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 Bihou-Nabera, Iwona Wlodarska, Christine Cabrol, Pascaleine Talmant, Philippe Bernard, Anne Hagemejer, 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 Brx gene. The gene was named BTL (Brx-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 (CLL). 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 regimen 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 ETV6F1a (5′-RACE) and ETV6F1a (3′-RACE) experiments and the oligonucleotide 465 for the 5′-RACE experiments and the oligonucleotide 465 for the 3′-RACE experiments (the sequence of all PCR primers is given at the end of this section). For the 5′-RACE experiments, the first-strand cDNA was tailed with deoxyadenosine triphosphate (dATP). Second-strand synthesis was performed using Klenow DNA polymerase (GIBCO-BRL) and the primers 466 (5′-RACE) and ETV6F1a (3′-RACE). Anchored PCR was performed for 35 cycles with primers ETV6R3b-467 (5′-RACE) and ETV6F1a-467 (3′-RACE). Nested PCR was performed with the primers ETV6R2-468 (5′-RACE) and ETV6F1b-468 (3′-RACE). PCR products were cloned into pGEM-T Easy (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 BTLF2 – 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 primer 468. On 10% of this cDNA, PCR was performed with the primer 465. On 10% of this cDNA, PCR was performed with the primer 468.

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

<table>
<thead>
<tr>
<th>Sex/Age</th>
<th>WBC (×10^9/L)</th>
<th>Pt</th>
<th>BM Blasts (%)</th>
<th>Blasts (%)</th>
<th>Blasts (%)</th>
<th>Diagnosis (FAB)</th>
<th>Treatment</th>
<th>Response and Survival (mo)</th>
<th>Cytogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/76</td>
<td>5.0</td>
<td>150</td>
<td>0</td>
<td>24</td>
<td>Myeloid/TVG-1A cell leukemia</td>
<td>Intensive chemotherapy</td>
<td>CR1 (B+)</td>
<td>BM</td>
</tr>
<tr>
<td>2</td>
<td>M/70</td>
<td>36.0</td>
<td>NA</td>
<td>80</td>
<td>70</td>
<td>AML-M0</td>
<td>Chemotherapy</td>
<td>Death at induction</td>
<td>BM</td>
</tr>
<tr>
<td>3</td>
<td>M/81</td>
<td>23.3</td>
<td>49</td>
<td>57</td>
<td>60</td>
<td>AML-M0</td>
<td>Mild chemotherapy (supportive)</td>
<td>NR (3+)</td>
<td>BM</td>
</tr>
<tr>
<td>4</td>
<td>M/54</td>
<td>5.8</td>
<td>7</td>
<td>57</td>
<td>20</td>
<td>AML-M0</td>
<td>Intensive chemotherapy</td>
<td>CR1 (26)</td>
<td>BM</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.

**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 Brx gene (Brain specific X-linked gene, Accession no. Y11896). Therefore, the gene was named BTL (for BRX-like Translocated in Leukemia). In addition, 2 Cae-norhabditis 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 clones 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 KS62 and U937 cells, 1 single transcript of approximately 1.4 kb was...
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 (Δ, 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.
BTL-ETV6 FUSION IN AML-M0 WITH t(4;12) was found to span the break-myeloid immunophenotype and were diagnosed as AML-M0. q12;p13) were collected. These 3 cases showed a very immature expression to the case described above, 3 other cases with a t(4;12)(q11-q12;p13) were collected. The 3 cases strongly suggest that it is involved in the leukemogenic process. The fusion protein contains both the HLH oligomerization domain and the ETS DNA binding domain of ETV6, which is most similar to the MN1-ETV6 fusion.8 No specific domains are present in BTL, which would shed light on its possible function. A striking feature of BTL is the presence of a hydrophobic stretch of 23 amino acids, which is also present in the BTL-ETV6 fusion protein. It remains to be investigated whether BTL and BTL-ETV6 are indeed membrane bound proteins and whether this is related to the potential oncogenic properties of this fusion protein.

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