Chromosome Aberrations in Bone Marrow Cells of C3H/He Mice at an Early Stage after Whole-Body Irradiation

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(Received, September 16, 1997)
(Revision received, November 10, 1997)
(Accepted, November 19, 1997)

Radiation/Mouse/Chromosome aberration/Chromosome 2/Acute myeloid leukemia

Murine acute myeloid leukemia is characterized by chromosome 2 aberrations, and genesis of the marker chromosome 2 by radiation is suspected to be an initiating event of radiation leukemogenesis. A detailed analysis of the type and frequency of chromosome 2 aberrations in murine bone marrow cells at an early stage after irradiation is provided here. A total of 40 male C3H/He mice was exposed to 140Cs γ-ray at a dose of 1, 2 or 3 Gy, and sacrificed 24 hours after irradiation. Metaphase samples prepared from bone marrow cells were Q-banded for karyotyping or painted with DNA probes specific to chromosome 2. In 5 mice analyzed by karyotyping, one mouse showed high frequency of the marker aberrations as well as other chromosome 2 aberrations. Chromosome painting analysis for the rest of the mice also detected 3 animals showing significantly high frequencies of chromosome 2 aberrations. Dose-dependence of the frequencies was observed even among those mice that tended to be sensitive. The results indicated that there was a subgroup of mice carrying hypersensitive chromosome 2. The subgroup could be leukemia-sensitive if radiation-induced chromosome aberrations are responsible for an early change in myeloid leukemogenesis.

INTRODUCTION

Significant progress in understanding cytogenetic and genetic nature of cancer has been made. Various types of tumor-specific chromosome aberrations were found, and rearranged genes in those aberrations were identified for many tumors1,2). Although these changes are also considered to be critical for radiation-induced cancers, the mechanism of cancer induction by radiation is still unclear. It is not well understood how radiation becomes involved in a multi-step process of oncogenesis3,4).

To know and understand the role of radiation in cancer induction, prospective studies are required in appropriate animal models looking at tumor-specific markers. Acute myeloid leukemia (AML) in some strains of mice provides a good model for that purpose. RFM, CBA, SJL/J and C3H/He strains are known to develop AML after irradiation2,31. Cytogenetic and microsatellite marker analyses identified consistent chromosome 2 aberrations for more than 90% of the AML cases in those strains8,101. Some prospective studies detected an excess number of chromosome 2 aberrations in bone marrow cells of irradiated mice.

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long before the overt leukemia came to appear. Consistency and early presence of chromosome 2 aberration in murine AML suggest that the aberration is a candidate for an initiating event of leukemogenesis. If so, radiation may initiate the cells directly by causing chromosome aberrations. To date, however, few studies have given attention to chromosome 2 aberrations in murine bone marrow cells at early stages after irradiation, and details of initial aberrations are not known.

Studying chromosome aberrations at an early stage after irradiation is problematical. The aberrations are not as simple as those in selected cells at later stages. In the usual karyotyping analysis of banded metaphases, all the aberrations cannot be scored since some cells are precluded by the presence of complex rearrangements. The analysis only gives information on relative involvement of each chromosome in the aberrations. However, the disadvantage of the karyotyping analysis can be compensated by the use of chromosome painting, which enables the scoring of complex interchange-type aberrations. Although chromosome painting is unable to detect small deletions or inversions, they are not the majority of the aberrations. Therefore, the technique provides good estimates for the aberration frequencies.

Here we present a systematic study of chromosome 2 aberrations in bone marrow cells of C3H/He mice at 24 hours after irradiation, adopting both the karyotyping analysis and the chromosome painting analysis. The experiments demonstrate that the AML marker chromosome 2 is found even in the first mitoses after irradiation. A subgroup of mice has been found to have high sensitivity in chromosome 2 aberrations, and its meaning for radiation leukemogenesis is also discussed.

MATERIALS AND METHODS

Irradiation of Mice

Male C3H/HeNCrj mice aged 8 weeks were purchased from Charles River Japan. The mice were housed under conventional conditions and allowed free access to food and water. They received a single whole-body exposure at 57 ± 1 days of age from a 75.4 TBq 137Cs γ-radiation source. For the karyotyping analysis, 5 mice received a dose of 3.0 Gy, and another group of 5 mice were treated as controls (0 Gy). For the chromosome painting analysis, a total of 35 mice were irradiated at a dose of 1.0, 2.0 or 3.0 Gy. Dose rate of each exposure was 0.2 Gy/min.

Preparation of Metaphase Samples

The mice were sacrificed 24 hours after irradiation. Femurs were taken out, washed in PBS and flushed with RPMI 1640 (Bio Whittaker) in order to extract bone marrow cells. The cell suspension was filtered through stainless steel mesh and pipetted well to dissociate fragments. The cells were collected by centrifugation at 1000 rpm for 5 minutes, incubated in 0.075M KCl for 30 minutes at room temperature and then fixed with multiple changes of methanol / acetic acid (3:1 v/v). Fixed cells were dropped onto clean and cold slides under humid conditions to serve as specimens for the karyotyping analysis and the chromosome painting analysis.

Full Karyotyping

Double staining with DAPI [4',6-diamidino-2-phenylindol] and actinomycin D was employed to produce high-quality banded chromosomes. The specimens were first stained with 0.6 μg/ml DAPI in
Mcllvaine's buffer (pH 7) for 20 minutes, rinsed with distilled water and then counterstained with 0.3 mg/ml actinomycin D in 0.01M sodium phosphate buffer containing 1mM EDTA (pH7) for 20 minutes. Once rinsed with distilled water, they were mounted in Mcllvaine's buffer (pH 7). In this technique, contrast of the Q-banding pattern of DAPI, which binds to DNA of A-T base pairs specifically, is significantly enhanced by actinomycin D, G-C specific fluorescence absorber21). Banded chromosomes were photographed on Fuji MINI COPY film and negatives were scanned with a film scanner, MICROTEK ScanMaker 35t, which was connected to a Macintosh computer. Incorporated metaphase images were karyotyped on the Macintosh using Adobe Photoshop software. Fifty cells were analyzed for each mouse, and chromosome-type aberrations in diploid cells were scored. Chromosomal breakpoints were identified according to standard idiogram of the banding patterns28'.

Chromosome Painting

Biotin-labelled probes for mouse chromosome 2 were used for chromosome painting. Metaphase chromosome spreads were denatured by immersing the slides in 70% formamide / 2 x SSC at 70°C for 2 minutes, dehydrated through ice-cold ethanol series and hybridized with the probes. For the mice receiving a dose of 3 Gy, probes of CLONTECH were used. For the dose group of 1 and 2 Gy, however, probes of Cambio were applied since the CLONTECH probes had come to be unavailable due to a termination of the supply.

[CLONTECH probe] The probes were denatured at 75°C for 5 minutes and incubated at 37°C for 90-120 minutes. A volume of 15 µl of probe was applied to each slide, and hybridization was carried out at 37°C for 24 hours. After hybridization, the slides were washed three times in 55% formamide/2 x SSC for 5 minutes and three times in 1 x SSC for 5 minutes, at 43°C, followed by 4 x SSC for more than 5 minutes at room temperature.

[Cambio probe] The probes were denatured at 65°C for 10 minutes and incubated at 37°C for 30-60 minutes. A volume of 20 µl of probe was applied to each slide, and hybridization was carried out at 42°C for 16 hours. After hybridization, the slides were washed twice in 50% formamide / 0.5 x SSC for 5 minutes and twice in 2 x SSC for 5 minutes, at 42°C.

Then the slides were incubated with 4 x SSC/3% bovine serum albumin, fraction V (Sigma) / 0.1% Tween-20 (Sigma) at 37°C for 20 minutes to block non-specific binding of reporter molecules. Detection of the biotinylated probes was achieved by incubating the slides with 5 µg/ml fluorescein avidin-DCS (Vector Laboratories) at 37°C for 20 minutes followed by three washes in 4 x SSC / 0.1% Tween-20 at 42-45°C for 3 minutes. Amplification of the signal was performed by reincubation with 5 µg/ml biotinylated goat anti-avidin D (Vector Laboratories) at 37°C for 20 minutes and another round of fluorescein avidin-DCS with the interval washes. The signals were amplified once for Cambio probe or twice for CLONTECH probe. The slides were finally stained with 0.2 µg/ml DAPI in Mcllvaine’s buffer (pH 7) for 20 minutes, rinsed with distilled water and mounted in VECTASHIELD mounting medium (Vector Laboratories) containing 1-2 µg/ml propidium iodide. A Nikon OPTIPHOT-2, equipped with B2 and UV filter sets, was the epifluorescence microscope used to examine the preparations. Chromosome aberrations in diploid cells were recorded according to PAINT nomenclature system25', but deletions were also scored.

Data Analysis

Inter-individual variation in aberration frequencies was statistically analyzed. Chi-square tests were
used for the tests of independence, and Smirnov-Grubbs outlier tests were applied to check extraneous values. Significance level of the tests was set to be 5%.

In the karyotyping analysis, the number of the breakpoints were analyzed considering RCL (relative corrected length) of chromosomes. The aberrations were classified into three categories; interchanges, intrachanges and terminal deletions. The number of chromosome 2 breakpoints was compared to a value expected from RCL for each aberration category. The values of RCL were calculated according to Savage, using published chromosome lengths. Dose-responses of the aberration frequencies were examined with the data of the painting analysis. Linear, linear-quadratic and quadratic models were tested. Binomial regression was employed for fitting the models to observed data assuming that the distribution of the number of the aberrations was binomial \( (n, p_i) \) where \( n_i \) is the number of the observed cells in the \( i \)-th mouse of dose group \( j \) and \( p_i \) is a probability that the aberration occurs. The parameters were determined by the method of maximum-likelihood using the SAS GENMOD Procedure.

RESULTS

Relative Sensitivity of Chromosome 2

DAPI / actinomycin D double staining produced clear QFH-banding pattern as shown in Fig.1 (a). Contrast of the bands was further improved when photographed on the MINI COPY film (Fig. 1 (b)), and thus the technique provided substantial quality to identify chromosomal breakpoints in abnormal karyotypes.

In the karyotyping analysis, a total of 101 cells had structural aberrations in 250 cells of 5 irradiated mice while 3 abnormal cells were found in 5 control mice with the same number of total cells. The aberrations of the control group were two chromatid breaks and one terminal deletion, but no aberration was found on chromosome 2. In the 101 aberrant cells of the irradiated group, 238 chromosomal breakpoints were identified, in which 16 were located on chromosome 2. The number of the breakpoints in each irradiated mouse is summarized in Table 1. A chi-square test showed statistically significant differences among mice \( (p = 0.045) \), and the significance appeared to be attributed to the high frequency of chromosome 2 breaks in Mouse E. Although the Smirnov-Grubbs test did not identify Mouse E as an outlier, binomial probability of obtaining the observed frequency was quite small \( (\approx 0.026) \) provided that the overall frequency \( (16 / 238) \) was a population parameter. Hence data of Mouse A, B, C and D were pooled, and Mouse E was analyzed separately. Fig. 2 shows the relative number of the breakpoints on each chromosome for (a) a total of 200 cells of Mice A-D and (b) 82 cells of Mouse E. Data of Mouse E includes 32 metaphases additionally analyzed. Chromosome 2 kept by far the largest percentage of the breakpoints in Mouse E while such a large value was not found in any chromosome of Mice A–D. Thus chromosome 2 of Mouse E is considered to be hypersensitive to radiation.

Types of Chromosome 2 Aberrations

Table 2 shows karyotypes of the cells carrying chromosome 2 aberrations. Some of the cells had the same aberrations as the marker chromosomes of murine AMLs, and they were shown in italics in the table. Mouse E had 5 marker chromosomes in 82 karyotyped cells while Mice A–D had 2 in 200 cells as a total. Although Mouse E had more chromosome 2 aberrations than the other mice, 6 out of 10 cells in Table 2
Fig. 1. Microphotographs of metaphase spreads. (a) and (b): QFH-banding pattern produced by DAPI/actinomycin D double staining; (a) original color image, and (b) high-contrast monochrome image. (c) and (d): A reciprocal translocation detected by chromosome painting; (c) fluorescein-painted chromosome 2 with PI counterstain, and (d) DAPI counterstain. (e) and (f): A deletion detected by chromosome painting; (e) fluorescein-painted chromosome 2 with PI counterstain, and (f) DAPI counterstain.
had no aberration other than chromosome 2. This reflects hypersensitivity of chromosome 2 in Mouse E, which was found in Fig. 2 (b).

Chromosome 2 was involved in various types of the aberrations, but in Mouse E, 70% of these

| Mouse ID | Chromosome 2 | Others | Total |
|----------|--------------|--------|-------|
| A        | 4 (5.6%)     | 68     | 72    |
| B        | 1 (2.3%)     | 42     | 43    |
| C        | 4 (8.2%)     | 45     | 49    |
| D        | 0 (0.0%)     | 30     | 30    |
| E        | 7 (15.9%)    | 37     | 44    |
| **Total**| **16**       | **222**| **238**|

Fig. 2. Relative involvement of each chromosome in the aberrations. (a) Distribution of 193 breakpoints in 200 karyotyped cells of Mice A, B, C and D. (b) Distribution of 78 breakpoints in 82 karyotyped cells of Mouse E. For Mouse E, 32 cells were karyotyped in addition to initial 50 cells.
aberrations were deletions, including a substantial number of interstitial deletions. These findings were endorsed by an analysis based on RCL (relative corrected length) where the observed/expected (O/E) ratios of chromosome 2 breakpoints in Mouse E were 3 / 3.2 for interchanges (translocations and dicentrics), 6 / 1.0 for intrachanges (interstitial deletions and inversions) and 4 / 1.6 for terminal deletions. Since the O/E ratios in Mouse A-D did not exceed 1.0 for any aberration category, chromosome 2 in Mouse E seemed to be more involved in deletions, especially interstitial deletions, than expected from its relative length.

With regard to the distribution of chromosome 2 breakpoints in Mouse E, a cluster was found in the 2F region, in which 5 out of 13 breakpoints were located. However, a meaningful statistical test was not applicable as a result of the small number of breakpoints.

Table 2. Karyotypes of the cells carrying chromosome 2 aberrations

| Mouse ID | Karyotype |
|----------|-----------|
| A        | 39, XY, –1, t(2;4)(q17;17), t(2;6)(q17;6), del(17)(q17) |
|          | 40, XY, del(2)(B) |
|          | 40, XY, del(2)(C1), del(2)(E2), del(2)(E2), del(2)(E2), del(2)(E2) |
| B        | 36, XY, t(2;10)(D;B4), del(5)(B1), t(6;17)(C1;C1), del(7)(D1or2), del(7)(D1or2), del(7)(D1or2), t(6;16)(C1;C1), del(7)(C) |
| C        | 39, XY, del(2)(D;F), del(2)(D;F) |
|          | 39, XY, dic(2)(A3;ter), del(2)(C1), del(2)(C1) |
|          | 41, XY, t(2;4)(q17;6), t(2;4)(q17;6), t(2;4)(q17;6) |
|          | 39, XY, dic(2)(A3;ter), del(2)(D;F), del(2)(D;F), del(2)(D;F), del(2)(D;F) |
|          | 40, XY, del(2)(C1) |
|          | 40, XY, del(2)(C1) |
| E        | 40, XY, del(2)(E2F1) |
|          | 40, XY, del(2)(E2F1) |
|          | 40, XY, del(2)(E2F1) |
|          | 40, XY, del(2)(E2F1) |
|          | 40, XY, del(2)(E2F1) |

*82 metaphases were karyotyped for Mouse E, 50 metaphases each for the other mice.

Aberrations of chromosome 2 identical to AML markers were shown in italics.

Frequencies of Chromosome 2 Aberrations

Figure 1 (c)–(f) shows samples of painted chromosome 2. The use of DAPI along with PI counterstain helped to distinguish between symmetrical and asymmetrical interchanges by highlighting centromeres. Not only interchanges such as reciprocal translocations (Fig. 1 (c) and (d)), but also deletions were detectable if large segments were lost (Fig. 1 (e) and (f)). It was impossible, however, to differentiate between interstitial deletions and terminal deletions.

Table 3 summarizes the results of the chromosome painting analysis. Aberrations of painted chromosome 2 were observed in every dose group, and their frequencies increased with dose. For the purpose of quantitative analysis, we selected frequencies of the following aberration categories as indices; the aberrant cells, deletions and color junctions. Color junction is a junction between painted and unpainted segments, and its score can be an index for intercalation-type aberrations. Figure 3 illustrates the dose-response relationship. Each
Table 3. The number of the aberrations detected by chromosome 2-specific composite probes

| Dose (Gy) | Number of mice observed | Metaphases | Aberrant cells | t | dic | ins | del | ace | color junctions |
|-----------|-------------------------|------------|----------------|---|-----|-----|-----|-----|----------------|
| 1.0       | 10                      | 1571       | 19 (1.2%)      | 6 (0.004) | 4 (0.003) | 1 (0.001) | 9 (0.006) | 9 (0.006) | 15 (0.010) |
| 2.0       | 10                      | 1393       | 84 (6.0%)      | 41 (0.029) | 12 (0.009) | 1 (0.001) | 35 (0.025) | 56 (0.040) | 68 (0.049) |
| 3.0       | 15                      | 1096       | 99 (9.9%)      | 48 (0.044) | 28 (0.026) | 5 (0.005) | 45 (0.041) | 54 (0.049) | 101 (0.092) |
| Total     | 35                      | 4060       | 202            | 95          | 44             | 7             | 89          | 119          | 184          |

*Cells carrying structural aberrations of chromosome 2.

*Including acentric fragments, acentric rings and dots.

Fig. 3. Relationship between whole-body dose and frequency of chromosome 2 aberrations. Each point corresponds to an individual mouse. Three mice showing significantly large number of aberrant cells are distinguished with black square markers (Mice 1g, 2b and 3a).
marker corresponds to an individual mouse. Despite the considerable inter-individual differences, the graphs in Fig. 3 exhibited simple dose-responses. Linear, linear-quadratic and quadratic models with no intercept were fitted to the data since no chromosome 2 aberration was found in the karyotyping analysis of the control mice. For any index, a quadratic curve provided a better fit than a linear model, but a linear-quadratic model did not improve the fit significantly. Consequently the dose-response curves were quadratic for all the indices on the statistical basis.

Comparing the frequencies of the aberrant cells between individuals, three mice (Mouse ID: 1g, 2b and 3a, with the numbers representing doses in Gy as shown in Fig. 3) seemed to have higher aberration frequencies than the other mice in the same dose group. The chi-square test could not detect inter-individual differences in any dose group due to small proportions of possible outliers. One-sided Smirnov-Grubbs tests, however, identified the Mice 1g, 2b and 3a as outliers for the frequencies of the aberrant cells and deletions. When Mice 2b and 3a were examined with painting probes for chromosome 1, the frequencies of the aberrant cells were about half of those in the chromosome 2-painted analysis while the frequencies of color junctions were comparable (data not shown). These data suggest that Mice 1g, 2b and 3a in the painting analysis had the same pattern of the aberrations as Mouse E in the karyotyping analysis, i.e. hypersensitivity of chromosome 2, especially in deletions.

DISCUSSION

In this study, metaphase samples of murine bone marrow cells were cytogenetically investigated at 24 hours after irradiation. At that time point, majority of the karyotyped cells were regarded to be in the first mitotic stage after irradiation since no artificial stimulation or harvesting of the cells was employed. It was confirmed by the presence of chromatid aberrations in some fraction of metaphases. Although AMLs are considered to originate from primitive cells in hematopoietic system, relative involvement of chromosomes in the aberrations was reported to be stable irrespective of time after irradiation when clonal aberrations were excluded. Therefore, chromosome aberrations observed at 24 hours after irradiation presumably reflect initial radiation damage of the target cells for myeloid leukemogenesis.

Chromosome 2 aberrations as a marker of murine AML were reported in several studies, and most of the cases have deletions of one homologue. The karyotyping analysis in our study demonstrated that these marker chromosomes can be actually produced by radiation. In 5 mice studied, Mouse E showed high frequency of the marker aberrations as well as other chromosome 2 aberrations. The excess number of chromosome 2 aberrations mainly resulted from interstitial deletions, particular aberration category of the AML markers. Although the proportion of the marker-carrying cells was about 6% even in Mouse E, the cells possibly develop into AML if chromosome 2 aberrant cells have proliferative advantages. Additionally, it is important for leukemogenesis that those cells are not severely damaged to escape cell deaths. Considering this point also, Mouse E seemed to have an advantage. As a result of the hypersensitivity of chromosome 2, four out of five marker-carrying cells of Mouse E had no structural aberrations in any other chromosomes while those in the other mice were accompanied by multiple aberrations. Therefore, production of the marker chromosome 2 in viable cells by radiation was more probable in Mouse E than the other mice. If initial chromosome aberrations are responsible for an early step of myeloid leukemogenesis, Mouse E would be highly sensitive to radiation-induced AML.
High frequency of chromosome 2 aberrations was also found in three mice in the painting analysis, and these mice showed the same characteristics of chromosome 2 aberrations as Mouse E in the karyotyping analysis, i.e. high frequency of deletions. From the results it was suggested that there was a subgroup of mice having hypersensitive chromosome 2. Totally, 4 such animals were found in 40 mice, hence the sensitive subgroup accounted for 10% of the whole population. If the sensitive subgroup could lead to AML with high probability, and if the rest of population is almost insensitive to the disease, no more than 10% of mice would be expected to develop AML. However, the cumulative AML incidence in C3H/He mice was reported to be higher, 22.7% at a dose of 3 Gy\(^3\). Discrepancy between the figures implies that the “sensitive subgroup theory” does not seem to provide a reasonable explanation for the AML incidence. Nevertheless, our ongoing study indicates apparently lower incidence of radiation-induced AML in C3H/HeNCrj strain that was used in the present study. Thus, the reported data alone cannot rule out the sensitive subgroup theory. Further inspection will be required to clarify this point.

With regard to the dose-response, aberration frequencies were well fitted by the quadratic model for any aberration index. The dose-response for deletions is generally expected to be linear or linear quadratic. The discrepancy might be attributable to multi-break aberrations such as incomplete translocations or interstitial deletions. Inclusion of these aberrations in deletions can bend the dose-response curve upwardly at higher doses. Conversely, undetectability of small deletions can modify the curve downwardly. We sometimes found metaphases bearing painted small fragments as only aberrations at doses of 1 and 2 Gy, but never at 3 Gy. This implies the downward effect might be significant at lower doses. Combination of these factors could lead to quadratic dose-response for deletions even if it is linear or linear-quadratic in nature. For color junctions, the fit seems to be consistent with a study on human lymphocytes which revealed that the frequency increased with the square of dose\(^2\). Furthermore, the dose-responses seemed to be retained with different curvature even among the sensitive subgroup. Therefore, these mice do not have higher background level of chromosome 2 aberrations, but higher radiosensitivity of chromosome 2.

Hypersensitivity of chromosome 2 was also reported in other in vivo studies. Hayata and Dutrillaux found high frequency of chromosome 2 rearrangements in bone marrow cells of C3H/He mice at 1–8 months after x-irradiation. The frequency was not as remarkable as the mice of hypersensitive chromosome 2 in our study, but they might have highly sensitive subpopulation since inter-individual differences were noted\(^7\). More obvious data were reported for CBA/H strain as Breckon, et al. showed a very large number of chromosome 2 breakpoints in bone marrow cells at 5 and 8 days after x-irradiation\(^19\,20\). The hypersensitivity of chromosome 2 was confirmed by Bouffler, et al. for both later and earlier sampling times, including 24 hours after irradiation. For CBA/H, however, deletions were relatively rare in chromosome 2 aberrations scored at an early stage after irradiation. Moreover, they found no significant inter-individual variation, and hypersensitivity of chromosome 2 was observed in all mice\(^21\,22\). In contrast, Rithidech, et al. reported evident inter-individual difference for CBA/Ca mice, a closely related substrain of CBA/H. In their study, chromosome 2 aberrations were observed in 20–30% of individuals in the population. The inter-individual variation in CBA/Ca was more straightforward than that of our study because the rest of the population had no chromosome 2 aberration\(^23\). The discrepancy between studies is mainly attributable to the strain or substrain difference, but other factors such as age of mice and irradiation condition might play some roles.

Distribution of the breakpoints on chromosome 2 is another subject of importance. In our study, 2F region of Mouse E appeared to be a cluster of chromosomal breaks. F region of chromosome 2 was reported to be one of the hot spots of radiation-induced chromosome aberrations in CBA/H bone marrow cell\(^19\,20\,22\).
This region was also identified as a fragile site in chemically treated cultured cells. For CBA/H, molecular mechanism of chromosome 2 hypersensitivity was examined, and interstitial telomere sequence arrays were considered to present at the hot spots. In our study, however, the PRINS (oligonucleotide-primed in situ DNA synthesis) with (TTAGGG)4 primer did not detect any interstitial signals on chromosome 2 in Mouse E (data not shown). Even if small telomeric repeats could not be detected by the PRINS technique, a small block of the repeat sequence is unlikely to have a special property in the genome. Alternatively, other repeat sequences such as (CCG)n of human FRAXA or epigenetic factors might be influential. In any case, intense studies will be needed to work out how the hypersensitivity is expressed only in subpopulation of the inbred mice.

In conclusion, we investigated initial cytogenetic damage of bone marrow cells in C3H/He mice, and provided basic data regarding frequencies and types of chromosome 2 aberrations. A subgroup of mice showed hypersensitivity of chromosome 2, and the subgroup could be critical to radiation-induced AML if initial chromosome aberrations are responsible for an early step of myeloid leukemogenesis.

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