.0012–0.0026) |

Our recent reports (*14–17*) showed the linear dose-response relationships of translocations and dicentric chromosomes in splenic lymphocytes from the mice irradiated at a low-dose rate of 20 mGy/day for 400 days. Dose dependent increases in the frequency of these chromosome aberrations have also been successfully detected in mice irradiated at a lower 1 mGy/day dose rate.

In the present study, we examined chromosome aberrations in the mice irradiated at an extremely low-dose rate of 0.05 mGy/day (=18.25 mGy/year), which approximates the occupational dose limit of 100 mSv/5 years (*18*) and is comparable to the dose rates found in the high background radiation areas (*10*).

## MATERIALS AND METHODS

*Animals, Animal Husbandry and Irradiation*

A total of 144 SPF female C3H/HeN mice, purchased from CLEA Japan, Inc. (Shizuoka, Japan), were used in this study. The mice were kept in animal rooms under SPF environmental conditions (*19*) and the irradiated group was exposed to $^{137}$Cs gamma rays continuously at a low dose rate of 0.05 mGy/day from 8 weeks (53–56 days) of age, as described previously (*17, 19*). Radiation exposure was continuous for 22 h a day. The remaining 2 h were used to clean the animal rooms, change cages, and provide a fresh supply of food and water. Splenic lymphocytes were collected from 7 or 10 mice at 0, 125, 200, 300, 400, 500, 600, and 700 days (53–56, 178, 255–256, 353–360, 457–460, 556–565, 656–670, and 761–774 days of age, respectively) from the start of irradiation alongside age-matched non-irradiated controls (Table 1). All experiments were conducted according to legal regulations in Japan and following the guidelines for Animal Experiments of the IES.

*Justification for selection of mouse strain and sex.* We elected to use female C3H/HeN mice in this study for ease of comparison, since our previously reported studies (*14–17*) also used the same strain.

*Justification of radiation dose selection.* We further reduced the dose rate to 0.05 mGy/day since we have previously detected radiation effects after long-term irradiation at the low-dose rates of 20 and 1 mGy/day.

*Chromosome Analyses*

On predetermined sacrifice days (see above), the mice were euthanized with an overdose of isofluorane (Isoflu®, DS Pharma Animal Health, Osaka, Japan) and their spleens were collected under aseptic conditions. Mice harboring neoplasms or having enlarged spleens at the time of sacrifice were excluded from the study to eliminate the influence of abnormal cell proliferations. Three mice were removed from the 700-day irradiated group and 4 mice were removed from the age-matched non-irradiated controls.

Processing of splenic lymphocytes for chromosome analyses has been described previously (*17*) except for a shorter culture time of 44 h. We analyzed 1,014 to 3,225 metaphases from each mouse for detecting translocations and dicentric chromosomes. Insertions and inversions were counted as translocations. When more than 3 cells had the same chromosome aberration, they were judged as clonal cells according to ISCN 2013 (*20*) and were counted as 1 cell. A one-way or reciprocal translocation between 2 chromosomes was scored as 1 translocation, whereas three-way translocations involving 3 chromosomes were scored as 2 translocations (*21*).

*Statistical Analyses*

The effects of mouse age and radiation exposure on the frequency of chromosome aberrations (translocations or dicentric chromosomes) were analyzed by use of the generalized linear model (GLM) with a Poisson distribution and a log-link function. In the GLM, mouse age (at sacrifice) and irradiation (i.e., dose rate) were included as explanatory variables, and the frequency of chromosome aberrations in each mouse was included as a response variable. Since there is a possibility that the frequency of chromosome aberrations could depend on the number of cells examined, the number of cells from each mouse was included in the model as an off-set term to correct this dependency. Thus, our model is expressed as:

$$Y = \exp(\mathrm{b} + \mathrm{a0} \times t + \mathrm{a1} \times r + \log(N))$$

where $Y$ is the number of chromosome aberrations (i.e., translocations or dicentric chromosomes) in cells, $t$ is the age in days, $r$ is the dose rate (it is 0 for the non-irradiated control group and 0.05 for the irradiated group), a0 and a1 are the regression coefficients, and b is the intercept. $N$ is the total number of cells examined. The tests were conducted with R software, version 3.6.1 (*22*).

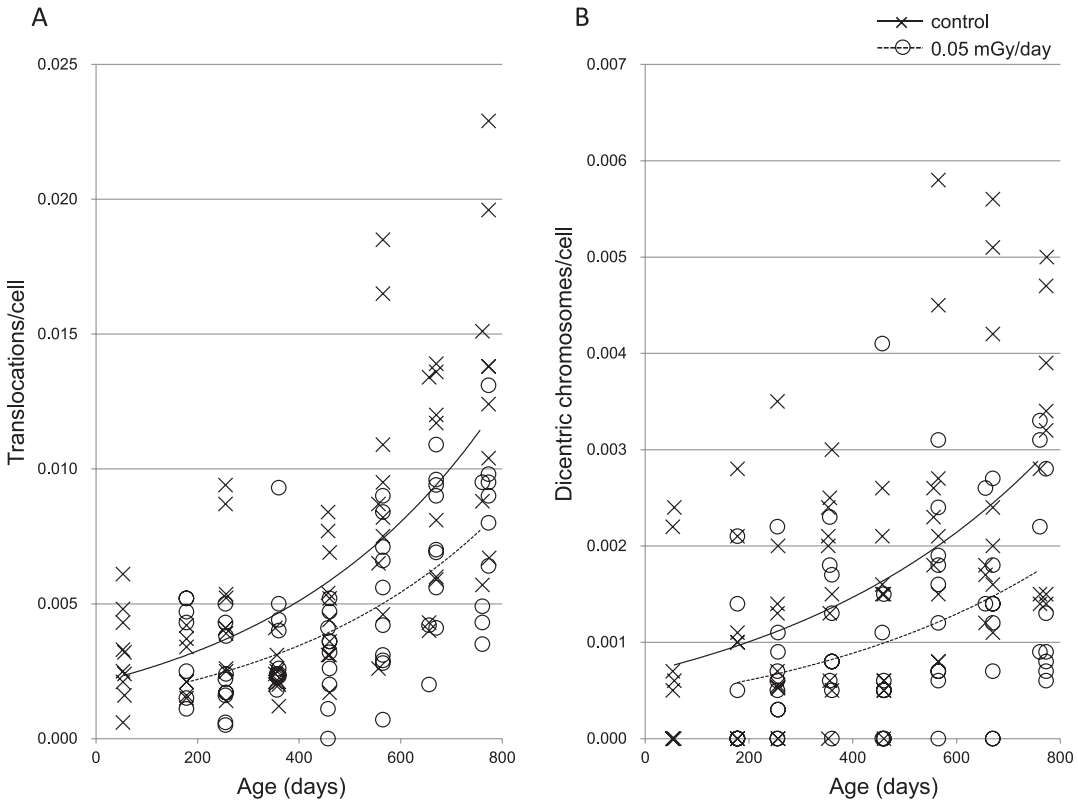


**FIG. 1.** Frequencies of chromosome aberrations in splenic lymphocytes from female C3H mice continuously irradiated with gamma rays at 0.05 mGy/day from 56 days of age compared with age-matched non-irradiated controls. Each data point indicates the frequency of translocations (panel A) or dicentric chromosomes (panel B) in an individual mouse. The curves are based on the results of the analysis using the generalized linear model (GLM) wherein the control is indicated by the solid line and the 0.05 mGy/day group is indicated by the dotted line.

## RESULTS

SPF female C3H/HeN mice were continuously exposed to gamma rays at a very low-dose rate of 0.05 mGy/day for up to about 700 days. Using M-FISH technology, we examined the change, over time, in the frequency of chromosomal aberrations in the splenic lymphocytes, specifically translocations [considered relatively stable over multiple cell generations (*23*)] and dicentric chromosomes (considered unstable). We used 7 or 10 mice per sacrifice point and analyzed 1,014 to 3,225 cells per mouse. The results are summarized in Table 1 and Fig. 1, where the statistical unit is the individual mouse rather than a cell. Since Fig. 1 is very crowded with the data points of individual mice, the mean values are plotted in Supplementary Fig. S1 (https://doi.org/10.1667/RADE-RADE-21-00159.1.S1).

As the mice aged, we observed that the frequencies of chromosome aberrations increased regardless of exposure to radiation. Curiously, the frequencies of translocations and dicentric chromosomes in the irradiated mice appeared to be lower than those of the non-irradiated control mice. The translocation frequencies in the irradiated mice were significantly lower compared to the age-matched non-irradiated control mice at the days 200, 500 and 700 of irradiation [the 95% confidence interval (CI) of the irradiated mice was lower than that of the non-irradiated controls without overlapping at each of these points]. We therefore analyzed the effects of two variables, mouse age and radiation exposure, on the frequency of translocations and of dicentric chromosomes, using the generalized linear model (GLM). Table 2 shows that the effects of both age and irradiation were highly significant ($P < 0.001$). We concluded that under our experimental conditions, continuous radiation exposure at a very low-dose rate of 0.05 mGy resulted in a decrease, not increase, in the frequency of chromosome aberrations, contrary to our findings for the higher dose rates of 1 mGy/day and 20 mGy/day (*14–17*).

## DISCUSSION

The linear no-threshold (LNT) model for biological effects of radiation has long been used as basis for radiation protection (*1*). Even though it is now understood that there exist some human epidemiological studies that do not fit this model, and that confirmation of this model by epidemiological studies does not seem to be possible due to severe statistical limitations at very low doses and at very

**TABLE 2**
**Effects of Mouse Age and Low-Dose-Rate Gamma-Ray Exposure on the Frequencies of Translocations and Dicentric Chromosomes in Splenic Lymphocytes from Female C3H Mice**

| | Variable | Coefficient | S.E. | Z value | P value |
|---|---|---|---|---|---|
| Translocation | Mouse age | 0.00226 | 0.00014 | 16.24 | $P < 0.001$ |
| | Irradiation* | –7.85 | 1.167 | –6.73 | $P < 0.001$ |
| Dicentric chromosome | Mouse age | 0.00189 | 0.00027 | 7.14 | $P < 0.001$ |
| | Irradiation* | –10.07 | 2.308 | –4.36 | $P < 0.001$ |

* Irradiation is a variable, where it takes 0 for the non-irradiated control group and 0.05 for the irradiated group.

low-dose rates, this model is still recognized as extremely useful for radiation protection purposes. In the range of very low doses and very low-dose rates, mechanistic studies using biological methodologies may supplement the results of human epidemiological studies. In the early days, the data used for theoretical considerations of radiation dose response or dose-rate response relationships were mostly those of chromosome aberrations.

The most important of the classical views based on these early theoretical considerations is the explanation of the dose-rate effect on chromosome aberrations: Many previous studies (*1*), including ours (*14–17*), have shown that the frequency of chromosome aberrations induced by low-LET irradiation can be described by a linear dose-response relationship in the low-dose rate or low-dose range, and by a linear-quadratic relationship in the high-dose rate and high-dose range, with a clear dose-rate effect. On the other hand, the frequency of radiation-induced DNA lesions, specifically DNA double-strand breaks, which are the major source of chromosome aberrations, seems to be linearly proportional to dose, down to very low doses (*24*). The lower estimated frequencies of DNA double-strand breaks in the samples irradiated at low-dose rates compared to those in the samples irradiated at high dose rates have been attributed to ligation (repair) of the breaks during the long irradiation periods (*25*, *26*), rather than decreased induction of the breaks at low-dose rates. The discrepancy between the presence of the dose-rate effect on chromosome aberrations and its absence with regards to the induction of DNA double-strand breaks can be consistently explained by the mechanism wherein multiple DNA double-strand breaks, when present simultaneously and in close proximity within a cell, can be illegitimately ligated by a DNA repair system called non-homologous end joining (*27*), resulting in chromosome aberrations (*1, 28*). This suggests that ligation of the DNA double-strand breaks induced by low-dose rate or low-dose radiation is, in most cases, macroscopically legitimate, and contributes to the maintenance of a normal karyotype. From the viewpoint of radiation protection, however, it should be noted here that even in these cases, the ligation points of chromosomes may microscopically contain very small mutations (deletions, additions, or base substitutions) due to the chemical structure of radiation-induced DNA strand breaks (*29*) and the nature of non-homologous end joining (*27*, *30*).

At present, despite the increasing availability of various molecular and cellular biological data, chromosome aberration studies continue to remain important (*31*). In our study, we performed long-term irradiation, at an extremely low-dose rate, of laboratory mice under strictly controlled environmental conditions in an effort to lessen disturbance by various factors, and examined the changes in the frequency of chromosome aberrations over time in a large number of cells from several individual animals. We consider the results of this study are very useful for supplementing the results of human epidemiological studies.

We believe that the results indicate that the exposure to radiation at a very low-dose rate of 0.05 mGy/day decreases, rather than increases, the frequency of chromosome aberrations. This is distinctly different from findings of studies that used dose rates of 1 mGy/day or more (*1*), including ours (*14–17*), where the frequency of chromosome aberrations shows dose-dependent increases, following dose-response relationships that can be interpreted as linear or linear quadratic. It would be inappropriate, however, to comment on the validity of the current radiation protection system based on the LNT model, except that the system does not seem to underestimate risks at low-dose rates.

Non-linear dose response findings have prior, sometimes contradictory, examples in the literature associated with varied explanations such as: 1. in cells irradiated at low-dose rates, the amount of DNA damage, which reflects the equilibrium between the induction by radiation and the elimination by repair, does not follow a canonical dose-response relationship (*26*); 2. at low doses or dose rates, some of the cellular DNA repair systems are activated (adaptive response) (*32, 33*); 3. at low-dose rates, the activation of some of the cellular DNA repair systems is diminished (inverse dose-rate effect) (*34*); 4. selective apoptosis, wherein damaged or mutated cells are selectively killed (*35*); 5. radiation induces genomic instability (*36*), but the resulting cellular response does not follow the usual dose dependence; 6. non-irradiated cells receive signals from neighboring irradiated cells (bystander effects) (*37*), but the response of the cells that have received signals does not follow the usual dose dependence; and, 7. in tissues in vivo, undamaged or unmutated cells outcompete damaged or mutated cells and selectively proliferate (*38*).

Our results are in best agreement with the second, fourth, and seventh examples of the non-linear dose-response findings and their explanations. Studies of chromosome aberrations in the high background radiation areas (*10*) refer to the second explanation in particular. Further experiments however are required to attribute the decrease in the chromosome aberration frequency in the present study to these or other mechanisms.

The statistical analyses found in Table 2 show that the effect of radiation on the frequencies of chromosome aberrations, as well as the effect of another factor, aging, is highly significant: Chromosome aberrations increase with age, especially later in lifespan. Comparing the results of the non-irradiated groups in this study with those in previous reports on mice (*39*) and humans (*40*) suggests that the frequencies of chromosome aberrations in non-irradiated controls are of the same magnitude in mice and humans, and that the trends of increase with age are similar between the two species. The rate of increase, however, is much faster in mice than in humans, which may correlate with a large difference in lifespan (*41*). There is a recent report (*42*) showing a strong inverse relationship between the rate of spontaneous somatic small mutations (detected by sequencing) and the lifespan across various mammalian species. It is not clear, however, whether such a relationship applies to chromosome aberrations, as the DNA repair systems responsible for small mutations and those responsible for chromosome aberrations are not necessarily the same (*43*). In addition, it has been pointed out that gene mutations arise frequently in younger ages, whereas cytogenetic mutations accumulate mainly in older ages (*39*). There have been some reports (*44–46*) that the mechanisms ensuring genome stability are less stringent in rodent cells than in human cells, which may account for the differences in the rates of the age-dependent increases in chromosome aberrations as well as small mutations between mice and humans. On the other hand, it should also be noted that high-dose-rate radiation seems to induce chromosome aberrations with similar efficiencies in mice and in humans (*47*). Comprehensive elucidation of the molecular and cellular mechanisms underlying the similarities and differences in the frequencies of spontaneous and induced chromosome aberrations and other mutations and their age-related changes in short-lived and long-lived mammals is of great importance, considering the role of the chromosome aberration research in the establishment of the current radiation protection system.

The pathway from radiation exposure to the appearance of health effects such as cancer (adverse outcome) is composed of many steps or events. When approaching the issues of the appropriateness of the LNT model and the magnitude of the dose-rate effect from biological and mechanistic studies, a systematic quantification-oriented method, such as the use of adverse outcome pathways, should be introduced (*48*), and then the quantification of each event that constitutes the pathway should be conducted. Chromosome aberrations have been generally considered as the outcome of relatively early events, such as induction of DNA damage by radiation and DNA repair in cells, in the pathway. However, some ambitious studies have used the chromosome aberration analysis to understand the dynamics of damaged or mutated cells in tissues or in bodies and their later outcomes (*49*). We surmise that our results may also reflect the selective removal or proliferation of specific cell populations within tissues or bodies, as well as early events occurring within cells. The identification of the mechanisms or pathways that lead to non-canonical dose-response or dose-rate-response relationships at very low doses or dose rates, such as the one we have reported here, will be beneficial in improving the system of radiation protection.

## SUPPLEMENTARY INFORMATION

Supplementary Fig. S1. Frequencies of chromosome aberrations in splenic lymphocytes from female C3H mice continuously irradiated with gamma rays at 0.05 mGy/day from 56 days of age compared with age-matched non-irradiated controls. Each data point indicates the mean (with 95% CI) of the frequencies of translocations (panel A) or dicentric chromosomes (panel B) in each experiment group (the same data as shown in Table 1). The curves are completely the same as those in Fig. 1 (calculated from the data of individual mice rather than the means).

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## REFERENCES

1. National Research Council. Health risks from exposure to low levels of ionizing radiation: BEIR VII Phase 2. Washington, DC: The National Academies Press; 2006.
2. Preston RJ, Boice JD, Jr., Brill AB, Chakraborty R, Conolly R, Hoffman FO, et al. Uncertainties in estimating health risks associated with exposure to ionising radiation. J Radiol Prot 2013; 33:573-88.
3. Breneman JW, Ramsey MJ, Lee DA, Eveleth GG, Minkler JL, Tucker JD. The development of chromosome-specific composite DNA probes for the mouse and their application to chromosome painting. Chromosoma 1993; 102:591-98.
4. Salassidis H, Braselmann ND, Okladnikova S, Pressl G, Stephan G, Snigiryova M, et al. Analysis of symmetrical translocations for retrospective biodosimetry in radiation workers of the Mayak nuclear-industrial complex (Southern Urals) using FISH-chromosome painting. Int J Radiat Biol 1998; 74:431-39.
5. Tawn EJ, Curwen GB, Jonas P, Gillies M, Hodgson L, Cadwell KK. Chromosome aberrations determined by FISH in radiation workers from the Sellafield nuclear facility. Radiat Res 2015; 184:296-303.
6. Bauchinger M, Salassidis K, Braselmann H, Vozilova A, Pressl S,

Stephan G, et al. FISH-based analysis of stable translocations in a Techa River population. Int J Radiat Biol 1998; 73:605-12.

7. Jiang T, Hayata I, Wang C, Nakai S, Yao S, Yuan Y, et al. Dose-effect relationship of dicentric and ring chromosomes in lymphocytes of individuals living in the high background radiation areas in China. J Radiat Res 2000; 41 Suppl:63-8.

8. Barcinski MA, Do Ceu Abreu M, De Almeida JC, Naya JM, Fonseca LG, Castro LE. Cytogenetic investigation in a Brazilian population living in an area of high natural radioactivity. Am J Hum Genet 1975; 27:802-6.

9. Zhang W, Wang C, Chen D, Minamihisamatsu M, Morishima H, Yuan Y, et al., Imperceptible effect of radiation based on stable type chromosome aberrations accumulated in the lymphocytes of residents in the high background radiation area in China. J Radiat Res 2003; 44:69-74.

10. Karuppasamy CV, Ramachandran EN, Kumar V, Kumar PR, Koya P, Jaikrishan G, et al. Frequency of chromosome aberrations among adult male individuals from high and normal level natural radiation areas of Kerala in the southwest coast of India. Mutat Res Genet Toxicol Environ Mutagen 2018; 828:23-9.

11. Lloyd DC, Edwards AA, Leonard A, Deknudt GL, Verschaeve L, Natarajan AT, et al. Chromosomal aberrations in human lymphocytes induced in vitro by very low doses of X-rays. Int J Radiat Biol 1992; 61:335-43.

12. Iwasaki T, Takashima Y, Suzuki T, Yoshida MA, Hayata I. The dose response of chromosome aberrations in human lymphocytes induced in vitro by very low-dose γ rays. Radiat Res 2010; 175:208-13.

13. Tanaka I.B. III, Tanaka S, Kohda A, Takai D, Nakamura S, Ono T, et al. Experimental studies on the biological effects of chronic low dose-rate radiation exposure in mice: overview of the studies at the Institute for Environmental Sciences. Int J Radiat Biol 2018; 94:423-33.

14. Tanaka K, Kohda A, Toyokawa T, Ichinohe K, Oghiso Y. Chromosome aberration frequencies and chromosome instability in mice after long-term exposure to low-dose-rate gamma-irradiation. Mutat Res 2008; 657:19-25.

15. Tanaka K, Kohda A, Satoh K, Toyokawa T, Ichinohe K, Ohtaki M, et al. Dose-rate effectiveness for unstable-type chromosome aberrations detected in mice after continuous irradiation with low-dose-rate gamma rays. Radiat Res 2009; 171:290-301.

16. Tanaka K, Kohda A, Satoh K. Dose-rate effects and dose and dose-rate effectiveness factor on frequencies of chromosome aberrations in splenic lymphocytes from mice continuously exposed to low-dose-rate gamma-radiation. J Radiol Prot 2013; 33:61-70.

17. Tanaka K, Satoh K, Kohda A. Dose and dose-rate response of lymphocyte chromosome aberrations in mice chronically irradiated within a low-dose-rate range after age adjustment. Radiat Prot Dosimetry 2014; 159:38-45.

18. The 2007 Recommendations of the International Commission on Radiological Protection. ICRP publication 103. Ann ICRP 2007; 37:1-332.

19. Tanaka S, Tanaka IB III, Sasagawa S, Ichinohe K, Takabatake T, Matsushita S, et al. No lengthening of life span in mice continuously exposed to gamma rays at very low dose rates. Radiat Res 2003; 160:376-79.

20. Shaffer LG, McGowan-Jordan J, Schmid M. ISCN 2013: An international system for human cytogenetic nomenclature (2013): Karger Medical and Scientific Publishers; 2013.

21. Fomina J, Darroudi F, Boei JJ, Natarajan AT. Discrimination between complete and incomplete chromosome exchanges in X-irradiated human lymphocytes using FISH with pan-centromeric and chromosome specific DNA probes in combination with telomeric PNA probe. Int J Radiat Biol 2000; 76:807-13.

22. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing 2019. [cited 2021Aug 10]. Available from: https://www.R-project.org/.

23. Tucker JD. Low-dose ionizing radiation and chromosome translocations: a review of the major considerations for human biological dosimetry. Mutat Res 2008; 659:211-20.

24. Rothkamm K, Löbrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. Proc Natl Acad Sci U S A 2003; 100: 5057-62.

25. Ishizaki K, Hayashi Y, Nakamura H, Yasui Y, Komatsu K, Tachibana A. No induction of p53 phosphorylation and few focus formation of phosphorylated H2AX suggest efficient repair of DNA damage during chronic low-dose-rate irradiation in human cells. J Radiat Res 2004; 45: 521-5.

26. Ulyanenko S, Pustovalova M, Koryakin S, Beketov E, Lychagin A, Ulyanenko L, et al. Formation of γH2AX and pATM Foci in human mesenchymal stem cells exposed to low dose-rate gamma-radiation. Int J Mol Sci 2019; 20(11):2645.

27. Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu Rev Biochem 2010; 79: 181-211.

28. Savage JR, Insight into sites. Mutat Res 1996; 366: 81-95.

29. Henner WD, Grunberg SM, Haseltine WA. Sites and structure of gamma radiation-induced DNA strand breaks. J Biol Chem 1982; 257:11750-4.

30. Duncan JR, Lieber MR, Adachi N, Wahl RL. Radiation dose does matter: mechanistic insights into DNA damage and repair support the linear no-threshold model of low-dose radiation health risks. J Nucl Med 2018; 59:1014-16.

31. Cornforth MN, Loucas BD. A Cytogenetic profile of radiation damage. Radiat Res 2019; 191:1-19.

32. Wolff S, The adaptive response in radiobiology: evolving insights and implications. Environ Health Perspect 1998; 106 Suppl 1:277-83.

33. Mitchel RE. The dose window for radiation-induced protective adaptive responses. Dose Response 2009; 8:192-208.

34. Vilenchik MM, Knudson AG, Jr. Inverse radiation dose-rate effects on somatic and germ-line mutations and DNA damage rates. Proc Natl Acad Sci USA 2000; 97:5381-6.

35. Bauer G. Low dose radiation and intercellular induction of apoptosis: potential implications for the control of oncogenesis. Int J Radiat Biol 2007; 83:873-88.

36. Morgan WF. Radiation-induced genomic instability. Health Phys 2011; 100:280-1.

37. Morgan WF, Sowa MB. Non-targeted bystander effects induced by ionizing radiation. Mutat Res 2007; 616:159-64.

38. Niwa O, Barcellos-Hoff MH, Globus RK, Harrison JD, Hendry JH, Jacob P, et al. ICRP Publication 131: Stem Cell Biology with Respect to Carcinogenesis Aspects of Radiological Protection. Ann ICRP 2015; 44:7-357.

39. Tucker JD, Spruill MD, Ramsey MJ, Director AD, Nath J, Frequency of spontaneous chromosome aberrations in mice: effects of age. Mutat Res 1999; 425, 135-41.

40. Sigurdson AJ, Ha M, Hauptmann M, Bhatti P, Sram RJ, Beskid O, et al. International study of factors affecting human chromosome translocations. Mutat Res 2008; 652:112-21.

41. Flurkey K, Currer JM, Harrison D. Mouse models in aging research. The mouse in biomedical research 2007; 637-72.

42. Cagan A, Baez-Ortega A, Brzozowska N, Abascal F, Coorens THH, Sanders MA, et al. Somatic mutation rates scale with lifespan across mammals. Nature 2022; 604:517-24.

43. Friedberg EC, Walker GC, Siede W, Wood RD. DNA repair and mutagenesis: American Society for Microbiology Press 2005.

44. Kung AL, Sherwood SW, Schimke RT. Cell line-specific differences in the control of cell cycle progression in the absence of mitosis. Proc Natl Acad Sci U S A 1990; 87:9553-7.

45. Hanawalt PC, Revisiting the rodent repairadox. Environ Mol Mutagen 2001; 38:89-96.

46. Rangarajan A, Weinberg RA, Opinion: Comparative biology of

mouse versus human cells: modelling human cancer in mice. Nat Rev Cancer 2003; 3:952-9.

47. Kligerman AD, Halperin EC, Erexson GL, Honore G, Westbrook-Collins B, Allen JW. A cytogenetic comparison of the responses of mouse and human peripheral blood lymphocytes to $^{60}$Co gamma radiation. Radiat Res 1988; 115:334-46.

48. Preston RJ, Ruhm W, Azzam EI, Boice JD, Bouffler S, Held KD, et al. Adverse outcome pathways, key events, and radiation risk assessment. Int J Radiat Biol 2021; 97:804-14.

49. Hamasaki K, Landes RD, Noda A, Nakamura N, Kodama Y. Irradiation at different fetal stages results in different translocation frequencies in adult mouse thyroid cells. Radiat Res 2016; 186:360-6.