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NO EVIDENCE FOR THE *IN VIVO* INDUCTION OF GENOMIC INSTABILITY BY LOW DOSES OF ^{137}CS GAMMA RAYS IN BONE MARROW CELLS OF BALB/CJ AND C57BL/6J MICE

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□ In spite of extensive research, assessment of potential health risks associated with exposure to low-dose (≤ 0.1 Gy) radiation is still challenging. We evaluated the *in vivo* induction of genomic instability, expressed as late-occurring chromosome aberrations, in bone-marrow cells of two strains of mouse with different genetic background, *i.e.* the radiosensitive BALB/cJ and the radioresistant C57BL/6J strains following a whole-body exposure to varying doses of ^{137}Cs gamma rays (0, 0.05, 0.1, and 1.0 Gy). A total of five mice per dose per strain were sacrificed at various times post-irradiation up to 6 months for sample collections. Three-color fluorescence *in situ* hybridization for mouse chromosomes 1, 2, and 3 was used for the analysis of stable-aberrations in metaphase-cells. All other visible gross structural-abnormalities involving non-painted-chromosomes were also evaluated on the same metaphase-cells used for scoring the stable-aberrations of painted-chromosomes. Our new data demonstrated in bone-marrow cells from both strains that low doses of low LET-radiation (as low as 0.05 Gy) are incapable of inducing genomic instability but are capable of reducing specific aberration-types below the spontaneous rate with time post-irradiation. However, the results showed the induction of genomic instability by 1.0 Gy of ^{137}Cs gamma rays in the radiosensitive strain only.

Keywords: low dose, gamma rays, mouse, genomic instability, cytogenetics

INTRODUCTION

It has been suggested that genomic instability is a key event in carcinogenesis, although the mechanisms by which initial damage leading to the instability of the genome remain unclear (Morgan *et al.*1996; Mothersill and Seymour 1998; Little 2003; Morgan 2003; Brooks 2005). The phenotypes of genomic instability can be expressed as delayed mutations, reproductive death, and chromosomal instability. Radiation-induced genomic instability determined as delayed chromosomal instability was extensively described in mouse (CBA/H) or human hematopoi-

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etic stem cells (Kadhim *et al.*1992; Kadhim *et al.*1994; Kadhim *et al.*1995), and human lymphocytes, following exposure *in vitro* to high linear energy transfer (LET) radiation, *i.e.* α-particles from plutonium-238 or those generated from a 4MV Van de Graaff accelerator. The majority of aberrations were of the chromatid-type, indicative of the damage occurring *de novo* in the subsequent cell cycles. Experiments using an *in vitro* irradiation/*in vivo* analysis strategy also showed a persistence of chromosomal instability *in vivo* for up to one year by ^{238}Pu α-particles in bone-marrow (BM) cells isolated from different strains of exposed mice (Watson *et al.*1996). The authors also reported higher radiosensitivity in CBA/H and DBA/2 mice than in C57BL/6 mice, suggesting the influence of genetic factors on radiation-induced genomic instability. In contrast to these studies, no delayed chromosomal instability was found in BM cells collected from the CBA/H mouse following exposure either *in vivo* or *in vitro* to the short-lived bone-seeking ^{224}Ra α-particles (3.6 day half-life) (Bouffler *et al.*2001). Differences in experimental design might have contributed to this discordant result. These included differences in the α-emitting radionuclides, culture systems, transplantation, and cytogenetic methods for analyzing chromosomal damage, *i.e.* G-banding (Kadhim *et al.*1992; Kadhim *et al.*1994; Kadhim *et al.*1995) *vs* a three-color FISH for chromosomes 1, 2, and 3 (Bouffler *et al.*2001). It should be noted that the numbers of mice per harvest time (*i.e.* one mouse in the control group and ranging from two to four mice in the treated groups) included in the *in vivo* study using a three-color FISH are relatively small.

Low LET radiation, at doses ranging from 3.0 to 10 Gy, is also capable of inducing genomic instability or late-occurring chromosomal damage *in vitro* (Holmberg *et al.*1993; Marder and Morgan 1993; Kadhim *et al.*1995). A similar finding has been observed *in vivo* by examining BM cells or lymphocytes collected from C57BL/6 mice (Kligerman *et al.*1990) or BALB/c mice (Jagetia 1993) exposed to 3.0 to 4.0 Gy of X rays. Subsequently, G-banding analysis was used to examine the fate of mouse chromosome 2 (chr2) in BM cells sequentially collected from CBA/CaJ mice exposed to 2.0 Gy of X rays (Rithidech *et al.*1995). An increase in cells acquiring stable aberrations involving chr2 was detected up to 24 months. In addition to chr2, both non-clonal and clonal aberrations involving several other chromosomes were observed in BM cells of exposed mice, suggesting the induction of genomic instability by low LET radiation. Similar findings of the hypermutability of mouse chr2 following exposure to low LET radiation have also been described by other groups of investigators (Ban *et al.*1997; Bouffler *et al.*1997; Xiao *et al.*1999). Further, chr2 lesions have been well recognized to be an important genetic event in the development of radiation-induced myeloid leukemia (rML) (Hayata *et al.*1979; Cox *et al.*1991; Bouffler *et al.*1996; Ban *et al.*1997; Rithidech *et al.*1999; Xiao *et al.*1999; MacDonald *et al.*2001;

Jawad *et al.*2006; Kanda *et al.*2008; Peng *et al.*2009). However, it is possible that the lesions on mouse chr2 acquired in rML cells may not be the lesions that are responsible for the induction of genomic instability in exposed cells at early time post-irradiation. Of note, it has been suggested that genetic susceptibility to radiation-induced hematopoietic neoplasms is not associated with the sensitivity to radiation-induced genomic instability (Boulton *et al.*2001). Further evidence for complex multigenic inheritance of rML susceptibility in mice has been found on several other chromosomes (*e.g.* chr8, chr13, and chr18) (Darakhshan *et al.*2006).

By means of FISH with DNA probes for several mouse chromosomes, a persistence of stable aberrations was detected in BM cells and lymphocytes collected from mice exposed *in vivo* to a single dose of X or γ rays at doses ranging from 0.5 to 7.0 Gy (Hande *et al.*1996; Spruill *et al.*1996; Bouffler *et al.*1997; Xiao *et al.*1999; Giver *et al.*2000). It has also been suggested that aging and clonal expansion contribute to the persistence of translocations (Giver *et al.*2000; Spruill *et al.*2000), and that genetic factors influence the translocation frequency in hematopoietic cells (Giver *et al.*2000). Recently, using the mouse *in vivo* micronucleus (MN) assay in blood erythrocytes, we found increases in the frequencies of MN in polychromatic erythrocytes (PCEs) of irradiated CBA/CaJ mice (the radiosensitive strain), but not C57BL/6J mice (the radioresistant strain), at 3 months post-exposure to ^{137}Cs γ rays (0, 0.5, 1.0 and 3.0 Gy, at the dose rate of 0.72 Gy/min) or 1 GeV ^{56}Fe ions (0, 0.1, 0.5 and 1.0 Gy, at the dose rate of 1.0 Gy/min) (Rithidech *et al.*2009). This finding indicates the potential induction of genomic instability in hematopoietic cells of CBA/CaJ (but not C57BL/6J) mice by both types of radiation. However, it is unclear whether such deleterious effects will be detected at doses less than or equal to about 0.05 Gy/year, the existing limit for radiation exposure in the workplace, of low LET radiation (*e.g.* X and γ rays).

In this study, we determined the effects of low doses (less than or equal to 0.1 Gy) of low -LET radiation (^{137}Cs γ rays) on the type and the frequency of initial and late-occurring chromosome aberrations induced *in vivo* in BM cells collected at different times up to 6 mos following irradiation from two strains of mouse, *i.e.* BALB/cJ and C57BL/6J mice. Chromosome aberrations were used as a marker for radiation induced genomic instability. Multicolor fluorescence *in situ* hybridization (FISH) of mouse chr1, chr2, and chr3 was used to determine their involvement in the *in vivo* induction of stable chromosomal exchanges (or rearrangements) by low-dose radiation. Using this approach, exchanges between each of the three painted chromosomes or those involving one of the painted chromosomes and a non-painted chromosome (designated as nonP) can be easily scored. Only three chromosomes were used for the FISH method in this study because of the unavailability, at that time when the study was carried out, of the combinatorial multicolor fluorescence *in*

situ hybridization (mFISH) probes for the whole genome of the mouse. The reasons for choosing mouse chr1, chr2, and chr3 for the analysis of chromosome rearrangements by means of FISH are as follows: (*a*) they are the largest three chromosomes of the mouse genome, and (*b*) the existing *in vivo* databases indicate the persistent abnormalities of these three mouse chromosomes following exposure to high doses of low LET radiation (Rithidech *et al.*1995; Hande *et al.*1996; Spruill *et al.*1996; Bouffler *et al.*1997; Xiao *et al.*1999; Giver *et al.*2000). However, these three mouse chromosomes represent approximately 19% of the whole mouse genome DNA (Disteche *et al.*1981). Hence, in order to accurately assess the total frequencies of chromosome aberrations induced by low-dose ^{137}Cs γ rays in mouse BM cells, all other gross structural abnormalities (breaks and exchanges) involving all nonP chromosomes were also determined on the same metaphase cells that were used for scoring stable chromosome aberrations involving painted chromosomes.

MATERIALS AND METHODS

Animals and Radiation Exposure

Male BALB/cJ and C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). They were 8-10 weeks old at the time of delivery and were acclimatized for 2 weeks prior to γ-irradiation. Since these male mice were not littermates, it was important that they were housed one in a cage to prevent fighting or cannibalism. Purina rodent chow and sterile drinking water were available to the mice *ad libitum*. The light cycle was 12 hr light/12 hr dark. Mice were housed and cared for in a facility accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All animal handling procedures were performed under the approved guidelines by the Institutional Animal Care and Use Committee (IACUC) of Stony Brook University.

Four groups of 20 mice (10-12 weeks old at exposure) of each strain were given a whole body dose to 0, 0.05, 0.1, and 1.0 Gy of ^{137}Cs γ rays (at the dose rate of 0.75 Gy/min) using the Gamma Cell40 (Atomic Energy of Canada, Ltd, Ontario, Canada) located in the Division of the Laboratory Animal Resources of Stony Brook University. A high dose of 1.0 Gy was used as a positive control. The radiation doses mentioned in this study were the average total-body doses. Dosimetry of the Gamma Cell40 is routinely performed by MDS Nordion (Ontario, Canada). To ensure the accurate exposure time for each dose of ^{137}Cs γ rays, the exposure time was determined manually, in addition to setting the built-in automatic timing (on/off) switch. The shutter opening and closing time, signaled by a unique built-in sound, was excluded from the exposure time. γ-irradiation procedures described previously (Rithidech *et al.*2005) were followed. Briefly, each unanesthetized mouse was placed in the ven-

tilated circular holder to minimize its movement during exposure so the whole body would receive the radiation dose uniformly (95% confidence level). Likewise, unirradiated controls were placed in the ventilated circular holder but received 0 Gy of ^{137}Cs γ rays.

Collection of BM Cells

There were four harvest times following irradiation, *i.e.* 1 hr, 4 hr, 1 mo, and 6 mos. At each harvest time, BM cells were collected from five mice per dose for the analysis of chromosome aberrations. We collected BM cells from each mouse by flushing both femurs and tibiae with 10 mL of McCoys' 5A medium (Invitrogen, Grand Island, NY). Usually, about 30-40 X 10^6 cells were obtained from each mouse.

Cytogenetic Assay

Methods for culturing and harvesting metaphase cells for cytogenetic analysis are modified from those previously described (Rithidech *et al.*1993; Rithidech *et al.*1995; Rithidech *et al.*2007). Briefly, BM cultures were established in McCoys' 5A medium supplemented with 15% fetal bovine serum, 1% penicillin/streptomycin, 1% L-glutamine, and 10% Interleukin-3. For each mouse at each harvest time, a total of four cultures were set up in 15-mL conical tubes. Each culture contained approximately 8 X 10^6 BM cells in 5-mL complete medium.

For BM cells harvested at early times (1 and 4 hr) post-irradiation, we obtained metaphase chromosomes by the addition of colcemid (0.2 μg/mL) into freshly prepared BM cultures that were incubated in a water bath at 37^{0}C for 2 hr. Of note, the incubation time was short to ensure the accurate measurement of the type and the number of chromatid- or G2-type aberrations occurring at 1 and 4 hr post-irradiation (see additional information in the Chromosome Aberration Scoring section below). Further, if the culture time was prolonged (*e.g.* 24 hr), the heavily damage cells might have been lost due to their inability to survive a subsequent cell division. This phenomenon would result in obtaining inaccurate information on the frequency of initial chromosome aberrations induced by radiation. For the measurement of late-occurring chromosome aberrations (BM cells harvested at 1 and 6 mos following irradiation), a short-term (24-hr) culture was applied. This protocol has routinely been used in our laboratory (Rithidech *et al.*2007) because it consistently provides a high yield of metaphase cells needed for the analysis of chromosome aberrations, in particular the stable-type aberrations and clones of aberrant cells that survive cell division. It is worth noting that a subset of BM cells might have undergone a cell cycle during the 24-hr incubation in which a dilution out of aberrations (in particular breaks and/or unstable-type aberrations incapable of surviving cell division) may have occurred. This, in turn, may have inconsequentially changed

the absolute numbers of abnormal cells. However, such aberrations may be irrelevant to the induction of genomic instability and an eventual neoplastic transformation of hematopoietic cells.

A standard cytogenetic method using freshly prepared Carnoy's solution (3:1 vol/vol of absolute methanol:glacial acetic acid) as fixative (Moorhead and Nowell 1964) was applied to harvest metaphase BM cells. After 2-3 washings in fixative, we stored the BM cells at 4°C (in approximately 5 mL of fixative) until use for slide preparation for the analysis of chromosomal damage. After the second wash in fixative, one test slide was made for all treatment groups to check the quality of metaphase chromosomes. This test slide also was used for the determination of the mitotic index (MI) after being stained in 10% Giemsa for 8 min. The MI was determined on coded slides by counting the number of mitotic cells among at least 1,000 cells per mouse and expressed in percentage.

Fluorescence *in situ* Hybridization (FISH) Assay

One-week old slides were routinely used for the FISH assay. Each slide was stained with concentrated paint probes for chromosomes 1, 2, and 3 (purchased from Vysis/Cambio, Inc., Cambridge, UK) simultaneously as previously suggested (Bouffler *et al.*2001) but with some modifications. Briefly, microscopic slides containing well spread metaphase chromosomes and a high MI were denatured in 70% formamide (diluted with 2X SSC, pH 7.0) at 70°C for 2 min. Thereafter, each slide was dehydrated in a -20°C ethanol series, *i.e.* 70%, 85%, 95% (2 min each), air dried for 20 min, heated to 37°C, and hybridized with a total of 25 µL of a solution containing 16 µL of hybridization mix, 1 µL of biotin-labeled chromosome 3 probe, 1 µL of FITC-labeled chromosome 2 probe, 1 µL each of biotin- and FITC-labeled chromosome 1 probe, and 5 µL of 1 mg/mL mouse Cot1 DNA (Invitrogen, Grand Island, NY). A 22 X 22 mm coverslip was placed on top of the slide and sealed with rubber cement. The slides were incubated in a moisture chamber at 42°C for about 48 hrs before being washed in 2X SSC, followed by two 15-min washes in 2X SSC /50% formamide at 43°C and two washes in 2X SSC (10 min each) prior to the fluorescent signal detection. Biotin-labeled probes were detected with alternating layers of avidin-Texas Red and biotinylated goat anti-avidin D antibody (Vector Laboratories, Burlingame, CA), and FITC-labeled probes were detected with alternating layers of rabbit IgG-FITC (Molecular Probes, Eugene, OR) and anti-rabbit IgG (Vector Laboratories, Burlingame, CA). Three layers of each fluorescence detecting reagent were applied and finally all other metaphase chromosomes were counterstained blue with Vectashield anti-fade containing 400 ng/mL 4',6-diamidino-2-phenylidonle (DAPI) (Vector Laboratories, Burlingame, CA). Using this protocol, chr3 appeared red, chr2 appeared green, chr1 appeared yellow (or a speckled mixture of red/green), and

all other chromosomes were blue. Metaphase images were captured and stored using a digital imaging ISIS system (MetaSystems Group, Inc, Watertown, MA) with a cooled CCD camera equipped with a special FISH software program.

Chromosome Aberration Scoring

All slides were coded before scoring. Chromosome aberrations were scored in metaphase cells using the criteria previously suggested (Tucker *et al.*1997). All other chromatid- or chromosome-type aberrations and gross structural abnormalities involving nonP chromosomes were determined simultaneously in the same metaphase cells that were used for scoring stable chromosome aberrations involving painted chromosomes. All chromosomal fragments were scored as one break, regardless of whether they appeared as double or single fragments with clear displacement of the broken segment. Gaps (those with a discontinuity shorter than the chromatid width or non-displacement) were recorded separately. The number of cells with chromosomal damage (abnormal cells) for each treatment group was also recorded.

To determine the number of initial breaks at the early harvest times (1 and 4 hr post-irradiation), a single chromatid fragment was scored as one chromatid break. However, since one iso-chromatid break results from the breakage at the same region of two chromatids of a chromosome (with or without acentric fragments), two chromatid breaks were scored to represent one iso-chromatid break. At late time-points, however, the breakage at the same region of two chromatids of a chromosome was scored as one chromosome break. The criterion for determining a clone of cells suggested previously (Rowley and Potter 1976) was used, *i.e.* two or more cells with the same structural abnormalities on the same chromosomes in each individual mouse.

Statistical Analysis

The average square root transformation (ASQRT, $\sqrt{X} + \sqrt{(X+1)}$ where X is the observed frequency of each type of aberration) was applied to each animal's measured aberration frequency to achieve reasonable normality and reasonably homogeneous inter-animal variability within treatment combination groups (Whorton 1985). The analysis of variance (ANOVA) methods appropriate for two factor factorial experiments were used to evaluate the resulting chromosome data for the main or overall effects of time, radiation dose, and their interaction. Analyses were conducted separately for each strain. One factor was time post-exposure and the other factor was radiation dose-level. A p value of ≤ 0.05 was considered statistically significant.

RESULTS

Mitotic Index (MI)

Figures 1a (BALB/cJ mice) and 1b (C57BL/6J mice) showed significant reduction in the percentage of mitotic cells in response to a high dose (1.0 Gy) of ^{137}Cs γ rays, related to the corresponding unirradiated controls and those in the 0.05 Gy-exposed group, at 1 and 4 hr post-irradiation (*p<0.01, student's t-test*). The reduction in MI, perhaps due to cell cycle arrest or cell killing, was more pronounced in irradiated BABL/cJ mice as compared to C57BL/6J mice (*i.e.* a 3-fold to a 2-fold reduction in BALB/cJ and C57BL6/J mice, respectively). Our data demonstrated that the MI assay may be useful for detecting differences in radiosensitivity of mice with different genetic backgrounds at the early time post-exposure. However, the MI assay is unable to distinguish between surviving cells with or without damage. The levels of MI in mice (both strains) exposed to 1.0 Gy of ^{137}Cs γ rays returned to the levels detected in the corresponding unirradiated controls at 1 and 6 mos post-irradiation.

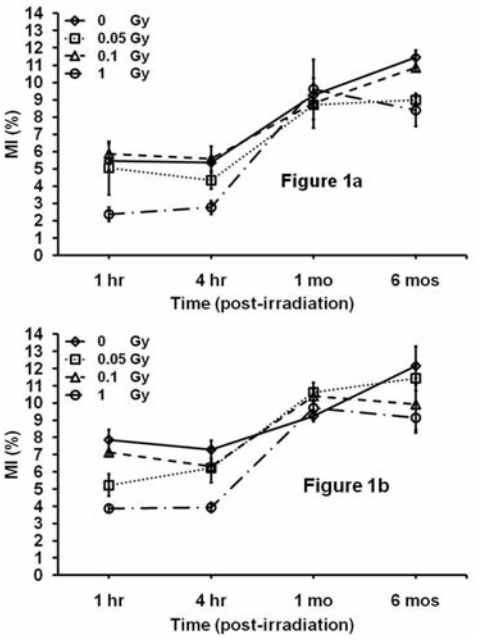


FIGURE 1. a) Dose response for MI as a function of time following irradiation in BM cells of exposed male BALB/cJ mice. The error bars represent standard error (S.E.) of the mean from 5 mice per dose at each harvest time. b) Dose response for MI as a function of time following irradiation in BM cells of exposed male C57BL/6J mice. The error bars represent standard error (S.E.) of the mean from 5 mice per dose at each harvest time.

Chromosome Aberrations

Early time-points (1 and 4 hr post-irradiation)

Tables 1a and 1b show the details of pooled raw data of each aberration type (*i.e.* abnormal cells, breaks, and exchanges) and the chromosome(s), both painted and nonP ones, involved in every aberration type determined at 1 and 4 hr from each group of exposed BALB/cJ mice, respectively. Likewise, Tables 2a and 2b show the details of such cytogenetic data from each group of exposed C57BL/6J mice. Figures 2a and 2b provide the frequencies of each type of chromatid (G2) aberration per 100 cells scored (± S.E.), including abnormal cells, detected in BM cells collected from BALB/cJ mice at 1 and 4 hr post-irradiation, respectively. The numbers presented on the graphs were the ASQRT numbers which also were used for evaluating the statistical significance (see the Method Section). Such data for C57BL/6J mice are shown in Figures 3a and 3b.

The majority of aberrations were chromatid breaks with or without the presence of acentric fragments. Chromatid exchanges included translocations (Robertsonian or centric fusion, reciprocal and incomplete types) and dicentrics. Most of the translocations belonged to the incomplete one-way type because fragments were missing, regardless of radiation dose, harvest time, or strain of mouse. These findings were similar to those reported previously in an *in vivo* study of γ- or ^{56}Fe-ion-irradiated CBA/CaJ mice (Rithidech *et al.*2007) or those observed in *in vitro* ^{56}Fe-ion-irradiated human lymphocytes (Durante *et al.*2002). These translocations occurred between either two of the painted chromosomes or between one of the painted chromosomes and a nonP one. There was no indication of the non-random involvement of specific chromosomes (either painted or nonP) in any particular type of the aberrations detected at these early time-points in both strains of mouse. Dicentrics and Robertsonian translocations (RT) were infrequently found. The resulting data showed no increases, related to the corresponding unirradiated controls, in the frequencies of abnormal cells or any type of chromatid-type aberrations (shown in Figures 2a, 2b, 3a, and 3b) in the 0.05 Gy-exposed mice at 1 and 4 hr post-irradiation in both strains of mouse. In contrast, there was significant damage ($p<0.05$) in BM cells of mice exposed to 0.1 or 1.0 Gy of ^{137}Cs γ rays.

Late time-points (1 and 6 mos post-irradiation)

Tables 3a and 3b show the details of pooled raw data of each aberration type (i.e. abnormal cells, breaks, and exchanges) and the chromosome(s), both painted and nonP ones, involved in every aberration type from each group of exposed BALB/cJ mice determined at 1 and 6 mos, respectively. As well, Tables 4a and 4b show the details of the cytogenetic data from each group of exposed C57BL/6J mice. The frequencies (ASQRT values) of each type of chromosomal damage per 100 cells

TABLE 1A. Cytogenetic data from bone marrow cells collected at 1 hr after exposure of male BALB/cJ mice to varying low doses of ^{137}Cs γ rays, including a high dose of 1.0 Gy serving as a reference dose [*t* Translocation (incomplete type), *RT* Robertsonian translocation, *dic* Dicentric, *nonP* non-painted chromosome]

Dose (Gy)	Total number of cells scored	Total number of abnormal cells	Total number of chromatid breaks	Chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of iso-chromatid breaks	Iso-chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of exchanges	Chromatid Exchanges (all types)
0	473	5	4	1	0	1	2	0	0	0	0	0	1	RT(nonP;nonP)
0.05	483	7	6	2	1	1	2	1	1	0	0	0	0	0
0.10	857	16	9	3	2	1	3	7	1	3	0	3	2	dic(1;nonP), t(nonP;2)
1.00	44	14	5	2	2	0	1	7	0	2	1	4	2	t(nonP;3),t(nonP;2)

TABLE 1B. Cytogenetic data from bone marrow cells collected at 4 hr after exposure of male BALB/cJ mice to varying low doses of ^{137}Cs γ rays, including a high dose of 1.0 Gy serving as a reference dose [*t* Translocation (incomplete type), *nonP* non-painted chromosome]

Dose (Gy)	Total number of cells scored	Total number of abnormal cells	Total number of chromatid breaks	Chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of iso-chromatid breaks	Iso-chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of exchanges	Chromatid Exchanges (all types)
0	432	6	3	1	0	1	1	2	1	0	1	0	1	t(nonP;1)
0.05	403	11	4	3	1	0	0	4	2	0	1	1	3	t(2;nonP), t(3;1), t(1;2)
0.10	431	17	10	4	1	1	4	3	0	3	0	0	4	t(3;2), t(1;2), t(2;nonP), t(3;nonP)
1.00	41	12	3	1	0	0	2	7	3	0	0	4	2	t(2;nonP), t(3;nonP)

TABLE 2A. Cytogenetic data from bone marrow cells collected at 1 hr after exposure of male C57BL/6J mice to varying low doses of ^{137}Cs γ rays, including a high dose of 1.0 Gy serving as a reference dose [*t* Translocation (incomplete type), *dic* Dicentric, *nonP* non-painted chromosome]

Dose (Gy)	Total number of cells scored	Total number of abnormal cells	Total number of chromatid breaks	Chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of iso-chromatid breaks	Iso-chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of exchanges	Chromatid Exchanges (all types)
0	1,427	36	11	1	0	1	9	20	5	1	2	12	7	t(nonP;1), t(nonP;2), t(nonP;3), t(nonP;3), t(3;nonP), dic(nonP;nonP)
0.05	1,320	18	9	3	0	2	4	7	3	2	1	1	4	t(nonP;1), t(nonP;1), t(nonP;1), t(nonP;3)
0.10	1,553	70	51	11	14	3	23	24	12	1	3	8	10	t(1;2), t(1;2), t(2;1), t(3;2), t(nonP;1), t(nonP;1), t(nonP;1), t(3;nonP), t(3;nonP), dic(2;nonP)
1.00	117	26	14	2	2	2	8	11	3	1	1	6	6	t(2;3), t(1;nonP), t(1;nonP), t(nonP;1), t(nonP;3), t(nonP;3)

TABLE 2B. Cytogenetic data from bone marrow cells collected at 4 hr after exposure of male C57BL/6J mice to varying low doses of ^{137}Cs γ rays, including a high dose of 1.0 Gy serving as a reference dose [*t* Translocation (incomplete type), *recip t* Reciprocal translocation, *dic* Dicentric, *nonP* non-painted chromosome]

Dose (Gy)	Total number of cells scored	Total number of abnormal cells	Total number of chromatid breaks	Chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of iso-chromatid breaks	Iso-chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of exchanges	Chromatid Exchanges (all types)
0	1,076	23	16	1	1	1	13	5	3	0	1	1	3	recip t(1;3), t(3;nonP), acentric-ring(1)
0.05	1,304	39	19	4	3	1	11	17	9	1	0	7	4	t(nonP;1), t(nonP;1), t(nonP;2), dic(nonP;nonP)
0.10	910	44	35	4	0	1	30	9	2	1	1	5	7	t(nonP;1), t(nonP;2), t(nonP;3), t(nonP;1), t(nonP;3), dic(nonP;nonP), dic(nonP;nonP)
1.00	316	72	54	3	2	3	46	12	3	0	0	9	13	t(1;3), t(2;1), t(3;2), t(1;nonP), t(1;nonP), t(2;nonP), t(3;nonP), t(nonP;3), t(nonP;3), dic(nonP;nonP), dic(nonP;nonP), dic(nonP;nonP), dic(nonP;nonP)

No evidence for in vivo induction of genomic instability

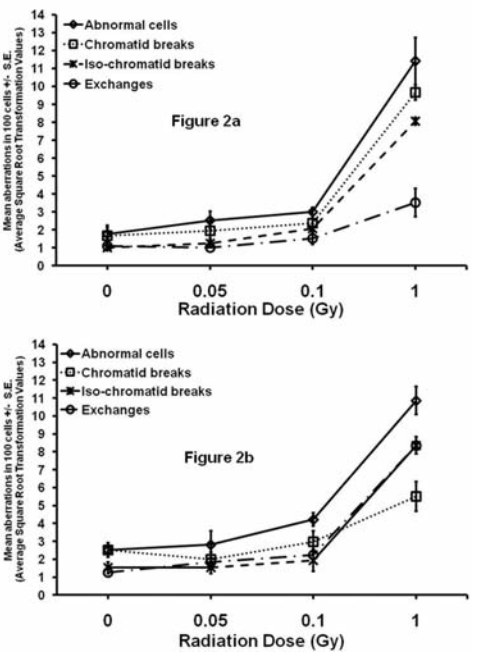


FIGURE 2. a) Frequencies of each type of aberration per 100 cells scored (±S.E.) detected in BM cells of BALB/cJ mice at 1 hr. The frequencies shown in each data point were the ASQRT values derived from five mice per treatment group, with one exception in a group of BALB/cJ mice exposed to 1.0 Gy of ^{137}Cs γ rays. Levels of statistically significant difference (p values ≤ 0.05, ↑ = increase) in the frequencies of each type of chromatid aberration in each exposed group related to those in the unirradiated controls are shown in the Table below (ns = non-significant).

Types of aberrations	P values		
	0.05 Gy	0.1 Gy	1 Gy
Abnormal cells	ns	0.05 (↑)	0.0002 (↑)
Chromatid breaks	ns	0.001 (↑)	0.0006 (↑)
Iso-chromatid breaks	ns	0.007 (↑)	0.0001 (↑)
Exchanges	ns	0.006 (↑)	0.00026 (↑)

b) Frequencies of each type of aberration per 100 cells scored (±S.E.) detected in BM cells of BALB/cJ mice at 4 hr. The frequencies shown in each data point were the ASQRT values derived from five mice per treatment group, with one exception in a group of BALB/cJ mice exposed to 1.0 Gy of ^{137}Cs γ rays. Levels of statistically significant difference (p values ≤ 0.05, ↑ = increase) in the frequencies of each type of chromatid aberration in each exposed group related to those in the unirradiated controls are shown in the Table below (ns = non-significant).

Types of aberrations	P values		
	0.05 Gy	0.1 Gy	1 Gy
Abnormal cells	ns	0.01 (↑)	0.0001 (↑)
Chromatid breaks	ns	0.05 (↑)	0.02 (↑)
Iso-chromatid breaks	ns	ns	0.0001 (↑)
Exchanges	ns	0.04 (↑)	0.0009 (↑)

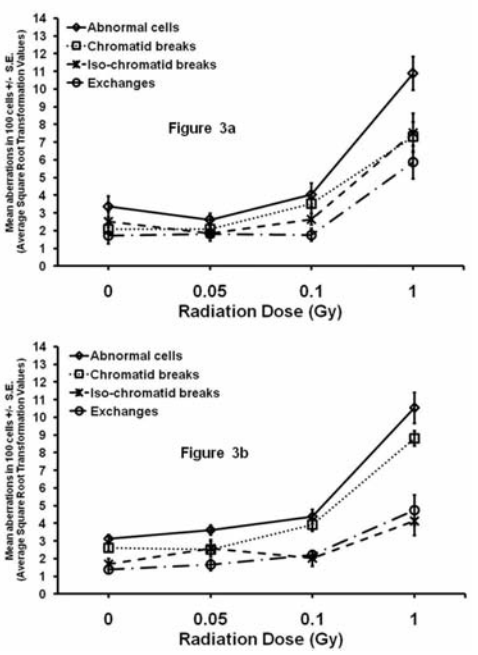


FIGURE 3. a) Frequencies of each type of aberration per 100 cells scored (±S.E.) detected in BM cells of C57BL/6J mice at 1 hr. The frequencies shown in each data point were the ASQRT values derived from five mice per treatment group. Levels of statistically significant difference (p value*s* ≤ 0.05, ↑ = increase) in the frequencies of each type of chromatid aberration in each exposed group related to those in the unirradiated controls are shown in the Table below (ns = non-significant).

Types of aberrations	P values		
	0.05 Gy	0.1 Gy	1 Gy
Abnormal cells	ns	ns	0.0002 (↑)
Chromatid breaks	ns	ns	0.001 (↑)
Iso-chromatid breaks	ns	ns	0.003 (↑)
Exchanges	ns	ns	0.003 (↑)

b) Frequencies of each type of aberration per 100 cells scored (±S.E.) detected in BM cells of C57BL/6J mice at 4 hr. The frequencies shown in each data point were the ASQRT values derived from five mice per treatment group. Levels of statistically significant difference (p value*s* ≤ 0.05, ↑ = increase) in the frequencies of each type of chromatid aberration in each exposed group related to those in the unirradiated controls are shown in the Table below (ns = non-significant).

Types of aberrations	P values		
	0.05 Gy	0.1 Gy	1 Gy
Abnormal cells	ns	0.02 (↑)	0.00003 (↑)
Chromatid breaks	ns	0.015 (↑)	0.00001 (↑)
Iso-chromatid breaks	ns	ns	0.01 (↑)
Exchanges	ns	0.03 (↑)	0.005 (↑)

TABLE 3A. Cytogenetic data from bone marrow cells collected at 1 mo after exposure of male BALB/cJ mice to varying low doses of ^{137}Cs γ rays, including a high dose of 1.0 Gy serving as a reference dose [*t* Translocation (incomplete type), *recip t* Reciprocal translocation, *RT* Robertsonian translocation, *dic* Dicentric, *ins* Insertion, *nonP* non-painted chromosome]

Dose (Gy)	Total number of cells scored	Total number of abnormal cells	Total number of chromatid breaks	Chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of iso-chromatid breaks	Iso-chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of exchanges	Chromatid Exchanges (all types)
0	1,727	12	4	0	0	0	4	8	3	0	0	5	5	t(1;2), t(1;3), recip t(1;2), recip t (3;1), RT(nonP;nonP)
0.05	1,979	29	12	6	1	3	2	11	3	2	4	2	6	t(1;2), t(2;nonP), RT(nonP;nonP), RT(1;3), RT(nonP;2), RT(nonP;1)
0.10	1,674	27	7	4	3	0	0	17	4	4	2	7	7	ins(1;nonP;1), t(nonP;2), recip t(2;nonP), recip t(2;nonP), t(2;nonP), t(1;2), RT(nonP;nonP)
1.00	1,134	50	16	2	2	2	10	26	5	4	6	11	17	t (1;2), t(1;3), t(1;nonP), t(2;nonP), t(3;nonP), t(nonP;2), dic(nonP;nonP), ins(nonP;3;nonP), recip t(2;3), recip t(1;2), recip t(2;nonP), recip t(2;nonP), recip t(2;nonP), recip t(2;nonP), recip t(2;nonP), RT(3;nonP), RT(2;nonP)

TABLE 3B. Cytogenetic data from bone marrow cells collected at 6 mos after exposure of male BALB/cJ mice to varying low doses of ^{137}Cs γ rays, including a high dose of 1.0 Gy serving as a reference dose [*t* Translocation (incomplete type), *recip t* Reciprocal translocation, *RT* Robertsonian translocation, *dic* Dicentric, *ins* Insertion, *nonP* non-painted chromosome]

Dose (Gy)	Total number of cells scored	Total number of abnormal cells	Total number of chromatid breaks	Chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of iso-chromatid breaks	Iso-chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of exchanges	Chromatid Exchanges (all types)
0	1,669	17	9	6	0	0	3	6	4	1	0	1	4	t(nonP;2),t(1;3), RT(nonP;nonP), t(nonP;1)
0.05	1,419	16	6	1	1	3	1	9	5	1	2	1	4	t(nonP;2), recip t(nonP;3), ins(1;3;1), RT(nonP;nonP)
0.10	2,052	37	15	7	0	3	5	16	7	3	4	2	6	t(nonP;1), t(nonP;1), t(nonP;3), RT(nonP;nonP), RT(nonP;nonP), t(1;nonP)
1.00	1,040	57	26	6	6	6	8	24	6	2	4	12	15	t(1;3), recip t(3;nonP), t(1;2), dic(nonP;nonP), t(nonP;1), t(nonP;3), recip t(2;nonP), recip t(1;nonP), recip t(2;nonP), dic(nonP;nonP), recip t(nonP;2), dic(nonP;nonP), RT(nonP;nonP), RT(nonP;nonP), RT(3;nonP)

TABLE 4A. Cytogenetic data from bone marrow cells collected at 1 mo after exposure of male C57BL/6J mice to varying low doses of ^{137}Cs γ rays, including a high dose of 1.0 Gy serving as a reference dose [*t* Translocation (incomplete type), *recip t* Reciprocal translocation, *RT* Robertsonian translocation, *dic* Dicentric, *ins* Insertion, *nonP* non-painted chromosome]

Dose (Gy)	Total number of cells scored	Total number of abnormal cells	Total number of chromatid breaks	Chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of iso-chromatid breaks	Iso-chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of exchanges	Chromatid Exchanges (all types)
0	1,221	77	43	7	0	3	33	29	7	0	0	22	10	t(nonP;1), t(nonP;1), t(nonP;2), t(nonP;3), t(nonP;3), t(nonP;3), t(nonP;3), dic(nonP;nonP), dic(nonP;nonP), dic(nonP;nonP)
0.05	1,153	40	22	1	1	2	18	19	0	0	0	19	3	dic(2;nonP), dic(3;nonP), RT(nonP;1)
0.10	1,340	85	35	2	0	3	30	42	2	0	3	37	12	t(3;nonP), t(nonP;nonP), dic(nonP;nonP), dic(nonP;nonP), dic(nonP;nonP), dic(nonP;nonP), RT(2;nonP), RT(2;nonP), RT(2;nonP), RT(3;nonP), RT(nonP;nonP), RT(nonP;nonP)
1.00	1118	88	36	3	2	0	31	45	4	0	3	38	14	recip t(1;2), t(1;nonP), t(1;nonP), t(nonP;2), t(nonP;3), t(3;nonP), dic(nonP;1), dic(nonP;2), dic(nonP;nonP), ins(nonP;1), RT(2;nonP), RT(3;nonP), RT(nonP;nonP), RT(nonP;nonP)

TABLE 4B. Cytogenetic data from bone marrow cells collected at 6 mos after exposure of male C57BL/6J mice to varying low doses of ^{137}Cs γ rays, including a high dose of 1.0 Gy serving as a reference dose [*t* Translocation (incomplete type), *RT* Robertsonian translocation, *dic* Dicentric, *ins* Insertion, *nonP* non-painted chromosome]

Dose (Gy)	Total number of cells scored	Total number of abnormal cells	Total number of chromatid breaks	Chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of iso-chromatid breaks	Iso-chromatid breaks (Chromosome involved) Chromosome (1)	(2)	(3)	(nonP)	Total number of exchanges	Chromatid Exchanges (all types)
0	1,962	49	39	4	2	3	30	23	7	0	0	16	4	t(nonP;1), t(nonP;3), dic(2;nonP), t(3;1)
0.05	2,236	42	35	3	0	1	31	9	0	0	1	8	5	t(3;1), t(nonP;2), dic(nonP;nonP), dic(nonP;nonP), ins(nonP;1)
0.10	1,577	48	29	6	1	3	19	16	6	0	1	9	6	t(1;3), t(1;3), t(nonP;1), t(nonP;2), dic(2;nonP), RT(nonP;nonP)
1.00	1,537	54	34	2	1	3	28	20	4	0	3	13	6	t(1;3), t(nonP;1), t(nonP;3), t(nonP;3), ins(1;nonP), ins(nonP;2)

scored (± S.E.), including the abnormal cells, are shown in Figures 4a and 4b for BALB/cJ mice and in Figures 5a and 5b for C57BL/6J mice.

The chromatid- and chromosome-types detected at 1 and 6 mos post-irradiation were breaks and exchanges (*i.e.* Robertsonian, reciprocal, one-way incomplete types, dicentrics, or inversions). There was no evidence of non-random involvement of a specific chromosome in any type of chromosome aberrations. Of note, it is possible that the frequencies of exchanges were underestimated in this study, in particular those reciprocal translocations involving two nonP chromosomes or inversions. The whole mouse genome mFISH, or spectral karyotyping (SKY), or the G-banding method is required for an accurate estimation of such abnormalities. With respect to RT, it is easily recognized even if it occurs between two nonP chromosomes because normal mouse chromosomes are telocentric. It also should be noted that it was impossible to determine whether a clone of cells existed without the use of the whole mouse genome mFISH, or SKY, or the G-band method, unless a clone of a specific type of aberration in a mouse occurred between two of the painted chromosomes in the same mouse.

Clearly, there were significant decreases in the frequencies of abnormal cells and chromosomal damage in BM cells collected from exposed mice (both strains) at both 1 and 6 mos post-irradiation, related to those observed in BM cells collected at early time-points. However, the residual levels of persistent chromosomal damage appeared to remain elevated significantly (related to the unirradiated-control level, $p<0.05$) for up to 6 mos in BM cells collected from BALB/cJ (not C57BL/6J) mice exposed to 1.0 Gy of ^{137}Cs γ rays. It also is important to note that significant reductions ($p<0.05$) were found in the frequencies of abnormal cells at 1 mo (Figure 5a, *at the arrow*), as well as in the frequencies of chromosome breaks at 6 mos in BM cells of C57BL/6J mice exposed to 0.05 Gy (Figure 5b, *at the arrow*). Further, there was a significant reduction ($p<0.05$) in the frequency of chromatid breaks in BM cells at 6 mos post-irradiation from the 0.05 Gy-exposed BALB/cJ mice (Figure 4b, *at the arrow*).

DISCUSSION

With the discovery of genomic instability, the focus has to shift with the changing paradigms in predicting health risk of radiation exposure. There is increasing evidence that induction of genomic instability is a critical event during the carcinogenesis process and that it requires: (*i*) multiple cell divisions between radiation exposure and the induction of genomic instability which make it necessary to measure these changes at both early and later times after radiation exposure as outlined in this study, and (*ii*) communication between cell/tissue and microenvironment during carcinogenesis (Barcellos-Hoff *et al.*2005). The change of paradigm in radiation biology strongly supports the idea that it is impor-

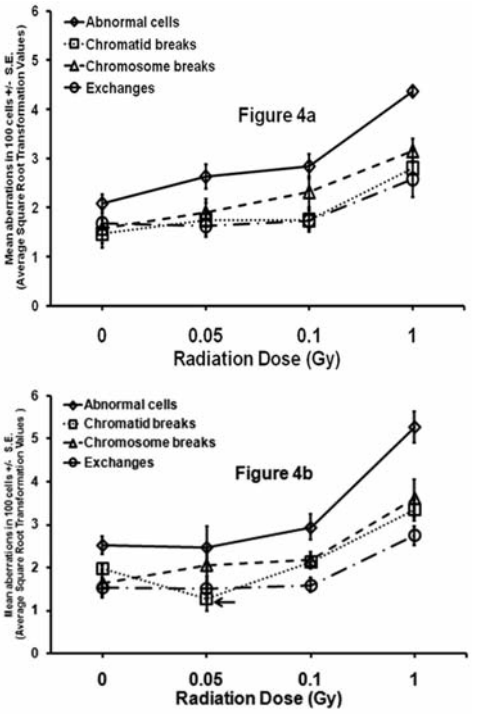


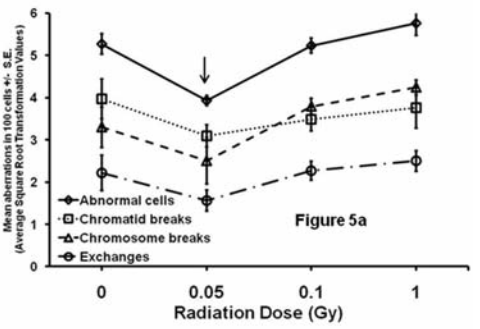
FIGURE 4. a) Frequencies of each type of aberration per 100 cells scored (±S.E.) detected in BM cells of BALB/cJ mice at 1 mo. The frequencies shown in each data point were the ASQRT values derived from five mice per treatment group. Levels of statistically significant difference (p values ≤ 0.05, ↑ = increase) in the frequencies of each type of aberration in each exposed group related to those in the corresponding unirradiated controls are shown in the Table below (ns = non-significant).

Types of aberrations	P values		
	0.05 Gy	0.1 Gy	1 Gy
Abnormal cells	ns	ns	0.000006 (↑)
Chromatid breaks	ns	ns	0.009 (↑)
Chromosome breaks	ns	ns	0.007 (↑)
Exchanges	ns	ns	0.003 (↑)

b) Frequencies of each type of aberration per 100 cells scored (±S.E.) detected in BM cells of BALB/cJ mice at 6 mos. The frequencies shown in each data point were the ASQRT values derived from five mice per treatment group. Levels of statistically significant difference (p values ≤ 0.05, ↑ = increase, ↓ = decrease) in the frequencies of each type of aberration in each exposed group related to those in the corresponding unirradiated controls are shown in the Table below (ns = non-significant).

Types of aberrations	P values		
	0.05 Gy	0.1 Gy	1 Gy
Abnormal cells	ns	ns	0.0002 (↑)
Chromatid breaks	0.04 (↓)	ns	0.0008 (↑)
Chromosome breaks	ns	ns	0.006 (↑)
Exchanges	ns	ns	0.005 (↑)

No evidence for in vivo induction of genomic instability



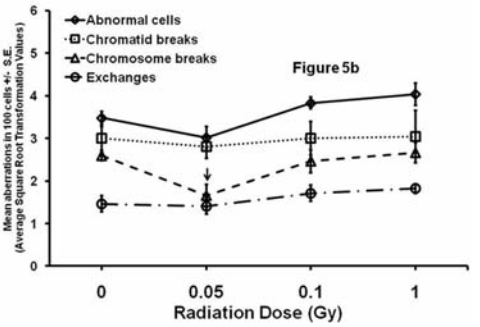


FIGURE 5. a) Frequencies of each type of aberration per 100 cells scored (±S.E.) detected in BM cells of C57BL/6J mice at 1 mo. The frequencies shown in each data point were the ASQRT values derived from five mice per treatment group. Levels of statistically significant difference (p values ≤ 0.05, ↓ = decrease) in the frequencies of each type of aberration in each exposed group related to those in the unirradiated controls are shown in the Table below (ns = non-significant).

Types of aberrations	P values		
	0.05 Gy	0.1 Gy	1 Gy
Abnormal cells	0.01 (↓)	ns	ns
Chromatid breaks	ns	ns	ns
Chromosome breaks	ns	ns	ns
Exchanges	ns	ns	ns

b) Frequencies of each type of aberration per 100 cells scored (±S.E.) detected in BM cells of C57BL/6J mice at 6 mos. The frequencies shown in each data point were the ASQRT values derived from five mice per treatment group. Levels of statistically significant difference (p values ≤ 0.05, ↓ = decrease) in the frequencies of each type of aberration in each exposed group related to those in the unirradiated controls are shown in the Table below (ns = non-significant).

Types of aberrations	P values		
	0.05 Gy	0.1 Gy	1 Gy
Abnormal cells	ns	ns	ns
Chromatid breaks	ns	ns	ns
Chromosome breaks	0.01 (↓)	ns	ns
Exchanges	ns	ns	ns

tant to measure the effects of radiation in total tissues like the BM and not on a specific cell type in the tissue or in tissue culture.

In this study, the total population of BM cells was selected for investigating the potential induction of genomic instability by low-dose radiation because these cells proliferate rapidly, express radiation-induced genomic instability both *in vitro* and *in vivo* as discussed previously, and are highly susceptible to the development of rML. The chromosome aberration assay was selected for the measurement of genomic instability induced by radiation at low (≤ 0.1 Gy) and high (1 Gy) dose levels because: (*a*) It has been shown to be the prime end-point in the evaluation of past radiation exposure (Bender *et al.*1988), and (*b*) Chromosome aberration has been proven to be the most relevant endpoint for detecting carcinogenic activity of suspect agents because of its strong correlation with the induction of cancers (Hagmar *et al.*2004). The extent, the frequency, and the type of chromosome aberrations in BM cells collected from exposed mice at 1 and 4 hr post-irradiation (chromatid- or G2-type aberrations) provide a measure of early response to radiation. The frequency of all types of chromosomal damage (both chromatid- and chromosome-type aberrations) at late time-points after irradiation offers a measure of *in vivo* induction of genomic instability. In addition, the frequency of stable exchanges (*i.e.* translocations) at late time-points reflects the fraction of surviving cells (carrying damage) that may be at an increased risk for subsequent neoplastic transformation.

The resulting data obtained from our study indicated that there were no increases in the frequencies of any type of aberration (both at the chromatid and chromosome levels) in BM cells from BALB/cJ or C57BL/6J mice exposed to 0.05 Gy of ^{137}Cs γ rays, relative to those found in the corresponding unirradiated controls. Instead, a significant reduction ($p<0.05$) in some types of abnormalities (*i.e.* abnormal cells, chromatid and chromosome breaks) was detected in BM cells of exposed BALB/cJ or C57BL/6J mice at 1 and 6 mos as compared to those in the corresponding unirradiated controls. These findings suggested that a low dose (as low as 0.05 Gy) of low LET radiation exhibited beneficial effects *in vivo*. Such protective effects of low-dose radiation have been widely observed (Wang and Cai 2000; Mitchel *et al.*2003; Feinendegen 2005; Ko *et al.*2006; Zeng *et al.*2006). Previously, we evaluated the protection factor [PROFAC; (Scott and Di Palma 2006)] of low doses of ^{137}Cs γ rays (*i.e.* 0.01, 0.05, and 0.1 Gy) *in vitro* by measuring the frequencies of MN in binucleated human lymphocytes (Rithidech and Scott 2008). We found that only the 0.01 Gy (but not 0.05 Gy) of ^{137}Cs γ rays exhibited protective effects *in vitro*. Differences in exposure conditions (*in vivo vs in vitro*) and the employed cell system (bone marrow *vs* lymphocytes) might have contributed to this disparity.

Our data also indicated a trend of persistent elevation in all types of chromosomal damage in cells collected from BALB/cJ mice (not

C57BL/6J mice) at 6 mos post-irradiation with 0.1 Gy of ^{137}Cs γ rays, although this increase was not statistically significant. It is clear, however, that there was a statistically significant increase ($p<0.05$) in the frequency of chromosome aberrations in cells collected at late time-points from BALB/cJ exposed to 1.0 Gy. In contrast, no increase in any type of chromosomal damage (including the number of abnormal cells) was detected in BM cells collected from C57BL/6J mice at late time-points following exposure regardless of radiation dose, suggesting no *in vivo* induction of sustained genomic instability in the C57BL/6J mouse by the radiation doses used in our study. The detection of differences in the response to radiation (in particular at the dose levels of 0.1 to 1.0 Gy) of BM cells from the BALB/cJ and C57BL/6J mouse presumably reflect differences in DNA repair capacity of these two strains as previously suggested (Okayasu *et al.*2000; Yu *et al.*2001). Nonetheless, other mechanisms may also contribute to the variation in response to radiation. These include differences in removal of damaged cells by apoptosis (Bauer 2007) or the cell turnover that removes damaged cells from the populations (Portess *et al.*2007). It also should be noted that the spontaneous rates of abnormal cells or chromosomal damage in the C57BL/6J mouse were higher than those of the BALB/cJ mouse, suggesting that the spontaneous rate of abnormalities is strain-dependent.

Of note, we (Rithidech *et al.*2005) measured levels of activated nuclear factor-κB (NF-κB, an important transcription factor) in a fraction of BM cells collected at 1 and 4 hr post-irradiation from the same individual BALB/cJ and C57BL/6J mice used for the analysis of chromosome aberrations in this study. We found a significant increase in levels of activated NF-κB in BM cells isolated from both mouse strains at 1 hr post-exposure to 0.1 and 1.0 Gy, but precipitously declined by 4 hr post-exposure (Rithidech *et al.*2005). The resulting data from this study showed no increase in the frequency of chromosomal damage in the 0.05-Gy-exposed mice of which no early (1 and 4 hr post-irradiation) signal of activated NF-κB was detected at any time point included in this study. Instead, a reduction in the frequencies of specific types of damage (*e.g.* abnormal cells, chromatid and chromosome breaks) was found. Taken together, the new set of data obtained from our laboratory supported a link between high levels of early NF-κB activation after irradiation and the *in vivo* induction of late occurring chromosomal damage (genomic instability). However, proper experimental approaches to test this hypothesis need to be designed in future studies.

Overall, the resulting data indicated no evidence for the induction of genomic instability following an acute exposure *in vivo* to a single dose of 0.05 Gy of ^{137}Cs γ rays in BM cells collected from BALB/cJ or C57BL/6J mice. Conversely, there was a suppression of specific aberration-types below the spontaneous rate after low-dose irradiation. Nonetheless, to

better mimic the low-dose exposure related to the public concern, future *in vivo* studies should be conducted to determine the biological effects of low-dose radiation (less than or equal 0.05 Gy) using chronic, or fractionated, or repeated exposure protocols.

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