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 homodimerize 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 SPL1/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

From the Assistance Publique–Hôpitaux de Paris (AP-HP) Service d'Hématologie Biologique, Hôpital Pitié-Salpêtrière, Paris, France; Institut National de la Santé et de la Recherche Médicale (INSERM), E0210, Paris, France; University Paris VI, Paris, France; Leukaemia Research Cytogenetics Group, Cancer Sciences Division, University of Southampton, United Kingdom; University Rene Descartes, Paris, France; AP-HP, Laboratoire de Cytogénétique, Hôpital Necker-Enfants Malades, Paris, France; Laboratoire de Génétique oncologique, Rouen, France; Laboratoire de Cytogénétique, Strasbourg, France; AP-HP, Laboratoire de Biochimie Génétique, Hôpital Robert Debré, Paris, France; Laboratoire de Génétique, Chambéry, France; Laboratoire de Génétique, Tours, France; and AP-HP, Laboratoire d'Hématologie, Hôpital Saint-Louis, Paris, France.


A complete list of the members of the Groupe Francophone de Cytogénétique Hématologique appears in “Appendix.”

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E.C. and L.R. contributed equally to this work.

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The online version of this article contains a data supplement.

Reprints: Florence Nguyen-Khac, Service d’Hématologie Biologique, Groupe Hospitalier Pitié-Salpêtrière, 47-83 Bd de l'Hôpital, 75013 Paris, France; e-mail: florence.nguyen@psi.aphp.fr.

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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. A comparable mechanism is observed previously described, using either bacterial artificial chromosomes (BACs) provided informed consent in accordance with the Declaration of Helsinki. Patient details are shown in Table 1. Samples were obtained after patients Study design

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 (RP11-270113) 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, CEBPG, and PEPD. 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 data 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

<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, × 10^9/L</th>
<th>Survival</th>
<th>Immunophenotype</th>
<th>Karyotype*</th>
</tr>
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<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;q11)],−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;q31),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>Pre-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,t(9;22)<a href="9">q34;q11</a>[46,idem,id<a href="p10">7</a>[2]/47,idem,+i(1;19)(q32;q13)[8]/48,idem,+i(6, t(8;9)[q7;11]),+der<a href="9;22">22</a>[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).
Figure 1. The CEBPA gene is targeted by the t(14;19) translocation. (A) A representative FISH image of the t(14;19)(q32;q13) in patient P5. The RP11-27013 probe produces a red signal on the normal chromosome 19 and a split signal between der(14) and der(19). A green signal from the IGH constant region-specific probe identifies chromosomes 14 and der(14). Images were visualized under a Leica DM RXA microscope equipped with a fluorescence epi-illumination 100×/130-0.60 oil-immersion objective lens (Leica, Rueil-Malmaison, France). Leica QFISH software was used to digitally acquire images after capturing them with a Photometrics Sensys camera (Roper Scientific, Evry, France). (B) Quantitative RT-PCR analysis of CEBPA expression in t(14;19) patients (P1–P6), 3 control patients with BCP-ALL and the human cell lines U937 (AML) and HEP3B (hepatocellular carcinoma) using gene expression assay no. Hs.00263372_s1 (Applied Biosystems, Foster City, CA). Data are presented as percentage of ABL expression. Note that for patient 1 the bar graph is not drawn to scale: the real value is 6672%. Comparable results were obtained when the GLU gene was used as a reference. Because CEBPA is composed of a single exon, control experiments were performed with omission of the reverse transcriptase from the reaction. The observed Ct values in control experiments were always several cycles higher than in the test experiments. Quantitative RT-PCR analyses of neighboring genes (CEBPG, PED, and LPR3) are shown in Figure S1. P1–P6 indicates t(14;19) samples; T1–T3, control BCP-ALL samples without a chromosome 19 abnormality. (C) Nucleotide sequence alignments of fusion CEBPA-Cmu transcripts isolated from patients P1 and P2; chromosome 19 sequences are indicated in uppercase; chromosome 14 sequences, lowercase. The JH segment is underlined on the germline chromosome 14 sequences and was identified as JH4 for der(14) and JH6 for der(19). The in silico analysis 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

Members of the Groupe Francophone de Cytogénétique Hématologique are as follows (unless otherwise indicated, institutions are located in France): Joris Andrieux, Laboratoire de Génétique Médicale, Hôpital Jeanne de Flandre, Lille; Nathalie Auger, Laboratoire de Cytogénétique, Département de biologie et de pathologie médicales, Institut Gustave Roussy, Villejuif; Hervé Avet-Loiseau, Laboratoire de Cytogénétique Hématologique, Plateau technique, Hôpital Dieu, Nantes; Laurence Baranger, Laboratoire de Génétique, Centre Hospitalier Regional Universitaire, Angers; Carole Barin, Unité de Génétique, Centre Hospitalier Universitaire Bretonneau, Tours; Christian Bastard, Département de Génétique, Centre Henri Becquerel, Rouen; Martine Becker, Service de Cytogénétique, Laboratoire Mériex, Lyon; Laurence Benattar, Service de Biologie du Développement, Hôpital Robert Debré, Paris; Roland Berger, Institut National de la Santé et de la Recherche Médicale (INSERM) EMI 02 10, Tour Pasteur Hôpital Necker, Paris; Alain Bernstein, Laboratoire de Génomique Cellulaire des Cancers, Unité mixte de recherche (UMR) Centre National de la Recherche Scientifique (CNRS) 8125, Institut Gustave Roussy, Villejuif; Chrystèle Bilhou-Nabera, Laboratoire de Cytogénétique, Service d’Hématologie, Hôpital Bicêtre,
References


8. Muehlematter, Unite de cytogenetique du cancer, Service de medicine, Reims; Odile Maarek, Laboratoire Central d'Hematologie, Hopital Saint-Louis, Paris; Lucienne Michaux, Jeanne-Marie Libouton, Genevieve Ameye, Centre de genetique (UCL), Brussels, Belgium; Hossain Mossafa, Laboratoire Pasteur-CERBA, Departement de Genetique Humaine, Val d'Oise; Marie-Joelle Mozzi-conacci, Laboratoire de Cytogenetique, Hematologique et Museleulaire, Departement de Biopathologie, Institut Paoli Calmettes, Marseilles; Dominique Muchemarter, Unité de cytogenetique du cancer, Service de genetique medecine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; Francine Mugneret, Laboratoire de Cytogenetique, Laboratoire de Cytogenetique, CHU Le Bocage, Dijon; Nathalie Nadal, Laboratoire d'hematologie - Pavillon de Biologie (niveau 1), CHU Hopital Nord, St Etienne; Florence Nguyen-Khac, Service d'Hematologie Biologique, Pr Merle-Beral, Pav Laveran, Groupe Hospitalier Pitie-Salpetriere, Paris; Jean-Pierre Pages, Laboratoire d'Hematologie et de Cytogenetique-Hopital Droussieux, Lyon; Dominique Penther, Laboratoire de Genetique Onco-Hematologique, centre de lutte contre le cancer Henri Becquerel, Rouen; Bernard Perissel, Laboratoire de Cytogenetique, Faculte de Medicine, Clermont-Ferrand; Christine Perot, Laboratoire de Cytogenetique, Hôpital Saint Antoine, Paris; Ghislaine Plessis, Laboratoire de Cytogenetique Postnatal, CHU Clemenceau, Caen; Helene Poirel, Cliniques Universitaires St-Luc, Centre de Geneique, Secteur Hematologie, Brussels, Belgium; Bruce Poppe, Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; Benoit Quilichini, Laboratoire de Cytogenetique, CHU La Timone, Marseille; Katrina Rack, Institut de Pathologie et de Geneique, Genipines, Belgium; Isabelle Radford-Weiss, Laboratoire de Cytogenetique, Hopital Necker-Enfants Malades, Paris; Sylvie Ramond, Laboratoire de Cytogenetique, Service d'Hematologie Biologique, Hôtel Dieu de Paris; Jean-Philippe Rault, Laboratoire de Biologie, Hôpital Legouest, Metz; Sophia Raynaud, Laboratoire de Genetique (niveau 3), Hopital de l'Archet, Nice; Aline Receveur, Laboratoire de Cytogenetique, Amiens, Paris; Serge Romana, Service de Genetique et Cytogenetique, Hopital Necker-Enfants Malades, Paris; Anna Rossi, CTS, Bois Guillaume; Michèle Schoenwald, Service d'Oncologie Medecinale, Centre Hospitalier Regional Orleans La Source, Orleans; Frank Spelman, Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; Claude Stoll, Laboratoire de Geneetique Medecine, Faculte de Medicine, Strasbourg; Stephanie Struski, Laboratoire d'Hematologie, Hôpital de Haute Pierre, Strasbourg; Pascale Tailmunt, Laboratoire de Cytogenetique Hematologique, Plateau technique, Hôtel Dieu, Nantes; Sylvie Taviaux, Laboratoire de Genetique Molleculaire et Chromosomique, Hôpital Arnaud de Villeneuve, Montpellier; Christine Terré, Laboratoire de Cytogenetique, Centre de Transfusion Sanguine, Le Chesnay; Isabelle Tiguad, Laboratoire de Cytogenetique, Centre Hospitalier Lyon Sud, Pierre Benite; Jacqueline Van Den Akker, Laboratoire de Cytogenetique, Hopital Saint Antoine, Paris; Michel Velmans, Service de Genetique, Hopital Necker-Enfants Malades, Paris; Christine Vetelien, Centre de genetique UCL, Brussels, Belgium; and Franck Viguie, Laboratoire de Cytogenetique, Service d'Hematologie Biologique, Hôtel Dieu de Paris.
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