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FISH-negative cryptic *PML–RARA* rearrangement detected by long-distance polymerase chain reaction and sequencing analyses: a case study and review of the literature

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Abstract Although a normal karyotype according to conventional cytogenetic analysis in association with cryptic t(15;17) has been infrequently reported in cases of acute promyelocytic leukemia (APL), a fluorescence in situ hybridization (FISH)-negative cryptic *PML–RARA* rearrangement is even more rare, with only 12 such APL cases of FISH-negative cryptic *PML–RARA* rearrangements in the literature. Reported here is an additional clinical APL case with a FISH-negative cryptic *PML–RARA* rearrangement, confirmed by long-distance DNA polymerase chain reaction method. Discussion includes a relevant literature review of similar cases. DNA-PCR can be a useful tool for the analysis of complex and cryptic rearrangements. © 2010 Elsevier Inc. All rights reserved.

1. Introduction

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) that is characterized by a unique morphology of predominant promyelocytes and a specific chromosomal rearrangement, t(15;17)(q22;q12), which is present in the majority of APL cases [1–3]. In a minority of APL cases, the t(15;17) is not detected through conventional cytogenetic analysis. Such cases, usually called cryptic or masked t(15;17), are known to occur through submicroscopic insertions of *PML* or *RARA*, or through more complex rearrangements [4,5]. These cryptic t(15;17) rearrangements can be detected by molecular methods, including fluorescence in situ hybridization (FISH), reverse transcriptase–polymerase chain reaction (RT-PCR), and sequencing analysis. The FISH approach is able to detect nearly all of the *PML–RARA* fusion signal of cryptic t(15;17), owing to the high resolution power of the method [5]. Nonetheless, in several recent reports the *PML–RARA* fusion genes were detected only by RT-PCR or sequencing analysis [6–14]. Here, we report a rare case of APL with cryptic *PML–RARA* rearrangement which was negative with both *PML–RARA* FISH and conventional karyotyping approaches, but positive with RT-PCR, sequencing, and long-distance PCR. We also present a brief review of the literature for APL with FISH-negative cryptic *PML–RARA* rearrangement.

2. Materials and methods

2.1. Case description

An 18-year-old Korean boy who had previously been in good health was brought to our hospital for evaluation of hematuria and hematochezia. The initial complete blood count showed a hemoglobin level of 9.5 g/dL (reference

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range, 12–16 g/dL), a platelet count of 37×10^9/L (reference range, 150–350 $\times 10^9$/L), and a white blood cell count of 16.5×10^9/L (reference range, 4–10 $\times 10^9$/L) with 7% segmented neutrophils, 4% lymphocytes, 5% monocytes, and 84% blasts and promyelocytes. The bone marrow aspirate showed hypercellular marrow replaced by blasts and promyelocytes with intense azurophilic granule and prominent nucleoli accounting for 94% of all nucleated cells. Flow cytometric analysis showed the blasts to be positive for CD13, CD33, CD45, and CD117 and negative for HLA-DR and CD34. The patient was diagnosed with APL. For further evaluation, chromosome, FISH, and multiplex RT-PCR with Hemavision kits (DNA Technology, Aarhus, Denmark) analyses were conducted, as well as long-distance DNA-PCR. The patient was then treated with all-trans retinoic acid (ATRA) and responded well.

2.2. Chromosome and FISH analyses

Chromosomes were prepared from 24-hour unstimulated bone marrow culture. Chromosomes were analyzed with GTG-banding, and the karyotypes were described according to ISCN 2009 [15].

The FISH analysis was performed according to the manufacturer's instructions with a Vysis PML–RARA dual-color, dual-fusion translocation FISH probe (Abbott Molecular, Des Plaines, IL). Under fluorescence microscopy, 500 interphase cells were scored for signal patterns. Metaphase FISH analysis was also performed, and representative cell images were captured using a computer-based imaging system.

2.3. RT-PCR, cloning, and sequencing

A multiplex gene rearrangement test was performed with a Hemavision kit (DNA Technology) according to the manufacturer's instructions. The kit is designed to detect 28 multiple rearrangements simultaneously, including more than 80 breakpoints or mRNA splice variants. The PCR analysis was performed in accordance with the manufacturer's guidelines (DNA Technology). The presence of a specific band in the first PCR (master PCR) prompted a split-out PCR with the corresponding set of split-out primers. Cloning and sequencing of the PCR product was performed in order to confirm the specific gene rearrangement. Sequence analysis was done using the Ensembl database (http://www.ensembl.org/index.html).

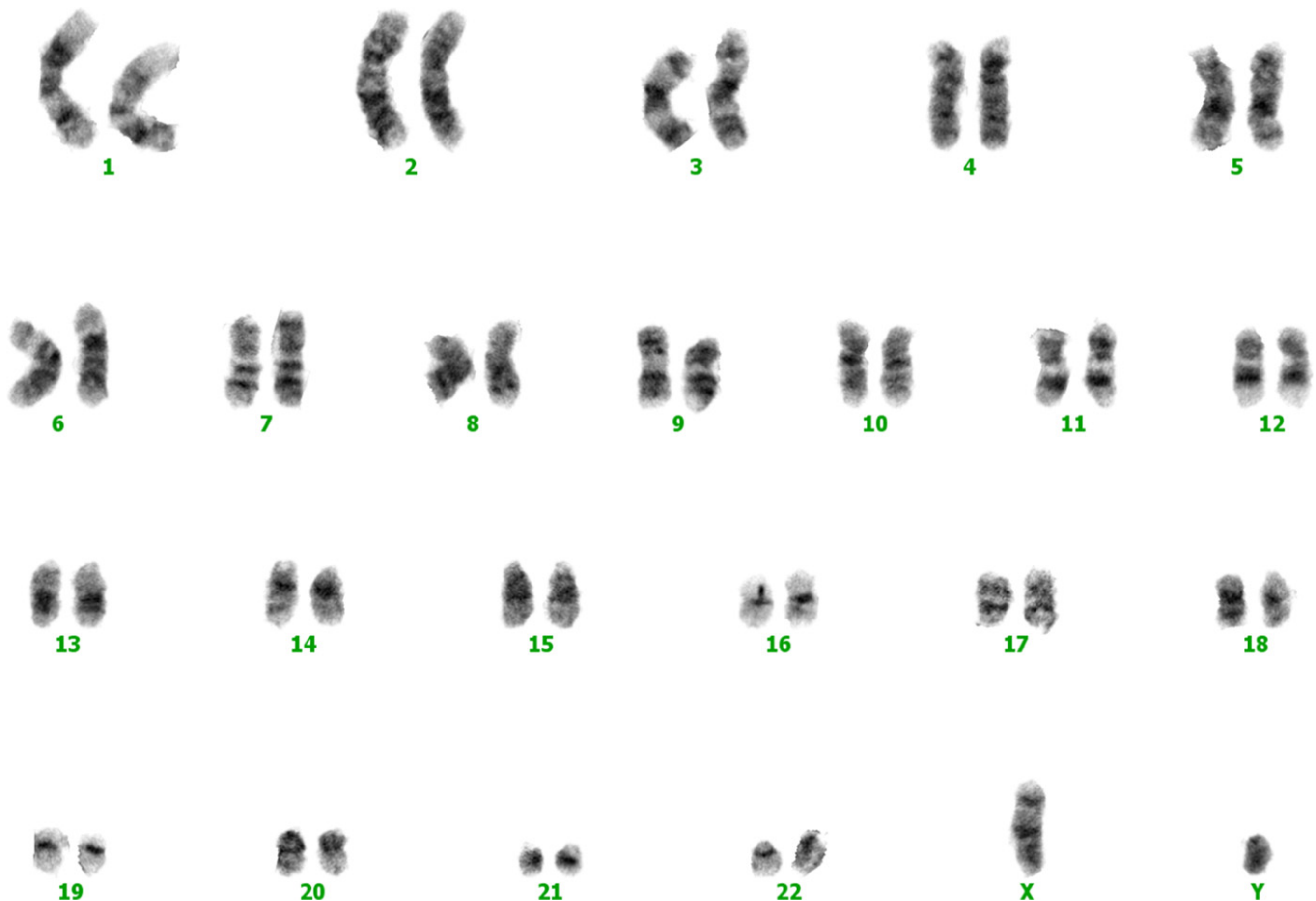

Fig. 1. Giemsa-banding karyogram of the bone marrow cells at diagnosis indicates the karyotype 46,XY.

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