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CASE REPORT

Diagnosis of variant *RARA* translocation using standard dual-color dual-fusion *PML/RARA* FISH probes: An illustrative report

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Abstract
Acute promyelocytic leukemia (APML) with variant *RARa* translocations comprises 1–2% of APML cases. However, the diagnosis of these cases is challenging as the routine practice includes fluorescence *in situ* hybridization (FISH) reverse transcription polymerase chain reaction targeting the *PML* and *RARA* genes to detect *PML/RARA* fusions. Here, we report a case highlighting the importance of atypical FISH signal patterns in standard dual-color dual-fusion *PML/RARa* FISH analysis complimented by karyotyping to detect these variant *RARA* translocations.

Introduction

Acute promyleocytic leukemia (APML) is characterized by accumulation of immature promyelocytes following a maturation block as a result of the rearrangement of the *RARa* gene; most commonly due to a reciprocal translocation between the long arms of the chromosomes 15 and 17. t(15;17) (q22;q22) results in the fusion of the *RARA* gene on 17q22 with the *PML* gene on chromosome 15q22 giving rise to the *PML/RARA* fusion gene product. The diagnosis of APML arising from reciprocal t(15;17) relies on morphological evaluation, immunophenotyping, and cytogenetic

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and/or molecular analysis. Fluorescence *in situ* hybridization (FISH) analysis using dual color dual-fusion probes is the most frequently used method to demonstrate the *PML/RARA* fusion at diagnosis, as it ensures early results with a short turnaround time.

Approximately 1–2% of APML patients do not harbor the *PML/RARA* fusions and show variant translocations involving the *RARA* gene and other partner genes [1,2]. As these variant translocations are rare, standard diagnostic laboratory algorithms do not include specific probes or primers to diagnose these rare variants. One strategy to identify these variants has been to use FISH analysis using a RARA break apart probe. The above-mentioned strategy can identify *RARA* gene rearrangements but does require metaphases to identify the partner chromosome or gene. Clinically, it is essential to identify the partners because patients with t(11;17) do not respond to the standard all-trans retinoic acid (ATRA) therapy and have to be treated with a more intensive acute myeloid leukemia protocol.

Systematic cytogenetic testing that includes karyotyping and FISH using the commonly available PML RARA dual-color dual-fusion probe can diagnose these rare *RARa* variants. Here, we report a case highlighting the importance of atypical FISH signal patterns in standard dual-color dual-fusion *PML/RARA* FISH analysis complimented by karyotyping to detect these variant *RARA* translocations.

Case report

A 27-year-old man presented to his general practitioner with a history of generalized weakness and low-grade fever in December 2015. His investigations at that time revealed leukocytosis with shift to left and mild thrombocytopenia. Subsequent bone marrow examination showed hypercellular marrow with >90% hypogranular promyelocytes, suggestive of APML. His *PML/RARa* transcript analysis by reverse transcription polymerase chain reaction (RT-PCR) done outside was negative. However, in view of morphological diagnosis, the patient was started with the single agent ATRA (80 mg daily). At 3 months after treatment with ATRA, his peripheral blood counts showed a decrease in leukocyte count, with 20% atypical cells. The patient at this time point was referred to us. At the time of his visit, he had mild weakness and had been treated with ATRA for the past 3 months. His general physical examination was unremarkable. The patient did not have bleeding or body rashes. His recent ultrasound examination of the whole abdomen showed mild splenomegaly (11.2 cm) and bilateral renal parenchymal disease. In view of the presence of atypical cells in the peripheral blood, a repeat bone marrow examination with conventional karyotyping, FISH for *PML/RARa* fusion, and *PML/RARa* transcript analysis by RT-PCR was advised.

His bone marrow examination revealed ~77% atypical cells/promyelocytes (medium- to large-sized cells with predominantly round nuclei, occasional prominent nucleoli, and moderate to abundant hypogranular cytoplasm; many of such cells were also exhibiting one to multiple Auer rods), favoring a diagnosis of APML. Flow cytometric immunophenotyping from bone marrow aspirates showed features consistent with APML (cells with moderate to high side scatter and dim to moderate CD45 coexpressing cMPO/CD117/CD13/CD33). RT-PCR for *PML/RARa* transcript was negative. FISH using dual-color dual-fusion *PML/RARA* probe (Vysis; Abbott, Illinois, USA) showed an additional spectrum green (*RARA*) signal. On metaphase FISH analysis, the extra spectrum green signal was observed to be on a different chromosome. A FISH report of a possible variant translocation was given. Consequently, cytogenetic analysis of 16 metaphases from bone marrow aspirate samples showed the presence of a neoplastic clone characterized by a balanced translocation between the long arms of chromosomes 11 and 17 in 13 metaphases. Ten of these metaphases showed loss of sex chromosome, Y. This is consistent with APML with variant *RARa* translocation involving the *ZBTB16* gene at 11q23 and *RARa* gene at 17q21 (Fig. 1). The patient was started with conventional 3 + 7 (daunorubicin + cytarabine) chemotherapy. Post-induction Day 14 bone marrow examination was suggestive of persistent disease with the presence of ~16% abnormal promyelocytes/blasts. However, the patient was clinically stable with negligible transfusion requirement. He was planned for allogenic transplant bridged by azacytidine treatment. The patient however opted out of definitive treatment and was lost to follow-up.

Discussion

To date, seven different translocation partners of *RARa* gene have been described with clinical and pathological findings similar to APML [3–6]. These include t(15;17)(q22;q21) *PML/RARA*, t(11;17)(q23;q21) *ZBTB16/RARA*, t(11;17)(q13;q21) *NUMA/RARA*, t(5;17)(q35;q21) *NPM/RARA*, der(17) *STAT5B/RARA*, der(17) *PRKAR1a/RARA*, t(X;17)(p11;q12) *BCOR/RARA*, and t(4;17)(q12;q21) *FIP1L1/RARA*. These findings are indicative of the key role of *RARa* in the pathogenesis of the disease. It is essential to identify the partner chromosomes taking part in the *RARA* gene rearrangement as patients harboring t(11;17)(q23;q21) *ZBTB16/RARA* and der(17) *STAT5B/RARA* rearrangements show poor response to ATRA [3].

Among these variants, t(11;17)(q23;q21), which fuses *ZBTB16* (formerly *PLZF* – promyelocytic leukemia zinc finger) with *RARa* and results in the production of the *ZBTB16/RARa* fusion protein and *RARa/ZBTB16* reciprocal fusion protein, comprises 0.8% of all cases of APML. It is the second most common translocation and was first described in 1994 [7–9]. The morphological findings in these variant APML cases were described and include the presence of regular round nuclei, abundant cytoplasm containing coarse granules, and usually a lack of Auer rods [10]. By contrast, our patient showed blasts with the presence of multiple Auer rods. These patients have poor prognosis in comparison to *PML/RARa*-positive patients.

Currently, the diagnosis of APML heavily relies on detection of *PML/RARa* fusion transcripts by RT-PCR. There is an inherent possibility with this methodology that a patient harboring any of these variant translocations (i.e., other than *PML/RARa*) would be missed. Herein lies the importance of conventional karyotyping and FISH. Conventional karyotyping would be helpful in picking up these variant translocations except in cases with cryptic translocations. FISH analysis using dual-color breakapart probes will also

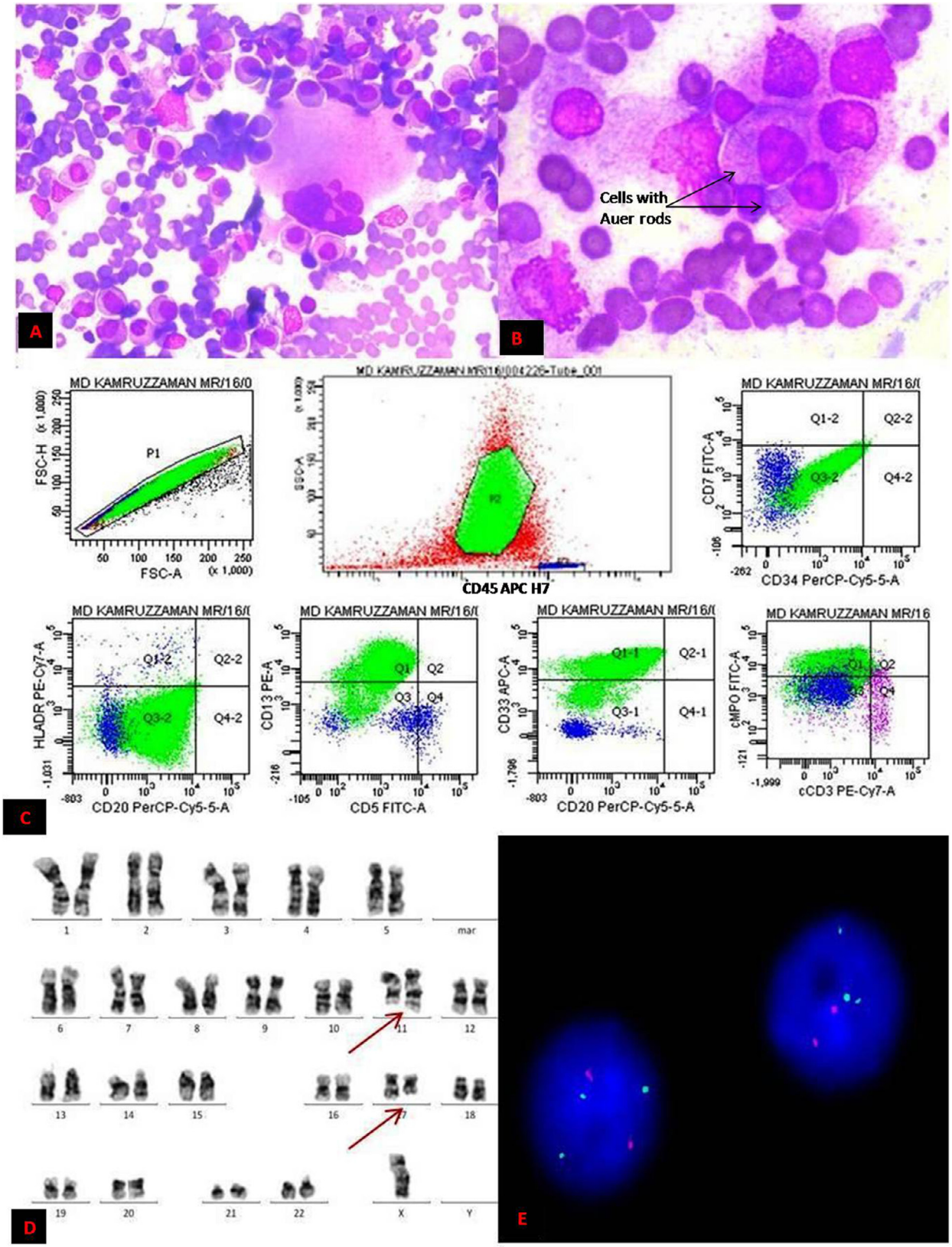

Fig. 1 (A) Photomicrograph with medium to large sized blasts with regular round nuclei and moderate amount of cytoplasm. (B) Blasts with multiple Auer rods. (C) Flow scatter plot (tumor population — green) — tumor cells with moderate CD45 and moderate to high side scatter showing positivity for CD13/CD33/cMPO, and negativity for expression of other markers including CD34/HLA-DR. (D) Bone marrow aspirate karyogram with t(11;17) — red arrows and loss of chromosome Y. (E) Dual-color dual-fusion fluorescence *in situ hybridization* analysis on interphase cells showing three green signals.

help in detecting splitting of *RARa* signals in interphase nuclei, as reported by others [4—6].

Although commercial fusion probes designed to detect *ZBTB16/RARA* fusions are not used commonly, the fusion can be identified through metaphase FISH analysis using a *RARA* breakapart probe or by studying metaphases using a the standard dual-color dual-fusion *PML/RARA* probe. In our patient, a metaphase FISH study matched with GTG-banded karyotype, helped identify the variant fusion, highlighting the importance of metaphase FISH in patients with atypical interphase FISH patterns. An additional *RARA* signal on FISH analysis using standard dual-color dual-fusion *PML/RARA* indicates the presence of a variant translocation and either FISH analysis using a *RARA* breakapart probe or metaphase analysis should be used to confirm the same.

This brief communication adds to the literature on this rare entity, highlighting the challenges in diagnosis and management, reiterating the poor prognosis associated with this entity.

Conflicts of interest

The authors declare that there is no conflict of interest related to this report.

References

[1] Grimwade D, Biondi A, Mozziconacci MJ, Hagemeijer A, Berger R, Neat M, et al. Characterization of acute promyelocytic leukemia cases lacking the classic t(15;17): results of the European Working Party. Blood 2000;96(4):1297—308.

[2] Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. Blood 2016;127:2391—405.

[3] Adams J, Nassiri M. Acute promyelocytic leukemia — a review and discussion of variants translocations. Arch Pathol Lab Med 2015;139:1308—13.

[4] Catalano A, Dawson MA, Somana K, Opat S, Schwarer A, Campbell LJ, et al. The PRKAR1A gene is fused to RARA in a new variant acute promyelocytic leukemia. Blood 2007;110 (12):4073—6.

[5] Kondo T, Mori A, Darmanin S, Hashino S, Tanaka J, Asaka M. The seventh pathogenic fusion gene FIP1L1-RARA was isolated from a t(4;17)-positive acute promyelocytic leukemia. Haematologica 2008;93(9):1414—6.

[6] Yamamoto Y, Tsuzuki S, Tsuzuki M, Handa K, Inaguma Y, Emi N. BCOR as a novel fusion partner of retinoic acid receptor alpha in a t(X;17)(p11;q12) variant of acute promyelocytic leukemia. Blood 2010;116(20):4274—83.

[7] Chen Z, Guidez F, Rousselot P, Agadir A, Chen SJ, Wang ZY, et al. PLZF-RAR alpha fusion proteins generated from the variant t(11;17)(q23;q21) translocation in acute promyelocytic leukemia inhibit ligand-dependent transactivation of wild-type retinoic acid receptors. Proc Nat Acad Sci USA 1994;91 (3):1178—82.

[8] Chen Z, Brand NJ, Chen A, Chen SJ, Tong JH, Wang ZY, et al. Fusion between a novel Kruppel-like zinc ¨ finger gene and the retinoic acid receptor-alpha locus due to a variant t(11;17) translocation associated with acute promyelocytic leukaemia. EMBO Journal 1993;12(3):1161—7.

[9] Licht JD, Chomienne C, Goy A, Chen A, Scott AA, Head DR, et al. Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukemia associated with translocation (11;17). Blood 1995;85(4):1083—94.

[10] Sainty D, Liso V, Cantu-Rajnoldi A, Head D, Mozziconacci MJ, Arnoulet C, et al. A new morphologic classification system for acute promyelocytic leukemia distinguishes cases with underlying *PLZF/RARA* gene rearrangements. Blood 2000;96:1287—96.