ELSEVIER

Cancer Genetics and Cytogenetics 184 (2008) 44–47

Cancer Genetics and Cytogenetics

Short communication

A new complex translocation t(5;17;15)(q11;q21;q22) in acute promyelocytic leukemia

Shori Abe[a,*], Idumi Ishikawa[b], Hideo Harigae[a], Tomohiro Sugawara[b]

[a]*Department of Rheumatology and Hematology, Tohoku University School of Medicine, 1–1 Seiryo-machi, Aobaku, Sendai, Japan*
[b]*Department of Internal Medicine, Osaki City Hospital, 2-3-10 Senjuji-machi, Furukawa, Osaki, Japan*

Received 14 February 2008; received in revised form 23 February 2008; accepted 3 March 2008

Abstract Acute promyelocytic leukemia (APL) is associated with the t(15;17)(q22;q21) translocation which causes the fusion of the retinoic acid alpha gene (*RARA*) on 17q21 to the promyelocytic leukemia gene (*PML*) on 15q22. The two chimeric genes, *PML/RARA* and *RARA/PML,* are thought to play a role in leukemogenesis. A small proportion of patients with APL have complex or simple variants of this translocation. We report the case of a 22-year-old woman with APL carrying a complex variant translocation t(5;17;15)(q11;q12;q22) confirmed by G-banding, reverse transcription polymerase chain reaction (RT-PCR), fluorescence in situ hybridization(FISH), and spectral karyotyping analysis (SKY). The patient achieved complete remission with all-trans retinoic acid treatment and chemotherapy. These results illustrate the usefulness of combined analysis consisting of G-banding, RT-PCR, FISH, and SKY methods to identify the *PML/RARA* fusion gene in cases with variant t(15;17).

1. Introduction

Acute promyelocytic leukemia is characterized by the specific chromosomal rearrangement t(15;17)(q22;q21) [1]. This translocation results in fusion of the retinoic acid alpha gene (*RARA*) on 17q21 to the promyelocytic leukemia gene (*PML*) on 15q22 [2,3]. The resultant *PML/RARA* fusion protein is supposed to have a fundamental role in leukemogenesis of APL [4].

A small number of APL cases lacking t(15;17) has been described, including three simple variant translocations, *ZBTB16/RARA* (previously *ZNF145/RARA*; alias *PLZF/RARA*) fusion in t(11;17)(q23;q21) [5], *NUMA1/RARA* (alias *NUMA/RARA*) fusion in t(11;17)(q13;q21) [6], and *NPM1/RARA* (alias *NPM/RARA*) in t(5;17)(q32;q21) [7]. Complex variant translocations, especially three-way translocations, are increasingly recognized [8–30]. Here we present a case of APL with a three-way complex variant of t(15;17) involving chromosome 5.

2. Materials and methods

2.1. Chromosomal analysis

Chromosomes were prepared from 24-hour unstimulated bone marrow culture. Cells were exposed to Colcemid for 1 hour and followed by hypotonic treatment for 40 minutes. A fixation procedure with ethanol and acetic acid (3:1) was performed. Chromosomes were analyzed with G-banded metaphase and identified according to ISCN 1995 [31].

2.2. Reverse transcription polymerase chain reaction analysis

Total cellular RNA was isolated from bone marrow mononuclear cells using a modified one-step acid guanidinium thiocyanate–phenol–chloroform method [32]. The reverse transcription reaction was performed on 1 μg of total RNA using random hexamer (Promega, Madison, WI) with SuperScript II RNAse-H reverse transcriptase (Gibco Laboratories, Grand Island, NY). Reverse transcriptase–polymerase chain reaction (RT-PCR) for the detection for *PML-RARA* fusion gene was performed as previously described [33].

* Corresponding author. Tel.: +81-22-717-7165; fax: +81-22-717-7168.
E-mail address: uhouho@snh.go.jp (S. Abe).

0165-4608/08/$ – see front matter
doi:10.1016/j.cancergencyto.2008.03.006

2.3. Fluorescence in situ hybridization

Cells kept in fixative at −20°C were used to prepare spreads. Fluorescence in situ hybridization (FISH) studies were performed using the a Vysis dual-color, dual-fusion probe for *PML/RARA* (Abbott Molecular, Des Plaines, IL) following the manufacturer's instructions. The chromosomes were identified by 4′,6-diamidino-2-phenylindole (DAPI) stain. Fluorescent signals were visualized and photographed with a Nikon microscope (Tokyo, Japan).

2.4. Spectral karyotyping

Slides for spectral karyotyping (SKY) analysis were prepared by using the fixed chromosome preparations as for G-banding analysis. SKY was performed according to the manufacturer's instructions, using the ASI SKY paint probe mixture (Applied Spectral Imaging, Carlsbad, CA). Briefly, the metaphase slides were treated with pepsin for 5 minutes at 37°C, fixed 1% formalin−PBS−$MgCl_2$ for 10 minutes at 37°C, and dehydrated through a graded 70, 80, and 100% ethanol sequence. Slides were then denatured in 70% formamide-2× SSC for 5 minutes at 72°C, and hybridized with denatured SKY probes for 48 hours at 37°C. Following this treatment, the slides were washed and stained with DAPI and analyzed using the SkyView imaging software (ASI).

3. Results and discussion

3.1. Case report

A 22-year-old woman was admitted to Osaki City Hospital because of gum bleeding in October 2006. She showed purpura in the upper and lower extremities. Peripheral blood examination showed pancytopenia (hemoglobin 10.5 g/dL, platelets 7×10^9/L, white blood cells 0.8×10^9/L with myeloblasts and promyelocytes). Coagulation tests revealed normal prothrombin time of 12.7 seconds (control, 13.2 seconds), prolonged an activated partial thromboplastin time of 34.1 seconds (control, 29.8 seconds), decreased fibrinogen (96 mg/dL), and increased fibrinogen degradation products (130 μg/mL). The bone marrow aspirate showed hypercellular marrow with 92% of promyelocytes with regular nuclei, many granules, mostly absence of Auer rods, an increased number of pseudo-Pelger-Hüet cells and strong myeloperoxidase activity. In an immunophenotypic analysis, the cells were positive for CD13 (82%), CD33 (98%), CD2 (38%), but negative for CD14, HLA-DR, and other lymphoid cell antigens. A diagnosis of APL, FAB M3v, was made according to the above data. The patient was successfully treated with all-trans retinoic acid (ATRA) and anthracycline-based chemotherapy and remained in complete remission after 12 months.

3.2. Cytogenetic analysis

The karyotype was interpreted as 46,XX,t(5;17;15)(q11;q21;q22)[8]/46,XX[12] (Fig. 1). By RT-PCR analysis, the *PML/RARA* transcript of the bcr2 subtype (long form) was detected (data not shown). *PML/RARA* fusion signal was detected on der(15) and *PML* signals on der(5) and *RARA* signals on der(17) by FISH analysis (Fig. 2). SKY analysis, performed to define the translocation, confirmed that the der(5) results from a translocation between chromosomes 5 and 15, the der(15) results from a translocation between chromosomes 15 and 17, and the der(17) results from a translocation between chromosomes 17 and 5 (Fig. 3).

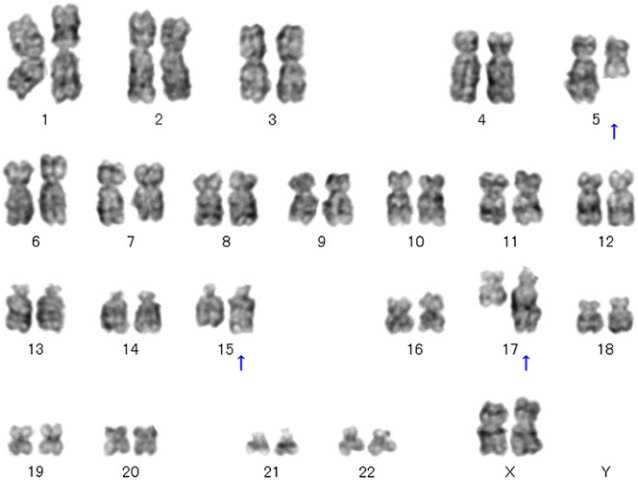

Fig. 1. G-banded metaphase cell of the bone marrow. A conventional karyotypic analysis of G-banding was performed and revealed chromosomal abnormality: 46,XX,t(5;17;15)(q11;q21;q22).

Complex variant translocations have been increasingly reported in APL. At least 33 cases with three-way complex

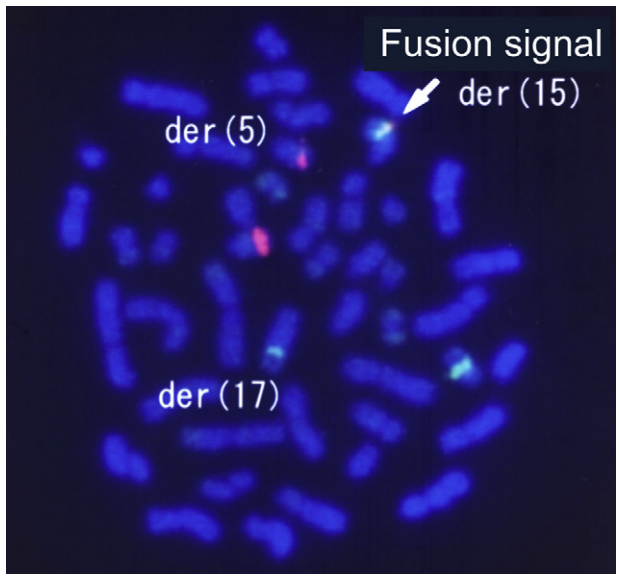

Fig. 2. Fluorescence in situ hybridization analysis. FISH analysis was performed as described in materials and methods. A *PML/RARA* fusion signal (yellow) was detected on der(15) and *PML* signals (red) on der(5) and *RARA* signals (green) on der(17).