

The novel three-way variant t(6;17;15)(p21;q21;q22) in acute promyelocytic leukemia with an FLT3-ITD mutation: A case report

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Abstract. Acute promyelocytic leukemia (APL) is characterized by the reciprocal translocation t(15;17)(q22;q21), resulting in the fusion of the promyelocytic leukemia gene at 15q22 with the retinoic acid receptor α at 17q21. Additionally, all patients with APL who have additional chromosome abnormalities (ACA) and gene mutations are resistant to all-trans retinoic acid (ATRA), the drug that causes disease regression specifically in patients with APL globally. The present study describes a case of a 19-year-old female with APL carrying a novel complex variant translocation t(6;17;15)(p21;q21;q22), add(7)(q32) and an FMS-related tyrosine kinase 3 internal tandem duplication (FLT3-ITD) mutation. Complete remission was attained following a course of chemotherapy with ATRA and arsenic trioxide. To the best of our knowledge, this is the first report of a novel three-way translocation of 6p21 and a FLT3-ITD mutation involved with APL.

Introduction

Acute promyelocytic leukemia (APL) is defined as a M3 subtype, according to the French-American-British classification, and is characterized by the translocation t(15;17)(q22;q21) (1,2). The promyelocytic leukemia/retinoic acid receptor α (PML/RAR α) oncoprotein is formed following the specific chromosomal translocation t(15;17)(q22;q21), and is considered to be responsible for the arrest of granulopoiesis

by directly inhibiting the transcription of retinoic acid target genes (3,4). All-trans retinoic acid (ATRA) is the first drug to cause disease regression in patients with APL, and interacts with the ligand-binding domain present on the RAR α moiety of the chimeric oncoprotein. ATRA causes transcriptional activation, as well as proteolytic degradation, resulting in the granulocyte differentiation of APL cells (5).

Previous studies indicated that patients with additional chromosome abnormalities (ACA) and gene mutations, such as FMS-related tyrosine kinase 3 internal tandem duplication (FLT3-ITD) and PML/RAR α mutations, do not respond to conventional treatment regimens, such as ATRA (6,7); therefore, molecular and cytogenetic characterization of variant translocations and mutations of key genes are important for understanding the pathogenesis of the disease and predicting the response to the ATRA treatment. In the present study, the case of a patient with APL with FLT3-ITD harboring the novel three-way variant translocation t(6;17;15)(p21;q21;q22) and ACA add(7)(q32) was described.

Case report

A 19-year-old female was referred to the First Affiliated Hospital of Nanchang University (Nanchang, China) with a 1-month history of the common cold and leukocytopenia in August 2012. At admission, peripheral blood examination demonstrated a white blood cell count of $60.19 \times 10^9/l$ (normal range, $4-10 \times 10^9/l$), with 67% immature white blood cells (normal range, <0.01%), a hemoglobin concentration of 89 g/l (normal range, 110-150 g/l) and a platelet count of $33 \times 10^9/l$ (normal range, $100-300 \times 10^9/l$). This patient had disseminated intravascular coagulation (DIC) with 1.10 g/l (normal range, 1.8-3.5 g/l) fibrinogen and 54.2 mg/l (normal range, 0.01-0.55 mg/l) fibrinogen degradation product (D-dimer). A bone marrow smear was performed and the procedure indicated hyperplasia; it contained 84% abnormal promyelocytes, numerous cytoplasmic azurophilic granules, occasional Auer rods and a number of them were faggot cells (Fig. 1). Immunophenotyping demonstrated that the leukemic cells were positive for clusters of differentiation (CD)9, CD13, CD15, CD33, CD38, CD64, CD117, CD123 and myeloperoxidase, but

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negative for CD34, human leukocyte antigen-antigen D related, CD2, and CD56. Chromosome analysis using GTG-banding indicated a karyotype of 46,XX,t(6;17;15)(p21;q21;q22),add(7)(q32) [17]/46, XX [3] (Fig. 2). Dual-color fluorescence *in situ* hybridization was conducted to detect PML/RAR α fusion by a specific probe of PML and RAR α . The results demonstrated the novel complex variant translocation t(6;17;15) (Fig. 3). Total RNA of the bone marrow were extracted by TRIzol[®] reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and reverse transcribed to complementary DNA (cDNA) with a QuantScript RT kit (cat. no. KR103; Tiangen Biotech Co., Ltd., Beijing, China), according to the manufacturer's protocols. The following polymerase chain reaction (PCR) with the specific primers (forward, 5'-CCGTCATAGGAA GTGAGGTCT-3', and reverse, 5'-GGCTGGGCACTATCT CTTCA-3') indicated long and short PML/RAR α transcripts, demonstrating the L-type PML/RAR α (data not shown) in the patient. Further molecular studies indicated the presence of an FLT3-ITD mutation. The genomic DNA of bone marrow extracted with a TIANamp Blood DNA kit (Tiangen Biotech Co., Ltd.), and PCR were detected at pre-denatured at 95°C for 5 min, followed by 30 cycles of denaturing at 95°C for 10 sec and annealing and elongation at 55°C for 20 sec and elongation at 72°C for 20 sec using the ABI2720 Thermal Cycler (Applied Biosystems; Thermo Fisher Scientific, Inc.) with specific primers: Forward, 5'-GCAATTTAGGTATGA AAGCCAGC-3', and reverse, 5'-CTTTCAGCATTTTGACGG CAACC-3'. The PCR products were separated by 3% agarose gel electrophoresis and analyzed using a gel imager (Peiqing Science & Technology Inc.) (Fig. 4). According to MICM classification, the patient was diagnosed with high risk APL (8).

The patient was then treated with ATRA combined with arsenic trioxide (ATO). Subsequently, differentiation of APL cells was morphologically observed and DIC improved immediately. Re-examination of the bone marrow smear with Wright-Giemsa staining [10 μ l bone marrow sample was spread on a slide to produce a smear and was dried at room temperature for 1 h. Each smear was stained in Wright-Giemsa Stain for 10 min at room temperature, and then they were rinsed with water. Following air-drying, the smear was inspected under a light microscope (Olympus BX51; Olympus Corporation, Tokyo, Japan; x1,000 magnification)] and immunophenotyping [100 μ l bone marrow sample incubated with antibodies CD9, CD33, CD13, CD117 and CD34 for 15 min in dark room, centrifuged at 200 x g at room temperature for 5 min following adding 200 μ l OptiLyse C Lysing solution (Beckman Coulter, Inc., Brea, CA, USA) for 5 min, and then detected using CYTOMICS FC500 (Beckman Coulter, Inc.)] after 1 month revealed complete remission. The patient received several courses of consolidation therapy and the development of illness was monitored by detecting the PML/RAR α chimeric transcript with reverse transcription-quantitative PCR (RT-qPCR). [For RT-qPCR, patient bone marrow RNA was reverse transcribed to cDNA as aforementioned and PML/RAR α transcript was detected with primers: Forward, 5'-GCAATTTAG GTATGAAAGCCAGC-3', and reverse, 5'-CTTTCAGCATTT TGACGGCAACC-3'; and fluorescein amidite (FAM)-labeled probe (Sangon Biotech Co., Ltd., Shanghai, China): 5'-CTG CTCTGGGTCTCAATGGCTGCCTCC-3'; ABL was used as the reference gene and detected with the primers: Forward,

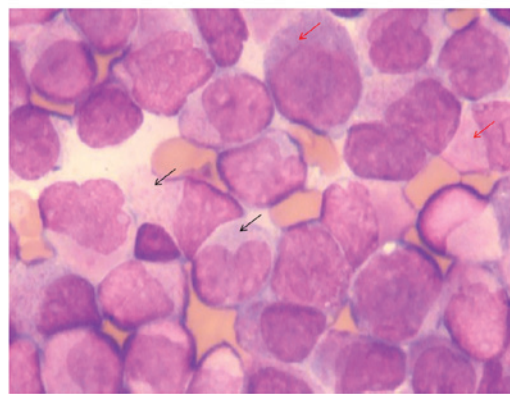


Figure 1. Bone marrow smear exhibiting abnormal promyelocytes with small cytoplasmic azurophilic granules (black arrow) and a number of Auerrods (red arrow).

5'-TCCATCTCGCTGAGATACGAAG-3', and reverse, 5'-ATG ATGAACCAACTCGGCCA-3'; and VIC-labeled probe 5'CAA CACTGCTTCTGATGGCAAGCTCTACG3'. RT-qPCR were detected at 50°C for 2 min and pre-denatured at 95°C for 3 min, followed by 40 cycles of denaturing at 95°C for 5 sec and annealing and elongation at 58°C for 30 sec using the ABI 7500 Real-time PCR system and the data were collected and analyzed with ABI 7500 software v2.3 (Applied Biosystems; Thermo Fisher Scientific, Inc.). The relative quantitation of PML/RAR α =(copiesPML/RAR α /copiesABL) x100% (9)] (Fig. 5). The patient had no recurrences within 5 years of treatment.

Discussion

To the best of our knowledge, the present case report is the first to describe an APL case containing a FLT3-ITD mutation with the novel three-way variant translocation t(6;17;15)(p21;q22;q21). The fusion gene PML/RAR α is observed in the majority of APL cases (10), whilst only a limited numbers of patients harbored complex variant translocations (11). Although no research has indicated an association between chromosome 6 and PML/RAR α , genes located on chromosome 6, such as tumor protein p53 pathway corepressor 1, NOTCH4 and B-cell lymphoma-2 antagonist/kill 1, are involved in the genesis and development of leukemia (12-15). FLT3 encodes a tyrosine kinase III receptor involved in hematopoietic malignancy types, and is associated with a poor prognosis and high rate of relapse (16-21); therefore, complex fusion and FLT3-ITD positive mutations are considered adverse prognostic factors. Conversely, in the present report, a patient with a poor prognosis successfully entered remission.

In this case, the age of onset was 20 years, which may have contributed to the patient's good prognosis. A recent study investigated the characteristics and outcomes of patients with acute myeloid leukemia treated in the Department of Leukemia of the MD Anderson Cancer Center (Houston, TX, USA) from 1965 to 2009 and determined that younger patients (<65 years old) have improved outcomes, compared with older patients (\geq 65 years old) (22). The patient received ATO during their hospitalization. Research conducted in 2011 indicated that ATRA combined with anthracycline chemotherapy was unable to ameliorate the



Figure 2. A G-banding karyotype of a bone marrow cell exhibiting 46, XX, t(6;17;15)(p21;q21;q22), add(7)(q32). Red arrows indicate the derivative chromosome of t(6;17;15)(p21;q21;q22); the red arrows indicate the derivative chromosome of add(7)(q32).

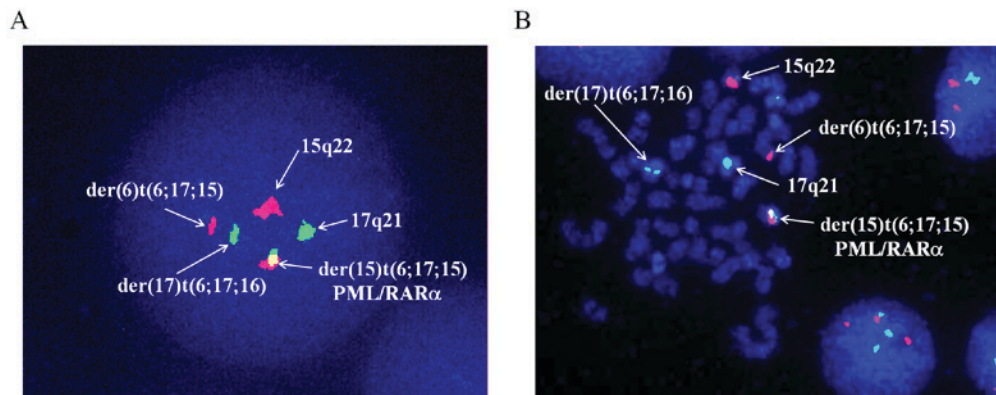


Figure 3. Dual-color fluorescence *in situ* hybridization analysis with PML/RAR α -specific probes 15q22 (red) and 17q21 (green) exhibiting a fusion signal in the acute promyelocytic leukemia cells of the patient. The images represent cells in (A) interphase and (B) metaphase. PML/RAR α , promyelocytic leukemia/retinoic acid receptor α ; der, derived chromosome.

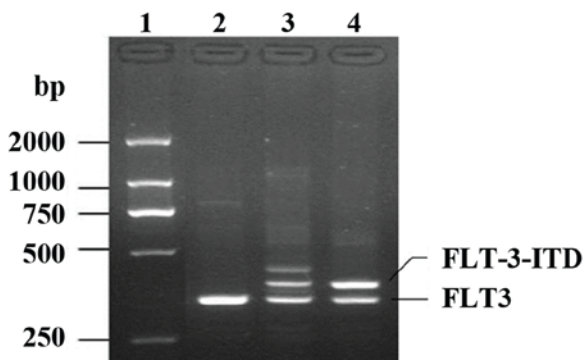


Figure 4. Detection of the FLT3-ITD mutation using the semi-quantitative polymerase chain reaction method. 1, DNA marker; 2, normal control; 3, positive control; 4, FLT3-ITD in the patient; FLT3-ITD, FMS-related tyrosine kinase 3 internal tandem duplication.

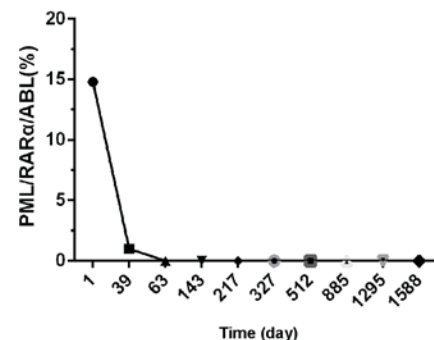


Figure 5. Dynamic monitoring of PML/RAR α expression in the patient undergoing therapy, via reverse transcription-quantitative polymerase chain reaction. ABL was used as the reference gene. PML/RAR α , promyelocytic leukemia/retinoic acid receptor α . PML/RAR α /ABL, the ratio of PML/RAR α to ABL.

progression of the patient with an FLT3-ITD positive mutation (23); however, previous studies have indicated that ATO may abrogate the unfavorable impact of FLT-ITD (6,24), accounting for an improved outcome for the patient. Additionally, disease compliance is critical for prognosis, which has been demonstrated in other diseases, including osteosarcoma, breast cancer and tumor associated neutrophils (25,26). Upon discharge, the patient revisited the hospital once a month for the first three months and every three months for the following nine months for follow-up. Subsequently, they revisited the hospital once a year until April 2018. The treatment of leukemia is multi-factorial and involves complex mechanisms, and it is considered that the effect of multiple factors is synergistic.

In conclusion, a patient with high-risk APL containing FLT3-ITD with the novel three-way variant translocation t(6;17;15)(p21;q21;q22) had favorable outcomes. The present case provides support for the hypothesis that treatment with ATRA and ATO results in an ameliorative effect for the outcome of a patient with an FLT-ITD mutation who did not respond to conventional treatments.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

ZLZ conceived and designed the study. YLZ, MJ and JHW performed the experiments. YLZ and ZLZ wrote the paper. SYL, SQL and LGW interpreted the data and provided final approval of the version to be published. All authors read and approved the manuscript.

Ethics approval and consent to participate

The patient provided written informed consent for the publication of this study and the study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Nanchang University (Nanchang, China).

Patient consent for publication

The patient provided written informed consent for the publication of this study.

Competing interests

The authors declare that they have no competing interests.

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