Distribution of breakpoints on chromatid-type aberration induced by three different radiations, in relation to fragile sites
Kimio Tanaka and Nanao Kamada

Abstract: Based on experimental evidence using human blood samples we able to suggest that ‘common fragile sites’ can be targets for different radiation sources. For that we used peripheral blood samples from three healthy adult donors and exposed them to $^{60}$Co γ-rays (2, 4 Gy) or neutrons (0.8, 2 Gy) at the G$_2$ stage. In another set, normal bone marrow cells from 19 adult donors were also exposed to $^{60}$Co γ-rays, tritiated water β-rays, and $^{252}$Cf neutrons and sampled 24 hr later. Chromatid breakpoint sites were identified using Wright's stain G-banding. Analyses of 912 breakpoint sites detected in lymphocytes and 545 breakpoint sites detected in bone marrow cells were within the same bands as 113 reported: (1) 41% – 52% of chromatid breakage sites had a chromosome band of “relative fragile sites” in both bone marrow cells and lymphocytes; (2) the breakage sites induced by different radiation sources were distributed in a similar pattern; (3) significantly higher numbers of breakpoint sites were found at 4q31, 2q35, 3p21, 5q31, 1q32, 2q31, 15q24 and 13q22 in lymphocytes, and at 3p21, 14q24, 17p13, 1q42, 7q22 and 9p11/q11 in bone marrow cells; (4) distribution of breakpoint sites was similar between lymphocytes and bone marrow cells except for certain breakpoints. Present study also revealed that a B-cell line established from lymphocytes with a history of $^{60}$Co γ-ray irradiation had more chromosome breakpoints at telomere regions than no-irradiated cell line, which indicating telomere protein might be associated with radiation-induced chromosome instability. This study provides information that will be useful for increasing our understanding of the mechanisms that underlie radiation-induced chromosome aberrations and will aid in assessing genetic and cancer risks in radiation-exposed human populations.

Keywords: γ-Rays, tritiated water, β-rays, neutrons, fragile sites, chromosome aberrations, chromosome instability.

Introduction
To date, 113 chromosomal fragile sites have been localized on human chromosomes (Sutherland & Ledbetter 1989), although the exact number of common fragile sites differs depending on the criteria. Of these, 89 have been termed common fragile sites, i.e., those which are commonly found in all individuals, and 26 have been termed rare or hereditary fragile sites; these are less commonly found and are inherited (Sutherland 1979; Yunis & Soreng 1984). Common fragile sites can be induced by antifolate agents, such as aphidicolin, fluorodeoxyuridine (FUdR), bromodeoxyuridine (BrdU), and distamycin (Yunis 1984; Sutherland 1988; Rao et al., 1988; Ban et al., 1995), which correspond well to the chromosome breakpoints of sister chromatid exchange (SCE) (Glover & Stein, 1987) and preferred sites for the integration of exogenous DNA (De Braekeleer et al., 1985a; Rassool et al., 1989; Thorland et al., 2000). Numerous studies have shown that those of chromosome aberration were associated with neoplastic and congenital diseases (Yunis, 1984b; De Braekeleer et al., 1985b; Le Beau, 1986; Sutherland, 1988; Hecht, 1988; Hori et al., 1988; Gümüs et al., 2002) and were suggested to be “hot spots” for chromosomal and gene recombination (Hastie & Allshire 1989), although relatively few known translocations and deletions involving common fragile sites have been reported (Huang et al., 1999; Schneider et al., 2008).

At present, 12 common fragile sites, including FRA3B, FRA7H and FRA16D, have been molecularly characterized (Ohta et al., 1996; Mishmar et al., 1998; Bednarek et al., 2000; Ried et al., 2000), and it was clarified that common fragile sites are specifically induced by replication stress caused by the inhibition of DNA polymerases (Le Beau et al., 1996; Lemoine et al., 2005). Unlike common fragile sites, rare fragile sites are caused by the expansion of trinucleotide repeat sequences (Dietrich et al., 1991; Kremer et al., 1991; Arlt et al., 2002). Replicabon is routinely arrested by a variety of stresses, like radiation (Berger et al., 2004). Some fragile sites have also been found to be targets of 16 diverse mutagens including $^{60}$Co γ-rays, although these agents damage the cells by different molecular pathways (Yunis et al., 1987). High LET radiation such as neutrons induces cluster damage on DNA within a very small, around 1-6 Mb, sized DNA region (Fakir et al. 2006), although the frequencies of chromatid-type aberration were not different between low and high LET radiations (Vral et al., 2002). Our present study was undertaken for the first time to identify the relationship between the location of the fragile site and the chromosomal breakpoint sites induced by radiation with different energy levels of $^{60}$Co γ-rays, tritiated water (HTO) β-rays, and californium $^{252}$Cf neutrons in cultured peripheral blood and bone marrow cells from adult donors.

Materials and Methods
Radiation sources and samples
Three kinds of radiation source, $^{60}$Co γ-rays (555 MBq/ml), $^{252}$Cf neutrons, and tritiated ($^3$H) water (HTO) β-rays (555 MBq/ml in HTO) (Amersham, USA), were used in the present study at the same dose rate of 200 mGy/min. A $^{252}$Cf source (3.1 mg) was installed in a linear accelerator facility at the Research Institute for Radiation Biology and Medicine, Hiroshima University. Buffy coat cells from samples of three healthy adult donors (two
males and one female) were used as the source of lymphocytes. Approximately 2x10^7 cells per flask cultured in 10 ml RPMI 1640 medium supplemented with 20% fetal calf serum and 0.2 μg/ml phytohemagglutinin (PHA) at 37°C for 44 to 45 h, in which most lymphocytes are in G2 stage of the cell-cycle phase, and then exposed to ^60^Co γ-rays (2 and 4 Gy) or to ^252^Cf neutrons (0.8 Gy and 2 Gy) at 37°C. The total doses of these radiation sources were fixed in order to maintain the relative biological effectiveness (RBE) at 2 to 3.3, which is the RBE value of ^252^Cf neutrons with respect to ^60^Co γ-rays, which were estimated by chromosome aberration yields (Nakamura & Sawada 1988; Tanaka et al., 2009). After exposure, cells were cultured for further 4-5 h at 37°C with medium change and washing 4 times; cell division was arrested with 0.02 μg/ml of colcemide. HTO exposure was not used in the lymphocyte experiment.

For bone marrow, mononuclear cells from 19 adult donors (10 males and 9 females) with no history of any hematological disease such as leukemia or myeloproliferative disorders, in complete remission after chemotherapy, and almost no blast cells in their bone marrow smear slides were separated by Ficoll-Hypaque sedimentation; 2x10^7 mononuclear cells were suspended in RPMI 1640 medium supplemented with 20% fetal calf serum in 50 ml flasks and exposed to different dose ranges from 0.25, 0.5, 1.0 and 2.0 Gy of ^60^Co γ-rays, 0.13, 0.27, 0.56 and 1.1 Gy of HTO β-rays, and 0.1, 0.2, 0.4, 0.8 and 1.2 Gy of ^252^Cf neutrons at 37°C.

Neutron and γ-rays doses were measured by the twin-chamber method. The method of irradiation and dose calculation of HTO β-rays were clearly shown in the previous paper (Tanaka et al., 1983). For HTO β-ray exposure, tritiated water (Amersham, USA) was diluted with RPMI 1640 medium to obtain 200 mGy/min. Bone marrow cells in RPMI medium containing 20% FCS in these culture flasks were exposed to a concentration of Bq (15 mCi/ml) of HTO at 37°C for different times (10-90 min) to obtain the respective radiation doses. After exposure, the supernatant of the first centrifugation was used to measure the physical dose with a scintillation counter and the exposure dose was calculated. Bone marrow cells were washed 4 times after exposure, and were then cultured for 24 h at 37°C because most bone marrow cells are in the first mitotic stage at this time. Establishment of B-cell lines and breakpoint distribution analysis in delayed chromosome aberrations

About 2x10^7 Go lymphocytes were separated by Ficoll sedimentation from peripheral blood of a healthy donor. After irradiation with ^60^Co-γ rays (555 MBq/ml) for 1 Gy at 200 mGy/min, half of cells were infected with Epstein-Barr virus (EBV) for 12h, and cultured for 1 week in 50 ml flask. The cell solution (5 ml) from the growing cells was again cultured in a 76-well microplate for several months. The growing colonies were isolated and transferred to 4 wells of a microplate for further 1-2 months for cell expansion (Tanaka et al., 2008). Finally, 8 cell lines were established from the irradiated lymphocytes. At the same time 8 cell lines were also established from non-irradiated lymphocytes from a same adult donor and they were used for a control B-cell line. Among the B-cell lines established, one each cell line (T8-1 from lymphocytes irradiated by ^60^Co-γ and T14-1 from non-irradiated lymphocytes) was selected and the two cell lines were maintained up to 30 passages for breakpoint distribution analysis. Medium was changed once a week. Chromatid type aberrations were observed at passage 30 of these cell lines and scored breakpoint site. After the irradiation with ^60^Co-γ rays, half of cells were cultured with 0.2 μg/ml PHA 37°C for 52 h for chromatid type analysis after several washing post the ^60^Co-γ irradiation. Distributions of breakpoint sites were compared between PHA stimulated T-lymphocytes for 52 h after ^60^Co-γ irradiation and two B-cell lines at passage 30, and also between the two B-cell lines (T8-1 and T14-1) with a history of ^60^Co-γ irradiation and T14-1 without a history of irradiation, aimed for evaluation of cytogenetical characteristics of late-arising (delayed) chromosome aberrations.

Scoring of chromosome aberrations

Metaphases, harvested for chromosome analyses after one-hour pretreatment of the culture with 0.02 μg/ml of colcemide (Invitrogen Corp., Carlsbad, CA, USA), were stained with Wright's solution. Only chromatid-type aberrations, such as breaks and exchanges, were scored in 500-1000 metaphases of each experimental series. Chromosome aberrations such as dicentric chromosomes and translocations were not used for the present study, and their breakpoints associated with chromatid-type aberrations were identified precisely on the photograph according to the International System of Human Chromosome Nomenclature, 1995 and 2005 (ISCN 1995, ISCN 2005). The chromatid gap was omitted for scoring because this aberration is not easily scored in G-banding metaphases stained with Wright's solution. The present study aimed to identify the relationship between the breakpoints and the location of 113 reported fragile sites taken from the 10th Human Gene Mapping Conference (Sutherland & Ledbetter, 1989) and radiation-induced breakpoints were compared among three different radiation sources. Lymphocytes and bone marrow cells without radiation exposure were also used. The relative length of each chromosomal band was calculated according to the previously determined results by majoring 300 bands of metaphase chromosomes (Francke, 1994). Both the results of the physical lengths of human chromosomes and the ratio of the long and short arms of chromosomes were used to calculate the
Fig. 1. Seven representative chromatid-type aberrations induced by $^{252}$Cf neutrons in human lymphocytes. Four breakpoints (a, b, c, and e) correspond with fragile sites. Chromatid breaks (d) and chromatid exchanges (f) Ctb, chromatid break; ctg, chromatid gap; cte, chromatid exchange.

Fig. 2. Distribution of breakpoints in chromatid aberrations induced by $^{60}$Co γ-rays and $^{252}$Cf neutrons in human lymphocytes. Each dot represents one breakpoint. Black arrow and open triangle below chromosome represents common fragile sites and rare fragile sites, respectively.

Statistical analysis

Based on the values of expected and observed breakpoints, relative risk (RR) was obtained, and the significance of RR was tested according to $\chi^2$ distribution using a test-based interval to know whether the 95% confidence interval overlapped (Miettinen, 1976).

Results

Lymphocytes

Metaphases showing radiation-induced breakpoints at the position of “relative fragile sites” in lymphocytes are shown in Fig. 1. After exposure to $^{60}$Co γ-rays or $^{252}$Cf neutrons, 91.7% and 83.4% of cells, respectively, contained chromatid breaks, 7.1% and 13.2% contained chromatid gaps, and 1.2% and 3.4%, respectively, had chromatid exchanges. Isochromatid breaks and gaps were observed at a higher incidence in $^{252}$Cf neutrons irradiation than in $^{60}$Co γ-rays. A total number of 305 breakpoints, detected in 280 of 1629 cells (17.2%) at 2 Gy exposure, and 157 breakpoints, detected in 136 of 400 observed cells (34.0%) at 4 Gy exposure to the $^{60}$Co γ-ray series, were mapped to the G-banded karyotype of human chromosome (Fig. 2) and compared with the location of fragile sites. A total of 336 breakpoints, detected in 296 of 1766 observed cells (16.8%) at 0.8 Gy exposure, and 114 breakpoints, detected in 91 of 356 observed cells (25.6%) at 2 Gy exposure for $^{252}$Cf neutrons, were also mapped (Fig. 2). The distribution of the breakpoints induced by $^{60}$Co γ-rays and $^{252}$Cf neutron exposure was found to be similar (Fig. 2). With both types of radiation exposure, the largest number of breakpoints was found at 3p21 and a large number of breakpoints were seen at 1p36, 2q33, 4q31.1, 7q22, 8q22 and 12q24.1.

Bone marrow cells

A total of 102 breakpoints of chromatid-type aberrations detected in 114 of 1386 cells exposed to $^{60}$Co γ-rays, 123 breakpoints detected in 114 of 1668 cells exposed to $^{252}$Cf neutrons, and 320 breakpoints detected in 223 of 2600 cells exposed to HTO β-rays at different doses were mapped (Fig. 3). The distribution of the breakpoints induced by $^{60}$Co γ-rays,
HTO β-rays and 252Cf neutron exposure in bone marrow cells was found to be almost identical (Fig. 3). With both types of radiation exposure, the largest number of breakpoints was found at 3p21 and a large number of breakpoints were seen at 1q42, 7q32, 9p11/q11, 10q24, 14q24 and 17p13. The breakpoint distribution for three radiation sources was similar. This distribution was also similar to that observed in lymphocytes, except for certain breakpoints.

The proportion of chromosome breakpoints pooled from different total doses and different radiation sources at fragile sites after radiation is summarized in Table 1. Between 41.2%-51.9% of breakpoints occurred in bands in which “relative fragile sites” have been located. In the present study, a “relative fragile site” was defined as a chromosomal band containing a fragile site. The significantly large number of chromosome bands at “relative fragile sites” and non-fragile sites, induced by all types of radiation in lymphocytes and bone marrow cells, among chromosome bands having more than 6 breakpoints are shown in Table 2. Expected breakpoints induced in each band area (all 330 bands) in G-band human chromosomes were calculated according to the size information of each band, DNA content of each chromosome and the ratio of long and short chromosome arms, on the assumption that the chromosome breakpoint is created randomly on the human chromosome in proportion to the size of the chromosome band and DNA content of each chromosome; therefore, in this analysis, chromosome breakpoints were generalized by combining all doses in all radiation sources.

The breakpoint distribution within “relative fragile sites” in certain band regions differed in lymphocytes and bone marrow cells. These sites were observed significantly highly at “relative fragile sites” of 3p21, 5q31 and 2q35 in both tissues, but some breakpoints correlated at 14q24 and 17p13 in bone marrow cells only, and another breakpoint was observed at 2q35 in lymphocytes only. On the other hand, although they were not statistical significance, many commonly found breakpoints were also recorded for other chromosome band regions, e.g., larger numbers of breaks were found

### Table 1. Localization of chromosome breakpoints towards three radiation sources in relation to “relative fragile sites”

<table>
<thead>
<tr>
<th>Tissues (Total dose; Gy)</th>
<th>No. of observed cells</th>
<th>No. of analyzed breakpoints</th>
<th>No. of breakpoints at “relative fragile sites”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co γ-rays (0.25, 0.5, 1.2 Gy)</td>
<td>1386</td>
<td>72</td>
<td>102(2)*</td>
</tr>
<tr>
<td>Cf neutrons (0.1, 0.2, 0.4, 0.8, 1.2 Gy)</td>
<td>1668</td>
<td>114</td>
<td>123(2)*</td>
</tr>
<tr>
<td>HTO β-rays (0.13, 0.27, 0.56, 1.11 Gy)</td>
<td>2600</td>
<td>223</td>
<td>320(2)*</td>
</tr>
<tr>
<td>Bone marrow cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co γ-rays (2, 4 Gy)</td>
<td>2029</td>
<td>416</td>
<td>462(10)*</td>
</tr>
<tr>
<td>Cf neutrons (0.8, 2 Gy)</td>
<td>2122</td>
<td>387</td>
<td>450(18)*</td>
</tr>
<tr>
<td>HTO β-rays (200 mGy/min)</td>
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</tr>
</tbody>
</table>

*number of breakpoints excluded from analysis due to non identified breakpoint, ctb: chromatid -type aberrations

at 4q31, 1p36, 12q24, 1p36, 12q24, 8q24, 8q22, 1q42, 2q33, 1q32, 17q21 and the centromeric region of chromosome 9 (9p11 and 9q11) in both tissues (Table 2). In contrast, “relative fragile sites” involving a rare fragile site region, which are indicated by the open triangle in Fig. 2-3 did not show significantly higher chromosomal breakpoints, except 12q24 and 8q24 in lymphocytes, and 8q24 in bone marrow cells.

Distribution of chromosome breakpoints in B-cell lines

Chromosome breakpoints associated with chromatid breaks and gaps were observed in two established B-cell lines (the γ-ray-irradiated T8-1 cell line and non-exposed T14-1 cell line) at passage 30 by Giemsa staining using Wright solution (Yunis, 1981). These cell lines had spontaneous chromatid type aberrations. The distribution was compared with that 60Co γ-ray-irradiated (200 mGy/min) lymphocytes derived from a same healthy adult, whose peripheral blood was used for establishing the B-cell lines. The γ-ray-
irradiated B-cell line had more breakpoint sites near the telomere region on chromosomes of 1, 2, 3, 5, 6, 9 and 11 (Fig. 4). On the other hand, breakpoint sites in T-lymphocytes exposed to $^{60}$Co $\gamma$-rays were distributed throughout the chromosomes and fragile sites had more breakpoints than other sites.

**Discussion**

Approximately 41 to 53% of chromosomal breakpoints were mapped on 113 “relative fragile sites” for all three kinds of radiation exposure, indicating that a significantly higher incidence of breakpoints occurred most specifically at the “relative fragile site” areas in both lymphocytes and bone marrow cells. The “relative fragile sites” in 3p21 and 5q31 band regions found in both lymphocytes and bone marrow cells were significantly related to the higher incidence of breakpoints induced by radiation. In contrast to the reports on treatment with anti-replication chemicals such as aphidicolin, FUdR, BrdU and 5-azacytidine, which has a more clustered distribution of breakpoints on specific fragile sites such as 3p14, 16q23, and 4q21, 9q34.1, and 4q12, 5p13, 5q15, 6q13, 9p21, 10q21, 13q21, and 1q12, 1q42, 9q12, 19q13, respectively (Yunis et al., 1987; Sutherland et al., 1998; Sutherland & Baker, 2003), radiation-induced chromosome breakage sites show more random distribution. Our finding suggests that some “relative fragile sites” can be target spots for radiation injury. Yunis et al. (1987) precisely identified 16 different mutagen-associated 110 chromatid breakpoints at the 650 Giemsa band level and reported that 55 of the 113 fragile sites were more general targets for a wide variety of mutagens, including $\gamma$-rays. $^{60}$Co $\gamma$-ray irradiation of 1,160 mGy induced 75 recurrent chromosome breakpoints such as 1q21.3 and 8q13.3. Most chromosome breakpoints identified in the present study were the same as those in Yunis group’s study, although they did not observe hot spots for $\gamma$-ray irradiation (Yunis et al., 1987).

Since the accuracy of fragile site localization in the chromosomal band region is quite limited by the Giemsa banding method and FISH chromosome painting method, we need more information on the sequences involved in these phenomena. There have been only a few molecular cytogenetical studies on the localization of breakpoints in the human chromosome. Close observation was first performed of the FISH painted human chromosome to know whether mutagen-induced breakpoints are non-randomly localized on the human chromosome and these groups proposed non-random distribution (Tucker & Senft, 1994; Luomahaara et al., 1999; Kiuru et al., 2000). So far, 12 fragile sites have been cloned and the cytogenetical features of chromatid or chromosomal breaks and gaps in 7 of the 12 fragile sites were visible by FISH along large genomic regions spanning hundreds to thousands of kilobases and the molecular basis for the expression of these fragile sites characterized (Zlotorynski et al., 2003; Schwartz et al., 2006). It has been hypothesized that the AT-rich chromosomal region leads to an accumulation of DNA damage and is a hot spot for chromosomal fragility, which might be consistent with the delayed replication site (Laird et al., 1987; Glover et al., 2006). Intragenomic heterogeneity in DNA repair and the chromatin structure may explain the different fragility of human chromosomes (Ouyang et al., 2005). Recent studies have shown that the cell-cycle checkpoint

<table>
<thead>
<tr>
<th>Lymphocyte</th>
<th>No. breakpoints</th>
<th>Bone marrow cell</th>
<th>No. breakpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Obs. Exp. RR&quot;Sig.&quot;</td>
<td>Location</td>
<td>Obs. Exp. RR&quot;Sig.&quot;</td>
</tr>
<tr>
<td>Relative fragile sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3p21*</td>
<td>21 7.71 2.72*</td>
<td>3p21*</td>
<td>19 4.61 4.12***</td>
</tr>
<tr>
<td>4q31*</td>
<td>18 4.48 0.101***</td>
<td>1q42</td>
<td>13 2.60 5.00***</td>
</tr>
<tr>
<td>1p36</td>
<td>16 7.89 2.03</td>
<td>1q42</td>
<td>12 2.45 4.90**</td>
</tr>
<tr>
<td>1q24</td>
<td>12 7.85 1.52</td>
<td>7q22</td>
<td>12 2.17 5.53***</td>
</tr>
<tr>
<td>7q32</td>
<td>11 5.53 1.98</td>
<td>1q13</td>
<td>10 4.69 2.13</td>
</tr>
<tr>
<td>8q24</td>
<td>10 8.15 1.22</td>
<td>1q13</td>
<td>9 1.69 5.33***</td>
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<td>9 1.52 5.92**</td>
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<td>7 4.03 1.74</td>
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<td>8q22</td>
<td>7 3.37 2.07</td>
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<td>7 2.20 3.18</td>
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<td>1q25</td>
<td>6 3.73 1.61</td>
<td>7q32</td>
<td>7 3.66 1.91</td>
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<td>6 1.95 3.07</td>
</tr>
<tr>
<td>5q33</td>
<td>6 2.91 2.06</td>
<td>3q25</td>
<td>6 2.26 2.65</td>
</tr>
<tr>
<td>7q36</td>
<td>6 3.78 1.59</td>
<td>8q24</td>
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<tr>
<td>9q22</td>
<td>6 4.59 1.31</td>
<td>2q37</td>
<td>6 1.87 3.21</td>
</tr>
</tbody>
</table>

**Non-fragile sites**

| 1q32        | 14 4.47 3.13* | 9p11/9q11   | 8 1.99 4.02* |
| 2q31        | 11 3.27 3.66* | 1q32        | 8 2.67 3.00 |
| 2q35        | 9 1.63 5.52** | 2q35        | 7 0.98 7.14*  |
| 1p31-32     | 9 7.15 1.25  | 1q24        | 7 1.50 4.64* |
| 2p21-22     | 8 4.22 1.89  | 1q24        | 6 2.90 2.07 |
| 6q25        | 8 3.45 2.32  | 4q21        | 6 2.46 2.44 |
| 15q24       | 8 2.12 3.77* | 1q24        | 6 2.50 2.40 |
| 6p21        | 7 6.19 1.13 |
| 13q22       | 7 1.98 3.54* |
| 17q21       | 7 1.84 1.67 |
| 6q21        | 6 2.51 2.39 |
| 9p11/9q11   | 6 3.33 1.80 |

*Chromosomal bands with $>6$ breakpoints are listed in bone marrow cells as well as in lymphocytes. **Underlined breakpoint location was common to cells of lymphocytes & bone marrow among breakpoints with more than 6. *Relative risk (RR) obtained was from the ratio of observed (Obs.) and Expected (Exp.) values using 912 and 545 breakpoints in lymphocytes and bone marrow cells, respectively. *Sig.: Statistical significance by $\chi^2$ test in p<0.05(*), p<0.01(**) and p<0.001 (***)) levels. Breakpoints in bold had a significantly higher incidence. **
protein ATR and its downstream target genes, such as BRAC1, SMC1 CHK1 and FANC D2, are important for maintaining chromosome stability at common fragile sites (Liu et al., 2000; Casper et al., 2002; Arlt et al., 2004, 2006; Howlett et al., 2005; Musio et al., 2005; Durkin et al., 2006).

Chromosome breakpoints induced by irradiation in both lymphocytes and bone marrow cells were not observed significantly higher at "relative fragile sites", including rare fragile site regions. Unlike common fragile sites, rare fragile sites are caused by the expansion of trinucleotide repeat sequences with CGC or AT repeats and segregate in a Mendelian manner (Dietrich et al., 1991; Kremer et al., 1991). Sequences from cloned rare fragile sites have been analyzed and no expanded dl- and tri-nucleotide repeat sequences have been identified within common fragile sites. The only sequence composition they share is the AT-rich sequence (Ikegami et al., 1978; Boldog et al., 1997; Shiraiishi et al., 2001; Howlett et al., 2005). Rare fragile sites are seen under the same conditions of folate or thymidylate stress used to induce common fragile sites, such as aphidicolin, an inhibitor of DNA polymerase (Glover et al., 1984). Microsatellite and minisatellite regions, which contain repeat sequences such as CGC, AT and TTAGGA are targets for radiation exposure (Jeffreys et al., 1997; Nakanishi et al., 1998; Yank et al., 2002).

The present study also showed that the distribution of breakpoints was similar for the three radiation sources (60 Co γ-rays, HTO β-rays and 252 Cf neutrons), although LET radiation, such as neutrons, is more clustered and structurally complex. The reason for this difference is still not clear, but chromatin structural specificities and the breakage specificity for chromatid-type aberrations might be due to intrinsic promoting factors present in the fragile site area. The same rate of chromatid breaks disappear through DNA rejoining after irradiation of 60 Co γ-rays and fast neutrons in human lymphocytes and the rejoining of chromatid breaks does not seem to be LET dependent (Vral et al., 2002) and their results suggest that a different mechanism underlies the formation of chromatid breaks in the S or G2 phase and chromosome breaks in G0 phase cells. More precise analysis of the DNA structure and gene expression of the breakpoint region involved in radiation damage will elucidate the role of fragile sites.

Breakpoint distribution within fragile sites in certain band regions differed in lymphocytes and bone marrow cells. Differences between the two tissues could be assessed because different stages of the cell-cycle were studied. These differences might also be due to different biological characteristics, such as differences in cell-cycle, replication processes, and nucleotide metabolism. Similar findings have been observed in aphidicolin-induced fragile sites in lymphocytes, bone marrow cells and fibroblasts (Furuya et al., 1989; Murano et al., 1989).

Breakage at fragile sites can initiate amplification of the intrachromosome in drug-resistant mutant CHO cells (Coquelle et al., 1997). It seems likely that loss of function driven by fragile site instability plays a role in cancer development (Mishmar et al., 1998; Huang et al., 1999;...
Bednarek et al., 2000; Reshmi et al., 2007). Comparing with more fragile sites observed on human chromosome just after irradiation, telomere site might be more important target for radiation-induced chromosome instability. Our previous B-cell line study on chromosome instability showed that several percentage of delayed chromosome aberrations occurred at telomere and subtelomere sites, which are not always involved in chromosome aberrations soon after irradiation (Tanaka et al., 2008). Present results on different distributions of chromatid breakpoints between fresh T-lymphocytes just after irradiation and B-cell lines established from irradiated lymphocytes at passages 30 also confirmed that the type of chromosome aberrations occurred as delayed chromosome aberrations long-term after irradiation were quite different from those just after exposed, although it remains a possible difference of radio-sensitivity between T- and B- lymphocytes. Telomere loss is a mechanism for chromosome instability in mouse embryonic stem cells and human cancer cells (Tanaka et al., 2001; Lo et al., 2002). Our study provides input in understanding the mechanisms of radiation-induced chromosomal aberrations and radiation-induced leukemias. This information will also aid in assessing genetic and cancer risks in radiation-exposed cases.

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