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Value of Oligonucleotide-Based Array Comparative Genomic Hybridization for Diagnosis of Acute Promyelocytic Leukemia in a Patient Negative for t(15;17)(q24.1;q21.2)/Promyelocytic Leukemia-Retinoic Acid Receptor, Alpha by Conventional Cytogenetics and Fluorescence In Situ Hybridization

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APL; FISH; POD stain; RT-PCR; tCGH array

Introduction

Acute promyelocytic leukemia (APL) is characterized by promyelocytic leukemia (*PML*)-retinoic acid receptor, alpha (*RARA*) fusion gene as a result of t(15;17)(q24.1;q21.2).¹ Current advances in APL therapy have dramatically improved patient outcome.^{2,3} However, patients with APL are at risk of potentially lethal coagulopathy if appropriate therapy is delayed.⁴

The t(15;17)(q24.1;q21.2) is detected using conventional cytogenetic analysis in approximately 90% of APL cases.^{5,6} A small subset of APL cases (4%) lack the classical t(15;17)(q24.1;q21.2) and have other complex translocations.^{1,2} Rare APL cases have submicroscopic insertions of *PML* or *RARA*, in which reverse transcription (RT) polymerase

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Disclosure

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chain reaction (PCR) detects *PML-RARA* transcripts and yet conventional cytogenetics and fluorescence in situ hybridization (FISH) results are negative.^{1,5,7-9}

Case Report

A 57-year-old Hispanic woman was transferred to our institution with cough, fatigue, and blood in her sputum. Laboratory studies showed: hemoglobin 112 g/L, white blood count $14.5 \times 10^9/L$, platelets $33 \times 10^9/L$, D-dimer $> 20.00 \mu g/mL$, fibrinogen 153 mg/dL, lactate dehydrogenase 1415 IU/L. A differential count of a peripheral blood smear showed 58% abnormal promyelocytes and 1% blasts. The abnormal promyelocytes had mostly bilobed nuclei and hypogranulated cytoplasm consistent with the microgranular variant. all-trans retinoic acid therapy was initiated.

Bone marrow (BM) examination was performed 4 days after the initiation of ATRA and showed a markedly hypercellular BM with most of the nucleated elements being promyelocytes (Figure 1A and B). Cytochemical staining showed that the promyelocytes were uniformly and strongly positive for myeloperoxidase. Anti-PML antibody assessed using immunofluorescence methods as previously described¹⁰ showed a finely, dispersed, and microgranular pattern consistent with the presence of *PML-RARA* fusion gene in most of the nucleated cells.

Flow cytometric studies were performed on BM aspirate material using methods described previously.¹¹ The promyelocytes have expressed CD13, CD15, CD33, CD34 (subset), CD64 (bright), CD117 (dim), HLA-DR (subset), and myeloperoxidase (bright), and were negative for CD2, CD3, CD5, CD7, CD10, CD14, CD19, CD20, CD41, CD56, and TdT. Conventional G-band karyotype analysis was performed on a BM aspirate specimen as described previously.¹¹ The karyotype was 46, XX[20]. FISH analysis was performed on interphase nuclei of peripheral blood and BM aspirate samples. Two different commercially available probes (both from Abbott Molecular, Inc/Vysis) were used: LSI *PML/RARA* dual color, dual fusion translocation probe and a LSI *RARA* dual color, breakpoint probe. A normal signal pattern was observed with both probes on peripheral blood and BM aspirate samples (Figure 1C and D).

Real-time quantitative RT-PCR was performed to detect *PML-RARA* transcripts, as previously described.¹² *PML-RARA* short fusion transcripts were detected in peripheral blood and BM aspirate samples with a percentage of 24.9% and 17.4%, respectively. The fms-related tyrosine kinase 3 (*FLT3*) gene was analyzed using methods described previously.¹² An internal tandem duplication of the *FLT3* gene was detected.

A modified oligonucleotide-based array comparative genomic hybridization (CGH) method designed to detect balanced translocations (known as translocation, tCGH) was performed using a 135K oligonucleotide (Roche-NimbleGen; based on UCSC 2006 hg18 assembly). The assay run, OncoChip AML/MDS panel 1 (Signature Genomic Laboratories), is targeted to detect 14 different balanced translocations, all with relevance to acute myeloid leukemia and/or myelodysplastic syndrome.^{13,14} The results showed t(15;17)(q24.1;q21.2). The breakpoint at 15q24.1 occurred within the alternative breakpoint cluster in intron 3 of *PML*.

The breakpoint at 17q21.2 occurred within intron 2 of *RARA* (Figure 2). Based on the *PML* breakpoint, this fusion gene predicts for the short form (bcr3) of *PML-RARA* to be transcribed, consistent with the RT-PCR results. Resolution for the precise location of the breakpoints is defined by the oligonucleotide coverage on the array, which for chromosome 15q24.1 was 159 base pair (bp) and for chromosome 17q21.2 was 120 bp.

The patient continued induction therapy for APL using ATRA, arsenic trioxide, and gemtuzumab ozogamicin, and achieved complete remission. At the last follow-up visit, 33 months after initial diagnosis, the patient remains in complete remission with no evidence of the disease and is not receiving any therapy. RT-PCR studies to assess for *PML-RARA* fusion transcripts performed every 3 months have been consistently negative.

Discussion

Conventional karyotypes and FISH analyses constitute key components in the standard workup for cases of APL. Thus, normal results for karyotype and FISH seen in the presence of positive results using RT-PCR, histochemistry, and clinical presentation create a conundrum for clinicians. Rapid decisions are important to the treatment of APL patients and consistent findings are relied on as assurance of this diagnosis. Flow cytometry can offer additional confirmation of APL and help further categorize patients.¹⁵ However, karyotype and FISH analysis provide evidence at the DNA level that a well described and diagnostically relevant translocation has indeed occurred. Though DNA sequencing has been shown to reveal cryptic *PML/RARA* rearrangements at the DNA level, the procedure remains expensive, takes significant time, and is dependent on extensive data analysis and DNA read depth to reveal such events.¹⁶ Alternatively, the more affordable and rapid traditional microarray assessment has been used to show the presence of a small copy gain within a *PML* gene inserted elsewhere in the genome in an RT-PCR positive case of APL,¹⁷ suggestive of a cryptic insertion event. However, detection of such a cryptic copy gain does not provide evidence of a genomic fusion event. The assay used in the analysis of the current case, a modified oligonucleotide-based tCGH, is designed to demonstrate the presence of a translocation in genomic DNA via linear amplification across the breakpoint. Thus, the technology provides genomic evidence of rearrangement and provides high resolution of the translocation breakpoints.

Others have reported rare cases of APL in which conventional cytogenetics and FISH were negative for t(15;17)(q24.1;q21.2) and *PML-RARA*, respectively, but RT-PCR was positive for *PML-RARA* transcripts. Kim and colleagues¹⁸ reported a single case of APL and reviewed 12 additional cases reported in the literature. They noted a high frequency of the short form of *PML-RARA* transcripts (8 of 13; 62%), a preference for women (9 of 13; 69%), and a high frequency of karyotypic abnormalities other than t(15;17)(q24.1;q21.2), in 9 of 13 (69%) patients; 4 of 13 patients in this review had died, perhaps emphasizing the point that the diagnosis of APL with cryptic t(15;17)(q24.1;q21.2) can be delayed and allow for life-threatening complications.¹⁸ Subsequently, Lewis and colleagues reported another male patient with a normal karyotype who was alive at last follow-up; no data on the type of *PML-RARA* transcripts or morphologic variant were included.¹⁹ Yang and colleagues used long distance PCR to identify *PML-RARA* in another case.⁹ Welch and colleagues reported a

woman with clinical and morphological features of APL, but karyotypic analysis showed a complex karyotype without t(15;17)(q24.1;q21.2) and FISH studies showed *RARA-PML*, but not *PML-RARA*.¹⁶ Whole-genome sequencing was employed to identify a novel insertion of a 77-kilobase (kb) segment of chromosome 15 into intron 2 of *RARA*, creating fusion that encodes short form *PML-RARA* transcripts.

Recently, microarray technology has been used in 2 reported cases of APL. Koshy et al reported an APL case presented with negative karyotyping and FISH analysis but positive in RT-PCR and single nucleotide polymorphism microarray for intragenic *PML* gene duplication.¹⁷ Use of a dual color dual fusion *PML-RARA* FISH probe identified a small extra signal of *PML* which appears to be located in either chromosome 19 or 20. However, *RARA* insert could not be detected using either *RARA* FISH probe or the array. The RT-PCR positive result for *PML-RARA* fusion was confirmed by Sanger sequencing across the *PML-RARA* breakpoint. The second case reported by Haoyue and colleagues was a *RARA* translocation variant of APL in which the patient was negative for *PML-RARA* according to karyotyping, FISH, and RT-PCR.²⁰ *RARA* dual-color break-apart FISH probe detect a submicroscopic deletion of the 30 end of 1 *RARA* gene. Nested RT-PCR and array-CGH have found the signal transducer and activator of transcription 5B (*STAT5b*)-*RARA* fusion transcript.

The precise mechanism of the inability to detect *PML/RARA* using FISH in the case we report, and in similar cases of cryptic t(15;17)(q24.1;q21.2) cases in the literature remains unclear. Because the FISH probes used in the LSI *PML/RARA* kit (Abbott/Vysis) are 239 kb for *PML* and 417 kb for *RARA*, in a case with *PML-RARA* as a result of a small insertion, 1 possible explanation is that the fluorescence signal generated by the fusion gene is too weak and blends in with the remainder of the probe.⁹ To avoid this pitfall, some authors recommend intensive reading of FISH results whereas others suggest increasing the sensitivity of the detection using single pass filters.^{9,21} Although these approaches are valuable and do increase sensitivity of detection of *PML-RARA* using FISH, these approaches cannot completely overcome the problem of cryptic t(15;17) in APL. Another possibility is that submicroscopic insertions of *PML* or *RARA* are too small for a FISH probe to hybridize.¹⁸

Conclusion

We have reported this case because it highlights the value of traditional morphologic assessment and anti-PML immunofluorescence staining when the diagnosis of APL is suspected. In addition, a unique aspect of this case report is that we employed translocation tCGH to prove the presence of t(15;17)(q24.1;q21.2), with breakpoints in intron 3 of *PML* and intron 2 of *RARA*.

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Clinical Practice Points

- Patients with acute promyelocytic leukemia (APL) are at risk of potentially lethal coagulopathy if appropriate therapy is delayed, and fluorescence in situ hybridization (FISH) studies for t(15;17)(q24.1;q21.2)/pro-myelocytic leukemia (*PML*)-retinoic acid receptor, alpha (*RARA*) have become a standard of care to rapidly establish the diagnosis. However, in a small subset of APL cases, FISH and conventional cytogenetic studies give a false negative result.
- If there is a discrepancy between the morphologic picture and cytogenetic/FISH results, other methods such as immunofluorescence staining with an anti-PML antibody or real-time quantitative reverse transcription polymerase chain reaction should be used.
- An oligonucleotide-based array comparative genomic hybridization method could be helpful to confirm t(15;17)(q24.1;q21.2).

Figure 1.

(A) Neoplastic Promyelocytes With Folded Nuclear Contours and Prominent Nucleoli (Magnification 31000). (B) Hypercellular Bone Marrow Biopsy Specimen Diffusely Infiltrated by Promyelocytes (Hematoxylin and Eosin; Magnification 3500). (C) Promyelocytic Leukemia (*PML*)-Retinoic Acid Receptor, Alpha (*RARA*) Dual Color, Dual Fusion FISH Probe With Normal Signals. Two Red Arrows Identify Separate Locations of *PML* Genes; 2 Green Arrowheads Indicate Separate Locations of *RARA* Genes. No Fusion Signal of *PML-RARA* is Detected. (D) *RARA* Breakapart Probe With Normal Fused Signals Detected on BM Sample. Two Yellow Arrows Indicate Intact Signals of *RARA* Genes on Both Chromosomes 17; no Split-Apart Signal is Detected

Figure 2.

(A) Promyelocytic Leukemia (*PML*) Breakpoint at Approximately Chr15:72103132-72103231. This Breakpoint Occurred Within the Alternative Breakpoint Cluster in Intron 3. (B) Retinoic Acid Receptor, Alpha (*RARA*) Breakpoint at Approximately Chr17:35743768-35743888. This Breakpoint Occurred Within Intron 2
Abbreviations: bp = base pair; kb = kilobase.