Fusion of EML1 to ABL1 in T-cell acute lymphoblastic leukemia with cryptic t(9;14)(q34;q32)

Kim De Keersmaecker, Carlos Graux, Maria D. Odero, Nicole Mentens, Riet Somers, Johan Maertens, Iwona Wlodarska, Peter Vandenberghe, Anne Hagemeijer, Peter Marynen, and Jan Cools

The **BCR-ABL1** fusion kinase is frequently associated with chronic myeloid leukemia and B-cell acute lymphoblastic leukemia but is rare in T-cell acute lymphoblastic leukemia (T-ALL). We recently identified **NUP214-ABL1** as a variant **ABL1** fusion gene in 6% of T-ALL patients. Here we describe the identification of another **ABL1** fusion, **EML1-ABL1**, in a T-ALL patient with a cryptic t(9;14)(q34;q32) associated with deletion of **CDKN2A** (**p16** and expression of **TLX1** (**HOX11**)). Echinoderm microtubule-associated protein–like 1–Abelson 1 (**EML1-ABL1**) is a constitutively phosphorylated tyrosine kinase that transforms Ba/F3 cells to growth factor–independent growth through activation of survival and proliferation pathways, including extracellular signal–related kinase 1/2 (**Erk1/2**), signal transducers and activators of transcription 5 (**Stat5**), and Lyn kinase. Deletion of the coiled-coil domain of **EML1** abrogated the transforming properties of the fusion kinase. **EML1-ABL1** and breakpoint cluster region (BCR)–**ABL1** were equally sensitive to the tyrosine kinase inhibitor imatinib. These data further demonstrate the involvement of **ABL1** fusions in the pathogenesis of T-ALL and identify **EML1-ABL1** as a novel therapeutic target of imatinib. (Blood. 2005;105:4849-4852) © 2005 by The American Society of Hematology

**Introduction**

T-cell acute lymphoblastic leukemia (T-ALL) is frequently characterized by chromosomal rearrangements leading to ectopic expression of transcription factors (including **TLX1**, **TLX3**, **LM01**, **LYL1**) or the generation of chimeric transcription factors (including **SIL-TALI** or **MLL** fusions). In addition, mutations in protein tyrosine kinases (**LCK** and **FLT3**) have also been identified in a small subset of T-ALL cases. In contrast to B-cell acute lymphoblastic leukemia (B-ALL), the **BCR-ABL1** oncogene is only rarely implicated in the pathogenesis of T-ALL, but we recently identified a variant **ABL1** fusion gene, **NUP214-ABL1**, in approximately 6% of T-ALL patients. **NUP214-ABL1** was highly associated with ectopic expression of **TLX1** or **TLX3** and deletion of **CDKN2A**. Here we report the identification and characterization of **EML1-ABL1**, another variant **ABL1** fusion gene that is generated by the t(9;14)(q34;q32), which is not detectable by standard cytogenetics.

**Study design**

**Patients**

A total of 116 T-ALL patients were screened for **ABL1** rearrangements. The 16-year-old female patient with a cryptic t(9;14) presented with very high leukocytosis (455 × 10⁹/L), with 99% blasts with the phenotype of cortical thymocytes, and normal karyotype. She is in first complete remission 15 months after diagnosis. This study was approved by the Ethical Committee of the Medical Faculty of the University of Leuven. Informed consent was obtained from all subjects.

**FISH**

Fluorescence in situ hybridization (FISH) was performed using standard protocols. Metaphases were hybridized up to 3 times using the LSI **BCR-ABL1** ES (Vysis, Downers Grove, IL) translocation probe or bacterial artificial chromosome (BAC) probes RP11-57C19 and RP11-83J21 (BACPAC Resources, Oakland, CA).

**RACE and PCR**

The 5′-rapid amplification of cDNA ends (5′-RACE) polymerase chain reaction (PCR) was performed as described previously. **Synthesis of cDNA** was performed with the **ABL1-R1** primer (5′-gcttgatagttggtc), followed by PCR with the **RACE** primers and the nested **ABL1-R2** (5′-acccatccctggtttagt) and **ABL1-R3** (5′-cggagttccacctgagattga). The presence of the **EML1-ABL1** fusion transcript was confirmed by reverse transcriptase–PCR (RT-PCR) using the primers **EML1-F** (5′-cactcactgggaggtgttt) and **ABL1-R2**. **EML1 expression** was detected using primers **EML1-F** (5′-tagaatagatctcgcgatggcactgtgttaccaaag) and **EML1-R** (5′-cagttgtgatgtagttgcttg). **ZNF384** was amplified as described previously. Detection of **TLX1**, **TLX2**, **TLX3**, and **NXX2-5** expression was performed as described.

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Constructs

The open reading frame of exon 1 to 17 of EML1 was amplified from an IMAGE clone (accession no. BC033043) with primers EML1-F1 (5'-ggaatgcacagctgagaacgccgacg) and EML1-R (5'-tagaatgcagcggctgtgtagaattc) and EML1 in which nucleotides 1 to 363 of the open reading frame were deleted (del EML1) was obtained by replacement of EML1-F1 by the EML1-F2 primer (5'-ggaatgcacagctgagaacgccgacg) and EML1-R (5'-tagaatgcagcggctgtgtagaattc). The ABL1 part was amplified from a BCR-ABL construct, using primers ABL1-F (Tyr5-5'-tagaatgcagcggctgtgtagaattc) and ABL1-R (Tyr204-5'-tagaatgcagcggctgtgtagaattc). The generated EML1/del EML1 parts were ligated together with the ABL1 fragment in the retroviral vector murine stem cell virus–puromycin (MSCV-puro; Clontech, Palo Alto, CA).

Cell culture and retroviral transduction

HEK 293T and Ba/F3 cells were cultured, transfected, and transduced as described previously. Transduced Ba/F3 cells were selected with puromycin (2.5 μg/mL) or neomycin (600 μg/mL medium). For Western blotting, Ba/F3 cells were incubated with imatinib for 90 minutes. For growth curves, 105 Ba/F3 cells were seeded in 1 mL medium and viable cells were counted on 3 consecutive days. For dose-response curves, 2 × 105 Ba/F3 cells were seeded in 1 mL medium and incubated in the presence of imatinib for 24 hours. Viable cell numbers were determined with the AQueousOne Solution (Promega, Madison, WI).

Western blotting

Total cell lysates were analyzed by standard procedures using the following antibodies: anti–phospho-ABL1 (Tyr412), anti-ABL1, anti-phospho–extracellular signal-related kinase 1/2 (anti-phospho-ERK1/2; Thr202/204), and anti-phospho–severe combined immunodeficiency repopulating cell (anti-phospho-SRC) family (Tyr 416; Cell Signaling, Beverly, MA); anti-Erk2, anti-phospho–signal transducers and activators of transcription 5 (anti-phospho-STAT5), anti-STAT5α, and anti-LYN (Santa Cruz Biotechnology, Santa Cruz, CA); antiphosphotyrosine, antibody; 4G10; Upstate Biotechnology, Lake Placid, NY); and antimonocyte/antirabbit peroxidase-labeled antibodies (AP Biotech, Uppsala, Sweden).

Results and discussion

In the process of screening 116 T-ALL patients for ABL1 gene rearrangements by FISH, we detected 6 cases with ABL1 amplification (5 were recently reported), and 1 case with a cryptic translocation t(9;14)q34;q32). Further investigation of the t(9;14) case confirmed that the breakpoint was in intron 1 of ABL1 (Figure 1A). RACE experiments revealed that the t(9;14) generated an in-frame fusion between exon 17 of EML1 (echinoderm microtubule-associated protein–like 1 gene) and exon 2 of ABL1 (Figure 1E).

EML1 was mapped within the Usher syndrome type 1a locus on 14q32 and encodes a protein with high similarity to the echinoderm microtubule-associated protein. Unlike other fusion partners of ABL1, EML1 seems to have a more restricted expression pattern,13,14 with the coiled-coil domain of EML1 being deleted (del EML1) was obtained by replacement of EML1-F1 by the EML1-F2 primer (5'-ggaatgcacagctgagaacgccgacg) and EML1-R (5'-tagaatgcagcggctgtgtagaattc). The ABL1 part was amplified from a BCR-ABL construct, using primers ABL1-F (5'-tagaatgcagcggctgtgtagaattc) and ABL1-R (5'-tagaatgcagcggctgtgtagaattc). The generated EML1/del EML1 parts were ligated together with the ABL1 fragment in the retroviral vector murine stem cell virus–puromycin (MSCV-puro; Clontech, Palo Alto, CA).

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the dimerization domain of ETV6 in the context of ETV6-ABL1.14 The importance of the coiled-coil domain in the context of BCR-ABL1 is less clear for transformation in vitro but is well demonstrated for its in vivo oncogenic properties.15,16

We next tested the sensitivity of EML1-ABL1 to imatinib, a selective inhibitor of ABL1 kinase activity.17 Imatinib concentrations required to inhibit proliferation of the EML1-ABL1- and BCR-ABL1-transformed Ba/F3 cells were comparable (50% inhibitory concentration [IC50] ~0.2 μM; Figure 2C). The effect of imatinib on EML1-ABL1–expressing Ba/F3 cells was assessed using an antiphosphotyrosine antibody. This confirmed that the major phosphorylated proteins were EML1-ABL1, Stat5, and Lyn and that phosphorylation of these proteins decreased with increasing dose of imatinib (Figure 2D). The phosphorylation status of Lyn, a recently identified critical downstream effector of BCR-ABL1 in B-ALL,18,19 was also determined by immunoprecipitation followed by detection of its phosphorylation on Tyr396 with anti–phospho-SRC. The blot was stripped and reprobed with anti-LYN.

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EML1-ABL1 FUSION IN T-ALL WITH t(9;14)(q34;q32)


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