**BRIEF REPORT**

**Fusion of a Novel Gene, BTL, to ETV6 in Acute Myeloid Leukemias With a t(4;12)(q11-q12:p13)**

By Jan Cools, Chrystèle Bilhou-Nabera, Iwona Wlodarska, Christine Cabrol, Pascaleine Talmant, Philippe Bernard, Anne Hagemeijer, and Peter Marynen

The ETV6 gene (also known as TEL) is the main target of chromosomal translocations affecting chromosome band 12p13. The rearrangements fuse ETV6 to a wide variety of partner genes in both myeloid and lymphoid malignancies. We report here 4 new cases of acute myeloid leukemia (AML) with very immature myeloblasts (French-American-British [FAB]-M0) and with a t(4;12)(q11-q12p13). In all cases, ETV6 was found recombined to a new gene, homologous to the mouse Brlx gene. The gene was named BTL (Brxl-like Translocated in Leukemia). Reverse transcriptase-polymerase chain reaction (RT-PCR) experiments indicate that the expression of the BTL-ETV6 transcript, but not of the reciprocal ETV6-BTL transcript, is a common finding in these leukemias. In contrast to the majority of other ETV6 fusions, both the complete helix-loop-helix (HLH) and ETS DNA binding domains of ETV6 are present in the predicted BTL-ETV6 fusion protein, and the chimeric gene is transcribed from the BTL promoter.

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**MATERIALS AND METHODS**

**Case reports.** Four patients (3 men, 1 woman), aged 54 to 81, presented with acute leukemia at 4 different hospitals. Clinical and cytogenetic data are summarized in Table 1. The patients had no particular antecedents, except for case 2, who was treated previously for chronic lymphocytic leukemia (PLL). The onset of the diseases was acute. Case 1 had extensive lymph node enlargement and was diagnosed as myeloid/NK cell leukemia, while the 3 other cases were classified as AML-M0. None presented with hepatosplenomegaly. The bone marrow showed dysplasia and blast cells of an undifferentiated phenotype. Remarkably, all cases showed the same very immature myeloid immunophenotype: CD7⁺, CD13⁺, CD33⁺. Response to intensive chemotherapeutic regimens for myeloid leukemia was obtained in 2 cases.

**Cloning of the (t(4;12) fusion.** Fluorescent in situ hybridization (FISH) was performed as described previously. First-strand cDNA for anchored polymerase chain reaction (PCR) (rapid amplification of cDNA ends [RACE]) was synthesized from 1 µg of total RNA using MuMLV-reverse transcriptase (GIBCO-BRL, Gaithersburg, MD) using, respectively, the oligonucleotide ETV6R3a, derived from exon 3 of ETV6, and 2 were performed on 1 µg of total RNA using the Titan RT-PCR reaction (Promega, Madison, WI) and sequenced.

Reverse transcriptase (RT)-PCR. RT-PCR experiments on cases 1 and 2 were performed on 1 µg of total RNA using the Titan RT-PCR system from Boehringer (Mannheim, Germany). The BTL-ETV6 tran-
script was detected using the primers BTLF1 – ETV6R3b for the first round PCR and primers BTL2 – ETV6R2 for the nested PCR. For detection of the ETV6-BTL transcript, the primers ETV6F1a – BTLR6a were used, followed by the nested primers ETV6F1b – BTLR6b. For cases 3 and 4, first-strand cDNA was generated as described above using the primer 465. On 10% of this cDNA, PCR was performed with the primers BTLF2 – ETV6R2 to detect the transcript and with the primers ETV6F1a – BTLR6a to amplify a 500-bp fragment of the ETV6 transcript and with the primers ETV6F1a – BTLR6a to detect the fusion transcript.

**Cloning of BTL**. A 500-bp PCR probe was generated from cDNA obtained from the K562 cell line by PCR using the primers BTLF2 and BTLR6a. A human fetal kidney cDNA library cloned into the lambda vector (Clontech, Palo Alto, CA) was screened using a standard protocol. Genomic PAC clones containing BTL were isolated by screening high-density filters carrying the RPCI1 and RPCI5 PAC libraries (Roswell Park Cancer Institute, Buffalo, NY).

**Oligonucleotides**. Oligonucleotides derived from ETV6 and BTL are indicated in Fig 1A.

ETV6F1a: 5’ TGGACATGCTGAGCTCCCTCTGT 3’; ETV6F1b: 5’ ACTCTGGCTCAGTGAGTTAAG 3’; ETV6R3a: 5’ GAAAGAAGATGATGCGG 3’; ETV6R3b: 5’ TCCCTGCTCCAGTAATTGTCAGCAGAAG 3’; ETV6R2: 5’ ACTGGAACAGAATGGGTGC 3’; BTL1: 5’ CAGAGTGGCGAGTTTGCAGC 3’; BTL2: 5’ GGTCTAGCTCACTATTGTTTTAAG 3’; BTL3: 5’ CTTGGCTACTTTTGGCGCT 3’; BTLR6a: 5’ CCACACAGTTCTGAGAAC 3’; BTLR6b: 5’ AATCTGGTCGAACATCTGTGTT 3’; BTLR4: 5’ TCCTCCATTAAACTTTCTC 3’; 465: 5’ CCAGTGACAGAGAGGTGAGGTCAGG 3’; 466: 5’ CCAGTGACAGAGAGGTGAGGTCAGG 3’.

**RESULTS AND DISCUSSION**

**Cloning of the t(4;12)**. Metaphases from case 1, a patient with myeloid/NK cell leukemia characterized by a t(4;12)(q11-q12;p13), were analyzed by FISH using probes covering, respectively, the 5’- and the 3’-end of ETV6.2,13 Cosmid 50F4 (intron 1-exon 2 of ETV6) showed split FISH signals (results not shown), indicating that the breakpoint occurred in intron 1 of ETV6. To identify the fusion partner of ETV6, 5’- and 3’-RACE experiments were performed on reverse transcribed RNA of the tumor cells. Nested oligonucleotides located, respectively, in exons 3 and 2 of ETV6 were used for 5’-RACE and oligonucleotides located in exon 1 were designed for the 3’-RACE experiments. Both experiments showed new sequences fused in frame to, respectively, exon 2 (5’-RACE) and exon 1 (3’-RACE) of ETV6. These sequences showed high similarity to the murine Btx gene (Brain specific X-linked gene, Accession no. Y11896). Therefore, the gene was named BTL (for Brx-like Translocated in Leukemia). In addition, 2 Caenorhabditis elegans predicted proteins from the cosmid W06E11 (accession no. U20862) were detected in the databases.

**Cloning of the BTL gene**. Oligonucleotides were designed to amplify a 500-bp fragment of the BTL cDNA. A human fetal kidney cDNA phage library was screened with this probe and 4 positive plaques were analyzed. A 1-kb consensus cDNA sequence, containing an open reading frame of 495 bp, was constructed and submitted to GenBank (accession no. AF159423). On a Northern blot carrying total RNA of K562 and U937 cells, 1 single transcript of approximately 1.4 kb was

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**Table 1. Clinical and Cytogenetic Data at Presentation and Relapse (Case 4)**

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex/Age</th>
<th>WBC (&lt;10^9/L)</th>
<th>Pt (×10^9/L)</th>
<th>Blasts (%)</th>
<th>Cytology</th>
<th>Blasts (%)</th>
<th>Immunophenotype</th>
<th>Diagnosis (FAB)</th>
<th>Treatment</th>
<th>Response and Survival (mo)</th>
<th>Tissue</th>
<th>Cyto stupidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/76</td>
<td>5.0</td>
<td>150</td>
<td>0</td>
<td>Hypercellular, dysgranulopoiesis, dysmegakaryopoiesis</td>
<td>24</td>
<td>CD2+, CD5+, CD7+, CD13+, CD56+, CD33+, cyMPO+</td>
<td>Myeloid/NK cell leukemia</td>
<td>Intensive chemotherapy</td>
<td>CR1 (8+)</td>
<td>BM</td>
<td>46, XX, t(4;12) [g12p13]</td>
</tr>
<tr>
<td>2</td>
<td>M/70</td>
<td>36.0</td>
<td>NA</td>
<td>80</td>
<td>Dry tap</td>
<td>NA</td>
<td>CD7+, CD13+, CD34+, HLA-DR+</td>
<td>AML-M0</td>
<td>Chemotherapy</td>
<td>Death at induction</td>
<td>BM</td>
<td>46, XY, t(4;12) [q11p13]</td>
</tr>
<tr>
<td>3</td>
<td>M/81</td>
<td>23.3</td>
<td>117</td>
<td>49</td>
<td>Hypercellular, dysgranulopoiesis, dyserythropoiesis</td>
<td>70</td>
<td>CD4+, CD7+, CD13+, CD33+, CD34+, CD56+, HLA-DR+</td>
<td>AML-M0</td>
<td>Mild chemotherapy (supportive)</td>
<td>NR (3+)</td>
<td>BM</td>
<td>46, XY, t(4;12) [g12p13]</td>
</tr>
<tr>
<td>4</td>
<td>M/54</td>
<td>5.8</td>
<td>7</td>
<td>57</td>
<td>Normocellular, dysgranulopoiesis</td>
<td>60</td>
<td>CD7+, CD13+, CD33+, CD34+</td>
<td>AML-M0</td>
<td>Intensive chemotherapy</td>
<td>CR1 (26)</td>
<td>BM</td>
<td>46, XY, t(4;12) [q11p13]</td>
</tr>
</tbody>
</table>

Abbreviations: Pt, platelet; WBC, white blood cell count; BM, bone marrow; LN, lymph node; NA, not available; CR1, CR2, first (second) complete remission; NR, no remission.
Fig 1. RT-PCR and schematic representation of the results. (A) Genomic structure of BTL and ETV6. The position of the PAC clones covering BTL was determined by Southern hybridization using oligonucleotides derived from the exon sequences (● positive by hybridization; ○ negative by hybridization). The genomic structure of ETV6 was taken from Baens et al.12 The different primers used for PCR are shown in italics above the respective genomic structures. Exons are represented by gray boxes. E, EcoRI; H, HindIII. (B) Detection of the BTL-ETV6 and ETV6-BTL transcripts in the 4 cases by RT-PCR. Only the results of the second-round PCR are shown. nc, negative control. (C) Schematic representation of the predicted fusion proteins. Sequences derived from ETV6 are drawn in italics. Arrowheads mark the boundaries between the ETV6 and BTL parts. HR, hydrophobic region of BTL; HLH, helix-loop-helix domain of ETV6; ETS, DNA binding domain of ETV6.
detected in both cell lines (results not shown), indicating that our 1-kb cDNA sequence is almost full-length.

The open reading frame of BTB predicts a protein of 165 amino acids that shows 49% similarity to the murine Brx protein. No similarity was found with any known functional protein domain, nor is there any information available about the function of Brx. Analysis of the amino acid sequence of BTB showed the presence of a hydrophobic stretch of 23 amino acids, which could represent a transmembrane domain.

The 500-bp BTB cDNA probe was also used to screen a genomic PAC library. Ten PAC clones were identified. The exact content of each PAC was investigated by hybridization with oligonucleotides derived from exons of the BTB gene (Fig 1A). Only PAC 238H24 contained the complete gene, whereas PAC 200D9 and PAC 1146G14 contained the 5’ end and the 3’ end, respectively, of BTB. Sequence analysis of EcoRI and HindIII subclones of these PACs showed the presence of 6 different exons. The exon sequences confirmed the consensus cDNA sequence. The length of introns 1 and 5 was measured by long range PCR. Intron 3 could not be amplified by PCR, most probably because it is more than 25 kb long. A map of the genomic structure of BTB is shown in Fig 1A.

Molecular characterization of new t(4;12) cases. In addition to the case described above, 3 other cases with a t(4;12)(q11-q13;p13) were collected. These 3 cases showed a very immature myeloid immunophenotype and were diagnosed as AML-M0.

To the case described above, 3 other cases with a t(4;12)(q11-q13;p13) have been reported in the literature.9,14,15 Interestingly, 4 of the AML cases reported by Harada et al show a similar immature immunophenotype (CD7+, CD13+, CD33+/CD34+) as our cases and for 2 of these cases, evidence was collected that ETV6 is involved. The PAC clones isolated by us for BTB should allow us to investigate whether the 4q breakpoint is homogeneous at the molecular level.

ACKNOWLEDGMENT

We thank Dr P. Van den Bergh and Dr G. Verhoeft (UZ Leuven, Leuven, Belgium) for providing us patient material and clinical data.

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