This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier’s archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright
Chromosome aberration frequencies and chromosome instability in mice after long-term exposure to low-dose-rate γ-irradiation

Kimio Tanaka a, Atsushi Kohda a, Takuo Toyokawa b, Kazuaki Ichinohe a, Yoichi Oghiso a

a Department of Radiobiology, Institute for Environmental Sciences, Hacchazwa 2-121, Takahoko, Rokkasho, Kamikita, Aomori 039-3213, Japan
b Tohoku Nuclear Corporation, Higashi-akamisawa 2-41-14, Misawa, Aomori 033-0024, Japan

1. Introduction

Medical radiologists and residents in radio-contaminated buildings in Taiwan, who have been chronically exposed, have a higher incidence of chromosome aberrations in peripheral blood lymphocytes than non-exposed individuals [1–3]. However, chronological changes of chromosome aberration rates related to accumulated doses of a low-dose-rate of radiation have not been well studied. C3H female specific pathogen-free mice (8 weeks of age) were chronically irradiated. Chromosome aberration rate in mouse splenocytes after long-term exposure to low-dose-rate (LDR) γ-rays was serially determined by conventional Giemsa method. Incidence of dicentrics and centric rings increased almost linearly up to 8000 mGy following irradiation for about 400 days at a LDR of 20 mGy/day. Clear dose-rate effects were observed in the chromosome aberration frequencies between dose rates of 20 mGy/day and 200 mGy/day. Furthermore, the frequencies of complex aberrations increased as accumulated doses increased in LDR irradiation. This trend was also observed for the incidences of micronuclei and trisomies of chromosomes 5, 13 and 18 in splenocytes, detected by micronucleus assay and metaphase fluorescence in situ hybridization (FISH) method, respectively. Incidences of 2–4 micronuclei and trisomy increased in mouse splenocytes after irradiation of 8000 mGy at a LDR of 20 mGy/day. These complex chromosome aberrations and numerical chromosome aberrations seem to be induced indirectly after radiation exposure and thus the results indicate that continuous γ-ray irradiation for 400 days at LDR of 20 mGy/day induced chromosome instability in mice. These results are important to evaluate the biological effects of long-term exposure to LDR radiation in humans.

© 2008 Elsevier B.V. All rights reserved.
of 200 mGy/22 h/day (10 mGy/h), up to 40 days, was used for comparison. Micronucleus assay and interphase FISH analysis using centromere probes of mouse chromosomes 5, 15 and 18 were also performed to detect cells with numerical chromosome changes in splenocytes after long-term irradiation at the LDR.

2. Materials and methods

2.1. Mice

Female, 8-week-old specific pathogen-free (SPF) C57/HeN mice (CLEA Japan, Inc., Shizuoka, Japan) were used for long- or short-term irradiation with 137Cs γ-rays irradiation starting from 8 weeks of age. They were kept in animal rooms under SPF barrier conditions at a room temperature of 23 ± 2°C, humidity of 50 ± 10% and a 12 h light/dark cycle. These experiments were approved by the appropriate Ethics Committee of our institute.

2.2. Radiation exposure

At least 5–7 mice were grouped for irradiation with each total dose together with age-matched, non-irradiated mice as controls. Groups of mice were irradiated with total doses of 100, 140, 250, 500, 750, 1000, 2000, 4000, 6000 and 8000 mGy at a dose rate of 20 mGy/22 h/day (0.91 mGy/h) for 5–400 days, and with total doses of 200, 400, 800, 1000, 2000, 4000 and 8000 mGy at a dose rate of 200 mGy/22 h/day (9.1 mGy/h) for 1–40 days using a 137Cs γ-ray irradiation device (74Gy and 740Gy respectively; Yoshizawa LA Co., Ltd., Japan). Mice were not exposed daily between weeks 8 and 12. The slides were treated with a fixative (4% formaldehyde) for 10 min and kept in phosphate buffered saline (PBS) for 12 h before chromosome analysis. For the conventional Giemsa staining method, chromosome preparations were made using a device able to automatically harvest metaphases. One microlitre of mouse DNA probe was mixed well with 9 μl of blocking solution [3% of bovine serum albumin (BSA) in 4× SSC and 0.1% Tween 20 (Nakarai, Osaka, Japan)] at 37°C for 30 min. To detect biotin, 150 μl of avidin–fluorescein ( Oncor, Gaithersburg, MD, USA) diluted 400 times with 4× SSC and 0.1% Tween 20 was applied to the slide, incubated at 43°C for 30 min, and then washed three times with washing solution (4× SSC and 0.1% Tween 20) at 43°C for 5 min. The slide was stained with propidium iodide (Oncor, Gaithersburg, MD, USA) solution (100ng/ml in 2× SSC) for 5 min at room temperature to stain the cell nucleus and chromosome, washed with 2× SSC, and coverslipped with 40g/ml of anti-fading solution (Oncor, Gaithersburg, MD, USA).

2.3. Spleen cell cultures

Mice were sacrificed under ether anesthesia immediately after the cessation of irradiation, and spleens were sterilly removed. All reagents were obtained from Sigma, St Louis, MI, USA except where noted. For chromosome analysis, spleen cells were isolated and cultured in RPMI 1640 medium (Invitrogen Corp., Carlsbad, CA, USA) containing lipo polysaccharide (LPS, 10 μg/ml), concanavalin A (ConA, 3 μg/ml) and 2-mercapto-ethanol (2-ME, 50μM) under 5% CO2 atmosphere with 95% humidity at 37°C. For micronucleus assay, cytochalasin B (0.5μg/ml) was added 24 h before harvesting to obtain binucleated cells.

2.4. Conventional Giemsa staining method

The conventional Giemsa staining method was suitable for the analysis of large numbers of chromosome metaphases of LDR-irradiated mice, because the procedure is simple and rapid. Colcemide (Invitrogen Corp., Carlsbad, CA, USA) at a final concentration of 0.002μg/ml was added for the last 2h of culture to collect metaphase cells. Cells were treated by hypotonic solution with 0.075M KCl for 8 min and centrifuged. The supernatant was removed and the cells were fixed with Carnoy’s solution (methanol and acetic acid = 3:1). Fixed cell suspensions were stored at −20°C for chromosome analyses. For the conventional Giemsa staining method, chromosome preparations were made using a device able to automatically control humidity (ADSTEC, Ciba, Japan), and stained with Giemsa solution. Chromosome aberrations such as dicentric chromosomes (Dic), centric ring chromosomes (Cr), acentric ring chromosomes (Ra), fragments, minutes (min) and deletions (del), hyperoids and chromatid-type aberrations such as gaps (gtp), breaks (ctb) and exchanges (exc) were observed in 500–1000 metaphases per mouse under a light microscope (Olympus BX50, Olympus, Tokyo, Japan). At least 500–1000 metaphases were scored per mouse in both irradiated and age-matched control groups. Deletion was defined as a chromosome piece with centromere less than the size of mouse chromosome 20.

2.5. Micronucleus assay in spleen and bone marrow

Three to five mice were analyzed in each experimental group at the total doses of 5000, 6000 and 8000 mGy with irradiation at a dose rate of 20 mGy/day by micronucleus assay to observe micronucleus-positive cells under the microscope (Olympus BX50, Olympus, Tokyo, Japan). Micronucleus-positive cells in about 5000–7000 polychromatic erythrocytes only of the bone marrow cells per mouse were scored under the microscope. Cells with 2–4 micronuclei were also scored.

2.6. Interphase fluorescence in situ hybridization (FISH) analysis

Five mice were analyzed at 8000 mGy with irradiation of the 20 mGy/day dose rate by the FISH method using centromere probes of chromosomes 5, 15 and 18 to observe the numerical chromosome aberration rate in splenocytes. Five non-irradiated control mice of the same age as irradiated mice were used as a control. About 3000 cells were enumerated to observe cells with one and three signals in the interphase nucleus, which were scored as monosomy and trisomy, respectively. For FISH analysis, slides were hybridized with a mouse centromere probe (STAR, Mouse Chromosome Probes, Cambio, Cambridge, UK) to detect the mouse centromere. One microlitre of mouse DNA probe was mixed well with 9 μl of hybridization buffer and denatured by heating at 80°C for 10 min. The chromosome slide was treated by denaturing the solution (70% of formamide in 2× SSC solution) at 75°C for 5 min and dehydrated in 70%, 85% and 95% ethanol. Formamide was purchased from Roche Diagnostic, Indianapolis, IN, USA. After air drying, the mouse denatured DNA probe solution was laid on a part of the slide and hybridized at 37°C overnight in a humidity chamber. After hybridization, the slide was washed three times with washing solution (50% of formamide in 2× SSC solution) at 43°C for 5 min, and three times with washing solution (0.8× SSC) at 61°C for 5 min. The slide was then treated with 150μl of blocking solution (3% of bovine serum albumin (BSA) in 4× SSC and 0.1% Tween 20 (Nakarai, Osaka, Japan)) at 37°C for 30 min. To detect biotin, 150 μl of avidin–fluorescein ( Oncor, Gaithersburg, MD, USA) diluted 400 times with 4× SSC and 0.1% Tween 20 was applied to the slide, incubated at 43°C for 30 min, and then washed three times with washing solution (4× SSC and 0.1% Tween 20) at 43°C for 5 min. The slide was stained with propidium iodide (Oncor, Gaithersburg, MD, USA) solution (100ng/ml in 2× SSC) for 5 min at room temperature to stain the cell nucleus and chromosome, washed with 2× SSC, and coverslipped with 40g/ml of anti-fading solution (Oncor, Gaithersburg, MD, USA).

2.7. Statistical analysis

The standard error for yields of chromosome aberrations was estimated by regression analysis. Alpha and beta coefficient values in the linear regression lines or linear quadratic regression curves were estimated by regression analysis. Incidences of chromosome aberrations and micronuclei were compared at each total dose between irradiated groups and non-irradiated control groups at 8 weeks of age, and were tested for statistical significance using Student t-test.

3. Results and discussion

3.1. Incidences of dicentric and centric ring chromosomes

Total aberrations of dicentrics and centric rings in mice exposed to γ-rays at a dose rate of either 200 mGy/day or 20 mGy/day, and age-matched, non-irradiated control mice were estimated. The frequencies of aberrations per 100 cells at 8000 mGy at 20 mGy/day were 17.2 times higher than those of non-irradiated mice, and increased as accumulated doses increased (Table 1, Fig. 1). Dose–response relationships between the incidences of dicentrics and centric rings and total accumulated doses up to 8000 mGy at both dose rates of 200 mGy/day and 20 mGy/day were obtained, respectively. The dose–response curves for both 200 mGy/day and 20 mGy/day were almost linear up to 8000 mGy, indicating that these aberrations increased with accumulated dose for both dose rates. The rate of chromosome aberrations also slightly increased with the accumulated dose of irradiation of 200 mGy/day and 20 mGy/day (Table 1). The equations obtained are shown in Fig. 1 for 20 mGy/day and 200 mGy/day. The value of 0.086 at 0 Gy was obtained as the mean number of aberrations of dicentric and centric rings per 100 cells from the observation of 25 non-irradiated 8 weeks old mice at the beginning of irradiation.

The incidence of aberrations as dicentric and centric ring chromosomes were compared at two different dose rates (200 mGy/day
Table 1

<table>
<thead>
<tr>
<th>Dosage (mGy/day)</th>
<th>Number of cells (number of mice)</th>
<th>Complexity (Cu/cell)</th>
<th>Dicentric (Ra/100 cells: mean ± S.E.)</th>
<th>Centric ring (Fg/100 cells: mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>20</td>
<td>0.09 ± 0.02</td>
<td>1.13 ± 0.04</td>
<td>1.0 ± 0.01</td>
</tr>
<tr>
<td>100</td>
<td>8</td>
<td>0.06 ± 0.02</td>
<td>1.32 ± 0.04</td>
<td>1.0 ± 0.01</td>
</tr>
<tr>
<td>140</td>
<td>7</td>
<td>0.08 ± 0.04</td>
<td>1.12 ± 0.04</td>
<td>1.0 ± 0.01</td>
</tr>
<tr>
<td>250</td>
<td>6</td>
<td>0.08 ± 0.04</td>
<td>1.33 ± 0.06</td>
<td>1.0 ± 0.01</td>
</tr>
<tr>
<td>400</td>
<td>4</td>
<td>0.06 ± 0.02</td>
<td>1.33 ± 0.06</td>
<td>1.0 ± 0.01</td>
</tr>
<tr>
<td>500</td>
<td>5</td>
<td>0.06 ± 0.02</td>
<td>1.44 ± 0.04</td>
<td>1.0 ± 0.01</td>
</tr>
<tr>
<td>600</td>
<td>6</td>
<td>0.06 ± 0.02</td>
<td>1.44 ± 0.04</td>
<td>1.0 ± 0.01</td>
</tr>
<tr>
<td>750</td>
<td>7</td>
<td>0.06 ± 0.02</td>
<td>1.56 ± 0.04</td>
<td>1.0 ± 0.01</td>
</tr>
<tr>
<td>800</td>
<td>8</td>
<td>0.06 ± 0.02</td>
<td>1.56 ± 0.04</td>
<td>1.0 ± 0.01</td>
</tr>
</tbody>
</table>

The values were statistically higher than that of 0 mGy (p<0.05 in regression line shown by and were almost constant in younger or older SPF mice, as in the bone marrow, thymus and lymph nodes of mice continuously exposed to low-dose radiation. It is possible that the spleen is a reservoir for lymphocyte populations, which could migrate into the spleen from other tissues, including the bone marrow, thymus and lymph nodes of mice continuously irradiated for a long period. Another possibility is that a long-lived lymphocyte subset may be relevant for the linear increase of dicentric and centric ring chromosomes.

Since it is well known that chromosome aberration rates increase with aging [19], we previously analyzed the relationship between unstable-type chromosome aberration rates and the age of mice in 109 SPF C3H/HeN female mice. In the analysis, unstable-type aberration was defined as total aberrations of Dic, Rc and Fg. Unstable-type aberration rates did not increase and were almost constant in younger or older SPF mice, as in the bone marrow, thymus and lymph nodes of mice continuously irradiated for a long period. Another possibility is that a long-lived lymphocyte subset may be relevant for the linear increase of dicentric and centric ring chromosomes.

Fig. 1. Frequencies of dicentrics and centric rings from mice after irradiation of less than 8000 mGy at LDR of 20 mGy/day (■) and 200 mGy/day (□). Each dot indicates the value obtained from individual mice.
at several doses to identify the existence of any dose-rate effect.

3.2. Chromosome instability detected by unstable-type aberrations

The complexity of chromosome aberrations in terms of the number of unstable-type aberrations per cell will be a suitable cytogenetic index for evaluating chromosome instability after prolonged LDR irradiation. Chromosome aberrations such as Dic, Rc, Ra, Fg, min and del were scored and the total number of aberrations per cell was calculated using only aberrant cells in each mouse. Complexity was determined as [total number of unstable-type aberrations; (Dic + Rc + Ra + Fg + min + del)/total number of abnormal cells with chromosome-type aberrations]. The results are summarized in Table 1 and Fig. 2a and b. Their complexity started to increase from more than 200 mGy in the irradiation at 200 mGy/day and from more than 500 mGy in the irradiation at 20 mGy/day, and both increased with the accumulated dose and their numbers became saturated to be almost identical at 8000 mGy (Fig. 2a). Then, with irradiation at both 20 mGy/day and 200 mGy/day, they started to increase from early days, 8 days and 1 day after initial irradiation, respectively (Fig. 2b). On the other hand, our preliminary observation of translocation in spleen cells from mice with prolonged irradiation at 20 mGy/day showed that the complexity increased after 200 days after initial irradiation (more than 4000 mGy). The number of aberrations per cell was not different between unstable-type aberrations and translocations. Chromosome complexity was also compared at the same irradiation period within 40 days between dose rates of 20 mGy/day and 200 mGy/day (Fig. 2b). Complex aberrations were enhanced by irradiation with a dose rate of 200 mGy/day than 20 mGy/day, indicating an influence of dose rate on chromosome instability.

7. On the other hand, the incidences of hyperploidy and complexity of chromatid-type aberrations, which are determined as [total number of (ctg + ctb + exc)/total number of abnormal cells with chromatid-type aberrations] did not increase with accumulated dose (Table 1), although these two types of chromosome aberrations have been used as suitable markers for establishing chromosome instability [4,5,20,21].

3.3. Chromosome instability detected by micronucleus assay

Then we observed micronucleus incidence in splenocyte and bone marrow cells in mice after long-term irradiation at an LDR of 20 mGy/day, and found that the pattern of chronological changes of micronucleus incidence was quite different from that of chromosome aberrations. Micronucleus incidence in splenocytes increased with age. Micronucleus incidence in mitogen-stimulated splenocytes started increasing after more than 306 days (Table 2), but the incidence was not significantly different between irradiated mice and age-matched non-irradiated mice. However, the numbers of cells with 2–3 micronuclei out of 10,000 observed cells were significantly higher in mice irradiated with 8000 mGy than age-matched non-exposed mice. Although the number of micronuclei in 5000–7000 polychromatic erythroblasts in bone marrow from
each mouse was not higher than in non-exposed mice (Table 2), the number of cells with 2–3 micronuclei in bone marrow erythroblasts was higher in 8000 mGy-exposed mice at LDR.

3.4. Chromosome instability detected by interphase FISH method

Trisomic splenocytes were also observed by the interphase FISH method to demonstrate chromosome instability in splenocytes from mice at a LDR of 20 mGy/day for 8000 mGy. The FISH method using three mouse centromere probes of chromosomes 5, 13 and 18 showed that splenocytes in the exposed mice had a significantly higher incidence of 3 signals in the nucleus, also indicating higher incidence of trisomies of chromosomes of 5, 13 and 18, than in non-exposed mice (Table 3). One signal in the nucleus, indicating monosomy of chromosomes 5, 13 and 18, was also observed at a higher incidence in irradiated mice, but cells showing one signal were excluded from the present analysis, because they are unconfirmed due to too much high background FISH signal (about 3%) in the nucleus from non-irradiated mice. FISH analysis of splenocytes showed that mice exposed to 8000 mGy by LDR irradiation had a higher percentage of trisomy cells. These numerical chromosome aberrations seem to be induced indirectly after radiation exposure and thus the results indicate the possibility that prolonged γ-ray irradiation at LDR of 20 mGy/day induces delayed (late-arising) chromosomal instability in mice. Numerical aberrations are induced directly by more than 2000–4000 mGy of acute X-ray irradiation with high-dose-rate in human peripheral blood [22]. These results will be useful for risk assessment of LDR radiation exposure.

3.5. Chromosome instability induced by chronic radiation at LDR

A micronucleus forms from either a broken chromosome or from chromosome loss during the step of chromosome segregation at the M phase, and induces cells with a chromosome deletion or aneuploid cells. Of general concern is that these numerical chromosome aberrations obtained by the FISH method and micronucleus assay are not induced directly by radiation exposure. These results indicate that prolonged 137Cs γ-ray irradiation with LDR of 20 mGy/day for 5000–8000 mGy induced delayed chromosome instability in splenocytes and bone marrow cells from mice. We have, for the first time, demonstrated in mice splenocytes that LDR long-term irradiation with γ-rays can induce chromosome instability in vivo. Cells with heavy DNA damage may be eliminated by apoptosis, whereas cells able to survive upon exposure may have a higher probability of developing a mutation, and cells surviving after ionizing radiation lead to genomic (chromosome) instability. Radiation-induced genomic instability arising at a late stage is demonstrated by increased rates of mutation, decreased cell survival, and various chromosome aberrations, such as chromosome-type aberrations, chromatid-type aberrations, and micronuclei. An acute low dose to mice in vivo induced high incidences of chromatid breaks and fragments and polyploid cells in bone marrow cells after birth [23]. Persistent chromatid-type aberrations were commonly observed in cells long-term cultured from haematopoetic and solid mouse tissues exposed to γ-rays [24–27]. Lung cells from rats injected with superoxide dismutase showed a reduced number of micronuclei, which indicates the involvement of oxygen radicals in chromosome instability [28]. Cells having chromatid-type aberrations result in one chromosomal deletion and one normal cell at the next cell division. Since unstable-type chromosome aberrations such as dicentric chromosomes are unstable and do not persist through the next cell cycle, a high percentage of these aberrations occurring by irradiation are eliminated after many cell generations. From these experiments, it was concluded that unstable-type aberrations, chromatid-type aberrations and polyploid cells are likely to be suitable markers for detecting chromosome instability in vivo after long-term LDR irradiation, although the precise classification of direct radiation-induced aberrations and delayed chromosome aberrations could be difficult. Complex chromosome aberrations having several aberrant chromosomes per cell are usually induced by high LET radiation and rarely induced by a low dose of low LET radiation. Peripheral blood lymphocytes from residents exposed to prolonged low-dose irradiation in radio-contaminated buildings in Taiwan had a higher frequency of variable type chromosome aberrations [3]. A clone with complex karyotype aberrations developed in a cultured human lymphocyte cell line during cell growth after γ-ray irradiation [9]. Thus, the complexity of unstable-type or stable-type chromosome aberrations will also be used as a suitable cytogenetic marker of chromosome instability.

Radiation-induced chromosome or genetic instability has been demonstrated mostly using acute irradiation at high-dose-rates. Few studies have addressed whether low-dose or LDR radiation can induce chromosome or genomic instability long-term after irradiation. Clones established from irradiated human lymphocytes by 1 Gy at a fairly LDR of 24 mGy/h showed chromosome instability with chromosome breaks and rearrangements [29]. The authors also stated that chromosome instability showed no dose-rate effects at 1 Gy with a high-dose-rate (4500 mGy/h) and LDR (24 mGy/h). On the other hand, low-dose radiation (100–500 mGy) did not induce chromosome instability in a rodent-human hybrid cell line after X-ray irradiation of at least 5.52 mGy/h by X-rays [30]. Abrahamsson-Zetterberg et al. [31] reported exposure to radiation with a dose rate of 20–2000 mGy/h of mouse embryos, which showed no increase of chromosome instability at 35 days after birth using a micronucleus assay combined with flow cytometry. This indicates that LDR radiation doses might permit sufficient DNA repair, however, our present observation of exposure to 20 mGy/day (0.91 mGy/h), which is much lower than reported by others, clearly showed chromosome instability. As reasons for the discrepant

---

### Table 3

<table>
<thead>
<tr>
<th>Dose (mGy)</th>
<th>Number of mice</th>
<th>Age (Days)</th>
<th>Total of observed cells</th>
<th>Observed chromosomes</th>
<th>Observed cells</th>
<th>Total cells with FISH signals of three chromosomes</th>
<th>Number of cells with 3 signals per 1000 cells (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>456</td>
<td>49,011</td>
<td>Chr.18  Chr.13  Chr.5</td>
<td>16,216  16,689  16,106</td>
<td>1945  46,784  205  78  0</td>
<td>4.18 ± 0.76</td>
</tr>
<tr>
<td>8000</td>
<td>5</td>
<td>456</td>
<td>49,354</td>
<td>Chr.18  Chr.13  Chr.5</td>
<td>16,597  16,272  16,485</td>
<td>3128  45,726  375  123  2</td>
<td>7.60 ± 2.47</td>
</tr>
</tbody>
</table>

1 The value was statistically higher than that of 0 Gy ($p < 0.05$ by t-test).
results, the much longer irradiation period (accumulated dose) and different developmental stage at the time of irradiation used in present or previous in utero studies [31–33], might have an influence.

3.6. Plausible mechanisms for chromosome instability

The mechanisms for chromosome instability induced by long-term LDR irradiation in vivo, observed in the present study, are unknown. However, these observations raised the possibility that radiation-induced genomic (chromosome) instability in vivo might not only result from direct DNA (chromosomal) damage by radiation, but also that persistent production of damaged cells in each cell generation might result from the production of reactive oxygen (ROS) or reactive nitrogen species (RNS), or inflammatory cytokines released by macrophages or other cells in tissue [24,34–36]. This also shows that genomic (chromosome) instability can arise from interactions between irradiated cells and non-irradiated cells, which is now known as a bystander effect by radiation exposure. It is well documented that cells exposed extracellularly to superoxide and ROS display high levels of chromosome aberrations [37], particularly chromatid-type aberrations [38]. Further precise analyses will be necessary to elucidate in vivo conditions in mice irradiated long-term at a LDR.

Because dicentric and centric ring chromosomes are eliminated at each cell division after irradiation due to their unique morphology with two centromeres on one chromosome, the increase of dicentric and centric ring chromosomes in cells having translocations increase with the increase of total accumulated dose of LDR irradiation. Cells with chromosome instability can persist for a longer period after irradiation and some form a benign or malignant clone, which may develop into neoplastic transformation, implicating the biological significance of continuous, long-term exposure to LDR radiation. Information on the incidence of chromosome aberration frequencies and chromosome instability in persistent translocations will become more important for low-dose radiation-induced risk in humans.

Conflicts of interest

None.

Acknowledgement

This study was performed under contract with the Aomori Prefectural Government, Japan.

References


[37] B.E. Lehnert, E.H. Goodwin, Extracellular factors(s) following exposure to α particle can cause sister chromatid exchanges in normal human cells, Cancer Res. 57 (1997) 2164–2171.
