II. BIOLOGICAL EFFECTS

Cytogenetic and Molecular Changes in Leukemia Found among Atomic Bomb Survivors

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Chromosome aberrations/Leukemia/Atomic bomb survivors/bcr-gene/ras-gene

Seventy five radiation-related leukemias (acute non-lymphocytic) in Hiroshima including 16 patients exposed to more than one Gray were cytogenetically examined. Statistical analysis of the data on the frequencies of chromosomal aberrations in survivors according to the bone marrow doses of DS86 estimation revealed that heavily exposed patients tended to have significantly higher aberration rates as compared with non-exposed patients. Furthermore, the chromosomal aberrations in the survivors were observed to be of a more complex nature and had characteristic findings of secondary leukemia. These observations therefore suggest that patients with a history of heavy exposure to atomic bomb radiation exhibit leukemic cells that originated from a stem cell which had been damaged by irradiation at the time of bombing and had been involved in the complex chromosome abnormalities.

Molecular biological studies on transforming genes in acute and chronic leukemia and the bcr gene in chronic myelocytic leukemia have been performed in exposed and non-exposed groups. So far, no distinctive differences have been observed in the frequency and the sites of point mutations in N- and K-ras genes or in the rearrangement of the bcr gene, for a final conclusion of the specificity of radiation induced leukemia. Further retrospective studies require patient DNAs that developed in the early period of the atomic bomb exposure.

INTRODUCTION

It has been shown\(^1\) that the incidence of leukemia is high among atomic bomb survivors. Since the first report by Ford\(^2\) on human chromosomes in leukemia, many reports have been presented. The relation between radiation and chromosome aberration is a very interesting problem from the viewpoint of the leukemogenic mechanism.

In this presentation, chromosome abnormalities and molecular biological changes found in leukemia patients with a history of atomic bomb exposure are reviewed in comparison with the findings in non-exposed leukemia patients.

1. Cytogenetic Changes
   i. Acute Leukemia

There are a number of reports on chromosome studies of peripheral blood from apparently

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healthy A-bomb survivors\(^6\)\(^{-9}\). It has been demonstrated that radiation-induced chromosome aberrations have persisted in the circulating lymphocytes of survivors more than three decades after radiation exposure, and that the chromosome-aberration frequency is, in general, proportional to the dose received. A high incidence of chromosome abnormalities of bone marrow cells has also been demonstrated in survivors with heavy exposure but without any hematological disorders at the time of examination. Chromosome aberration rates among bone marrow cells and T and B lymphocytes were found to be almost the same\(^{10}\). Kamada et al. have demonstrated annual changes of abnormal clones, representing the evidence that one abnormal clone after another showed dominant changes during several years, and have speculated that more active and more malignant clones leading to leukemia would appear in the bone marrow\(^{10}\).

The first report on chromosome aberrations in acute leukemia among atomic bomb survivors in Hiroshima appeared in 1969\(^{11}\). Kamada conducted chromosome studies on acute leukemia patients with a history of atomic bomb exposure and observed six abnormal karyotypes in ten exposed cases as well as ten abnormalities in 26 non-exposed cases. The type of chromosome aberrations varied between cases. No abnormal karyotypes characteristic of acute leukemia were observed in the exposed group. He extended his observations to 47 acute leukemia cases directly exposed to the atomic bomb and found 12 of 13 leukemia patients exposed within 1 km had abnormal clones in their bone marrow, showing a higher incidence of abnormal clones (92.3\%) than the non-exposed group (67.3\%) in 238 leukemia patients. According to his recent results\(^{12}\) including 76 cases with a history of exposure analysed by radiation doses, some interesting findings were found among the exposed group. That is, patients who had been exposed to more than one Gray of bone marrow dose (DS86 dosimetry system) demonstrated a significantly higher incidence of chromosome aberrations in the leukemic cells. They had more complex types of chromosome aberrations and a higher incidence of aberrations in chromosomes 5 or 7, as compared with 261 non-exposed patients (Table 1).

Concerning chromosome abnormalities of leukemias found in Nagasaki survivors, Tomonaga et al.\(^{13,14}\) reported ten cases exposed within 4 km from the hypocenter, in which two patients who were exposed within 1.2 km had chromosome abnormalities. Though the number of cases examined in Nagasaki is smaller, leukemia patients with a history of heavy exposure seem to have a higher incidence of chromosome abnormalities. The reasons why acute leukemia patients with a history of heavy exposure manifest more complex chromosome aberrations in their leukemia cells is obscure. However, the previously mentioned findings in healthy survivors with a history of heavy exposure who have a high incidence of chromosome aberrations in bone marrow cells may give some suggestions. There is the possibility that the leukemic cells originated from a stem cell which had been damaged by irradiation at the time of bombing and was involved in the complex chromosome abnormalities.

Abnormalities in chromosomes 5 and 7 were frequently reported in myelodysplastic syndrome and secondary leukemia\(^{15-18}\). A high frequency of abnormalities in chromosomes 5 and 7 among leukemias in heavily exposed survivors indicates that leukemias induced by atomic bomb exposure have the same cytogenetic characteristics found in secondary leukemia induced by therapeutic irradiation or chemical drugs for cancer treatments.
Table 1. Chromosome aberrations in acute non-lymphocytic leukemia found among atomic bomb survivors

|                          | Controls\(^1\) | 0 rad\(^2\) | 0.1–0.99 G | 1.0 G-
|--------------------------|----------------|-------------|------------|--------
| No. of cases examined    | 261            | 25          | 34         | 16     |
| % of cases with chr. aber.| 60.9           | 56.0        | 52.9       | 100\(^2\) |
| % of cases with more than| 26.4           | 24.0        | 41.8       | 75\(^3\) |
| 3 chr. aber.             |                |             |            |        |
| % of cases with abnormalities in chrs. 5 or 7 | 21.6 | 22.2 | 9.5 | 75\(^4\) |

1) leukemia patients born before August 5, 1945.  
2) statistically significant (p<0.001), compared with the controls.  
3) statistically significant (p<0.05), compared with other groups.  
4) statistically significant (p<0.01), compared with other groups.  
5) bone marrow dose by means of DS86 dosimetry system given by RERF\(^{12}\).

ii. Chronic Myelocytic Leukemia

In 1963, Tanaka et al.\(^{19}\) first reported a case with a history of atomic bomb exposure and with the Philadelphia chromosome which is specific for the disease. Kinugasa et al.\(^{20}\) reported a case which developed 11 years after the exposure to atomic bomb and showed 17% of cell with Ph\(^1\) chromosomes and hyperdiploidy in the blastic phase.

Tough\(^{21}\) made a comparison on the clinical and hematological findings, survival time and cytogenetic findings between 8 cases of CML who had received X-ray treatment for ankylosing spondylitis and some cases without any radiation history and noted that for the first three above items there was no difference whatsoever between groups, and also that there was no decisive difference between the groups with regard to chromosome findings.

In 1969 Kamada\(^{11}\) described clinical and cytogenetic findings of six A-bomb exposed and 8 non-exposed patients with CML. Tsuchimoto et al.\(^{22}\) reported chromosome, clinical and hematological findings in 27 patients with CML, comparing 12 atomic-bomb survivors with 15 non-exposed patients. Ph\(^1\) chromosomes were found in marrow cells from 14 patients with a frequency ranging from 62 to 100 per cent of the leukemic cells. No difference was observed cytogenetically between the atomic bomb and non-exposed groups.

Kamada et al. extended their observations to 36 cases directly exposed to the atomic bomb, compared with 111 non-exposed cases\(^{23}\). All cases, irrespective of atomic-bomb exposure, showed Ph\(^1\) chromosome. There was no statistical difference in the incidence of the cases with
Table 2. Point mutation of N- and K-ras genes in exposed and non-exposed groups

<table>
<thead>
<tr>
<th></th>
<th>number of cases examined</th>
<th>mutated ras gene</th>
<th>mutated codon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N-ras</td>
<td>K-ras</td>
</tr>
<tr>
<td>acute leukemia exposed</td>
<td>17</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>non-exposed</td>
<td>19</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>chronic myelocytic leukemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>exposed</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>non-exposed</td>
<td>25</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

* a double mutation in single case.

additional chromosome abnormalities at the time of diagnosis of chronic phase of the disease (Table 2). Tomonaga and Sadamori et al. reported 6 exposed and 112 non-exposed cases in Nagasaki. All patients in the exposed and 97% of patients in the non-exposed group had Ph chromosomes with no statistical difference between the two groups.

So far no cytogenetic differences in CML were found among the radiation exposed cases from either Hiroshima or Nagasaki.

2. Molecular Changes
   i. Transforming genes in acute and chronic leukemias

The molecular genetic analysis of tumor development has been made possible by a combination of rapid technical progress in molecular biology and the fusion of ideas and perspectives from the fields of virology and chemical carcinogenesis. One of the most exciting developments has been the demonstration that proto-oncogenes exist in mutated or activated forms in non-virus-infected cells transformed by chemicals or isolated from human tumors. It was shown that purified DNA from many tumors but not from equivalent normal tissues, could cause morphological transformation of recipient NIH/3T3 cells. In the vast majority of cases, the gene responsible for the transforming properties of DNA from tumors is a member of the ras family of proto-oncogenes. Three different members of the ras gene family have been identified as biologically active transforming genes in neoplasm DNA: H-ras, K-ras, and N-ras. These genes have been detected in many different types of neoplasms, including carcinomas, sarcomas, lymphomas, and leukemias of myeloid and lymphoid origin. Thus, it appears that ras genes can contribute to the development of neoplasms.

To clarify the mechanism of leukemia induced by radiation, the transforming activity of DNAs extracted from leukemic cells of acute and chronic leukemias of atomic bomb survivors was examined by the in vivo selection assay and the polymerase chain reaction. N- and K-ras gene mutations were detected in three acute and two chronic myelocytic leukemia patients in the exposed group. So far, no significant differences in the incidence of the mutated ras gene and
of the specific site of codons was observed between the exposed and non-exposed\textsuperscript{32}).

Guerrero I, et al. reported a consistent point mutation at codon 12 of the K-ras gene in radiation induced mouse lymphoma\textsuperscript{33}). Transforming ras genes have also been detected in rat mammary carcinomas induced by treatment with nitroso-methylurea\textsuperscript{34}). In this case, a point mutation was found at codon 12 of H-ras gene, resulting in the replacement of glycine with glutamic acid in the p21 protein product.

Further examination of point mutations in leukemias and cancers among survivors would be necessary for a final conclusion, using polymerase chain reaction (PCR) for specimens from malignant tissues which developed in the early period of atomic bomb exposure.

ii. bcr gene in chronic myelocytic leukemia

The Philadelphia chromosome detected in over 95\% of patients with chronic myelocytic

\textbf{Fig. 1.} Breakpoint of \textit{bcr} gene in chronic myelocytic leukemia of exposed and non-exposed group. open circles: CML patients in exposed group solid circles: CML patients in non-exposed group
leukemia (CML) is generated by a reciprocal 9q34;22q11 translocation. This recombination event fuses a portion of chromosome 9 containing the entire c-abl gene to a region on chromosome 22 called the breakpoint cluster region (bcr)\textsuperscript{35}. Tanaka et al.\textsuperscript{36} conducted molecular studies on seven CML patients with a history of atomic bomb exposure in comparison with 14 non-exposed CML patients. All patients, irrespective of radiation exposure, had rearrangements of the bcr gene in the leukemic cells. Further analysis of break points within the bcr gene demonstrated no distinct difference between the exposed and the non-exposed groups (Figure 1). They examined CML patients exposed within 2 km who developed CML in the 1980's, 35–44 years after exposure. Most atomic bomb-related CML had developed by 1970. Again, as in the transforming gene, retrospective study is required for a final conclusion.

REFERENCES

13. Tomonaga, Y., Sadamori, N., Tagawa, M., Kusano, M., Nishio, K., Yao, E. and Ishimaru, M. Cyto genetic studies on leukemia and preleukemia in atomic bomb survivors – Structural abnormalities of chromosomes as leukemia inducing factors (further report) –.


