SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 12: 4717-4719, 2016

A complex translocation (3;17;15) in acute promyelocytic leukemia confirmed by fluorescence *in situ* hybridization

YANMING WANG[1,2], JUNJIE MA[2], XINGUANG LIU[1], RIMING LIU[2], LINGLING XU[2], LI WANG[2], JIANNONG CEN[3] and XIAOXIA CHU[2]

[1]Department of Hematology, Qilu Hospital of Shandong University, Jinan, Shandong 250012; [2]Department of Hematology, Yantai Yuhuangding Hospital, Yantai, Shandong 264000; [3]Jiangsu Institute of Hematology, Collaborative Innovation Center of Hematology, Key laboratory of Thrombosis and Hemostasis of Ministry of Health, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006, P.R. China

Received April 18, 2015; Accepted July 28, 2016

DOI: 10.3892/ol.2016.5280

Abstract. Acute promyelocytic leukemia (APL) is typified by t(15;17)(q22;q21), generating the promyelocytic leukemia (PML) gene at 15q22 with the retinoic acid α-receptor (RARA) gene at 17q21. The PML-RARA fusion gene is believed to play a vital role in leukemogenesis. A sizeable minority of patients with complex variants of APL have been reported. The present study reports the case of a 33-year-old male with APL carrying a potential complex translocation. The initial symptom was bleeding gums. Chromosomal analysis of the bone marrow cells revealed an atypical 17q aberration. Fluorescence *in situ* hybridization further indicated that 92% of analyzed cells were positive for the PML-RARA fusion gene. The patient experienced complete remission following treatment with arsenic trioxide and chemotherapy. The atypical translocations in acute promyelocytic leukemia require further investigation.

Introduction

Acute promyelocytic leukemia (APL), a distinct subtype of acute myeloid leukemia, is defined by a specific balanced translocation, t(15;17), leading to the fusion of the promyelocytic leukemia (PML) and retinoic acid receptor-α (RARA) genes (1,2). The PML-RARA gene is supposed to play a vital role in the pathophysiological process of APL (3), and patients with the fusion gene could benefit from treatment with all-trans retinoic acid (ATRA) and AS_2O_3.

As supported by fluorescence *in situ* hybridization (FISH) assessment, complex variant translocations of 15;17 have been increasingly reported in APL, while the majority of complex variants of APL have revealed three-way translocations (4,5). The current study presents the fifth three-way translocation involving chromosomes 3;17;15 (6-8), which may be the second breakpoint involving the long arm of chromosome 3 reported thus far. Written informed consent was obtained from the patient for the publication of this study.

Case report

A 33-year-old male with no significant previous medical history was admitted to Yantai Yuhuangding Hospital (Yantai, Shandong, China) in July 2014 due to bleeding gums. A peripheral blood examination showed the following: Hemoglobin, 10.2 g/dl (normal, 13.0-17.5 g/dl); white blood cell count, 2.14×10^9/l (normal, $3.5\text{-}9.5 \times 10^9$/l), with 46% atypical promyelocytes packed with numerous azurophilic granules; and platelet count, 28×10^9/l (normal, $125\text{-}350 \times 10^9$/l). Coagulation tests revealed a prothrombin time of 19.4 sec (normal, 15 sec), an activated partial thromboplastin time of 41.8 sec (normal, 40 sec) and a fibrinogen level of 67 mg/dl. Bone marrow was markedly hypercellular, with 84.5% atypical promyelocytes. The promyelocytes were stained by allophycocyanin (APC)-conjugated cluster of differentiation (CD)33 (dilution, 1:2; catalog no. 340474), phycoerythrin (PE)-conjugated CD123 (dilution, 1:10; catalog no. 340545), APC-conjugated CD38 (dilution, 1:2; catalog no. 345807), PE-conjugated CD13 (dilution, 1:5; catalog no. 347837) and PerCP-conjugated CD45 (dilution, 1:5; catalog no. 347464). All of the antibodies were monoclonal, composed of mouse immunoglobulin G1 heavy chains and κ light chains, diluted with phosphate-buffered saline and purchased from BD Biosciences (Franklin Lakes, NJ, USA). The immunophenotype was $CD33^+$, $CD123^+$, $CD117^+$, $CD38^+$ and $CD13^+$. The PML-RARA (L-form) rearrangement was confirmed by reverse transcription polymerase chain reaction (RT-PCR), and a diagnosis of APL [M3 in the French-American-British classification (9)] accompanying disseminated intravascular coagulation (DIC) was formed. Due to ATRA (20 mg/m^2/day) intolerance, the patient started induction therapy with arsenic trioxide alone at a daily dose of 10 mg for 35 days, to induce remission through PML/RARA degradation, and cryoprecipitate transfusion to

Correspondence to: Dr Xiaoxia Chu, Department of Hematology, Yantai Yuhuangding Hospital, 20 Yuhuangding East Road, Yantai, Shandong 264000, P.R. China
E-mail: lucychu66@163.com

Key words: complex translocation, t(3,15,17), acute promyelocytic leukemia

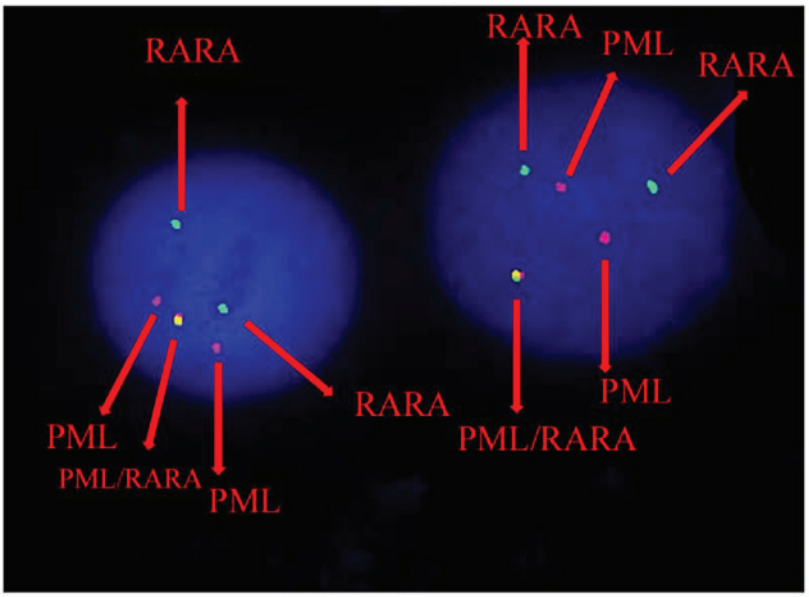

Figure 1. Fluorescence *in situ* hybridization analysis. Interphase cells demonstrating one PML/RARA fusion signal, two pink signals (PML), and two green signals (RARA), constistent with the karyogram der(3)t(3;17;15)(q25;q21);q24),der(15)t(3;17;15),der(17)der(17)(q11q12).

control the DIC (accumulated dose, 83 units). At 15 days after therapy initiation, the atypical promyelocytes started to differentiate. However, 27 days after therapy, the white blood cells reached a level of $144x10^9$/l with no evidence of APL differentiation syndrome. Coinciding with this, symptoms of high cerebrospinal fluid pressure emerged. The patient subsequently received daunorubicin (20 mg for 3 days) and dexamethasone (10 mg for 3 days) treatment, as well as intrathecal methotrexate (12 mg), following which the white blood cell counts gradually decreased to normal. Molecular remission was achieved 10 days after the administration of the chemotherapy. The treatment was continued with 3 courses of medial-dose cytosine arabinoside. The patient currently remains in complete remission.

Karyotypic analysis and FISH. Cytogenetic analysis was performed on Giemsa-banded chromosome preparations. The karyotype was presented according to the 2005 International System for Human Cytogenetic Nomenclature (10). As the metaphases from abnormal cells were often of poor quality and resolution, it was difficult to cytogenetically identify the characteristic PML/RARA fusion gene. FISH was performed using the Vysis dual-color, dual-fusion probe (Abbott Molecular, Des Plaines, IL, USA) following the manufacturer's instructions. Fluorescent signals were visualized and images were captured using a fluorescence microscope. The FISH analysis revealed the 46,XY,der(3)t(3;17;15)(q25;q21;q24), der(15)t(3;17;15), der(17)der(17)(q11q12) karyotype (Fig. 1).

RT-PCR analysis. Total RNA was extracted from mononuclear cells in a bone marrow sample obtained from the patient using TRIzol reagent (Gibco; Thermo Fisher Scientific, Inc.), and reversed transcription was performed. PCR for the PML-RARA fusion gene was performed as previously described (6). An initial denaturation at 94˚C for 5 min was followed by 40 cycles at 94˚C for 15 sec and 60˚C for 60 sec. The sequences of the PCR primers were as follows: PML-RARA forward, 5'-CCGTCATAGGAAGTGAGGTCT-3' and reverse, 5'-GGCTGGGCACTATCTCTTCA-3'; and GAPDH forward, 5'-TGGAGATAACACTCTAAGCATAACTAAAGGT-3' and reverse, 5'-TGGAGATAACACTCTAAGCATAACTAAAGGT-3'. The results demonstrated that only the L-type chimeric transcription was expressed, while the RARA/PML was not expressed.

Discussion

To date, >35 cases with three-way complex translocations have been reported (6-8,11-30). Among these translocations, 9 recurrent breakpoints have been identified, including 2q21 (5,14,26), 19p13 (5,24), 3p21 (7,8), 4q21 (13,15), 11q13 (18,21), 1p36 (16,19,22), 20p13 (23,31), 6p21 (27) and Xq13 (11,25). As a supplement, the present study reported another case of an APL patient harboring a three-way translocation involving chromosomes 3;17;15. This case provided a similar survival outcome of this complex translocation compared with the previous 4 cases (6,8).

According to FISH analysis, the complex translocation can be assessed as follows: 15q24-qter translocated to 3q25; a small piece of chromosome 17 (17q11-q22) to 15q24; and 3q25-qter connected to 17q22 that has been located in der(15)(q24).

To date, 4 cases of three-way translocation regarding t(3;17;15) have been reported (6,8). However, the breakpoint in the present case occurred in a novel area of the long arm of chromosome 3. The previous 3 cases harboring 3;17;15, reported in 2009, 1980 and 1994 (6-8) reported survival times of 6 days, 33 months and 14 months, respectively. In the present study, the patient presented with the typical morphological and clinical features of APL, and exhibited a good response to (arsenic trioxide) and chemotherapy treatment, as observed in typical APL. Comigrating RARA/PML rearrangements were present in 2 cases from previous reports (8), which differed from the present case. On the basis of RT-PCR analysis, there were only PML-RARA fusion transcripts and the RARA/PML fusion

gene was not expressed in this case. This was inconsistent with the opinion that the majority of cases of APL lacking the t(15;17) are still associated with the formation of the RARA/PML fusion gene (27).

The poor prognosis of the previously reported 3 cases with the three-way t(3;17;15) appears to be associated with early mortality, which may due to the absence of targeted therapy and the comigrating RARA/PML rearrangements. The inference is that the involvement of the 3q25 region has no clear effect on disease outcome, further supporting the aforementioned notions. It remains unclear if the aberration can affect clinical consequences. A close clinical follow-up will be important in the present patient and in other cases of APL with a three-way t(3;17;15).

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