RAPID COMMUNICATION

Breakpoints at 11q23 in Infant Leukemias With the t(11;19)(q23;p13) Are Clustered


We have analyzed a series of nine infant leukemias that carry a t(11;19)(q23;p13). They had the morphologic features of acute lymphoblastic leukemia (ALL) and expressed markers typical of B-cell progenitor ALL or pre-B ALL; one coexpressed myeloid markers in addition to lymphoid markers (biphenotypic). Two probes (P/S4 and 98.40) subcloned from a yeast artificial chromosome (YAC) known to span the breakpoint in the t(4;11) were used to investigate DNA isolated from the leukemic cells of these patients. A total of approximately 15 kb of genomic DNA in the vicinity of the probes was examined by conventional Southern blot analysis using a series of restriction enzymes. In eight of the nine cases, the breakpoint could be mapped to an approximately 10-kb BamH1 fragment disclosed by hybridization to the P/S4 probe.

"advertisement"

CHROMOSOME BAND 11q23 is the common breakpoint region for a number of different reciprocal translocations associated with acute leukemia, including chromosome partners 4q21, 19p13, 6q27, and 9p21. Other chromosomal regions have also been reported, but at a much lower incidence. These translocations are amongst the most common leukemia-associated chromosome aberrations, particularly in infant leukemia where the incidence may be as high as 70%; altogether they constitute approximately 2% of the total cases of acute lymphoblastic leukemia (ALL).3

The clinical features of the infant leukemias associated with the different translocations are broadly similar.4,6 Patients usually present with a high white blood cell (WBC) count and there is a female sex bias. Phenotypically, the blast cells are progenitors of B-cell lineage, although some cases coexpress myeloid antigens in addition to lymphoid antigens. The initial response to chemotherapy can be rapid; however, an early relapse (less than 6 months remission) resistant to further treatment is common. However, there are important clinical distinctions that have been noted between the different translocations. The t(11;19), t(9;11), and t(11;17) may be seen in acute myeloid leukemias (AMLs), often with a monocytic component; this is in contrast to the t(4;11), which is only rarely observed in AML.2 The t(6;11) has only been reported in AML.

Therapy-related myelodysplasia is often associated with the t(9;11) and it has been suggested that chromosomal damage may play a role in the etiology of this translocation.

The CD3D gene has been shown to lie close to the breakpoint by pulsed field gel electrophoresis (PFGE) of DNA from the RS411 cell line and patient samples that carry a t(4;11).10,11 Chromosomal in situ hybridization has been used to identify a YAC, harbored in the yeast clone yB22B2, which spans the breakpoint in the t(4;11), t(6;11), t(9;11), and t(11;19).12 Further subcloning of this YAC has allowed the identification of unique probes.

Methods looking at relatively large portions of DNA, including PFGE and fluorescent in situ hybridization (FISH), have been applied in the investigation of several cases. Although evidence suggests that there may be some heterogeneity of breakpoints involving the 11q23 region, the majority of leukemia-associated breakpoints appear to occur within a similar region.

Some 11q23 breaks are clearly distinct. A cell line, RCK8 derived from a case of NHL carrying the t(11;14)(q23;q11), has been analyzed and the breakpoint was found to lay outside this region of DNA. Further characterization of this breakpoint has mapped it telomeric to other breaks on 11q23.13 The breakpoint in a T-ALL with a similar translocation has also been shown to lie outside the region encompassed by the YAC described above.14

More precise mapping of the breakpoint position of other translocations into 11q23 have also suggested some heterogeneity. While two t(11;19) translocation breakpoints can be localized to the region of 11q23 encompassed by the yB22B2 YAC,12,14 mapping by PFGE has shown that, at least in some cases, the breakpoint maps more distal to the CD3D gene than those of the t(4;11) and closer to the PBGD gene.10,15 A single t(11;19) case has been investigated by FISH with a series of cosmids mapping to 11q23.16 Here, the cosmids could be used to distinguish between more distal breakpoint in an AML case with a t(11;19) and the apparent cluster of breakpoints in a case each of t(4;11), t(6;11), and t(9;11). In contrast, recent studies17,18 have shown clustering of breakpoints in a large proportion of, but not all, patient samples harboring translocations that included t(4;11), t(9;11), t(11;19), and other variants involving 11q23. The phenotypes and clinical history of the above leukemias varied considerably or were not described in detail.
Heterogeneity has also been reported with respect to the region of chromosome 19, which can be translocated to the derivative 11 partner. A breakpoint centromeric to the insulin receptor locus at 19p13 was identified in a case of AML whereas the breakpoints were telomeric to the insulin receptor in two cases of ALL.

Therefore, to address the question of whether there are heterogeneous breakpoints at 11q23 with defined clinical characteristics, we have confined our studies to a series of infant leukemia cases carrying translocations into 11q23 with an emphasis on those containing the t(11;19).

MATERIALS AND METHODS

Clinical and laboratory investigations. Mononuclear cell preparations isolated from either peripheral blood or bone marrow (BM) were analyzed using direct immunofluorescence and a panel of antibodies. The panel included CD10, CD19, anti–HLA-DR, CD2, CD7, CD13, CD33, and CD34. The immunophenotype deduced at presentation is given in Table 1. Cytospin preparations of blasts were stained for either TdT or cytoplasmic IgM using a direct immunofluorescence and a panel of antibodies. The panel included CD10, CD19, anti-HLA-DR, CD2, CD7, CD13, CD33, and CD34. The immunophenotype deduced at presentation is given in Table 1.

Cytogenetic investigations were performed according to standard protocols and have been previously reported for seven of the patients. The other two patients (cases 2 and 10) had the karyotype 46, XX, t(11;19)(q23;p13) in 54% or 60%, respectively, of the BM metaphases examined.

Southern analysis. Restriction enzyme analysis was performed using standard protocols on DNA prepared from stored mononuclear cell preparations obtained from either BM or peripheral blood from patients at presentation. Single-copy probes centromeric (98.40) and telomeric (P/S4) to the RS411 cell line breakpoint were isolated from lambda sub-clones of the yB22B2 YAC (P.D., manuscript in preparation). The localization of the probes is shown in Fig 1 together with a restriction map of the area.

RESULTS

Patients. The clinical details of the seven of the nine infants with ALL harboring t(11;19) karyotypic abnormality have been previously reported and are summarized in Table 1. Characteristically they have a high WBC count and poor prognosis associated with this translocation. Morphologically they are typical of ALL, either L1 or L2, and included cases that were phenotypically pro-B ALL, pre-B ALL, or biphentotypic (Table 1 and ref 8). The cytogenetic analysis showed that all cases had t(11;19)(q23;p13), although four (patient nos. 3, 6, 7, and 8) had additional cytogenetic alterations.

Table 1. Details of Nine Infant Leukemias Carrying the t(11;19)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (mos)</th>
<th>Sex</th>
<th>FAB</th>
<th>Immunophenotype</th>
<th>WBC × 10^3/L</th>
<th>Survival (mos)</th>
<th>Case No. in Ref 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4</td>
<td>F</td>
<td>L1</td>
<td>Pro-B</td>
<td>192</td>
<td>14</td>
<td>New patient</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>F</td>
<td>L2</td>
<td>Bipheno</td>
<td>497</td>
<td>&lt;1</td>
<td>Patient 1</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>F</td>
<td>L1</td>
<td>Pro-B</td>
<td>470</td>
<td>16</td>
<td>Patient 2</td>
</tr>
<tr>
<td>5*</td>
<td>3</td>
<td>F</td>
<td>L2</td>
<td>Pre-B</td>
<td>280</td>
<td>&lt;1</td>
<td>Patient 5</td>
</tr>
<tr>
<td>6</td>
<td>12.5</td>
<td>M</td>
<td>L1</td>
<td>Pro-B</td>
<td>1,018</td>
<td>&lt;1</td>
<td>Patient 6</td>
</tr>
<tr>
<td>7</td>
<td>0.5</td>
<td>M</td>
<td>L1</td>
<td>Pro-B</td>
<td>540</td>
<td>2</td>
<td>Patient 4</td>
</tr>
<tr>
<td>8</td>
<td>1.5</td>
<td>F</td>
<td>L1</td>
<td>Pro-B</td>
<td>722</td>
<td>&lt;1</td>
<td>Patient 3</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>F</td>
<td>L1</td>
<td>Pro-B</td>
<td>28.2</td>
<td>1.5</td>
<td>New patient</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>F</td>
<td>L1</td>
<td>Pro-B</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Pro-B, CD10+, CD19+ B-cell progenitor; Bipheno, % of CD19+ and CD33+ cells add up to more than 100% indicating coexpression of B-cell and myeloid markers; Pre-B, CD10+, CD19+ B-cell precursor.

*Patient in which rearrangement could not be detected with probes and enzymes used in this study.

Cytogenetic investigations were performed according to standard protocols and have been previously reported for seven of the patients. The other two patients (cases 2 and 10) had the karyotype 46, XX, t(11;19)(q23;p13) in 54% or 60%, respectively, of the BM metaphases examined.

Southern analysis. Restriction enzyme analysis was performed using standard protocols on DNA prepared from stored mononuclear cell preparations obtained from either BM or peripheral blood from patients at presentation. Single-copy probes centromeric (98.40) and telomeric (P/S4) to the RS411 cell line breakpoint were isolated from lambda sub-clones of the yB22B2 YAC (P.D., manuscript in preparation). The localization of the probes is shown in Fig 1 together with a restriction map of the area.
Restriction enzyme digests with SacI, BamHI plus HindIII, EcoRI, or XbaI of DNA isolated from the leukemic cells were electrophoretically separated and transferred to nylon membrane. The filters were then hybridized to either P/S4 or 98.40. P/S4 detects germline fragments of approximately 13 kb (SacI), 10 kb (BamHI), 4.5 kb (EcoRI), and 12 kb (XbaI). The probe 98.40 detects the same SacI and XbaI germline fragments, but distinct 5.5-kb BamHI/HindIII and 5.6-kb EcoRI fragments. The rearrangement pattern with each of the probes was used to map the breakpoints in the restriction map of the area (Fig 1). A summary of the rearranged fragments identified with each probe is given in Table 2.

In total, a region of approximately 15 kb of genomic DNA spanning the breakpoint was examined using the combination of two probes and four restriction enzymes. It was possible to detect rearrangements in eight of the nine cases in this region. Mapping of these breakpoints showed that the majority are clustered in an EcoRI fragment of 5.6 kb (Fig 1).

**DISCUSSION**

Chromosomal band 11q23 is the common breakpoint region involved in a number of leukemia-specific chromosomal translocations. These leukemias tend to be of B-cell lineage particularly when found in infants; however, they often express myeloid as well as lymphoid antigens. This has led to the hypothesis that a gene concerned with differentiation/lineage determination, located at 11q23, is deregulated by the various translocation partners. Alterna-
BREAKPOINTS AT 11q23

...}

tively, the 11q23 may contain a regulatory sequence that can act to deregulate potential oncogenes located on the other chromosomes involved in each translocation.

The different spectrum of leukemias in which the translocation is seen and the preliminary molecular characterization of the region has suggested the possibility of at least two distinct breakpoints at 11q23. The data obtained from this study when combined with that of Chen et al13 suggest that the majority of breakpoints in both the t(11;19) and the t(4;11) are clustered in a similar region (Fig 2) in infant ALLs.

However, there are cases where the breakpoint may be outside this region, as illustrated both by case no. 5 reported here and the case of Cherif et al.16 In the case (patient no. 5) we have analyzed, there were no major clinical features that distinguished it from the other eight cases, although the immunophenotype is characteristic of a slightly more mature B-cell precursor than the others, being the only case in the series which was CD10 positive (see case 5 in ref 8).

There has been speculation that t(11;19) may be different from other leukemia-associated breakpoints as determined by a few individual cases. This report suggests that when a series of leukemias with similar age and clinical characteristics are compared, the breakpoint in the majority of cases occurs within a small cluster region.

ACKNOWLEDGMENT

The authors thank Profs Judith Chessells and Mel Greaves for their continuing support and critical comments, and Dr J. Kersey for sharing data before publication.

REFERENCES

16. Cherif D, Der-Sarkissian H, Derre J, Tokino T, Nakamura Y, Berger R: The 11q23 breakpoint in acute leukemia with t(11;19)(q23;p13) is distal to those of t(4;11), t(6;11) and t(9;11). Genes Chrom Cancer 4:107, 1992
17. Cimino G, Moir DT, Canaani O, Williams K, Crist WM, Katzav S, Cannizzaro B, Nowell PC, Croce CM, Canaani E: Cloning of ALL-1, the locus involved in leukemias with the t(4;11)(q21;q23), (t;11)(q22q23), and (t;11)(q23;p13) chromosome translocations. Cancer Res 51:6712, 1991
Breakpoints at 11q23 in infant leukemias with the t(11;19)(q23;p13) are clustered

GJ Morgan, F Cotter, FE Katz, SA Ridge, P Domer, S Korsmeyer and LM Wiedemann