Identification of novel PIEZO1::CBFA2T3 and INO80C::SETBP1 fusion genes in an acute myeloid leukemia patient by RNA -seq

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Short Report

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Abstract

Fusion genes are recurrent molecular aberrations in acute myeloid leukemia, with significant diagnostic and therapeutic value. The identification of novel fusion genes provides advanced biomarkers for diagnosis and facilitates the discovery of drug targets. Here we report a male AML patient with presence of *PIEZO1::CBFA2T3* and *INO80C::SETBP1* fusion genes detected by RNA sequencing. As far as we know, it is the first time *PIEZO1::CBFA2T3* and *INO80C::SETBP1* are identified in AML patients.

1. Introduction

Fusion genes are recurrent molecular aberrations in acute myeloid leukemia (AML), with significant diagnostic and therapeutic value. It is rare in AML to bear coexisting pathogenic fusion genes. A large-cohort-based research screened 36 common fusion genes, and reported that only 0.3% of leukemia patients were positive for two concurrent pathogenic fusion genes [1]. In recent years, the application of high-throughput sequencing technologies makes identifying novel fusion genes more convenient and has revealed numerous rare pathogenic fusion genes in leukemia.

The *CBFA2T3* gene, located on chromosome 16q24.3, is a member of the *ETO* gene family and an important regulator in hematopoietic progenitor cell proliferation, erythropoiesis, and leukemia stem cell transformation [6–8]. *CBFA2T3* encodes a protein that contains four evolutionary conserved nervy homology regions (NHR 1–4), among which the NHR2 domain can interact with hematopoiesis-related transcription factor *RUNX1*, promoting acute lymphoblastic leukemia proliferation [2].

The *SETBP1* gene is mapped to chromosome 18q12.3, and encodes a protein that contains a SKI-homology domain, a SET-binding domain (SETBD), and 3 A-T hooks. *SETBP1* protein binds to oncoprotein *SET* and forms a heterodimer, inhibiting *PP2A* and enhancing leukemia cell proliferation. SETBP1 also modulates target genes at the transcriptional level by interacting with DNA via its A-T hooks [3–5]. Somatic gain of function mutations of the *SETBP1* homology domain has been extensively reported as a common driver mutation in myeloid malignancies[6].

Here we report an AML case identified with coexisting *PIEZO1::CBFA2T3* and *INO80C::SETBP1* fusion genes. To the best of our knowledge, it is the first time these two novel driver fusion genes were reported in AML patients.

2. Methods And Materials

2.1 case report

The patient, male, 64 years old, was transferred to our department on September 9th, 2019, with fatigue and consistent fever. Peripheral blood examination showed hemoglobin level of 67g/L, red blood cell (RBC) count of 2.2×10^{12}/L, white blood cell (WBC) count of 8.4×10^{9}/L, and platelet count of 7×10^{9}/L. Morphological examination of peripheral blood reported a blast cell ratio of 45% and the presence of

abnormal erythrocytes. Ultrasound found an enlarged spleen. To further clarify the diagnosis, bone marrow (BM) aspiration was performed and revealed 81.5% blast cells. The blast cells were positive for CD117, CD34, HLA-DR, CD36, and CD17; negative for CD19, CD56, CD22, and MPO. Metaphase analysis was unsuccessful. DNA sequencing of 58 frequently mutated genes in hematologic malignancies [7] identified *KRAS c.35G > A/p.G12D* and *PTPN11 c.1508G > T/p.G503V* mutations in this patient. He was diagnosed with AML FAB-M2 based on the patient's symptoms, clinical findings, and immunophenotyping results. Hemostasis and anti-inflammatory treatments were administered since the day of admission. However, the patient refused chemotherapy for financial reasons and was discharged from the hospital on September 19th ,2019, and passed away ten days later.

2.2 RNA sequencing

RNA-Seq was performed using RNA extracted from the BM sample by HiSeq 2,500 (Illumina, San Diego, CA, USA) to search for the potential fusion gene. Reads were mapped and processed by combining Arriba (v1.0.1)[8] and STAR-Fusion (v1.3.1) [9]to analyze the gene fusions.

2.3 Differential Expressed Genes

We utilized the DESeq2 R package to compare the expression data (HTSeq-Counts) of the patient with the control group of 50 healthy people. The threshold value was log 2-fold change (FC) > 1 and adjusted p value < 0.05. Normal control samples used for gene expression analysis were bone marrow samples from healthy donors. All of the samples used in this study were approved by the ethics committee at the 2nd Affiliated Hospital of Harbin Medical University.

2.4 Gene set enrichment analysis

Gene Set Enrichment Analysis (GSEA) of differentially expressed genes in RNA-Seq data were implemented by the ClusterProfiler R package[10].

3. Results

3.1 novel fusion gene PIEZO1::CBFA2T3 is associated with dysregulation of CBFA2T3

Whole transcriptome RNA sequencing (RNA-Seq) performed on the patient’s bone marrow sample detected a highly likely pathogenic fusion genes, *PIEZO1 exon1::CBFA2T3 exon2* (figure 1A). The novel *PIEZO1::CBFA2T3* fusion in this elderly male patient preserved the coding region of all functional domains of *CBFA2T3*, possibly acts as a driver in oncogenesis (Fig. 1A). To elucidate the pathogenic significance of *PIEZO1::CBFA2T3*, we screened four *CBFA2T3*-coexpressing genes in AML based on a previous study (up-regulating genes include *CDKN1A*, *JUP, KAT2A*, and the down-regulating gene *TYROBP*[11]) and compared the expression levels with a control set of 50 normal bone marrow (BM) samples. (Fig. 1C) Overall, these data support the *PIEZO1::CBFA2T3* fusion resulted in overexpression and dysregulation of *CBFA2T3* and played an essential role in leukemogenesis.

3.2 Identification of *INO80C::SETBP1* fusion gene and overexpression of *SETBP1*

RNA-Seq identified an in-frame *INO80C exon1 -SETBP1 exon3* fusion in this patient. Notably, the *INO80C::SETBP1* fusion protein comprises the intact functional domains of *SETBP1*, suggesting the dysregulated *SETBP1* function possibly contributes to oncogenesis (Fig. 1B). To confirm our speculation, we examined the patient's expression levels of a selected list of genes (*SETBP1* along with *SETBP1*-coexpress genes *HOXA9, HOXA10*, function-related genes *SET, PP2A*, and hematopoiesis-associated gene *MECOM* as well as oncogene *MYB*, which can be upregulated by *SETBP1* and mutated *SETBP1*, respectively[3, 12, 13]) with the expression levels of 50 healthy donors as a control set. Our findings indicated the aberrant upregulation of *SETBP1*. (Fig. 1C)

3.3 *MYC* and immune-related pathways are activated in this patient

RNA-Seq data analysis found the overexpression of patient-outcome-associated genes *WT1, GATA2*, and *MYC* [14–16]. Gene set enrichment analysis (GSEA) analysis was performed and the results indicated the activation of the *MYC* and *E2F* pathways, which is in concert with the upregulation of *MYC*. Other significantly dysregulated pathways include *TGFβ, IFNγ, TNFα*, and *IL2_STAT5*, suggesting a broad dysregulation of the immune microenvironment (Fig. 1D).

4. Discussion

Despite *CBFA2T3* fusions being rare, *CBFA2T3::GLIS2* has been reported in pediatric AML. *RUNX1::CBFA2T3, NFIA::CBFA2T3, and CTCF::CBFA2T3* have been reported in pediatric and adult AML cases. The splicing site of *CBFA2T3* in *CBFA2T3::GLIS2* is located at exon 10 or exon 11, and the splicing sites in other *CBFA2T3* fusions are commonly located at *exon 3, exon 4*, or *exon 5*[17–19]. Notably, the main functional domains are generally retained in previously reported *CBFA2T3* fusion genes, regardless of whether it acts as a 5' or 3' partner gene, which is in accordance with our findings and strongly suggests the promiscuous oncogenesis function of CBFA2T3 in these fusion scenarios.

Fusion genes often serve as prognostic determinant biomarkers and guide risk-adapted treatment in AML. *CBFA2T3* protein has almost the same functional structure as *RUNX1T1*, a frequent fusion partner of *RUNX1*. *RUNX1::CBFA2T3* fusion mimics the characteristics of *RUNX1::RUNX1T1*, leading to a favorable prognosis[20]. However, *CBFA2T3::GLIS2*, a recurrent fusion in pediatric AML, correlates with a poor outcome[21]. Steinauer et al. found that the expression level of *CBFA2T3* can predict patient-specific outcomes in AML, which is congruent with our findings[11]. Somatic *SETBP1* mutations are related to inferior outcomes in myeloid malignancies [22–24]. Moreover, *INO80C*, the *SETBP1* fusion partner, also potentially impacts prognosis. *INO80C*, which regulates chromatin remodeling by interacting with transcription factor YY1, is part of the *YY1-EGR1-INO80C* network that can act as a prognostic biomarker of AML[25].

5. Conclusions

In summary, we report a case of AML bearing two previously undescribed fusion genes, *PIEZO1::CBFA2T3* and *INO80C::SETBP1*. RNA-Seq data and *in silico* analysis strongly support the leukemogenesis of these two novel fusion genes. The synergetic function of concurrent molecular lesions and their impact on patients' prognosis are worth exploring.

Declarations

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Competing Interests

The authors declare no conflict of interest.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent to participate

Informed consent was obtained from the patient's family member.

Consent to publish

Informed consent was obtained from the patient's family member for publication of this study.

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Figures

Figure 1

identification of PIEZO1::CBFA2T3 and INO80C::SETBP1 fusions in an AML patient.

(A) RNA-seq showed in-frame fusion transcripts of PIEZO1 exon 1 to CBFA2T3 exon 2 and schematic diagram of the predicted PIEZO1::CBFA2T3 fusion protein. The breakpoint is indicated by the red dash line. (B) RNA-seq showed in-frame fusion transcripts of INO80C exon 1 to SETBP1 exon 3 and schematic diagram of the predicted INO80C::SETBP1 fusion protein. The breakpoint is indicated by the red dash line (C) Box plot of the expression levels of CBFA2T3, SETBP1 and other related genes between the patient (left) and 50 healthy people (right) by differential analysis. TPM: Transcripts per Kilobase Million. (D) The most significantly dysregulated pathways that revealed by gene set enrichment analysis.