

A novel recurrent EP300 ZNF384 gene fusion in B-cell precursor acute lymphoblastic leukemia

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AUTHOR CONTRIBUTIONS

CT designed the research, analyzed the data and wrote the paper; IS supervised the work; PVR, SC, RP and CDB performed experiments; GZ provided AML cell lines, reagents and analyzed the data; SC and DB commented on the paper. SC and RF provided AML samples. AA provided T-ALL samples. CT, PVR, SC assembled the figures.

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⁷Dedicated to the cherished memory of Alberto Gulino.

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A novel recurrent *EP300–ZNF384* gene fusion in B-cell precursor acute lymphoblastic leukemia

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In pediatric patients with B-cell precursor acute lymphoblastic leukemia (BCP-ALL), approximately three-quarters harbor well-characterized, clinically relevant chromosomal alterations, including hyperdiploidy, hypodiploidy, t(12;21) *ETV6/RUNX1*, t(1;19) *E2A/PBX1*, t(9;22) *BCR/ABL1* and the rearrangement of *MLL* at 11q23, and they can facilitate diagnosis, risk stratification and targeted therapy.^{1,2} In the remaining patients, however, major pathogenic or driver gene abnormalities and their association with the clinical outcome have yet to be fully clarified. As recent advanced genomic studies using next-generation sequencing have identified a number of novel fusion genes and stratified a high-risk subtype in BCP-ALL,^{3–5} unknown genetic alterations that constitute characteristic subgroups may still exist in the remaining patients. We therefore intended to investigate unknown fusion genes in BCP-ALL by using next-generation sequencing.

As a consequence of whole transcriptome sequencing performed on complementary DNA from 55 selected samples of pediatric BCP-ALL patients without conventional genetic abnormalities (Supplementary Information), an *EP300–ZNF384* fusion gene was identified in two patients (Cases 1 and 2) as a repeatable and plausible candidate fusion gene (Figure 1a). The 372-bp fragment of the *EP300–ZNF384* fusion cDNA was amplified by RT-PCR using a pair of specific primers, and Sanger sequencing of the PCR products revealed a sequence of the products identical to that obtained by whole transcriptome sequencing (Supplementary Figure 1). The presence of *EP300–ZNF384* fusion in Case 1 was further confirmed by FISH using a combination of appropriate probes for *EP300* and *ZNF384*, respectively (Figure 1b). We screened a further 346 of pediatric ALL cases by RT-PCR, and identified 4 additional patients with *EP300–ZNF384* fusion (Cases 3–6, Supplementary Figure 1). All six patients were BCP-ALL without conventional cytogenetic abnormalities. Our RNA samples

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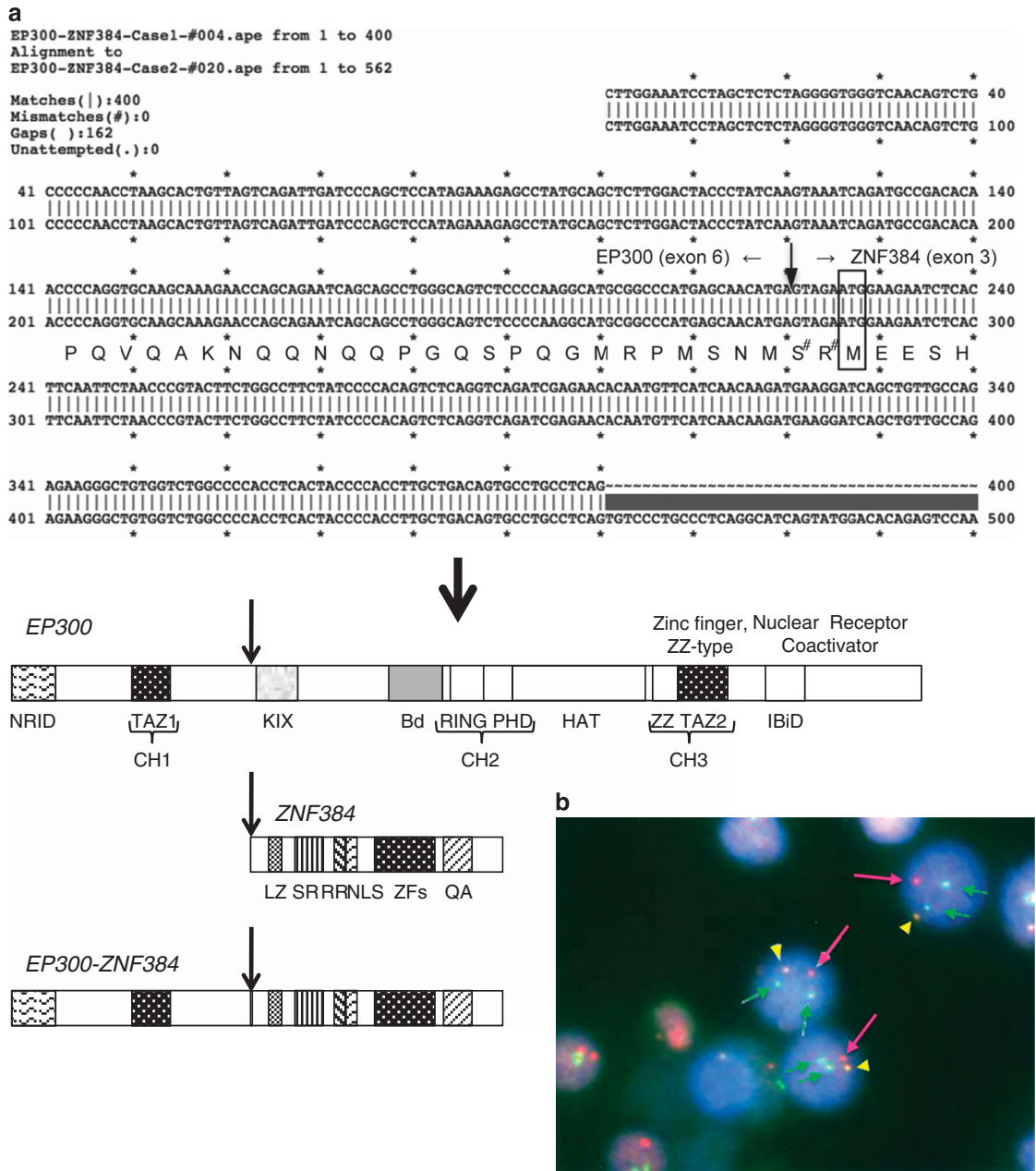


Figure 1. Detection of the *EP300-ZNF384* fusion gene. **(a)** Two split sequences of *EP300-ZNF384* obtained from two individual patients by whole transcriptome sequencing were aligned. Complementary DNA showing fusion of the intron following *EP300* exon 6 and the intron preceding the first coding exon (exon 3) of *ZNF384*. Amino-acid sequences of the predicted *EP300-ZNF384* fusion protein are presented. Arrows indicate the fusion points. The box indicates the initiation codon of *ZNF384*. #, inserted amino acids originating from the 5' untranslated 5-bp sequence of *ZNF384*. The predicted *EP300-ZNF384* protein is schematically indicated. NRID, nuclear receptor interaction domain; TAZ1, transcriptional adaptor zinc-finger domain 1 (also known as the cysteine-histidine-rich (CH) region (CH1)); KIX, kinase-inducible domain of CREB-interacting domain; Bd, bromodomain; RING, 'really interesting new gene' domain; PHD, plant homeodomain; HAT, histone acetyltransferase domain; ZZ, ZZ-type zinc-finger domain; TAZ2, transcriptional-adaptor zinc-finger domain 2 (the RING-PHD segment is also known as the CH2 region, and the ZZ-TAZ2 domain as the CH3 region); IBiD, IRF3-binding domain; LZ, leucine-rich domain; SR, serine-rich domain; PR, proline-rich domain; NLS, nuclear localization signal; ZFs, Kruppel-type C2H2 zinc-finger domains; QA, Gln-Ala repeat. **(b)** The results of FISH using the *EP300* probe (green) and *ZNF384* probe (red) are indicated. The fusion signals are indicated by yellow arrowheads.

used in this study were obtained from the patients consisted of an uncontinuous series of 68 BCP-ALL without conventional cytogenetic abnormalities and a continuous series of 333 ALL patients, including 291 BCP-ALL patients (133 patients without conventional cytogenetic abnormalities, Supplementary Information). As three out of six cases of *EP300-ZNF384*+ BCP-ALL (Cases 2-4) belong to later group, the frequency of *EP300-ZNF384* expression in BCP-ALL was estimated as 1.03% (0.90% in total ALL, 2.26% in

BCP-ALL without conventional cytogenetic abnormalities) in our cohort.

EP300 encodes the E1A-binding protein p300 (EP300), a transcriptional co-activator closely related to CREB-binding protein (CBP) and with the ability to interact with a wide spectrum of transcription factors and histone acetyltransferase activity.⁶ On the other hand, the *zinc-finger protein 384* (*ZNF384*) gene encodes a transcription factor that regulates promoters of the extracellular

Table 1. Clinical findings of patients

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age at initial diagnosis	7	13	7	10	17	15
Gender	M	M	M	F	M	F
Methods	RNAseq, PCR, FISH	RNAseq, PCR	PCR	PCR	PCR	PCR
Samples obtained at:	Second and third relapse	Newly diagnosed	Newly diagnosed	Newly diagnosed	Newly diagnosed	Newly diagnosed
Current status	In fourth CR	In first CR	In first CR	In first CR	In first CR	In first CR
Duration of event-free survival	4y 4m	8y 6m+	10y 1m+	9y 5m+	2y 1m+	4y 2m+
Duration of overall survival	10y 11m+	Same as above	Same as above	Same as above	Same as above	Same as above
Study	Others	L0416/0616	L0416/0616	L0416/0616	Others	Others
Initial WBC	17 380	10 500	14 600	2600	2300	5000
Day 8 blasts (per ml)	66	162	80	0	0	0
CNS involvement	No	No	No	No	No	No
Risk classification	IR	IR	IR	IR	IR	IR
Cytogenetics	46,XY, t(9;10)(p13;p13) 20/20	46,XY (20/20)	46,XY (20/20)	46,XX (20/20)	46XY (10/10)	46XX (20/20)

Abbreviations: CNS, central nervous system; CR, complete remission; F, female; FISH, fluorescence *in situ* hybridization; IR, intermediate risk group; M, male; m, months; WBC, white blood cell; y, years. Clinical findings of patients with the *EP300-ZNF384* fusion gene are summarized.

matrix genes.⁷ Chromosomal translocations involving *EP300* fused to either *Monocytic leukemia zinc-finger protein (MOZ)* or *Mixed lineage leukemia (MLL)* genes are associated with acute myeloid leukemia.^{8,9} *ZNF384* is known to be involved in ALL through fusion with the *TET* family gene, such as the *Ewing sarcoma breakpoint region 1 (EWSR1)* gene, *TATA box binding protein-associated factor (TAF15)* and *transcription factor 3 (TCF3 or E2A)*.^{10,11} Although the mutations of both *EP300* and *ZNF384* are known to be involved in the development of leukemia, this is the first report on the *EP300-ZNF384* fusion gene.

The resulting sequence of the *EP300-ZNF384* fusion gene (Figure 1a) revealed that exon 6 of *EP300* was fused to exon 3 (the first coding exon) of *ZNF384*. The fusion point of *ZNF384* is the same as those of previously reported *ZNF384*-related fusion genes between *TAF15* and *E2A* genes.^{10,11} The 5-bp sequence upstream of the initiation codon in *ZNF384* is included, while the *EP300-ZNF384* fusion transcripts were predicted to encode a fusion protein with in-frame joining between the N-terminal portion of *EP300* and the entire *ZNF384* protein. A schematic representation of the *EP300-ZNF384* fusion protein predicted to encode a 110-kDa protein with 1027 amino acids^{10,12} is shown in Figure 1a. The *EP300-ZNF384* fusion protein retains the transcriptional adapter zinc-finger 1 (TAZ1) domain in the cysteine-histidine-rich region 1 (CH1) of *EP300*, but it lacks the other domains such as the histone acetyltransferase (HAT) domain to be included in *EP300*. On the other hand, the complete *ZNF384* protein is retained in the *EP300-ZNF384* fusion.

The clinical findings of *EP300-ZNF384* fusion-positive patients are summarized in Table 1. The patients were aged between 7 and 17 years (mean: 11.5 ± 3.8), comprised four males and two females, and were all classified into an intermediate risk group at the initial diagnosis based on an advanced age.¹³ Their initial white blood cell count ranged from 2300 to 17 330 (mean: 8730 ± 5849). All patients showed a good response to steroid monotherapy, using the cutoff of 1000/μl for the blast count in peripheral blood on day 8.¹³ All six patients achieved complete hematological remission after induction therapy. Four patients (Cases 2, 3, 4 and 6) completed the entire protocol and maintained complete remission for 4–10 years. One patient (Case 5) received maintenance therapy and was in complete remission for 2 years and 1 month. The remaining patient (Case 1) also completed the initial therapy, but he relapsed in the off-therapy period, and he is now in his fourth complete remission after a second bone marrow transplantation.

Importantly, the predicted chromosomal translocation t(12;22)(p13;q13) was not detected in all six cases by conventional G-banding, and five patients (Cases 2–6) showed a normal karyotype on cytogenetic analysis. The remaining patient (Case 1), who had relapsed three times, consistently showed 46,XY, t

(9;10)(p13;p13) from the initial diagnosis through to the third relapse, whereas corresponding gene fusion was not identified by whole transcriptome sequencing. As FISH confirmed the presence of *EP300-ZNF384* translocation in Case 1 and 954 among 1000 cells showed positive fusion signals, it is suggested that translocation leading to *EP300-ZNF384* is difficult to detect with conventional G-banding.

All six *EP300-ZNF384*+ cases revealed dull or negative expression of CD10 based on immunophenotypic examination (Supplementary Figure 2, Supplementary Table). They also exhibited negative expression of cytoplasmic μ chain. In addition, each case aberrantly expressed one or more myeloid antigen(s), including CD13 and CD33. Therefore, most BCP-ALL patients carrying the *EP300-ZNF384* fusion gene may be predictable based on their characteristic immunophenotype.

The *EP300-ZNF384* fusion and conventional cytogenetic abnormalities are mutually exclusive. FISH analysis revealed that a vast majority of leukemic blasts express the fusion. The above data suggest the involvement of this fusion gene in the development of BCP-ALL, whereas the functional role of this fusion gene is not yet known. As we present in Figure 1a, the *EP300-ZNF384* fusion protein is predicted to lack the HAT domain of *EP300*. It has been suggested that the deregulation of acetylation could lead to a disruption in the balance between proliferation and differentiation during hematopoiesis and *in vivo* structure–function analysis of *EP300* by others demonstrated that the loss of HAT activity results in an increase in the numbers and proliferative potential of hematopoietic progenitors and stem cells.¹⁴ Furthermore, it has been reported that some B-cell lymphoma harbor frequent structural alterations inactivating CREBBP or *EP300* as a major pathogenetic mechanism, reading to specific defects in acetylation-mediated inactivation of the BCL6 oncoprotein and activation of the p53 tumor suppressor.¹⁵ Therefore, the N-terminal portion of *EP300* without the HAT domains in the *EP300-ZNF384* fusion may be involved in the development of ALL by deregulating acetylation, but further functional evaluation is necessary.

In contrast, the complete *ZNF384* sequence is retained in the *EP300-ZNF384* fusion, being the same as in cases of other *ZNF384*-related recurrent fusions observed in acute leukemia.^{10,11} Both *EWSR1-ZNF384* and *TAF15-ZNF384* have transforming properties in NIH3T3 cells, but do not alter expressions of known *ZNF384* target genes.¹⁰ The functions of the *ZNF384* gene in these fusion genes remain unclear and should also be investigated in the future.

In conclusion, we identified a novel recurrent fusion gene between the *EP300* and *ZNF384* genes in children with BCP-ALL. Further studies involving a large series of patients should be conducted to elucidate the oncogenic properties of *EP300-ZNF384*

and confirm the clinical and biological features of patients with BCP-ALL harboring the *EP300–ZNF384* fusion gene.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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