Brief report

Overexpression of CEBPA resulting from the translocation t(14;19)(q32;q13) of human precursor B acute lymphoblastic leukemia


Subtle variation in the expression or function of a small group of transcription factors can drive leukemogenesis. The CEBPA protein is known to regulate the balance between cell proliferation and differentiation during early hematopoietic development and myeloid differentiation. In human myeloid leukemia, CEBPA is frequently inactivated by mutation and indirect and posttranslational mechanisms, in keeping with tumor suppressor properties. We report that CEBPA is activated by juxtaposition to the immunoglobulin gene enhancer upon its rearrangement with the immunoglobulin heavy-chain locus in precursor B-cell acute lymphoblastic leukemia harboring t(14;19)(q32;q13). Overexpression of apparently normal CEBPA RNA or protein was observed in 6 patients. These data indicate that CEBPA may exhibit oncogenic as well as tumor suppressor properties in human leukemogenesis. (Blood. 2006;108:3560-3563)

Introduction

The CCAAT/enhancer-binding protein (CEBP) belongs to a subfamily of transcription factors sharing a basic region–leucine zipper (bZIP) motif within their carboxy terminal end. They can homo- or heterodimerize through the bZIP motif. Alternative splicing and multiple translational initiation sites add further complexity to the family picture.1 CEBPA, the founder member of the family, is encoded from a gene composed of a single exon localized to the chromosomal band 19q13. Expression studies and knock-out experiments have demonstrated its essential role in the control of balance between proliferation and differentiation in a range of tissues, including hematopoietic stem cells and different stages of myeloid differentiation.2-4 CEBPA controls the expression of myeloid genes and interacts with many protein partners such as CDK2, CDK4, CDKN1A/p21, E2F, and the SPI1/PU.1 transcription factor. The interaction with E2F is essential for the repression of MYC expression and induction of granulocytic differentiation. Besides the full-length 42-kDa protein, an internal translational initiation site leads to the synthesis of a smaller 30-kDa product. This product fails to inhibit E2F and to down-regulate MYC and acts as a dominant-negative form.5,6

In line with its essential role in myeloid differentiation, inactivation of CEBPA is observed in human myeloid leukemic samples.1 Mutations of CEBPA are observed in about 8% of acute myeloid leukemia (AML). They comprise 2 classes: mutations within the carboxyterminal part of the protein, resulting in the functional inactivation of the transcription factor; and mutations that occur within the 5’ part of the gene, allowing the synthesis of only the short, dominant-negative 30-kDa CEBPA protein. The latter situation is also observed in rare constitutive mutations of CEBPA, which are associated with the occurrence of familial AML. Both constitutive and somatic mutations are frequently associated with mutation or loss of the second copy of the CEBPA gene, related to progression of the oncogenic process.7,8

More frequently, CEBPA is indirectly inactivated in AML.1 Patients with t(8;21)(q22;q22) express the RUNX1-ETO fusion protein, which disrupts the positive autoregulation of the CEBPA...
promoter, suppressing CEBPA protein expression. The RUNX1-MDS1-EVI1 fusion oncogene, expressed in patients with t(3;21)(q26;q22), and the CBFB-MYH11 fusion protein, expressed as a result of inv(16)(p13q22), indirectly suppress CEBPA protein expression through translational inhibition of the CEBPA mRNA and loss of CEBPA protein.1 A comparable mechanism is observed as a result of t(3;21)(q26;q22), and the CBFB-MYH11 fusion protein, expressed in patients with MDS1-EVI1 fusion oncoprotein, expressed in patients with BCP-ALL, in which the breakpoint on chromosome 19 differs from the t(14;19)(q32;q13) identified in mature B-cell malignancies.9 Together, these data support a tumor suppressor role for the CEBPA gene in myeloid malignancies.

Chromosomal translocations are frequently observed in hematologic malignancies, representing an important step in the leukemic transformation process. A number of chromosomal translocations, particularly in lymphoid malignancies, result in ectopic or enhanced expression of genes located within the vicinity of the chromosomal breakpoints.10 This leads to overexpression of a usually normal sequence, although functionally significant mutations have been reported.11

In human precursor-B acute lymphoblastic leukemia (BCP-ALL), translocations involving the immunoglobulin heavy-chain locus (IGH) at chromosomal band 14q32 is a rare but recurrent event. One such translocation, t(14;19)(q32;q13), has been previously described in 6 patients with BCP-ALL, in which the breakpoint on chromosome 19 differs from the t(14;19)(q32;q13) identified in mature B-cell malignancies.12 The Groupe Francophone de Cyto´ge´ne´tique He´matologique (GFCH) has collected 8 additional patients for hematologic, cytoge´netic, and molecular studies. Here we report that this translocation involves the CEBPA gene on chromosome 19, resulting in a marked up-regulation of its expression and the production of an apparently normal CEBPA protein.

**Study design**

Patient details are shown in Table 1. Samples were obtained after patients provided informed consent in accordance with the Declaration of Helsinki. Fluorescence in situ hybridization (FISH) analysis was performed as previously described,13 using either bacterial artificial chromosomes (BACs) or commercially available IGH probes (LSI IGH; Abbott Diagnostics, Rungis, France).14 Immunologic, molecular, and quantitative polymerase chain reaction (PCR) analyses were performed as previously described.15,16 The fusion CEBPA-Cmu transcript was amplified using standard reverse transcription (RT)–PCR techniques with the primers CEBPA3 (AGGGGTGGGCAATAGGGACTT) and C1 (CCAACGGCCACGCTGCTC).

**Results and discussion**

Conventional chromosomal analysis identified 8 patients with t(14;19)(q32;q13). Apart from 1 child, the patients were adults (median, 38 years; range, 22-76 years) with ALL, ranging from pro-B to pre-B immunophenotype. Myeloid markers tested were essentially negative (Table S1, available at the Blood website; see the Supplemental Materials link at the top of the online article). Data from the Leukaemia Research Acute Leukemia Cytogenetics Database has shown an incidence of less than 0.5% and approximately 2% for this translocation in B-lineage childhood and adult ALL, respectively. The involvement of the IGH locus was confirmed by FISH (data not shown). Extensive FISH mapping identified a single BAC (RP11-270I13) containing sequences that encompassed the breakpoint within 19q13. Figure 1A shows a representative image from patient P5, which was similar in all 8 patients. No material was available for further study of patients P7 and P8. The clustering of the chromosome 19 breakpoints within the sequences covered by a single BAC indicated the recurrent targeting of an individual gene.

To investigate the potential partner gene, we evaluated the expression by quantitative PCR of 4 genes located in this region of chromosome 19: LRP3, CEBPA, CEBSG, and PEPO. The expression levels were normalized against ABL and compared with the human cell line U937. The candidate oncogene was CEBPA. Its level of expression was at least 2.5 times higher in t(14;19) than control BCP-ALL samples, with a number of t(14;19) patients expressing extremely high levels of CEBPA mRNA (Figure 1B; Figure S2 for the 3 other genes).

Recent data17 have indicated that the t(14;19) translocation breakpoint may be located within the 3′ untranslated region (UTR) of CEBPA, suggesting a fusion transcript between CEBPA and the IGH constant region. RT-PCR analysis of 6 patients (P1 to P6) with primers from Cmu and CEBPA allowed specific amplification of material from patients P1 and P2 only. Direct sequencing demonstrated the fusion of Cmu-JH to the 3′ UTR of CEBPA, establishing

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**Table 1. Hematologic, individual, and cytogenetic data of patients with t(14;19)(q32;q13)**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age, y</th>
<th>WBC count, × 10^9/L (% blasts)</th>
<th>Hb level, g/L</th>
<th>Platelet count, × 10^11/L</th>
<th>Survival</th>
<th>Immunophenotype</th>
<th>Karyotype*</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>F</td>
<td>7</td>
<td>13.4 (92)</td>
<td>75</td>
<td>167</td>
<td>12 y</td>
<td>Early pre-B/pre-B?†</td>
<td>46, XX, t(14;19)(q32;q13)(2)/45, idem, der(16)t (16;17)(q11;21), v-17(14)</td>
</tr>
<tr>
<td>P2</td>
<td>F</td>
<td>26</td>
<td>4.5 (55)</td>
<td>100</td>
<td>50</td>
<td>Unknown</td>
<td>Early pre-B/pre-B?†</td>
<td>46, XX, dup(1)(q21;31), t(14;19)(q32;q13)(2)</td>
</tr>
<tr>
<td>P3</td>
<td>M</td>
<td>22</td>
<td>6.8 (26)</td>
<td>53</td>
<td>17</td>
<td>5 mo (d)</td>
<td>Early pre-B (CD10+, lgc−)</td>
<td>46,XY,t(14;19)(q32;q13)(9)</td>
</tr>
<tr>
<td>P4</td>
<td>M</td>
<td>40</td>
<td>4.9 (67)</td>
<td>55</td>
<td>27</td>
<td>5 mo (d)</td>
<td>Pro-B (CD10+, lgc−)</td>
<td>46,XY,t(14;19)(q32;q13)(12)/46, idem, idic(8)(p11)(6)</td>
</tr>
<tr>
<td>P5</td>
<td>F</td>
<td>38</td>
<td>16.2 (72)</td>
<td>104</td>
<td>37</td>
<td>3 mo</td>
<td>Early pre-B (CD10+, lgc−)†</td>
<td>46,XX, del(7)(p15), t(14;19)(q32;q13)(20)</td>
</tr>
<tr>
<td>P6</td>
<td>M</td>
<td>76</td>
<td>11 (77)</td>
<td>126</td>
<td>57</td>
<td>5 mo (d)</td>
<td>Pre-B (CD10+, lgc−)†</td>
<td>46,XY,t(14;19)(q32;q13)(7)</td>
</tr>
<tr>
<td>P7</td>
<td>F</td>
<td>38</td>
<td>94 (46)</td>
<td>120</td>
<td>168</td>
<td>22 d (d)</td>
<td>Early pre-B (CD10+, lgc−)§</td>
<td>46,XX,del(9)(22)/q34;11)(9/46, idem, id(7)p10)(2)/47; idem, +8; t(14;19)(q32;q13)(8/48, idem, +6, t(8;9)(p22;q11), +der(22)/19)(22;9)(21)</td>
</tr>
<tr>
<td>P8</td>
<td>F</td>
<td>41</td>
<td>1.5 (16)</td>
<td>82</td>
<td>145</td>
<td>9 mo</td>
<td>Early pre-B/pre-B?†</td>
<td>46,XX,t(14;19)(q32;q13)(10)</td>
</tr>
</tbody>
</table>

WBC indicates white blood cell; Hb, hemoglobin; (d), dead; and lgc, cytoplasmic IgM.

*Normal population omitted from the karyotype.
†CD10−, lgc not done.
‡All analyzed myeloid markers (myeloperoxidase, CD13, CD33, CD117, CD65) were negative for all patients, except for P5 (CD13+).
§All karyotypes were analyzed from bone marrow, except for P7 (peripheral blood).
equivocably that CEBPA was the gene involved in the t(14;19) (Figure 1C).

To confirm the expression of CEBPA at the protein level, whole-cell extracts were analyzed by Western blotting. The CEBPA protein was present as the 2 usual species, p42 and p30, of similar size to the U937 positive control (Figure 1D; Figure S3). Thus, apparently normal CEBPA proteins are expressed in patients with t(14;19). This was expected, as no mutation of the CEBPA gene was observed in patients (data not shown).

Our results indicate that CEBPA may act as an oncogene in lymphoid malignancies, in contrast to its role as a tumor suppressor in myeloid leukemia. A preliminary report has indicated that additional members of the CEBP family are involved in translocations with IGH in BCP-ALL, which may also exhibit oncogenic properties.17 Interestingly, our patients with CEBPA overexpression did not express myeloid markers, such as CD13 or myeloperoxidase (MPO), suggesting that only a subset of the known CEBPA target genes, mainly defined in a myeloid context, would be activated in a lymphoid context. Their identification would be of great interest. Another possibility is that CEBPA exerts its effect through its interaction with other transcription factors, thereby deregulating a different set of genes. This example from CEBPA strongly supports the emerging paradigm that both an increase or decrease in gene dosage may contribute to the pathogenesis of leukemia.4

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Appendix

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References


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