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## Letter to the Editor

### Molecular methods for genomic analyses of variant *PML-RARA* or other *RARA*-related chromosomal translocations in acute promyelocytic leukemia

**TO THE EDITOR:** We read an interesting paper by Palta *et al.* in a recent issue of the Korean Journal of Hematology titled, “*ZBTB16-RARA* variant of acute promyelocytic leukemia with tuberculosis: a case report and review of literature” [1]. We would like to add some comments to their article and suggest additional molecular methods to confirm variant translocations in acute promyelocytic leukemia (APL).

Apart from its characteristic morphology, APL is strongly correlated with presence of the *PML-RARA* fusion gene due to a t(15;17) translocation. Patients displaying cryptic *PML-RARA* gene rearrangements or other translocations involving *RARA* are very rare. Since *RARA* generates chimeric fusion genes with various partners such as *ZBTB16* at 11q23, *NUMA1* at 11q13, *NPM1* at 5q35, *FIP1L1* at 4q12, and *STAT5b* at 17q11.2 (Fig. 1), confirmatory molecular methods to detect gene rearrangements are necessary when unique or unusual chromosomal translocations are found in APL cases. Palta *et al.* reported a case of APL accompanied by tuberculosis where cytogenetic study showed a variant chromosomal translocation, t(11;17)(q23;q21). First, although they describe this case as APL with t(11;17)(q23;q21) through bone marrow (BM), immunophenotyping, and cytogenetic studies, we noted an absence of results based on molecular methods. To demonstrate the existence of the *ZBTB16-RARA* fusion gene, a single specific reverse transcriptase-polymerase chain reaction (RT-PCR) with a specific primer to detect the gene rearrangement or multiplex RT-PCR (e.g. HemaVision) to detect *ZBTB16-RARA* could

have provided an accurate molecular diagnostic result. Second, the authors' reference to a paper by Kang *et al.* was puzzling despite the fact that it was cited as a similar case with chromosomal translocations at a similar location, in cases of AML with chromosomal translocation t(11;17) [2]. In the first case, cytogenetic analysis from BM aspirate showed t(11;17)(q23;q21) in 18 out of 20 cells while the *MLL* probe in fluorescence *in situ* hybridization (FISH) showed split-out signals in 98.5% of interphase cells. The second case also showed t(11;17)(q23;q25) in the chromosome study while the FISH assay showed split-out signals in 79% of interphase cells. However, normal fluorescence patterns were found in 200 cells in additional FISH assays when a dual-color single-fusion *PML-RARA* probe was used for both cases, which led to the conclusion that the *MLL* gene was involved in the 11q23 breakpoint and *RARA* was not involved in the 17q breakpoint. Hence, the authors'

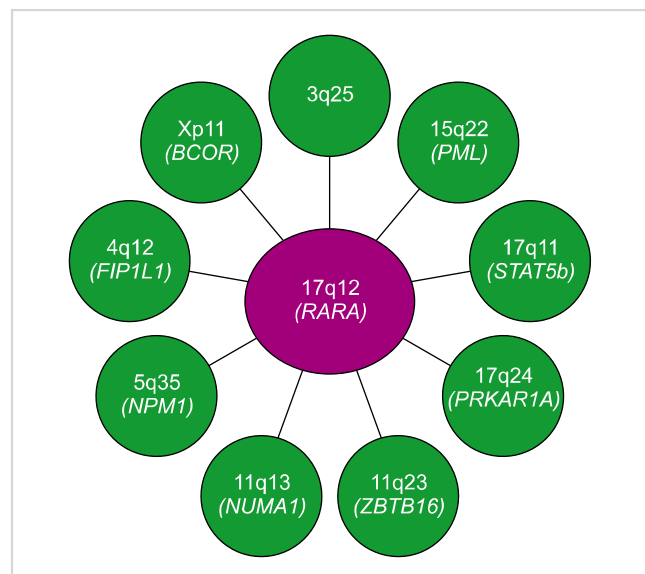


Fig. 1. Various *RARA* partner genes in acute promyelocytic leukemias.

citation of this study to explain the *ZBTB16-RARA* fusion gene appears inadequate, and a molecular study could unambiguously identify the involvement of *ZBTB16* at 11q23 in their case.

We suggest the authors conduct RT-PCR analysis of the *ZBTB16-RARA* fusion gene if any remnant RNA or cDNA samples from their APL patient are available. However, if only genomic DNA is available, analyzing the genomic breakpoints by multiplex long distance-polymerase chain reaction (LD-PCR) would be helpful. LD-PCR or long distance inverse (LDI)-PCR can identify known or unknown partner genes, monitor minimal residual disease (MRD), and identify multi-way chromosomal translocations such as three-way or four-way translocations [3]. We have recently used LD-PCR to confirm APL cases showing unusual karyotypes or discrepant results between conventional cytogenetics and molecular studies. More precisely, we identified a novel *PML-ADAMTS17-RARA* rearrangement and confirmed the genomic fusion breakpoints of cryptic *PML-RARA* rearrangement in 2 APL patients [3-5].

In conclusion, detection of leukemia-associated fusion genes is important due to their direct relevance to diagnosis, MRD assessment, and treatment response. Since APL patients with *ZBTB16-RARA* are resistant to all-trans-retinoic acid (ATRA), molecular methods with higher resolution than chromosome studies must be conducted to detect fusion genes and facilitate selection of the most appropriate treatment. We believe that molecular techniques such as FISH, RT-PCR, and LD-PCR should be used in addition to morphology and cytogenetic studies in APL diagnosis and evaluation so that atypical *PML-RARA* or *RARA* variant gene rearrangements are not missed.

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