Chromosome Translocations Involving Band 7q35 or 7p15 in Childhood T-Cell Leukemia/Lymphoma

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In a chromosome study in childhood T-cell leukemia/lymphoma, we found t(7;11)(q35:p13) in 2 patients, t(7;14)(q35;q11) in one patient, and t(7;14)(p15;q32) in 1 patient. Southern blotting and in situ chromosomal hybridization studies in one patient with the t(7;11) demonstrated that both alleles of the T-cell antigen receptor β-subunit gene (TCRB) were rearranged, and that one TCRB allele had relocated from 7q35 to the fusion point in band p13 of the involved chromosome 11 (11p-). These findings suggest that juxtaposition of TCRB with the putative oncogene tcl-2 located in band 11p13 may be a critical step toward development of this T-cell leukemia/lymphoma. In the other two translocations, all breakpoints were sites for lymphocyte function genes, ie, 7q35 for TCRB, 14q11 for T-cell antigen receptor α-subunit gene (TCRA), 7p15 for T-cell antigen receptor α-subunit gene (TCRG), and 14q32 for immunoglobulin heavy-chain gene (IGH). Thus, the findings in these cases allow us to expand the above hypothesis and propose that the juxtaposition of TCRB or TCRG with tcl-2, TCRA, or IGH through chromosomal translocation may activate a mechanism for the genesis of T-cell leukemia/lymphoma with these chromosome translocations.

A TRANSLOCATION with a break in chromosome band 14q32, the site for the immunoglobulin heavy chain gene (IGH), is directly involved in the development of B-cell leukemia/lymphoma.1 A growing amount of data also exist on T-cell leukemia/lymphoma with a translocation or inversion having a break in band 14q11 where the T-cell antigen receptor α-subunit gene (TCRA) resides.2-6 Although the T-cell antigen receptor β (TCRB) and γ (TCRG) subunit genes have been mapped in 7q35 and 7p15,7,8, respectively, reports on T-cell leukemia/lymphoma with a translocation or inversion having a break in 7q35 or 7p15 are scarce.9,10 We report four patients with 7q35 or 7p15 translocation-associated T-cell leukemia/lymphoma. The breakpoint of the translocation partner was 11p13, 14q11, or 14q32. By applying Southern blotting and in situ chromosomal hybridization techniques to leukaemic cells with t(7;11)(q35;p13), we proved that both TCRB alleles were rearranged, and that one TCRB allele had relocated to the fusion point of the abnormal chromosome 11 formed by the translocation. These findings indicate that the putative oncogene tcl-2 in band 11p1311 may also be activated through the juxtaposition with TCRB. The chromosome translocations that we report suggest that the juxtaposition of either TCRB or TCRG and one of tcl-2, TCRA, and IGH may be an important step toward genesis of T-cell leukemia/lymphoma.

MATERIALS AND METHODS

Patients. Chromosomes from 25 children with T-cell leukemia/lymphoma were studied at Saitama Cancer Center or Hokkaido University Hospital between May 1982 and May 1987. Four of these patients were the subjects of this report. Detailed clinical and cytogenetic data on patient 1 have been reported elsewhere.12 Patients 1 and 4 were diagnosed as having lymphoblastic lymphoma by tonsil or lymph node (LN) biopsy on the basis of the Working Formulation.13 Patients 2 and 3 were diagnosed as having acute lymphoblastic leukemia (ALL)-L1 by bone marrow (BM) smear on the basis of French-American-British (FAB) classification.14

Cytogenetic studies. Samples for chromosome studies were obtained from BM, peripheral blood (PB), or LN before chemotherapy. Cells were cultured for 24 hours without stimulation. Chromosomes were analyzed with regular Giemsa staining and Q-banding methods. Karyotypes were described according to ISCN (1985).15 Clonality was defined by using the criteria of the First International Workshop on Chromosomes in Leukemia (1977).16

Immunophenotyping. Immunophenotypes of malignant cells obtained from PB, BM, or LN of the four patients were evaluated before chemotherapy. The cells were examined by a direct immunofluorescence method for surface immunoglobulin and by rosette formation for sheep erythrocyte receptor. Cell surface antigens were examined by an indirect immunofluorescence method using several monoclonal antibodies: T6(CD1), T11(CD2), T3/Leu-4(CD3), T4/Leu-3(CD4), T1/Leu-1(CD5), and T8/Leu-2(CD8) for T-cell markers, B4(CD19) and B1(CD20) for B-cell markers; JSI(CD10) for common ALL antigen (CALLA); and I2 for HLA-DR (Coulter Immunology, Hialeah, FL, and Becton Dickinson, Mountain View, CA).

Southern blot analysis. Total genomic DNA was extracted from peripheral leukemia cells of patient 2. Purified DNA was digested with various restriction enzymes, including BamH I, EcoRI, and HindIII, size-fractionated by electrophoresis through 0.5% to 0.9% agarose gels, and transferred onto nitrocellulose filters. Analyses of TCRB and TCRG were carried out by using a constant region (BglII-StuI) fragment of DNA probe isolated from the leukemic cell line Jurkat17 and a 0.7-kilobase (kb) Jrl (HindIII-EcoRI fragment)
probe isolated from a genomic library (pH60), respectively. Rearrangements of immunoglobulin gene were analyzed with a JH region (BamHI-HindIII fragment) probe. All probes were oligolabeled using 3P-dCTP (Amersham, Tokyo).

In situ chromosomal hybridization. In situ chromosomal hybridization was performed on leukemia cells of patient 2 as described previously. The entire plasmid containing the human Csl probe, a 3.5-kb EcoRI-HindIII constant region fragment was nicktranslated with 3H-dATP and 3H-dTTP (Amersham). A specific activity of 1 to 9 x 105 cpm/µg DNA was obtained after separation of the labeled DNA from the free nucleotides by spin-dialysis using Sepharose CL-6B. After hybridization, the slides were coated with Kodak NTB-2 emulsion, and kept in darkness at 4°C for 7 days. After development, chromosomes were stained with Wright’s solution and photographed. Then the metaphase cells were de-stained, Q-banded, and photographed again. The locations of the grains were determined by using two photographs thus prepared for each cell.

RESULTS

Clinical and laboratory data. Table 1 shows clinical and laboratory data for all patients, 3 boys and one girl with ages ranging from 7 to 14 years. They had no signs of ataxia telangiectasia. Two patients had mediastinal mass, and two had hepatosplenomegaly. Central nervous system (CNS) infiltration of neoplastic cells was not proved in any patient at the time of diagnosis. Patient 1 had normocellular bone marrow with normal differentials at the time of diagnosis, but developed leukemic transformation 8 days after diagnosis of lymphoblastic lymphoma. He had 5 months of remission and developed CNS involvement. He subsequently relapsed in the BM and died with progressive disease. Patients 3 and 4 entered complete remission, but patient 3 relapsed and died. Patient 2 had no response to chemotherapy and died 2 months after diagnosis. Thus, only patient 4 is now alive (November 1, 1987), 23 months after the diagnosis.

Cytogenetic data. Table 2 shows cytogenetic data on all patients. Patients 1 and 2 had a 7;11 translocation involving bands 1q35 and 11p13 (Fig 1). In addition to the t(7;11), some cells of patient 1 had a 9;22 translocation, and all cells of patient 2 had a del(5)(q31q35) and a del(9)(p22). Patients 3 and 4 had a 7;14 translocation involving bands 7q35 and 14q11 (Fig 2), and bands 7p15 and 14q32, respectively.

Immunophenotypic data. Table 1 shows immunophenotypic data for all patients. Malignant cells of all patients formed E rosettes and reacted with T1/Leu-1 antibodies. Cells of patients 1 and 3 were reactive with T4/Leu-3 and T8/Leu-2 antibodies, but those of patients 2 and 4 were not. Neoplastic cells of all patients examined showed no surface immunoglobulins and negative reactions to anti-CD13, CD19, CD20, and HLA-DR antibodies. Accordingly,

Table 1. Clinical and Laboratory Data at Diagnosis for Four Patients With T-cell Leukemia/Lymphoma and 1q35 or 7p15 Translocation

<table>
<thead>
<tr>
<th>Factor</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)/Sex</td>
<td>7/M</td>
<td>13/M</td>
<td>14/M</td>
<td>10/F</td>
</tr>
<tr>
<td>Mediastinal mass</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Spleen (cm)</td>
<td>0</td>
<td>10</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Liver (cm)</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.7†</td>
<td>12.7</td>
<td>10.9</td>
<td>10.4</td>
</tr>
<tr>
<td>Leukocytes (x 10^9/L)</td>
<td>72.8†</td>
<td>34.5</td>
<td>294.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Blasts in PB (%)</td>
<td>67†</td>
<td>79</td>
<td>92</td>
<td>0</td>
</tr>
<tr>
<td>Platelets (x 10^9/L)</td>
<td>180.0†</td>
<td>37.0</td>
<td>7.0</td>
<td>289.0</td>
</tr>
<tr>
<td>Immunophenotype (%) cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E rosettes</td>
<td>42†</td>
<td>26</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>CD1(T6)</td>
<td>ND</td>
<td>4</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>CD2(T11)</td>
<td>ND</td>
<td>46</td>
<td>98</td>
<td>90</td>
</tr>
<tr>
<td>CD3(T3/Leu-4)</td>
<td>ND</td>
<td>6</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>CD4(T4/Leu-3)</td>
<td>40†</td>
<td>5</td>
<td>63</td>
<td>16</td>
</tr>
<tr>
<td>CD5(T1/Leu-1)</td>
<td>80†</td>
<td>93</td>
<td>98</td>
<td>72</td>
</tr>
<tr>
<td>CD8(T8/Leu-2)</td>
<td>50†</td>
<td>11</td>
<td>92</td>
<td>3</td>
</tr>
<tr>
<td>CD10(J5)</td>
<td>0†</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Response to therapy</td>
<td>CR-R</td>
<td>NR</td>
<td>CR-R</td>
<td>CR</td>
</tr>
<tr>
<td>Survival (mo)</td>
<td>14</td>
<td>2</td>
<td>12</td>
<td>23†</td>
</tr>
</tbody>
</table>

CR, complete remission; R, relapse; NR, no response; ND, not done.
*Below the costal margin.
†Data on the eighth hospital day when the patient developed leukemic transformation of lymphoblastic lymphoma.
‡After survival: patient is still alive.
patients 1 and 3 were classified as having T-cell leukemia/lymphoma in the common or mid-thymocyte stage, and patients 2 and 4 as having leukemia/lymphoma in the early or immature thymocyte stage. Thus, although neoplastic cells of patients 1 and 2 had the same 7;11 translocation, they were in different stages of T-cell differentiation.

Southern hybridization analysis of TCRB, TCRG, and JH genes in leukemic cells with t(7;11). A combination of restriction enzymes EcoRI and HindIII, and Cβ1 probe showed both Jβ2 alleles to be rearranged. One rearranged band of TCRG was observed at 16.5 kb, between the 13-kb and 20-kb germline bands, in BamHI-digested DNA. Germ-line configuration of IGH was confirmed with the JH probe and the restriction enzymes BamHI and HindIII.

In situ hybridization analysis of TCRB gene in leukemic cells with t(7;11). Two hundred fifty-eight grains were counted on 145 leukemic metaphase cells of patient 2; 28 grains were on the normal chromosome 7, 3 were on the 7q+ chromosome, 5 were on the normal chromosome 11, 17 were on the 11p− chromosome, and 205 were on the other chromosomes. The clustering of the grains on the normal chromosome 7, the 11p− chromosome, and the short arm of the 11p− was significant (P < .001) by chi-square testing relative to the number that would be expected if there were a random distribution of grains over all chromosomes. No significant clustering of grains was observed on chromosomes other than the normal chromosome 7 or the 11p− chromosome. The location of each grain on the normal chromosome 7, the 7q+, the normal chromosome 11, and the 11p− is shown in Fig 3. Thus, TCRB in band 7q35 had relocated to the terminal portion of the short arm of the 11p− chromosome through the translocation.

DISCUSSION

In contrast to the increasing number of reports on T-cell leukemia/lymphoma with translocations or inversions having a break in 14q31, there have been few reports on T-cell leukemia/lymphoma with structural chromosome abnormalities involving 7q35 or 7p15. To our knowledge, none of the 3 translocations described in this report, ie, t(7;11)(q35;p13), t(7;14)(q35;q11), and t(7;14)(p15;q32), have been reported in association with T-cell leukemia/lymphoma.

Southern hybridization studies on leukemic cells with t(7;11)(q35;p13) demonstrated that both TCRB alleles were rearranged. One of the rearranged alleles may have represented the relocation of TCRB, as evidenced by in situ chromosomal hybridization. Whether the rearrangements of this and the other alleles were functional and productive of transcripts is not known. Rearrangement in both TCRB alleles was recently reported in two T-cell leukemia/lymphoma cell lines with t(7;9)(q36;q34), SUP-T1, and...
SUPT-3, TCRB was found to have relocated from 7q36 to the point of fusion of the involved chromosome 9, and juxtaposed with a DNA sequence in 9q34 of the neoplastic cells from SUPT-1 and SUPT-3. Moreover, a gene surrounding the breakpoint in 9q34.3 and productive of multiple transcripts was isolated and designated rec-3. These works suggested that the TCRB locus was involved in the chromosomal translocation, and also in the malignant process, of T lymphocytes.

In situ chromosomal hybridization studies in T-cell leukemia/lymphoma with t(11;14)(p13;q11), the variable and constant regions of TCRα were shown to have separated and were localized to the breakpoints on the 14q− and the 11p+ chromosomes, respectively. Thus, in the neoplastic cells with t(11;14)(p13;q11), the constant region of TCRα was juxtaposed with the DNA sequence tcl-2 within band 11p13, and the juxtaposition was believed to be the critical step in the pathogenesis of this T-cell neoplasm. Our findings in the case with t(7;14)(q35;p13) raise the possibility that the putative oncogene rec-2 in band 11p13 may be activated through juxtaposition not only with TCRα as already proposed, but also with TCRB.

Two other translocations, t(7;14)(q35;q11) and t(7;14)(p15;q32), seen in the neoplastic T-cells of our two patients have been reported in single T lymphocyte clones on normal individuals and patients with ataxia telangiectasia. An inverted chromosome 14, inv(14)(q11q32), has also been reported in T lymphocytes of normal individuals and patients with ataxia telangiectasia, and in neoplastic T cells. T lymphocytes may be particularly fragile at the chromosome sites of the functional genes that undergo rearrangement. In addition, the lymphocyte functional genes have certain homology to each other in the DNA sequence. As a result, chromosome translocations involving 7p15, 7q35, 14q11, or 14q32 may occur in the T lymphocytes of normal individuals. The cells carrying these translocations may have a selective advantage over chromosomally normal cells in patients with ataxia telangiectasia, in whom cells with such translocations were seen more frequently than in normal individuals.

In SUPT-1 with inv(14)(q11q32), a T-cell leukemia cell line, the variable region of IGH had joined the junction and constant regions of TCRα at the terminal portion of the 14q, and the variable and junction regions of TCRα had joined the variable and constant regions of IGH at the proximal portion of the 14q. The resultant fusion genes in both fusion points of the inversion 14 produce novel transcripts which may be involved in T Cell oncogenesis. Similar mechanisms may operate in the neoplastic T cells with t(7;14)(q35;q11) or t(7;14)(p15;q32), and TCRB and TCRG may have fused to TCRα and IGH in our patients 3 and 4, respectively. The findings of the t(7;14)(q35;q11) and t(7;14)(p15;q32) in the neoplastic T cells of our two patients suggest that T cells with these translocations may proliferate to be a large neoplastic clone of cells through a still unknown mechanism.

In conclusion, the juxtaposition of one lymphocyte functional gene with another such gene or an oncogene through chromosomal translocation may be an important step toward genesis of T-cell leukemia/lymphoma.

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