Myeloproliferative neoplasm with ETV6-ABL rearrangement conferred a granulocyte proliferation bias

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Abstract

Background: ETV6-ABL (TEL-ABL) fusion gene is a rare but recurrent genetic aberration found in hematologic malignancies including myeloproliferative neoplasm, acute myeloid leukemia and acute lymphoblastic leukemia. As a infrequent fusion gene, there is no special treatment guideline for the diseases it causes.

Methods: A case of a 42-year old man was reported, who presented fever with a markedly leukocytosis and CML-like marrow without BCR-ABL. Fusion gene was detected by RT-PCR and confirmed by direct sequencing. ETV6-ABL mRNA expressions in each cell population were determined by real-time RT-PCR in sorted peripheral blood cells.

Results: ETV6-ABL fusion gene was detected by RT-PCR in the bone marrow cells. The type A of ETV6-ABL was confirmed by direct sequencing. The ratio of ETV6-ABL fusion gene transcript level in polymorphonuclear was nearly 3.6 relative to total cells, which was significantly higher than other cells. His blood routine returned to normal after three months of treatment with imatinib at 400mg daily dose. After 6 months for TKI treatment, the ratio of ETV6-ABL/ABL decreased from 174.1% to 1.882%.

Conclusion: ETV6-ABL fusion gene conferred a granulocyte proliferation bias and responded to TKI therapy.

1. Background

Fusion genes resulting from chromosomal translocation play an important role in the occurrence and development of hematologic malignancies. T(9;22)/BCR-ABL is the first discovered chromosomal translocation and the characteristic of chronic myeloid leukemia (CML)[1]. ABL is located on chromosome 9q34. BCR is the most common of its multiple partner genes. Tyrosine kinase inhibitor, such as Imatinib, produced an excellent outcomes in patients suffered from CML [1]. BCR-ABL is a marker of malignant cloning, which was detected in granulocytes, erythrocytes, megakaryocytes and lymphocytes of CML patients.

ETV6-ABL (TEL-ABL) fusion gene is a rare but recurrent genetic aberration found in hematologic malignancies including myeloproliferative neoplasm (MPN), acute myeloid leukemia and acute
lymphoblastic leukemia. The existence of two different transcripts, type A and B, is an evidence for alternative splicing. Type A transcript includes the first four exons of ETV6, fused to exon 2 of ABL, while type B includes exons 1 to 5 of ETV6 fused to ABL exon 2.

We described a patient who had MPN with ETV6-ABL fusion gene. We identified the transcript subtype by PCR and sequence analysis, sorted different peripheral blood cell populations and explored the ETV6-ABL mRNA expression level in each proportion. This patient appeared to respond favorably to imatinib.

2. Case History
A 42-year old male came to our hospital had presented with leukocytosis for five months. He was admitted to the local hospital for dizziness and sudden deafness in December 2017, he went to local hospital three months later because of developing an unprovoked fever. Histopathology reported a myeloproliferative neoplasm suggestive for CML, but BCR-ABL/ABL-P210 was 0%. In order to seek further treatment, he came to our hospital in May 2018. Physical examination was unremarkable and without hepatosplenomegaly. The white blood cell (WBC) count was 48.2×10⁹/L (normal range, 4 to 10×10⁹/L) with 4% myelocyte, 9% metamyelocyte, 56% segmented, 12% band neutrophils, 6% monocytes, 6% lymphocytes, 2% eosinophils, and 5% basophils. Laboratory evaluation revealed hemoglobin at 109 g/L (normal range, 120 to 160 g/L), platelet at 424×10⁹/L (normal range, 100 to 300×10⁹/L) and LDH at 1511U/L (normal range, 0-247U/L). The bone marrow at diagnosis was markedly hypercellular with a marked granulocytic predominance and prominent eosinophilia. Megakaryocytes were markedly increased, atypical, and forming clusters. Fusion gene screening results showed that ETV6-ABL was positive. The karyotype was 46,XY,del(9)(q32)[20]. Based on the lab results, the patient was diagnosed with MPN with ETV6-ABL(+). His blood routine returned to normal after three months of treatment with imatinib at 400mg daily dose. It was worth mentioned that the copy number ratio of fusion gene decreased heavily after 6 months of TKI treatment, from 174.1% to 1.882%.

**Fig.1** Bone marrow aspirate smear (×100) revealed increased granulocytes with progressive maturation, increased eosinophils.
**Fig. 2** G-banded karyogram of bone marrow cells before treatment. The karyotype is 46,XY,del(9)(q32)[20]. Arrows indicate abnormal chromosomes.

3. Methods

**Sample processing**

Blood cells were separated into polymorphonuclear neutrophils and mononuclear fractions by standard density gradient centrifugation (Ficoll-Paque). Fluorescence-activated cell sorting was used to isolate monocytes (CD14+ cells), B cells (CD19+ cells), and T cells (CD3+ cells) from the mononuclear cell fraction[3].

**RNA isolation, reverse transcription, and multiplex-nested PCR**

Total RNA was isolated from the different blood cell populations by the using TRIzol reagent. cDNA was obtained by retrotranscription of 2ug of total RNA with oligo (dT) primer. The alternative splice variants (type A/B) were sequenced after multiplex-nested PCR.

**Quantification of ETV6-ABL fusion gene transcripts by real-time PCR**

Real-time PCR was performed to quantify ETV6-ABL fusion gene transcripts. Each quantitative RT-PCR was carried out in a 20-μl volume with TB Green Fast qPCR Mix, ROX Reference Dye II, appropriate primers and cDNA. The PCR cycled conditions listed in Table 1.

4. Results

**ETV6-ABL fusion gene type A transcripts was identified.**

Multiplex-nested PCR was performed to identify the type of ETV6-ABL fusion gene. To identify the translocation breakpoint, we sequenced the ETV6-ABL PCR product by Sanger sequencing analysis. A sequence data bank search showed that exon 4 of ETV6 was fused with ABL exon 2, confirmed the ETV6-ABL fusion transcript was type A (Fig.3).

**Fig. 3** Sequence analysis confirmed the fusion was ETV6-ABL type A.

**The distribution of ETV6-ABL fusion gene in hematopoietic cells.**
To investigate the distribution of ETV6-ABL fusion gene in hematopoietic cells, peripheral blood cells were obtained by venipuncture and separated into polymorphonuclear, monocytes, B cells, and T cells. The mRNA transcript levels of the ETV6-ABL in T cells, B cells, monocytes and polymorphonuclear were quantified by real-time RT-PCR.

As shown in Figure 4, the expression of ETV6-ABL in total cells is considered as control. Obviously, the ratio of ETV6-ABL in T cells is 0.02 relative to total cells. Similarly, the proportion of ETV6-ABL in B cells, monocytes and segmented granulocyte is 0.196, 0.005 and 3.599, respectively. This indicated that ETV6-ABL fusion gene conferred a granulocyte proliferation bias.

**Fig.4** ETV6-ABL mRNA expression in different cell populations

5. Discussion

In this patient, we identified the ETV6-ABL fusion gene appeared in his blood and identified the type A ETV6-ABL transcript by sequence analysis. The mRNA transcript levels of the ETV6-ABL were quantified in each hematopoietic lineage. We found that the level of ETV6-ABL transcript in polymorphonuclear was significantly higher than other cells. This indicated that ETV6-ABL fusion gene conferred a granulocyte proliferation bias.

Torsten Haferl et al[4]. reported that in CML patients with BCR-ABL, 34% of T lymphocytes and 32% of B lymphocytes could detecte the fusion gene. However, our study showed that in this patient, ETV6-ABL was mainly distributed in the polymorphonuclear cells, which seemed different from the distribution of BCR-ABL in CML patients.

Including our patient, we summarized 18 patients who had MPN with ETV6-ABL shown in Table 2. 12 patients received TKIs treatment and responses were found in all of them including three ones in blast phase with a transient response. It indicated that patients with ETV6-ABL fusion gene in chronic phase responded to TKIs favorably. Once disease progressed to blast phase, response to TKIs became limited. Similar to BCR-ABL, ETV6-ABL kinase could be targeted by TKIs. Currently, TKIs are standard treatment for CML. TKIs was also a treatment option for ETV6-ABL positive patients.
In summary, ETV6-ABL-positive MPN is a rare disease and responds to TKI. We suggested that all patients with CML-like features but lack of BCR-ABL fusion gene should be screened for ETV6-ABL and treated with TKIs if ETV6-ABL was positive. Similar cases deserve to be reported to allow further understanding of their molecular basis, disease history, and response to TKI therapy.

6. Conclusions
ETV6-ABL fusion gene conferred a granulocyte proliferation bias. MPN patients with ETV6-ABL positive had CML-like appearance and responded to TKIs therapy.

Declarations

Ethics approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki. Written informed consent for treatment and genetic testing was obtained from all patients.

Consent for publication
Written informed consent for publication was obtained from the patient.

Availability of data and materials
Not applicable

Competing interests
The authors declare that they have no competing interests.

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Authorship Contributions
Yuan Chen, Qian Liu and Hui Wei performed all experiments, analyzed data, and wrote the paper; Yan Li and Qing Rao assisted with data analysis; Hui Wei, Min Wang, and Jianxiang Wang designed the study.

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Not applicable

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Tables
Due to technical limitations, tables are only available as a download in the supplemental files section.

Figures

Figure 1

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Sequence analysis confirmed the fusion was ETV6-ABL type A.
Figure 4

ETV6-ABL mRNA expression in different cell populations
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Table 1.jpg