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Acute Lymphoblastic Leukemia Patient with Variant *ATF7IP/PDGFRB* Fusion and Favorable Response to Tyrosine Kinase Inhibitor Treatment: A Case Report

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Conflict of interest: None declared

Patient: Female, 14-month-old
Final Diagnosis: Acute lymphoblastic leukemia
Symptoms: Fever
Medication: —
Clinical Procedure: —
Specialty: Hematology

Objective: Rare disease

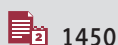
Background: Chromosomal translocations involving the *PDGFRB* gene have been reported in a broad spectrum of hematological malignancies. An *ATF7IP/PDGFRB* fusion was recently identified in a Philadelphia chromosome-like (Ph-like) B-progenitor acute lymphoblastic leukemia (B-ALL) patient. Here we report on a special case of a Ph-like ALL patient who had a variant *ATF7IP/PDGFRB* fusion.

Case Report: In this case, a variant fusion was created between *ATF7IP* exon 9 (instead of exon 13) and *PDGFRB* exon 11, resulting in the loss of 411 nucleotides and 137 amino acids in the *ATF7IP/PDGFRB* fusion cDNA and its encoded chimeric protein, respectively. Interestingly, *ATF7IP* has also been reported as a fusion partner of the *JAK2* kinase gene in Ph-like ALL, but all of the genomic breakpoints in *ATF7IP* in this fusion reported thus far occurred in intron 13. Enforced expression of the variant *ATF7IP/PDGFRB* fusion induced cytokine-independent growth and glucocorticoid resistance of BaF3 cells. Similar to the initially described *ATF7IP/PDGFRB*-bearing Ph-like ALL patient who was refractory to conventional chemotherapy but highly sensitive to tyrosine kinase inhibitor (TKI) monotherapy, our patient with a variant *ATF7IP/PDGFRB* fusion had a poor initial treatment response to chemotherapy but responded well to TKI-based therapy and is now doing well in continuous remission.

Conclusions: Ph-like ALL patient with an *ATF7IP/PDGFRB* fusion is rare, but can benefit from TKI therapy.

MeSH Keywords: Oncogene Fusion • Philadelphia Chromosome • Precursor Cell Lymphoblastic Leukemia-Lymphoma

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Background

Chromosomal translocations involving the *PDGFRB* gene have been reported in a broad spectrum of hematological malignancies, including both myeloid neoplasms and acute lymphoblastic leukemia (ALL) [1–3]. Myeloid neoplasms with *PDGFRB* rearrangements are genotypically and phenotypically diverse but typically present as a myeloproliferative neoplasm (MPN) with eosinophilia in the bone marrow and the peripheral blood [4]. Recently, *PDGFRB* rearrangements were reported in a special type of ALL named Philadelphia chromosome-like (Ph-like) ALL, which lacks the characteristic *BCR-ABL1* fusion gene but possesses a similar gene expression profile to that observed in Ph+ ALL [3,5,6]. So far, over 20 fusion partners of *PDGFRB* have been identified, of which five genes – namely *EBF1*, *SSBP2*, *TNIP1*, *ZEB2*, and *ATF7IP* – were reported to be fused with *PDGFRB* in Ph-like ALL [2,3,7]. *ATF7IP* as a novel *PDGFRB* fusion partner was initially identified in an eight-year-old boy with B-ALL by a Japanese group in 2014 [7]. This patient had a cryptic *PDGFRB* translocation revealed by the conventional cytogenetics, but mRNA sequence analysis identified an in-frame fusion transcript between exon 13 of *ATF7IP* and exon 11 of *PDGFRB*.

In our case, a 14-month-old girl with B-ALL, and a completely normal karyotype was observed by conventional cytogenetics. However, fluorescence *in situ* hybridization (FISH) analysis, using a breakapart probe set, demonstrated rearrangement of the *PDGFRB* gene, and next-generation genomic DNA

sequencing revealed that the *ATF7IP* gene was fused to *PDGFRB*. Unexpectedly, the breakpoint in *ATF7IP* was not located in intron 13 as described in the first reported case of a Ph-like ALL patient with the *ATF7IP/PDGFRB* fusion and as in other Ph-like ALL patients containing an *ATF7IP/JAK2* fusion gene [3,7], but rather in *ATF7IP* intron 9. Consequently, this rearrangement created a variant *ATF7IP/PDGFRB* chimeric transcript in which the fusion was produced between *ATF7IP* exon 9 (instead of 13) and *PDGFRB* exon 11. This variant fusion lacks 411 nucleotides and consequently 137 amino acids compared to the originally described *ATF7IP/PDGFRB* chimeric cDNA and protein.

Case Report

A 14-month-old female was hospitalized in October 2015 due to fever and skin petechiae. Laboratory test findings showed a white blood cell (WBC) count of $7.8 \times 10^9/L$ with 9% immature lymphocytes, a hemoglobin concentration of 7.6 g/dL, and a platelet count of $11 \times 10^9/L$. Bone marrow (BM) examination was consistent with ALL-L2. Immunophenotyping results showed a pre-B cell type ALL with the presence of CD19, CD10dim, CD20, CD22, HLA-DR, cCD79a, and cIgM. Karyotyping analysis demonstrated a normal karyotype of 46 XX (data not shown). FISH analysis failed to detect the *BCR/ABL1* fusion gene (Figure 1A); however, a distinct split signal was seen in the *PDGFRB* gene (Figure 1B). Further study with next-generation genomic DNA sequencing demonstrated fusion of the ninth intron of *PDGFRB* with the eleventh intron of the *ATF7IP*

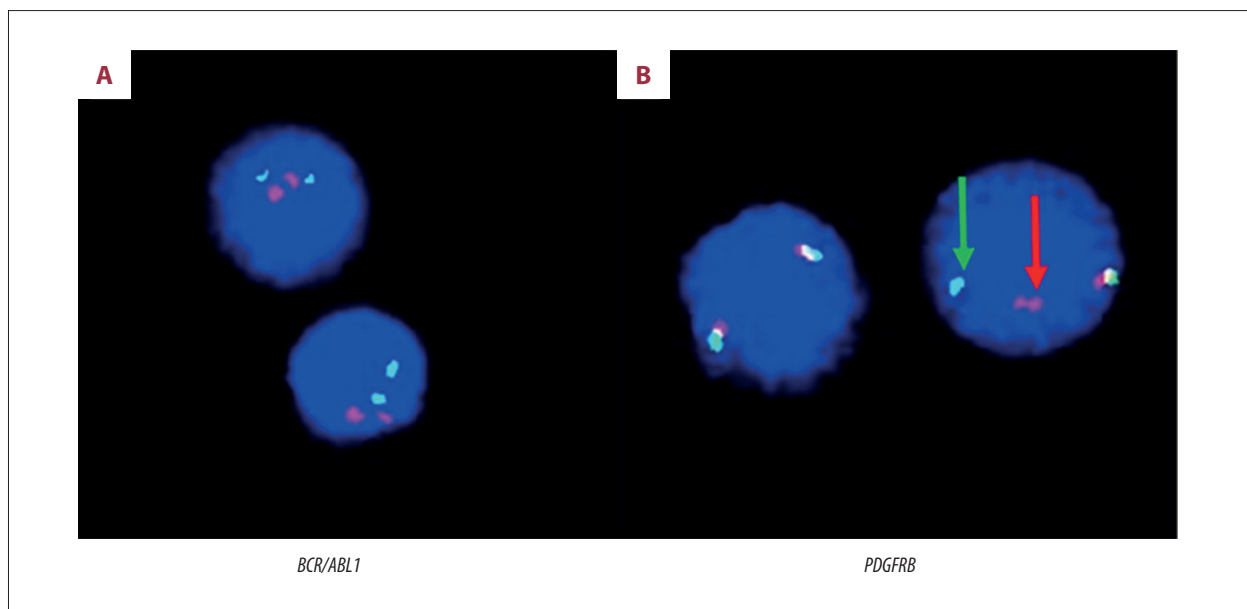


Figure 1. Results of FISH analysis. (A) No *BCR/ABL1* fusion was detected in the interphase nuclei of leukemia cells by the *BCR/ABL1* D-FISH probe (Vysis Inc., Downers Grove, IL, USA). (B) *PDGFRB* gene rearrangement was detected by the *PDGFRB* break-apart probe (Vysis). Co-localized red/green or yellow signals identify the normal *PDGFRB* gene. Split signals (a red and a green signal, as indicated by the red and green arrows) demonstrate *PDGFRB* gene translocation.

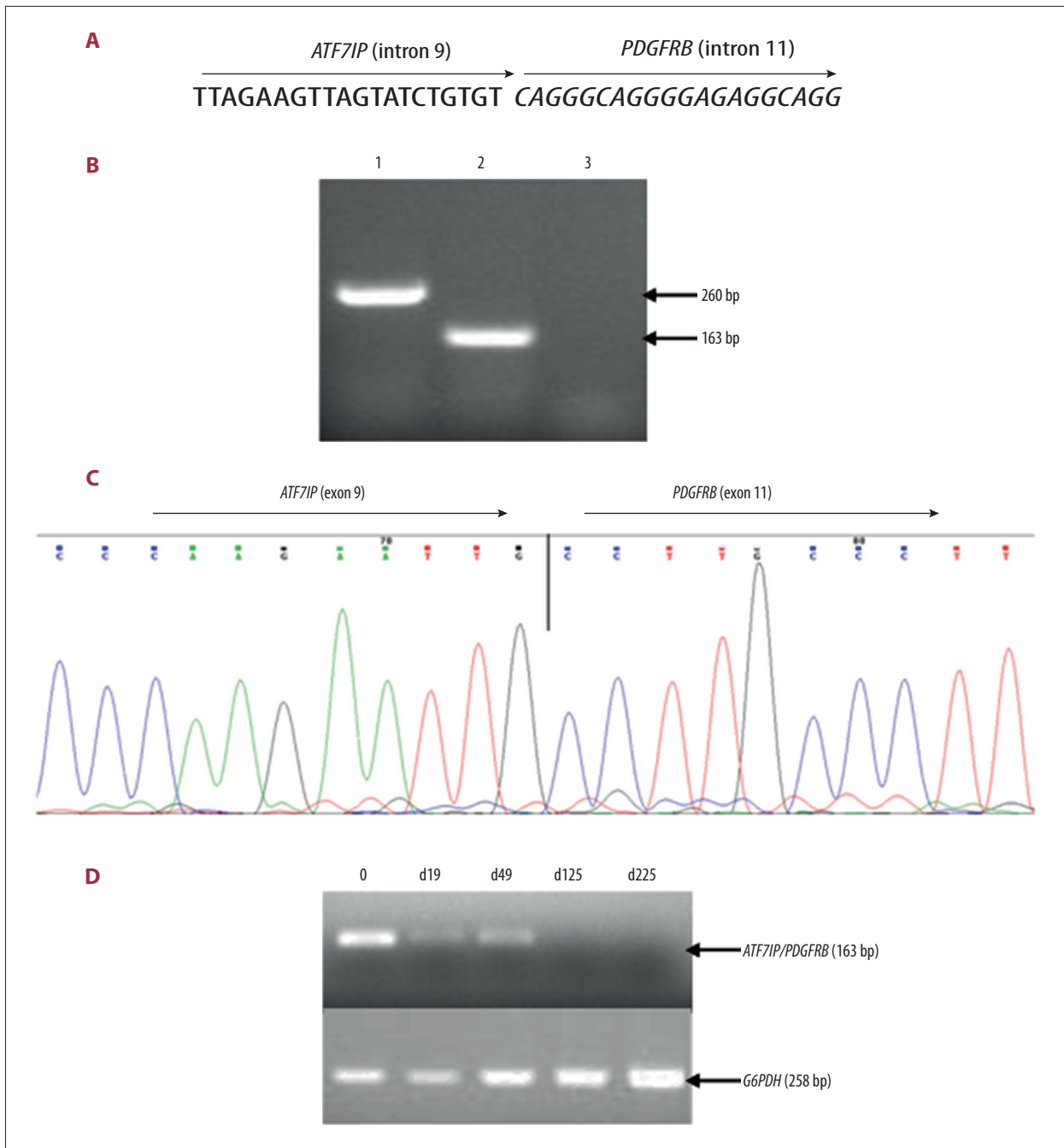


Figure 2. Genomic and cDNA fusion junction sequences of the *ATF7IP/PDGFRB* fusion gene and monitoring of minimal residual disease (MRD) by RT-PCR detection of the *ATF7IP/PDGFRB* fusion transcript. **(A)** Next-generation sequencing analysis indicated the genomic breakpoint site. **(B)** RT-PCR test detected the *ATF7IP/PDGFRB* fusion transcript. First round RT-PCR using primers of *ATF7IP*-F1 (ACCCTACTGCCAGTGCTGCAC) and *PDGFRB*-R (GATGGCTGAGATCACCACCAC) yielded a product of 260 bp (lane 1) and a semi-nested PCR using primers of *ATF7IP*-F2 (ACAGTGGGCCCATCAGGACTC) and *PDGFRB*-R amplified a product of 163 bp (lane 2). Lane 3 was a negative control. **(C)** Sequencing analysis of the RT-PCR product indicated the fusion junction of the *ATF7IP/PDGFRB* cDNA. **(D)** MRD monitoring by RT-PCR of the *ATF7IP/PDGFRB* transcript indicated that no fusion gene product could be detected after 125 days of treatment.

gene (Figure 2A). RT-PCR and sequence analysis of the resulting amplicon confirmed the expression of an *ATF7IP/PDGFRB* fusion transcript (Figure 2B, 2C).

Given these clinical and molecular findings, the patient was diagnosed with Ph-like B-ALL and treated with standard remission induction therapy of VDLP (vincristine, daunorubicin, L-asparaginase, and prednisone). At day 19, a BM examination indicated partial remission with 6.5% lymphoblasts, and a 40 mg dose of multi-targeting second generation tyrosine kinase inhibitor (TKI) dasatinib was administered daily thereafter together with the VDLP therapy. At day 49, BM examination showed complete remission (CR) and the minimal residual disease (MRD) test by flow cytometry (FACS) was negative. However, RT-PCR analysis remained positive for the *ATF7IP/PDGFRB* fusion. At day 125, both the *ATF7IP/PDGFRB* fusion gene expression by RT-PCR testing and the MRD analysis by FACS were negative (Figure 2D). The patient is now receiving the Chinese Children's Cancer Group (CCCG)-2015-ALL protocol plus dasatinib and remains free of molecularly detectable disease.

Discussion

Ph-like ALL is a recently recognized disease entity related to B-progenitor ALL [2,8]. It constitutes a distinct subtype of ALL characterized by a gene expression profile similar to that of *BCR/ABL1* positive ALL, a diverse range of genetic alterations that activate tyrosine kinase signaling, mutations of lymphoid transcription factor genes, and a poor outcome. In contrast to the incidence of Ph-positive ALL, which is known to be lower than 5% of childhood ALL but continually increases with age, comprising 20% to 30% of adults with ALL and with a peak incidence of 50% in patients over 50 years old [9], Ph-like ALL increases in frequency from 10% among children with standard risk and 13% with high risk ALL to 21% among adolescents and 27% among young adults with ALL but then decreases significantly with more advanced age, forming a bell-shaped curve [3,10,11].

Clinically, Ph-like ALL patients have higher leukocyte counts at presentation than those with non-Ph-like ALL and non-Ph+ ALL [3,8,9]. Ph-like ALL is more commonly seen in males than females and is associated with elevated levels of MRD at the end of induction therapy [3,12,13]. Across all age groups, both the median five-year event-free survival rates and the five-year overall survival rates were reported to be inferior to those in patients with non-Ph-like ALL [3,14]. The presence of Ph-like ALL was considered to be an independent prognostic factor in all age groups [10,14].

The molecular mechanism underlying the pathogenesis of Ph-like ALL involves characteristic kinase-activating alterations that are identified in 91% of patients, including rearrangements of

kinases as well as other proteins that transduce kinase signaling pathways such as *ABL1*, *ABL2*, *CRLF2*, *CSF1R*, *EPOR*, *JAK2*, *NTRK3*, *PDGFRB*, *PTK2B*, *TSLP*, or *TYK2*, and sequence mutations involving *FLT3*, *IL7R*, or *SH2B3* [3]. Engineered overexpression of *ABL*-class fusions (involving *ABL1*, *ABL2*, *CSF1R*, or *PDGFRB*) and *JAK* fusions in cell lines results in cytokine-independent proliferation and activation of downstream signaling pathways, both of which are inhibited by TKIs, consistent with the oncogenic role of the fusion kinases in the pathogenesis of Ph-like ALL [3,10,12]. *ABL*-class rearrangement is more common among children and predicted to respond to *ABL1* and other specific kinase inhibitors [10].

Fusion genes derived from *PDGFRB* play an important role in the pathogenesis of eosinophilia-associated myeloproliferative neoplasms (Eo-MPN) [1,4]. More than 20 *PDGFRB* fusion partners have been identified [2]. Reports indicate that the TKI imatinib is well-tolerated, and excellent long-term responses have been achieved in MPN patients with *PDGFRB* rearrangements, no matter the identity of the fusion partner [1]. So far, five different fusion partners have been reported in Ph-like ALL with *PDGFRB* rearrangements, of which only *TNIP1* has been observed in both MPN and ALL [3,7]. *ATF7IP* was recently identified as a fusion partner of *PDGFRB* in a childhood Ph-like ALL case [7], and it was previously reported to be fused to *JAK2* in four Ph-like ALL patients [3]. The breakpoints of *ATF7IP* in these five Ph-like ALL cases were all within intron 13. In our case, however, the breakpoint in *ATF7IP* was present in intron 9, causing a loss of 411 nucleotides from exons 9 to 13 in the variant fusion cDNA and consequently a loss of 137 amino acids in the fusion protein. Considering that in both cases the N-terminal domain of *ATF7IP* still contains the coiled coil structure that is known to contribute to the constitutive activation of kinase and cytokine receptor signaling, functionally there should be no difference in kinase activation and TKI responsiveness in patients with either the classic or variant *ATF7IP/PDGFRB* fusion. Our *in vitro* cell culture study indicated that enforced expression of the variant *ATF7IP/PDGFRB* fusion could not only transform BaF3 cells like *ATF7IP/PDGFRB* fusion as demonstrated by the Japanese investigators [15], but also induce the cells resistant to glucocorticoid treatment (Figure 3). Indeed, clinically both the Japanese boy with the classic *ATF7IP/PDGFRB* fusion and our patient showed excellent long-term responses to TKI therapy [16].

In our case, the patient's leukemic cells showed a normal karyotype by conventional cytogenetics. We note from the literature that a certain percentage of patients with *PDGFRB* fusions have a normal karyotype in MPN, MDS and ALL [2,7,17]. We suggest that FISH screening for *PDGFRB* rearrangement be strongly considered in such cases. As demonstrated herein in *BCR-ABL1*-negative B-progenitor ALL, Ph-like ALL with *PDGFRB* fusion is clinically refractory to conventional therapy but highly sensitive to TKI therapy.

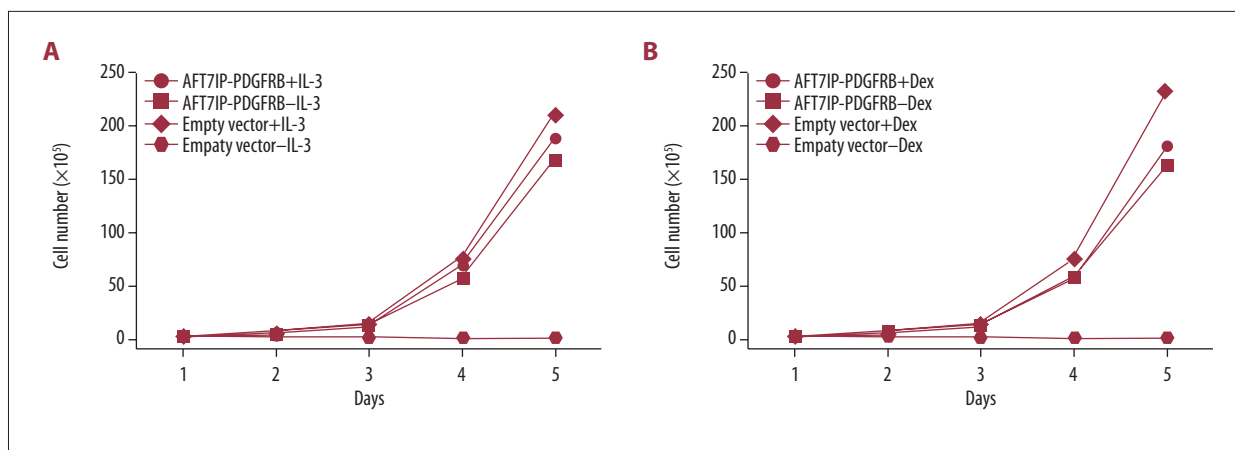


Figure 3. Enforced expression of variant *ATF7IP/PDGFRB* fusion induced cytokine-independent growth and glucocorticoid resistance of BaF3 cells. **(A)** Compared with the parental BaF3 cells which can grow in the media with IL-3 (10 ng/mL), the cells stably transfected with variant *ATF7IP/PDGFRB* fusion became IL-3-independent growth. **(B)** The transfected BaF3 cells acquired resistance to dexamethasone treatment, even under a high concentration of the drug (10 μM).

Conclusions

Although the variant *ATF7IP/PDGFRB* fusion lacks 411 nucleotides and 137 amino acids compared to the originally described *ATF7IP/PDGFRB* chimeric cDNA and protein, *in vitro* cell culture study indicated that both fusion genes could transform BaF3 cells and convert the cells to GC resistance. Clinically, both of the patients with the variant or the classical fusion were refractory to conventional therapy but highly sensitive to TKI therapy. We suggest that FISH analysis for *PDGFRB* rearrangement

be strongly considered as a supplementary screening test to identify the patients who might benefit from TKI treatment.

Acknowledgements

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Conflicts of interest

None.

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