RADIATION-INDUCED CHROMOSOME ABERRATIONS IN BONE MARROW CELLS LEADING TO ACUTE MYELOID LEUKEMIA IN MOUSE

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INTRODUCTION

It is well known that radiation-induced acute myeloid leukemia (RI-AML) in mice is characterized by deletion and/or rearrangement of chromosome 2 (1-5). While chromosome 2 has been suspected to be a target of RI-AML, radiation-sensitive site of the chromosome might be implicated in the leukemogenesis (3,6). There were few cytogenetical studies, however, focusing on chromosomal rearrangements shortly after irradiation, and little was known about the frequency and pattern of chromosome 2 aberrations during the early period. In this study, metaphase samples were prepared from whole-body irradiated mice 24 hours after irradiation, most of the cells considered to be in the first mitotic stage. Distribution of chromosomal breakpoints on the metaphase samples were analyzed to study the relationship between chromosome aberrations and RI-AML.

MATERIALS AND METHODS

Eight-week male C3H/He mice were exposed to a single dose of 3 Gy of γ-ray from a ^{137}Cs source, and sacrificed 24 hours after irradiation. Bone marrow cells were extracted from femurs, and metaphase samples were prepared without cultivating process. Chromosome banding was achieved by double staining with DAPI [4',6-diamidino-2-phenylindol] and actinomycin D, which enhances contrast of Q-band with DAPI (7). Banded metaphases were photographed and negatives were scanned with a film scanner connected to a Macintosh computer. Incorporated metaphase images were karyotyped on the Macintosh using Adobe Photoshop software. Chromosome-type aberrations were scored for 5 mice, and breakpoints were identified according to standard idiogram of the banding patterns (8).

RESULTS AND DISCUSSION

Table 1 shows frequencies of chromosome-type aberrations. A total of 250 metaphases was analyzed for 5 mice, and 232 breakpoints were observed in 101 aberrant cells. As an average,

Table 1. Frequencies of chromosome-type aberrations.

Mouse No.	Metaphases analyzed	Metaphases with structural aberrations	Number of breakpoints	Brekapoints per metaphase
#1	50	27 (54%)	70	1.40
#2	50	16 (32%)	43	0.86
#3	50	21 (42%)	46	0.92
#4	50	15 (30%)	29	0.58
#5	50	22 (44%)	44	0.88
Total	250	101 (40%)	232	0.93

approximately one breakpoint per cell was detected.

Number of the breakpoints in each mouse was graphed in Figure 1, where breakpoints on chromosome 2 were distinguished from the other chromosomes. Although there are statistical errors, Mouse #5 seemed to have more breaks on chromosome 2 than the other mice. Hence data of Mice #1 to #4 were pooled, and Mouse #5 was analyzed separately.

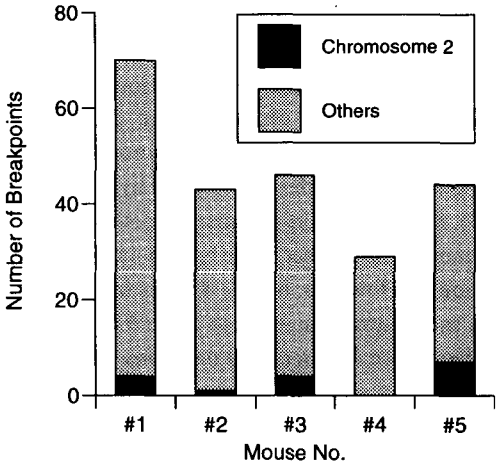

Figure 1. Number of chromosomal breakpoints in each mouse

Figure 2 shows the number of breakpoints in each chromosome for Mice #1 to #4 and Mouse #5. Data of Mouse #5 includes 32 metaphases additionally analyzed. The difference between two graphs is evident, and chromosome 2 of Mouse #5 is conspicuous. Mouse #5 had 13 breakpoints on chromosome 2 in 82 metaphases while Mice #1 to #4 had 9 in 200, 3.5 times higher in relative frequency.

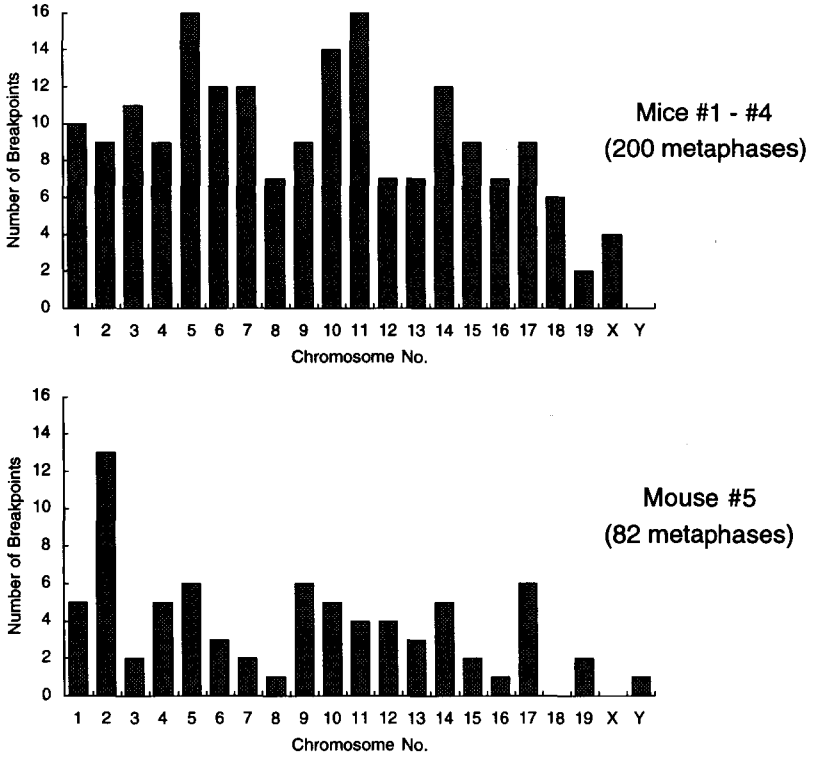

Figure 2. Number of chromosomal breakpoints in each chromosome

Figure 3 illustrates distribution of the breakpoints on chromosome 2. The breakpoints were classified into three aberration categories as described by Savage (9). So far, any sort of cluster is not evident although the number of the breakpoints is not enough. However, Mouse #5 was involved in more intrachanges than Mice #1 to #4. These were all interstitial deletions, typical aberration category of chromosome 2 in murine AML (1-5).

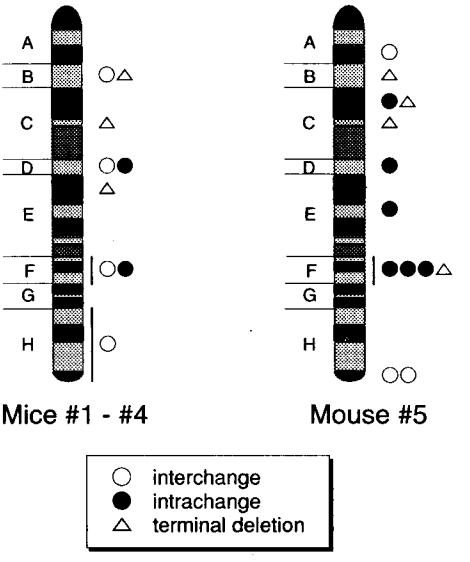

Figure 3. Distribution of breakpoints on chromosome 2

CONCLUSION

Our experiment detected a chromosome 2-sensitive mouse out of 5 mice studied. The result indicates inter-individual difference in chromosome aberration exists even in an inbred strain. It is inferred, if radiation-induced chromosome aberrations are responsible for murine leukemogenesis, there might be AML-sensitive individuals.

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