Chromosomal Instability in Acute Myelocytic Leukemia and Myelodysplastic Syndrome Patients among Atomic Bomb Survivors

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To clarify the mechanism of leukemogenesis in atomic bomb survivors, leukemic cells were investigated using fluorescence in situ hybridization (FISH) analysis on the basis of conventional G-banding in patients with a history of radiation exposure and also in de novo patients. Conventional G-banding showed higher incidences (p < 0.005) of structural and numerical abnormalities without any specific types of chromosome aberrations in the group exposed to a dose of more than one Gy, compared to the non-exposed group. FISH analysis revealed significantly higher incidences (P < 0.05) of subclones with monosomy 7 and deletion of the 20q13.2 region, which were not found in conventional cytogenetic analysis in the exposed group (more than one Gy) compared to the non-exposed controls. Furthermore, segmental jumping translocation (SJT) of the c-MYC gene region was observed only in the exposed group. These chromosomal instability suggested that the leukemic cells from the heavily exposed patients contained persistent cellular genetic instability which may strongly influence the development of leukemia in people exposed to radiation.

INTRODUCTION

From the results of a number of epidemiological studies1–3), it is well known that leukemia occurs more frequently among atomic bomb survivors than in the general population. Investigation of the cytogenetic and molecular biological characteristics of leukemia patients in the atomic bomb survivors is very important to clarify the mechanism of leukemogenesis in radiation-re-
lated leukemia.

Previously, we demonstrated a high frequency of chromosomal aberrations in bone marrow cells as well as peripheral T and B lymphocytes from proximally exposed healthy atomic bomb survivors; most aberrations were of stable types such as deletions, translocations and inversions related to the doses of radiation to which the patients had been exposed\textsuperscript{\textendash}6).

To clarify the mechanism of leukemogenesis of radiation-related leukemia, we investigated chromosomal changes in the leukemia cells from patients with a history of heavy exposure\textsuperscript{7,8). In the present study, we performed fluorescence \textit{in situ} hybridization (FISH) analysis for more precise examination of the chromosomal changes.

**MATERIALS AND METHODS**

**Patients**

In this study, 507 patients with acute myelocytic leukemia (AML) and 278 with myelodysplastic syndrome (MDS) during the 18 years from April 1978 to March 1996 were cytogenetically analyzed. These patients were diagnosed according to the French-American-British (FAB) classification. The control group included only patients who were born before August 1945 and had no history of exposure to therapeutic radiation. The radiation doses in the exposed patients had been estimated by Tentative 1965 Doses (T65D) dosimetry\textsuperscript{9) at the Radiation Effects Research Foundation (RERF) (later recalculated by Dosimetry System 1986 (DS86) system adjusted for our institute\textsuperscript{10}) before they developed leukemia. These patients were divided into four groups according to their exposure status; those who received more than 1.0 Gy (bone marrow dose), 0.01–0.99 Gy, less than 0.01 Gy and non-exposed. The number of patients in each group was 25 (15 patients of AML and 10 of MDS), 27 (11 patients of AML and 16 of MDS), 54 (36 patients of AML and 18 of MDS) and 679 (445 patients of AML and 234 of MDS), respectively. Twenty-five patients who had received more than 1.0 Gy and 679 \textit{de novo} patients were compared cytogenetically in this study.

For FISH study, 17 patients with a history of exposure to atomic bomb radiation including 11 AML (one patient each of M1, M3 and M6 and 8 of M2) and 6 MDS (one patient of RA, 3 of RAEB and 2 of RAEB-T), and 15 \textit{de novo} AML (6 of M1 and 9 of M2) were used. The exposure doses of exposed patients were as follows: 8 had been exposed to more than 1.0 Gy, and 9 had been exposed to between 0.01 and 0.99 Gy. \textit{De novo} AML patients were chosen at random from the M1 and M2 patients.

**Cytogenetic analysis**

All bone marrow samples from these patients were analyzed by G-banding, according to the previously reported method\textsuperscript{11,12). Chromosomes were studied from G-banded preparations using May-Giemsa’s stain. The karyotypes were determined according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995).
Fluorescence in situ hybridization

After completing the conventional chromosome analysis, the fixed samples were stored at −20°C for FISH analysis, which was essentially carried out as described previously. Six probes detecting abnormalities in chromosome number 5, 7, 8, 13, 17 and 20 were selected for FISH analysis for the precise examination, because their aberrations were more frequently observed among the patients with AML or MDS. The probes used were digoxigenin-labeled 5q31, centromeric chromosome 7(D7Z1), RB (13q14) and p53 (17p13) DNA probes (Oncor Science, MD, USA), and probes specific for c-MYC (8q24) and 20q13.2 directly labeled with Spectrum Orange (Vysis Doconers Grove, IL, USA). Hybridization signals were scored under a fluorescence microscope using absorption and excitation filters (Olympus, Tokyo, Japan). The percentages of abnormal cells with one signal or more than three signals were evaluated from a total of 200–700 scored nuclei.

The cut-off levels of 5q- / 5, −7, 13q- / −13, 17p- / −17 and 20q- / −20 positive patients were estimated from bone marrow cells of more than ten patients without hematological malignant diseases and evaluated as 11.5, 7.8, 7.1, 5.1 and 3.4% (mean ± 2SD ), respectively. The cut-off levels of 8q24 and 17p13 amplification, i.e. patients with three signals, were 3.1 and 3.8% (mean ± 2SD ). However, for the latter amplification we regarded those patients with obvious amplification, that is, more than 10.0% as positive.

Dual color FISH was applied to some patients to differentiate monosomy 20 and 20q deletion, and gain of chromosome 8 and genuine amplification of the c-MYC region, using region-specific or whole painting probes.

RESULTS

Conventional G-banding analysis

Conventional G-banding revealed higher incidences of chromosomal abnormalities without any specific types of structural or numerical changes in the group exposed to more than one Gy (Table 1). The incidences of abnormalities in chromosome 5 (P < 0.001), chromosome 13 (P < 0.005) and chromosome 20 (P < 0.0001) were significantly higher than those in the non-exposed group (χ²-test, Table 2). In the non-exposed AML group, 72 patients showed specific types of chromosome translocation ; 24 with t(8;21), 38 with t(15;17) and 10 with inversion 16. Hyperdiploidy was found in 63 patients, pseudodiploidy in 67, hypodiploidy in 67 and normal karyotype in 176. The non-exposed MDS patients consisted of 35 with hyperdiploidy, 39 with pseudodiploidy, 29 with hypodiploidy and 131 with normal karyotype. The average number of chromosomal aberrations among the heavily exposed group was 6.2 (4.6 structural and 1.6 numerical), whereas that in the non-exposed group was only 2.6 (1.5 structural and 1.1 numerical). The difference was significant (P < 0.005, t-test) between these two groups (Table 2).

Results of FISH analysis

The result of FISH on leukemic cells from 32 patients is shown in Table 3. The number of patients with chromosomal aberrations newly detected by FISH is presented in Table 4. Includ-
| patient No. | age<sup>b</sup> | estimated type of leukemia/MDS | ATB at Dx | karyotype<sup>a</sup> |
|------------|-----------------|-----------------------------|-----------|-----------------|
| 1          | 16              | M2                          | 61        | 46,X,Y,del(20)(p11)[13]/46,X,Y,idem,-7,+mar[7] |
| 2          | 31              | RAEB                        | 66        | 48,X,Y,del(5)(q12q31),+17,+20[43] |
| 3          | 25              | M5                          | 71        | 46,X,Y,del(1)(q23),del(2)(t;2)(q23;q5)\(\alpha \equiv 2\),del(11)(q21)[5]/47,X,Y,idem,+8[7] |
| 4          | 23              | M2                          | 72        | 48,X,Y,-1,+del(1)(p12p22)\(\alpha \equiv 2\)(8;18)(p11;pl1),-11,+der(18)(t;8;18)(p11;p11),del(20)(q11),+r[12] |
| 5          | 17              | M1                          | 63        | 44,XX,dup(1)(q25 l q44),del(3)(q21),del(4)(q25q28),del(5)(q13q32),-11,+t(11q),add(12)(q37),del(13)(q12q14),-14,-17,der(19)(t;9;11?;q13;7;HSR)[21] |
| 6          | 16              | M2                          | 60        | 46,X,Y,del(5)(q13q15),(t;3;18)(p21;q23),(t;3;21)(q25;q28)[4]/46,XX,t(11;11)[2]/46,XX[7] |
| 7          | 31              | RAEB                        | 71        | 45,XXi(1q),del(5)(q15),-10,der(14)(t;3;14)(q22;q22),-20,+mar[5]/46,XX,idem,+r[21] |
| 8          | 21              | RAEB                        | 71        | 46,X,Y,-1,del(5)(q21q33),+der(9)dic(9)(t;1;9)(q10;q34)t(9;?)(p22;?),del(20)(q11q13)[2] |
| 9          | 40              | M1                          | 74        | 47,X,Y,2q,-2q+,5q,+q(7)[46,XY][4] |
| 10         | 22              | M1                          | 70        | 46,XX,del(5)(q15q32),del(13)(q2q32)[3]/46,XX,idem,del(20)(q11q21)[8] |
| 11         | 39              | RAEB                        | 83        | 47,X,Y,der(1)(t;1;2)(p36q31),add(2)(q21),der(4)(t;4;10)(q25q24),der(11)(t;4;11)(q25q23),+13[8] |
| 12         | 37              | RAEB                        | 79        | 47,X,Y,+r(21)[10] |
| 13         | 3               | M2                          | 46        | 46,X,Y,+r(7)(t;1;7)(q21q22)[16]/46,XY[5] |
| 14         | 47              | M2                          | 80        | 42,X,Y,-4,-4,-5,-8,i(8q)[19]/46,XY,-8,i(8q),+22[3] |
| 15         | 21              | M1                          | 68        | 45,XXi(1;16)(p3;13)(t;2;2)(q33;q21),add(11)(q22),-12,del(20)(q11)[15] |
| 16         | 15              | M2                          | 59        | 45,XX,der(1)(t;1;13)p11q11,add(11)(q13),-13[5]/45,XX,-20[3]/46,XX[5] |
| 17         | 18              | RAEB-T                      | 66        | 43,XX,add(1)(q24),+del(1)(p22),der(3)(t;?;3)(q735;p11),del(5)(q13),del(7)(q11),add(12)(q24),-13,-15,-16,-18,add(21q22)[11] |
| 18         | 11              | M2                          | 53        | 42,X,Y,add(1)(p32),add(2)(q37),-5,del(7)(q32),i(8q),del(9)(q11q22),-13,-13,add(14)(q32),-15,-17,der(19)(t;11;19)(q42q13),-21,add(22)(p11),+mar,1,+mar,2,+mar3[18] |
| 19         | 40              | M6                          | 81        | 46,XX,del(7)(q11q22),del(20)(p12)[13] |
| 20         | 1               | M2                          | 42        | 46,XX[3] |
| 21         | 17              | RA                          | 64        | 46,XXi(9;13)(q22q34)[11]/46,XY,t(6;9)(q12q22),del(4)(q23q31),del(20)(q11)[2]/46,XY[6] |
| 22         | 30              | M1                          | 76        | 46,XX,del(1)p22p34,del(11)(q32q35),-20,+mar[19] |
| 23         | 2               | RAEB                        | 33        | 46,XX,-18,-20,+mar[1],+mar2[13]/46,XX[4] |
| 24         | 39              | M4                          | 57        | 46,XX,del(11)(q13)[15] |
| 25         | 31              | RAEB                        | 70        | 46,XX,-8[4]/46,XY[13] |

<sup>a</sup> Patient no. 2, 7, 14, 18 and 19 were previously published<sup>b</sup>.  
<sup>b</sup> ATB; at the time of bombing, Dx; diagnosis. Those exposed doses were later recalculated by DS86 system<sup>c</sup>.
Table 2. Chromosomal aberrations by conventional G-banding

| exposed doses | number of patients examined | number of patients with chromosomal aberration on aberrations / stemline | average number of chromosomal aberrations / stemline |
|---------------|-----------------------------|-------------------------------------------------------------------------|---------------------------------------------------|
| 1.0 Gy <      | 25                          | 10 5 5 2 10                                                           | 4.6 1.6 6.2                                       |
| 0.01–0.99 Gy  | 27                          | 3 1 1 1 1                                                           | 1.6 0.7 2.3                                       |
| < 0.01 Gy     | 54                          | 5 4 1 1 0                                                           | 1.4 0.5 1.9                                       |
| non-exposed   | 679                         | 88 68 27 48 32                                                         | 1.5 1.1 2.6                                       |

Table 3. Loss/deletion and amplification of genetic loci detected by FISH

| No. | patient | exposed | dose | 5q31<sup>a</sup> | 7 cen.<sup>a</sup> | 13q14<sup>a</sup> | 17p13<sup>a</sup> | 20q13.2<sup>a</sup> | 8q24<sup>b</sup> | 17p13<sup>a</sup> | amplifications |
|-----|---------|---------|------|----------------|------------------|----------------|----------------|----------------|--------------|----------------|----------------|
| 1   | 4 <     | 7.4     | 11.0<sup>b</sup> | 3.4     | 2.3   | 1.0   | 1.8   | 11.6           |
| 4   | 4 <     | 1.8     | 8.2   | 4.8     | 2.4   | 8.5<sup>a</sup> | 9.6   | 6.5            |
| 6   | 4 <     | 1.67    | 5.2   | 1.2     | NE    | NE    | 9.7<sup>c</sup> | 26.3           |
| 8   | 4 <     | 78.4<sup>b</sup> | 10.9 | 4.8     | 1.4   | 68.5<sup>c</sup> | 1.5   | 1.9            |
| 10  | 3.90    | 42.3<sup>b</sup> | 29.5 | 79.5<sup>b</sup> | 9.6   | 6.5   | 0.8   | 2.0            |
| 12  | 3.22    | 5.3     | 4.5   | 6.6     | 4.9   | 1.8   | 1.3   | 30.5           |
| 17  | 2.69    | 44.2<sup>b</sup> | 53.0 | 20.4<sup>d</sup> | 17.8  | 3.2   | 3.0   | 0.4            |
| 18  | 2.13    | 86.6<sup>b</sup> | 8.2  | 5.3     | 15.8<sup>b</sup> | 1.5   | 42.3<sup>c</sup> | 1.5            |
| 26  | 0.88    | 6.3     | 11.1  | 1.4     | 1.1   | 2.0   | 0.6   | 13.7           |
| 27  | 0.82    | 6.8     | 10.9  | 24.5    | 0.5   | NE    | NE    | 1.9            |
| 28  | 0.39    | 11.2    | 2.4   | 13.5    | 3.1   | 3.1   | 1.3   | 9.2            |
| 29  | 0.38    | 96.3<sup>b</sup> | 5.9  | 10.3<sup>b</sup> | 52.1  | 2.9   | 7.6<sup>c</sup> | 0.8            |
| 30  | 0.36    | 4.5     | 8.3   | 32.9<sup>b</sup> | 0.4   | 1.8   | 0.3   | 7.5            |
| 31  | 0.32    | 25.7    | 15.5  | 0.9     | 0.4   | 1.1   | 1.7   | 20.2           |
| 32  | 0.32    | 8.9     | 7.2   | 12.0    | 2.2   | 2.9   | 0.5   | 13.9           |
| 33  | 0.14    | 6.4     | 4.0   | 7.3     | 4.5   | 4.8   | 3.0   | 8.0            |
| 34  | 0.03    | 87.5<sup>b</sup> | 5.9  | 6.3     | 37.9<sup>b</sup> | 0.0   | 94.9<sup>b</sup> | 1.4            |
| 35  |         |         |       |         |       |       |       |                |
| 36  |         |         |       |         |       |       |       |                |
| 37  |         |         |       |         |       |       |       |                |
| 38  |         |         |       |         |       |       |       |                |
| 39  |         |         |       |         |       |       |       |                |
| 40  |         |         |       |         |       |       |       |                |
| 41  | non-    |         |       |         |       |       |       |                |
| 42  | exposed | 1.3     | 1.4   | 4.8     | 9.1   | 0.4   | 1.4   | 0.9            |
| 43  |         | 1.3     | 0.6   | 2.8     | 1.7   | 0.0   | 85.0<sup>b</sup> | 3.0            |
| 44  |         | 0.6     | 1.7   | 2.5     | 5.1   | 0.4   | 84.0<sup>b</sup> | 2.2            |
| 45  |         | 86.8<sup>b</sup> | 9.7  | 4.8     | 1.5   | 0.3   | 1.4   | 9.3            |
| 46  |         | 4.6     | 7.3   | 5.7     | 13.4  | 0.7   | 1.3   | 4.3            |
| 47  |         | 4.1     | 1.4   | 3.4     | 31.2<sup>b</sup> | 0.8   | 2.9   | 1.6            |
| 48  |         | 0.7     | 2.3   | 1.5     | 0.4   | 1.7   | 87.6<sup>b</sup> | 11.1           |
| 49  |         | 4.3     | 5.6   | 85.4<sup>b</sup> | 2.5   | 0.8   | 0.5   | 12.5           |

<sup>a</sup>Percentage of cells showing loss/deletion and amplification. NE; not examined. Boldfaced numerals indicate % of positive clones, first detected by FISH. The cut-off levels of 5q−/5, −7, del.(13q14), del.(17p13) and 20q−/20 positive patients were evaluated as 11.5, 7.8, 7.1, 5.1 and 3.4 (mean ± 2SD), respectively. The cut-off levels of amplification at 8q24 and 17p13 were evaluated as 3.1 and 10.0 (mean ± 2SD), respectively.

<sup>b</sup>The abnormality had already detected in the conventional karyotype.

<sup>c</sup>Amplification of 8q24 in these cases were due to segmental jumping translocation (SJT), not to trisomy 8.

<sup>d</sup>Monosomy 20 was revealed in this case, while the conventional analysis showed 20q-.
ing the patients already shown to be positive in conventional analysis, deletions of regions at 5q31 and 13q14 and amplification of the 17p13 region were more frequent among the exposed patients (Table 3). In contrast, deletion of 17p13 region was more common in the de novo AML patients than in the patients with radiation exposure. Ten exposed patients (58.8%), 6 of whom (patients 4, 6, 8, 10, 17 and 18) had been exposed to more than 1.0 Gy, and 4 de novo AML patients (26.7%) had subclones of monosomy 7 detected only by FISH; this difference was significant (P < 0.05, χ²-test). Conventional chromosome analysis revealed monosomy 7 in only one of 32 patients examined in this study. Furthermore, in two patients (patients 17 and 18), monosomy 7 appeared later on conventional examination. Abnormalities were found in chromosome 20 in three patients (patients 4, 8 and 10) in the exposed group (more than 1.0 Gy), one (patient 33) in the 0.01–0.99 Gy group and none in the de novo group. The difference between exposed and non-exposed groups was significant (P < 0.05, χ²-test). Amplification of c-MYC signals was observed in only three exposed patients forming segmental jumping translocation (SJT); i.e. the region containing c-MYC had migrated onto other chromosomes.

Eleven of 17 exposed leukemia patients showed more than two additional aberrations by FISH, but only 4 non-exposed patients had multiple aberrations (P < 0.05, χ²-test, Table 4).

**DISCUSSION**

The present study showed that leukemic cells from heavily exposed A-bomb survivors contained complex chromosome abnormalities accompanying subtle subclones with monosomy 7 or deletion of 20q only detected by FISH analysis.

Previously, we reported that the heavily exposed patients had more complex chromosomal abnormalities as revealed by conventional analysis in a radiation dose-dependent manner⁸, and speculated that leukemic cells in these patients may have originated from stem cells which had been damaged by irradiation at the time of exposure.

Using more sensitive FISH analysis, we detected higher frequencies of monosomy 7 as subclones, especially in the exposed patients (Table 3). The clone with monosomy 7 detected only by FISH was thought to be due to the inability of these cells to enter mitosis in vitro¹⁴,¹⁵ and to spread well at metaphase¹⁶. Our results indicated that monosomy 7-positive cells, especially in the exposed group, at metaphase were not scored in conventional analysis but could be de-

| exposed doses | number of the abnormality |
|---------------|--------------------------|
|               | 0 | 1 | 2 | 3 | 4 |
| 1.0 Gy        |   |   |   |   | 1 |
| 0.01–0.99 Gy  | 1 | 2 | 4 | 2 | 0 |
| non-exposed   | 3 | 8 | 3 | 1 | 0 |
tected only by FISH. Deletion and loss of chromosome 20 were also detected only among the heavily exposed group at a frequency of 26.7% (Table 3). Deletions of 20q were found in a number of myeloproliferative disorders (MPD)\textsuperscript{17,18}, in approximately 5% of patients with MDS and in some patients with AML\textsuperscript{19,20}. MDS and MPD frequently result from transformation of multipotent hematopoietic progenitors\textsuperscript{21}. Since the leukemia among atomic bomb survivors would also have arisen from transformation of stem cells damaged by radiation, we speculated that a target gene on 20q may play a role in the regulation of normal multipotent hematopoietic progenitors.

Segmental jumping translocation was found in only the exposed group (Table 3). The segmental jumping translocation is thought to be associated with the leukemogenesis of secondary leukemia, as one mechanism of secondary leukemia\textsuperscript{22}. On the other hand, it was experimentally demonstrated that the frequency of DNA amplification in tumors contrasted strongly with the rarity in normal human cells\textsuperscript{23}. It is predicted that regions containing genes concerned with the regulation of hyperplasia, i.e. proto-oncogenes, are amplified selectively and not at random in tumors\textsuperscript{24}. Our results suggested that leukemic cells of the exposed patients had acquired the abnormal ability to amplify DNA with high frequency, i.e. genetic instability induced in their stem cells by the radiation exposure.

The observation that the leukemic cells among the exposed patients had many complex abnormalities (Tables 2 and 4) supports this hypothesis, and this complexity could be regarded as a kind of chromosomal instability. Chromosomal instability in irradiated mammalian cells were observed to inherit through the progeny\textsuperscript{25,26}. In colorectal cancers of humans, either chromosomal instability or microsatellite instability were proved. They can arise through two distinct pathways\textsuperscript{27}. Microsatellite instability has been recently observed in hematopoietic neoplasias such as blastic crisis of chronic myelogenous leukemia (CML)\textsuperscript{28,29}, MDS\textsuperscript{29,30}, and therapy-related leukemia\textsuperscript{31}.

The results presented here suggested the existence of persistent cellular genetic instability induced by radiation. This cellular process could enhance the probability of subsequent mutational steps, leading to the malignant transformation of the progeny of the irradiated stem cells. For understanding the development of secondary leukemia related to radiation, further studies, especially on microsatellite instability, will be necessary on the patients with a history of radiation exposure.

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