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Case Report

Identification of a Novel *CSNK2A1-PDGFRB* Fusion Gene in a Patient with Myeloid Neoplasm with Eosinophilia

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Platelet-derived growth factor receptor beta (PDGFRB) rearrangements play an important role in the pathogenesis of eosinophilia-associated myeloid/lymphoid neoplasms. Up to now, more than 70 *PDGFRB* fusions have been identified. Here, a novel *PDGFRB* fusion gene *CSNK2A1-PDGFRB* has been identified in myeloproliferative neoplasm (MPN) with eosinophilia by RNA-sequencing, which has been verified by reverse transcription polymerase chain reaction and Sanger sequencing. The new *PDGFRB* fusion partner gene *CSNK2A1* encoded one of the two catalytic subunit of casein kinase II (*CK2*). To our knowledge, this is the first report on the involvement of *CSNK2A1* in fusion genes, especially fusion with another kinase *PDGFRB* in MPN. In addition, the *CSNK2A1-PDGFRB* fusion retained the entire kinase domain of PDGFRB and response to imatinib at low concentration. The patient with *CSNK2A1-PDGFRB* was sensitive to imatinib treatment and acquired sustained complete remission.

Key words CSNK2A1-PDGFRB, Myeloid neoplasms, RNA-seq, Imatinib

Introduction

On the base of the 2016 World Health Organization, myeloid/lymphoid neoplasms with eosinophilia are commonly related to rearrangements of *PDGFRA*, *PDGFRB*, or *FGFR1*, or *PCM1-JAK2* fusion gene [1]. The *PDGFRB* gene translocation is one of the most chromosomal aberrant in myeloid neoplasms associated with eosinophil [2], high results in the fusion of the 3′ kinase domain of *PDGFRB* to a 5′ region of the partner gene. So far more than 70 *PDGFRB* fusions have been reported, mostly reported in single case. Imatinib mesylate function as a tyrosine kinase inhibitor which can potently inhibit ABL kinase, which is equally against PDGFRB kinase, even at a low concentration [3-5]. Most of the patients with *PDGFRB* fusions show an outstanding long-term response to imatinib treatment at sub-micromolar concentrations [6].

Casein kinase II (*CK2*) is ubiquitously expressed, constitutively active serine/threonine protein kinase, which was involved in various cellular processes, including cell growth, survival, apoptosis, and circadian rhythm [7]. CK2 upregulated in a lot of malignancies including hematological cancers [8]. The CK2 tetramer consists of two catalytic CK2α and *CK2α′* subunits, as well as two regulatory CK2β subunits, with the composed patterns of α2β2, α′2β2 or αα′β2. CK2α was encoded by *CSNK2A1* gene (casein kinase II subunit α), which was predominantly studied likely because of its ubiquitous nature. But *CK2α′* expressed varied, particularly in brain [9]. All domains of CK2α are highly conserved throughout evolution, but *CK2* has low homology with other kinases [10]. In addition, *CK2α* knock out mice are lethal at E11 with multiple embryonic alterations [11]. Therefore, the importance and uniqueness of *CK2α* were highlighted. Here, we reported a new fusion gene involving *PDGFRB* and *CSNK2A1* in a patient with myeloproliferative neoplasm (MPN), who is extremely sensitive to imatinib mesy-late treatment. So far, this is the first report on the involvement of *CSNK2A1* in fusion genes in cancers.

Case Report

A 37-year-old man was admitted to local hospital with weight loss, night sweat, repeating fever for a week in April 2018. The patient's initial laboratory examination showed that leukocyte count was 12.48×10^9/L with 37% eosinophilia in the peripheral blood, hemoglobin concentration was 127 g/L and platelet count was 146×10^9/L. The initial bone marrow (BM) aspirates and biopsy showed hyper leukocytes and significantly increased eosinophils (8%), and neutrophil alkaline phosphatase score was 18 (Fig. 1A and B). Above all,

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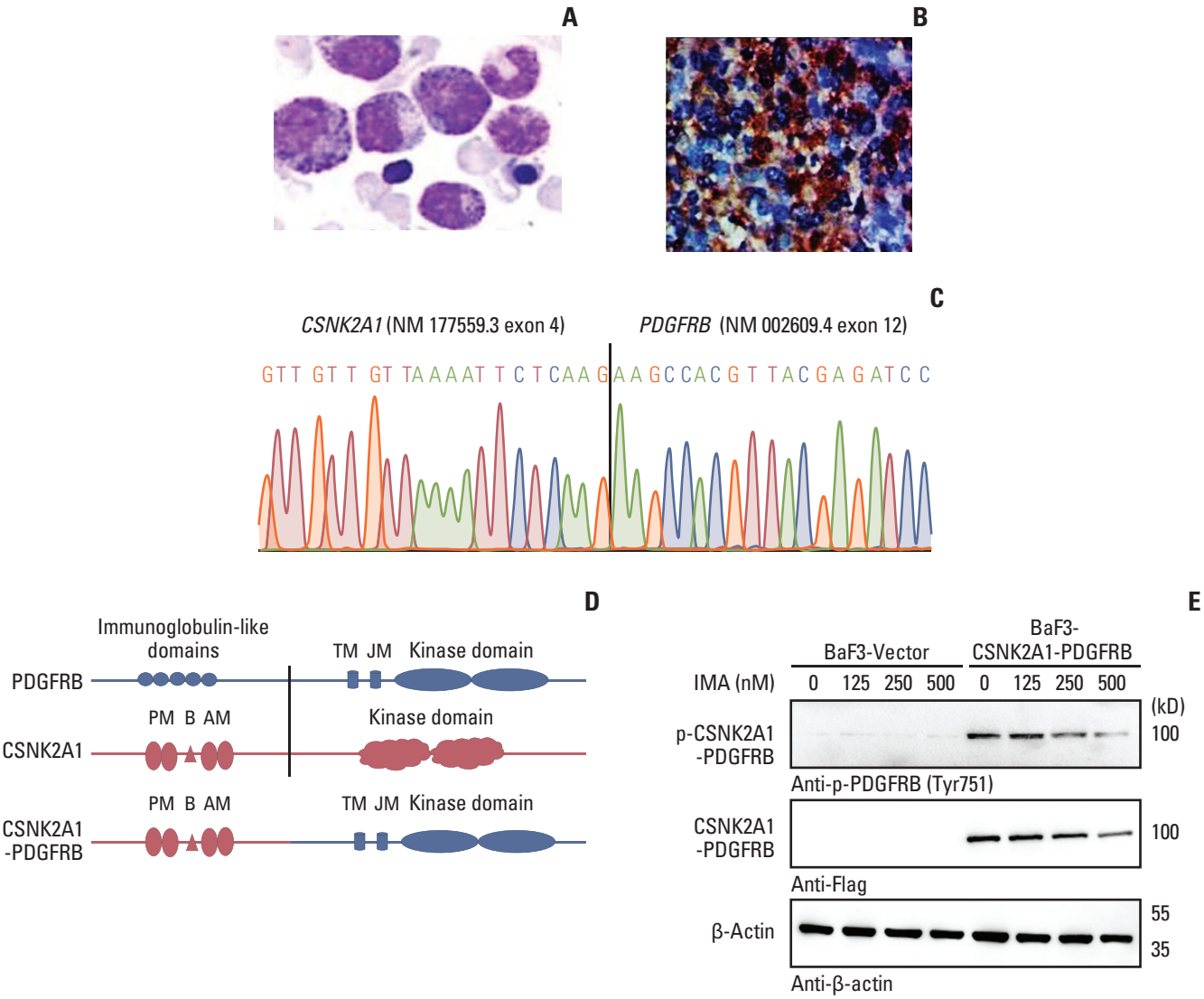

Fig. 1. Identification of novel *CSNK2A1-PDGFRB* fusions. (A) May-Grünwald-Giemsa staining showing several abnormal eosinophilia in the diagnostic bone marrow aspirate. (B) PDGFRB was stained in bone marrow of patient using immunohistochemistry. (C) Sanger sequencing revealed the fusion between exon 4 of the *CSNK2A1* gene (NM_177559.3) and exon 12 of the *PDGFRB* gene (NM_002609.4). (D) Fusion model of CSNK2A1-PDGFRB are shown. (E) Immunoblot analysis show CSNK2A1-PDGFRB is constitutively activated and is inhibited by imatinib in a concentration-dependent manner. AM, ATP binding domain; B, CK2B subunit binding domain; IMA, imatinib; JM, juxtamembrane domain; PM, polypeptide binding domain; TK, tyrosine kinase domain; TM, transmembrane domain.

these inspections were consistent with a diagnosis of MPN. Then the patient was administrated with 20 mg prednisone per day, but it showed no any effect with white blood cell (WBC) 15.1×10^9/L, hemoglobin 108 g/L, platelet 114×10^9/L, eosinophils 5.13×10^9/L (33.91%) in peripheral blood.

The suite of fluorescence *in situ* hybridization (FISH) assay on the BM aspirate was used to detect *BCR-ABL*, *PDGFRA*, *PDGFRB*, and *FGFR1* rearrangement. MPN FISH assay showed *PDGFRB* arrangement positive. The karyotype analysis of BM cells showed 46,XY[20].

Patient's RNA was extracted from BM cell by Trizol methods according to the manufacturer's protocol (Invitrogen, Waltham, MA) for RNA-sequencing (RNA-seq) in July 2018. RNA quality and concentration were estimated by Nano Drop ND-2000 (Thermo Fisher Scientific, Waltham, MA). Paired-end reads were generated from the complementary DNA (cDNA) libraries using an Illumina Next Seq 550 instrument (Illumina, San Diego, CA). Then we used star-fusion software to analyze the RNA-seq raw data [12] (Supplementary Material). Standard settings were applied for all

three tools and reads were aligned to the Genome Reference Consortium Human Build 37 (GRCh37). RNA-seq revealed that *PDGFRB* fused with *CSNK2A1* gene. To confirm the fusion, reverse transcription–polymerase chain reaction (RT-PCR) was performed with *CSNK2A1-PDGFRB* forward primer: 5′-GTGCCAAGCAGGGCCAGAGT-3′, reverse primer 5′-AGGGTGCGTCCCAGCACAAG-3′. The reciprocal *PDGFRB-CSNK2A1* fusion forward primer: 5′-TCAGAGCTGACACTGGTTCG-3′, reverse primer: 5′-GATGTTGGGACCTCCTCTCAA-3′. The PCR products was purified with PCR purification kit (Tiangen, Beijing, China) and sequenced by GENEWIZ Biotechnology Co., Ltd. (Suzhou, Jiangsu, China). The sequence was analyzed using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast).

Given the existence of PDGFRB fusion, he received imatinib therapy with 200 mg every day orally in September 2018. Two months later, the laboratory examination showed that WBC was 6.14×10^9/L with 2.8% eosinophilia in the peripheral blood (S1 Table). Finally, real-time quantitative reverse transcription PCR was performed to quantify the fusion gene and showed negative in 8 months after imatinib treatment. To date, the patient acquired sustained molecular complete remission for 2 years until the last follow-up.

Discussion

RNA targeted capture sequencing showed a fusion between *CSNK2A1* exon 4 (NM_177559.3) and *PDGFRB* exon 12 (NM_002609.4), forming a novel fusion gene *CSNK2A1-PDGFRB*. RT-PCR and Sanger sequencing has confirmed *CSNK2A1-PDGFRB* fusion transcripts (Fig. 1C). The reciprocal fusion transcript *PDGFRB-CSNK2A1* was negative by detection of RT-PCR. To our knowledge, this is the first case on *CSNK2A1* gene rearrangement in neoplasms, especially fusion with another kinase *PDGFRB* in hematological cancers. The fusion protein retained the transmembrane domain and the entire kinase domain of *PDGFRB* (Fig. 1D).

Whole cDNA in *CSNK2A1-PDGFRB* open reading frame was cloned and transduced to BaF3 cells. As a result, *CSNK2A1-PDGFRB* fusion protein is constitutively activated (Fig. 1E, panel 5). In addition, incubation of BaF3 cells transduced with *CSNK2A1-PDGFRB* with imatinib for 4 hours caused a concentration-dependent decrease of *CSNK2A1-PDGFRB* protein (Fig. 1E). The result suggested that imatinib might induce the degradation of PDGFRB fusion protein besides inhibition of its activation.

It is well known that dimerization results in activation of *PDGFRB* and its down signaling play a vital role in mitogenesis, cytoskeletal rearrangements, and chemotaxis [13]. Most partners have coiled-coil domains, which are required for dimerization or oligomerization of *PDGFRB* fusions. However, there is no coiled-coil motif in *CK2α*. In addition, lacking transmembrane domain or disrupting the WW-like domain in juxta membrane region of *PDGFRB* may also play a role in *PDGFRB* kinase activation and transformation properties. However, the fusion protein retained the transmembrane domain of *PDGFRB*. So that, there may be another unknown way to active kinase region of *PDGFRB*. Notably, *CK2α* harbored several regions referred to polypeptide binding domain and *CK2β* binding domain, which were retained in the *CSNK2A1-PDGFRB* fusion, and may be associated with dimerization or oligomerization of the *CSNK2A1-PDGFRB* fusion. Indeed, the kinase domain of *CSNK2A1-PDGFRB* was constitutively activated as shown in Fig. 1E and the patient with *CSNK2A1-PDGFRB* was sensitive to imatinib treatment.

Totally, we have identified a novel *PDGFRB* fusion gene with *CK2α* in an MPN by RNA-seq, which was extremely sensitive to imatinib. To our knowledge, it is the first report to find a *CK2α* rearrangement in neoplasm, especially fusion with another kinase *PDGFRB* in MPN.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (https://www.e-crt.org).

Ethical Statement

The study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University (No. 221 of 2019 LSP (application)) and was conducted following the Declaration of Helsinki.

Author Contributions

Conceived and designed the analysis: Chen S, Zeng Z, Ruan C.
Collected the data: Xu X.
Contributed data or analysis tools: Xu X, Lu Q, Wang Z, Cai P, Wang M, Ma L.
Performed the analysis: Xu X, Lu Q, Wang Z, Cai P, Zhang L.
Wrote the paper: Xu X, Zeng Z.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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