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LETTER TO THE EDITOR

Cytogenetically cryptic insertion of *PML* segment into *RARA* on chromosome 17q resulting *PML-RARA* fusion in acute promyelocytic leukemia

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Dear Editor,

Acute promyelocytic leukemia (APL) represents 5–8% of acute myeloid leukemias (AML), and is characterized by the classic t(15;17)(q24.1;q21.2) translocation resulting in fusion of the *PML* (15q24.1) and *RARA* (17q21.2) genes. Prompt diagnosis of APL is critical in management of patients with APL as an effective therapy is available to improve outcome. The diagnosis of APL is routinely confirmed by the presence of *PML-RARA* fusion using karyotype analysis, fluorescence in situ hybridization (FISH), or polymerase chain reaction (PCR) assay. The classic t(15;17) is present in approximately 90–95% of APL cases while other structural rearrangements including submicroscopically cryptic *PML-RARA* aberrations are seen in the remaining cases [1]. Of reported cytogenetically cryptic *PML-RARA* rearrangements, most were insertions of *RARA* on 17q into *PML* on 15q and only few were interstitial insertions of *PML* into *RARA*, and they were all readily detected by FISH [2, 3]. Moreover, APL with FISH negative and PCR positive for *PML-RARA* is very rare [4]. Here, we present an APL case with *PML-RARA* derived from a cytogenetically cryptic and FISH-negative interstitial insertion of *PML* into *RARA*.

A 23-year-old Caucasian woman presented with epistaxis and easy bruising. Initial hemogram revealed thrombocytopenia (55×10^3/mcL), anemia (11.2×10^3/mcL), and leukocytosis (15.4×10^3/mcL). Coagulation studies were grossly abnormal with INR of 1.7, fibrinogen 81 mg/dL, and quantitative D-Dimer > 20,000 ng/mL, consistent with disseminated intravascular coagulation (DIC). The peripheral smear showed that morphologically abnormal myeloblasts/promyelocytes represented ~ 59% of the leukocytes on differential. These cells had dense azurophilic granulation, ovoid nuclei, and only occasional forms contained rare Auer rods. The leukemic population expressed CD13, CD33 (partial), CD56 (partial, dim), CD64, and (c)MPO, and were negative for HLA-DR and CD34 by flow cytometry. Bone marrow biopsy showed a hypercellular marrow approaching 100% cellularity with ~ 70% involvement by blasts with bilobed/folded nuclei and more numerous Auer rods. APL was suspected and treatment with all-trans-retinoic acid (ATRA) and idarubicin was initiated on day 1 of admission.

A FISH analysis of bone marrow using the dual color, dual fusion *PML-RARA* probe, and dual color *RARA* break-apart probe (Abbott Molecular Inc., Abbott Park, IL) did not show classic signal patterns for *PML-RARA* or *RARA* rearrangement. Karyotyping study revealed trisomy 8 but not t(15;17)(15q24.1;17q21.2). Concurrent RT-PCR revealed *PML-RARA* fusion. In light of the positive PCR result, the FISH data was re-analyzed. Using the Spectrum Orange and chromomycin fluorescence filters to view *PML* signal and nuclei, respectively, a tiny *PML* signal (Fig. 1a) along with two *PML* signals in normal size was present in a subset of cells. When the same cells were examined with the Spectrum Green and chromomycin fluorescence filters, two *RARA* signals were seen (Fig. 1b). When the three filters were applied, a green (*RARA*) signal superimposed on a small red (*PML*) signal (Fig. 1c), resulting in a small red-green fusion signal. This was confirmed by metaphase FISH which showed

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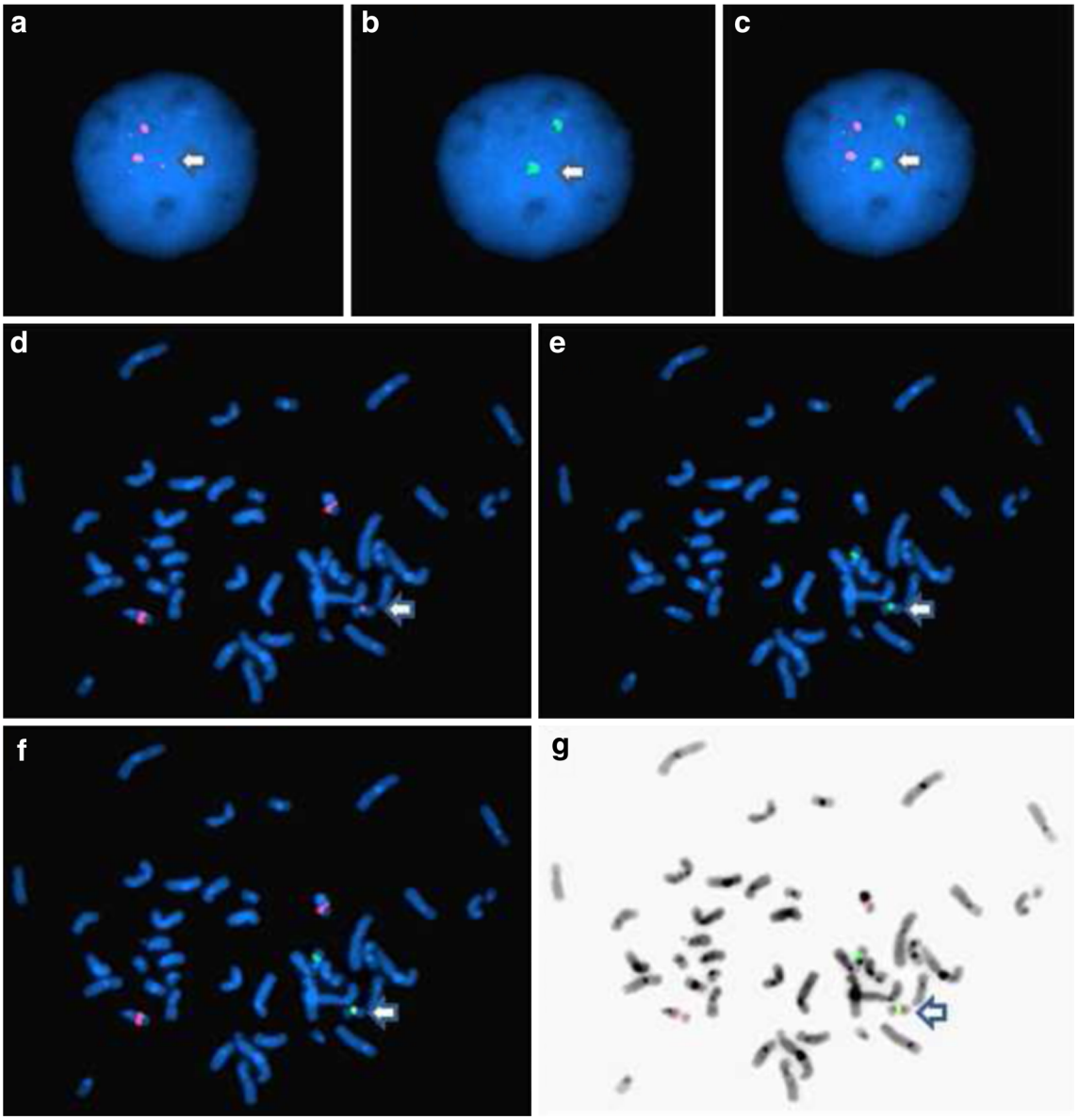

Fig. 1 *PML-RARA* FISH analysis. Interphase FISH (**a**–**c**), **a** Spectrum Orange filter showing a small *PML* signal (arrowed) along with two normal sized *PML* signals. **b** Spectrum Green filter showing one of normal sized *RARA* signals at the same location as small *PML* signal shown in (**a**). **c** Spectrum Orange and Spectrum Green filters showing a small *PML* signal was superimposed by *RARA* signal resulting in small red-green fusion in the middle (arrowed). Metaphase FISH (**d**–**f**). **d** Spectrum Orange filter showing a small *PML* signal (arrowed) on one chromosome 17 along with two normal size *PML* signals one chromosomes 15. **e** Spectrum Green filter showing one *RARA* (arrowed) on the same chromosome 17 as the small *PML* signal shown in (**d**). **f** Spectrum Orange and Spectrum Green filters showing a *RARA* signal superimposed to the small *PML* signal resulting in a tiny red-green fusion signal (arrowed). **g** Metaphase spread with DAPI staining

a small *PML* fragment was present in the *RARA* locus on chromosome 17 (Fig. 1d–f), demonstrating this being an interstitial insertion of *PML* into the *RARA* gene on 17q. The cytogenetic result is described as 47,XX,+8[17]/46,XX [3]. nuc ish(PMLx3, RARAx2)(RARA con PMLx1)[200], ish ins(17;15)(q21;q24q24)(RARA+,PML+; RARA+). ATRA and arsenic trioxide (ATO)-based chemotherapy was successful and a complete remission was achieved. The patient has remained in remission to date.

Promptly, molecular confirmation of APL diagnosis and timely institution of ATRA and ATO-based therapy is essential to reduce life-threatening complications such as DIC and to dramatically improve the outcome. Detection of *PML-RARA* fusion masked in cytogenetically cryptic rearrangement can be challenging. So far, there have been six APL patients with an insertion of *PML* into *RARA* on 17q who were reported before, and all were FISH positive for *PML-RARA* (Table 1) [1, 2,

Table 1 APL cases with *PML-RARA* derived from an interstitial insertion of *PML* into *RARA* on 17q

Case no.	Cytogenetic findings		*PML-RARA*		References
	Karyotype	Cryptic rearrangement	FISH	PCR	
1	46,XX,del(9)(q12q33)	yes	pos.	pos.	1
2	47,XY,+21/48,idem,+18	yes	pos.	n.d.	1
3	46,XX,ins(17;15)(q21;q21q22)	no	n.d.	pos.	1
4	46,XY,t(4;16)(p14;q22),t(9;12)(q22;q24),ins(17;15)(q21;q15q22)/46,idem,t(6;8)(q13;q22)	no	pos.	pos.	2
5	46,XX	yes	pos.	neg.	5
6	46,XX,i(17)(q10)	yes	pos.	pos.	6
7	47,XX,+8	yes	pos. at re-analysis	pos.	This study

pos. positive, *neg.* negative,*n.d.* not determined

5, 6]. To our knowledge, our patient is the first APL case with *PML-RARA* resulted from a submicroscopic interstitial insertion of *PML* into *RARA* on 17q that is not readily detectable by FISH. Our report highlights the importance of being cognizant of cryptic *PML-RARA* rearrangements in APL diagnosis. Careful investigation of atypical FISH data with various filter selections may aid in characterizing spatial relation between *PML* and *RARA* masked in cryptic rearrangements, and integration of RT-PCR into APL diagnostic work-up would improve sensitivity of *PML-RARA* detection.

Finally, compared to the classic t(15;17) that produces two fusion products, *PML-RARA* and its reciprocal fusion *RARA-PML*, the interstitial insertion described in this report yields only one fusion, *PML-RARA*. The fact that our patient was presented with pathologic and clinical features comparable with classic t(15;17)-APL supports the notion that the *PML-RARA* fusion is the primary fusion product for APL pathogenesis and progression [7].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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