Identification of a Novel \textit{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 \textit{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 \textit{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 \textit{CSNK2A1} in fusion genes, especially fusion with another kinase PDGFRB in MPN. In addition, the \textit{CSNK2A1-PDGFRB} fusion retained the entire kinase domain of PDGFRB and response to imatinib at low concentration. The patient with \textit{CSNK2A1-PDGFRB} was sensitive to imatinib treatment and acquired sustained complete remission.

\textbf{Key words} CSNK2A1-PDGFRB, Myeloid neoplasms, RNA-seq, Imatinib

\section*{Introduction}

On the base of the 2016 World Health Organization, myeloid/lymphoid neoplasms with eosinophilia are commonly related to rearrangements of \textit{PDGFRAn}, \textit{PDGFRB}, or \textit{FGFR1}, or \textit{PCMLJAK2} fusion gene [1]. The \textit{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 PDGFR 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 upregulation 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 \textit{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 \textit{PDGFRB} and \textit{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 \textit{CSNK2A1} in fusion genes in cancers.

\section*{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 127g/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, 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 127g/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,
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) $15.1 \times 10^9/L$, hemoglobin $108 \text{ g/L}$, platelet $114 \times 10^9/L$, eosinophils $5.13 \times 10^9/L$ (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

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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.
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′-GTGCGAACAGGGCCAGAGT-3′, reverse primer 5′-AGGGTGCTCCAGCACAGAG-3′. The reciprocal PDGFRB-CSNK2A1 fusion forward primer: 5′-TCAGACGTGACCTGTTGTCG-3′, reverse primer: 5′-GATGTTGGAGCTCCTCTCTCAA-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 6.14×10⁹/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 in peripheral blood (S1 Table). 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

Conflicts of Interest
Conflict of interest relevant to this article was not reported.

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References


