Additional PML and RARA Rearrangements Accompanying PML::RARA in a Case of Acute Promyelocytic Leukaemia with Myeloblast Morphology

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Based on cytoplasmic granule morphology, the World Health Organization classification recognizes two subtypes of acute promyelocytic leukemia (APL). The hypergranular variant is characterized by promyelocytes with cytoplasm densely packed with coarse granules; faggot cells are commonly observed. The hypogranular variant exhibits blasts with scant or absent granules, a pattern historically referred to as "M1-like APL."[1,2] We report a case of hypogranular APL harboring additional PML and RARA genomic rearrangements beyond the canonical PML::RARA fusion. This finding enhances our understanding of leukemogenesis in this disease entity.

A 29-year-old male patient was admitted with a one-month history of sore throat and gingival bleeding, accompanied by one week of chest tightness and dyspnea. His complete blood count revealed a white blood cell count of 49.84 × 10^9/L, hemoglobin of 76 g/L, platelets of 22 × 10^9/L, and C-reactive protein of 49.32 mg/L. Coagulation studies showed prolonged prothrombin time (PT, 16.8 s) and decreased fibrinogen (2.06 g/L), while activated partial thromboplastin time (APTT) and thrombin time were within normal limits. The D-dimer level was markedly elevated at 8060 ng/mL. Bone marrow aspiration demonstrated 81% blasts, suggesting a myeloid origin with some suspicious abnormal promyelocytes. Flow cytometry identified 89.12% blasts with the following immunophenotype: CD34 partial+, CD117 partial+, CD33 strongly+, CD13+, HLA-DR-, CD38-, CD11b-, CD10-, CD7-, CD36 partial+, CD56-, CD64-, CD4-, CD14-, CD15-, CD16-, cytoplasmic myeloperoxidase (MPO) dim+, cytoplasmic CD3-, and cytoplasmic CD79a-. Cytogenetic analysis showed a normal karyotype, while the *PML::RARA* fusion gene was positive. Next-generation sequencing (NGS) detected four distinct FLT3-ITD mutations and several other alterations (Supplementary Table 1). The diagnosis of APL was confirmed. Treatment included infection control, hemostatic agents, and component blood transfusions; however, the patient refused leukemia-specific therapy and self-discharged.

Ten days later, the patient was readmitted with fever. Complete blood count showed a white blood cell count of 74.90 × 10^9/L, hemoglobin of 51 g/L, and platelets of 32 × 10^9/L. Coagulation studies revealed PT of 18.4 s, fibrinogen of 1.46 g/L, and APTT of 24.6 s. After admission, he received hydroxyurea for leukocyte reduction and infection management; however, he died of cerebral hemorrhage on the second hospital day.

Targeted DNA-based NGS also identified two novel fusion events: an *RARA::intergenic RPLP1* fusion with breakpoints in intron 2 of *RARA* and the intergenic region upstream of *RPLP1* (Supplementary Figure 1a), and a *TBC1D2B* (intron 9)::*PML* (intron 3) fusion (Supplementary Figure 1b). To determine whether these fusions produced transcripts, RNA sequencing was performed. The *TBC1D2B::PML* fusion showed abundant supporting reads (Figure 1c), whereas only a single read of *RARA::intergenic RPLP1* was detected; it contained intronic sequences and was therefore interpreted as potential RNA contamination. The *TBC1D2B::PML* fusion was subsequently validated by polymerase chain reaction (Figure 1b) and Sanger sequencing, confirming a fusion between exon 9 of *TBC1D2B* and exon 4 of *PML*. However, a frameshift mutation was identified in the *PML* sequence (Figure 1d, e).

TBC1 domain family member 2B (TBC1D2B) is located at chromosome 15q24.3–q25.1. As a member of the Tre2-Bub2-Cdc16 (TBC) domain-containing GTPase-activating protein family (RABGAPs), TBC1D2B interacts with RAB22A and RAB22B.[3] RABGAPs play a central role in regulating RAB GTPase activity,[4] promoting the transition of RAB proteins from active GTP-bound to inactive GDP-bound states. When GTP is bound, RAB proteins associate with specific subsets

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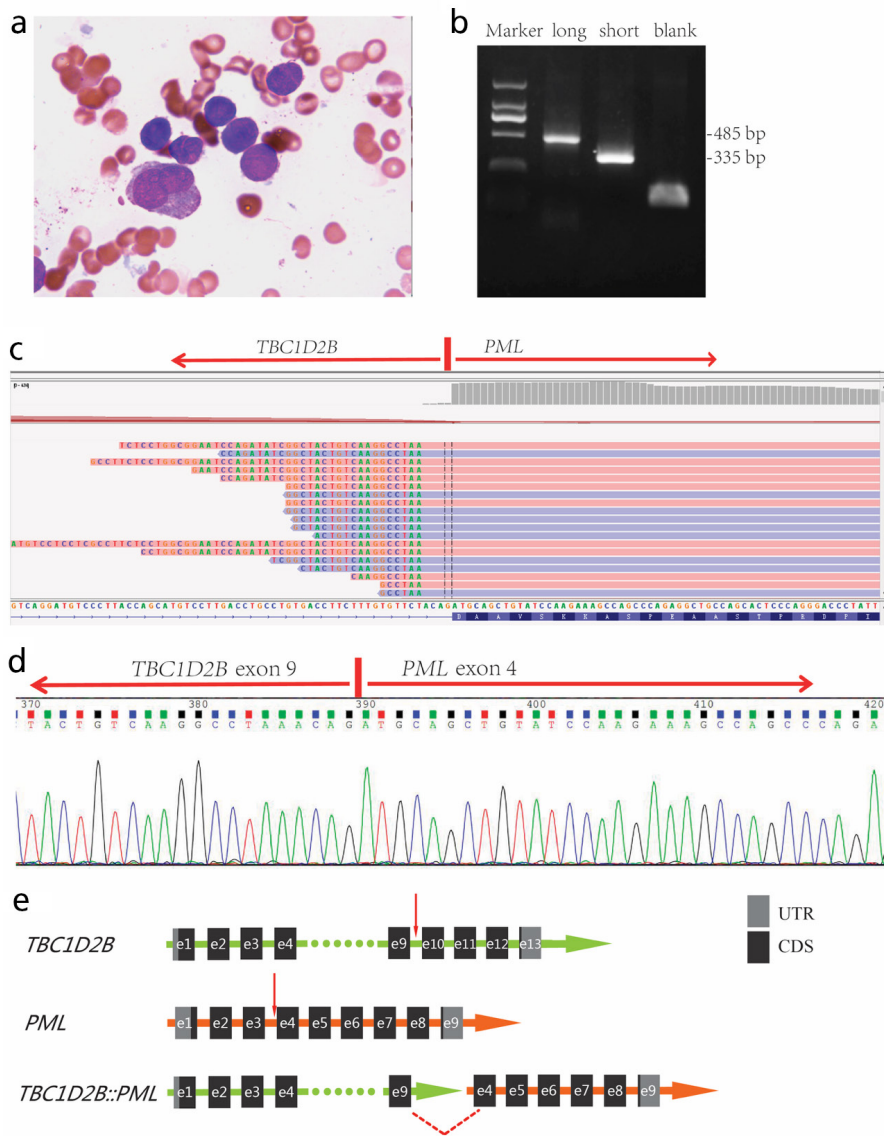

FIG. 1. Cytomorphology and molecular biological characteristics of the present case. (a) Bone marrow smear (1000×, Wright staining). The leukemic cells are medium-sized with irregular, sometimes folded nuclei, and contain a moderate amount of pale blue cytoplasm with sparse or fine granules. (b) Agarose gel electrophoresis. Two pairs of primers were designed, yielding two products of different lengths. The primers for the long product are: forward (*TBC1D2B*-exon 9): 5'-TCTGAAGACTCTCTACCTCACA-3', reverse (*PML*-exon 4): 5'-CAGGTCAACGTCAATAGGGT-3'; the primers for the short product are: forward (*TBC1D2B*-exon 9): 5'-CAGAAGGCGCTGGAGAAA-3', reverse (*PML*-exon 5): 5'-CTGACTGTACCACAGCCATAG-3'. (c) Junction reads from RNA-seq, screenshot from IGV (Integrative Genomics Viewer) browser. (d) Sanger sequencing of the PCR amplicons (e) Schematic of the *TBC1D2B*::*PML* fusion. The exon 9 of *TBC1D2B* (NM_144572.2) fuses with the exon 3 of *PML*(NM_033238.3). Red arrows indicate breakpoint sites. The green dotted lines represent that the display of partial sequences is omitted. UTR, untranslated region; CDS, coding sequence; PCR, polymerase chain reaction.

of intracellular vesicles, ultimately mediating vesicle tethering and fusion with target membranes.[5] This membrane trafficking pathway is involved in multiple cellular processes, including macropinocytosis, primary cilium formation, and synaptic function.[6] Additionally, TBC1D2B facilitates autophagy initiation through phase separation mechanisms.[7]

PAX5-PML represents the first reported fusion gene involving *PML* that is unrelated to *RARA*. The recurrent *PAX5-PML* fusion was identified in two pediatric acute lymphoblastic leukemia cases, corresponding to the chromosomal translocation t(9;15)(p13;q24). This in-frame fusion nearly retains the entire *PML* protein.[8] To our knowledge, *TBC1D2B::PML* constitutes the second reported *PML*-involving fusion gene not associated with *RARA*.

Transcriptome sequencing analysis yielded only a single read corresponding to *RARA::intergenic RPLP1*. This read was identical to the fusion detected by DNA sequencing, as both contained intronic

sequences. We postulate that this likely represents minor DNA contamination during RNA-seq library preparation. Consequently, the fusion between *RARA* and the 5′ intergenic region of *RPLP1* appears to lack transcriptional products.

The reciprocal *RARA::PML* fusion is observed in 70–80% of APL patients and is invariably accompanied by *PML::RARA*. In rare APL cases, *RARA::PML* exists as the sole cytogenetic abnormality.[9] Both partner genes in *RARA::PML* demonstrate breakpoints within their coding sequences (CDS), resulting in chimeric transcript formation. In our case, no *RARA::PML* transcript was detected. The *RARA::intergenic RPLP1* breakpoint is located proximal to the 5′ region of *PML*, suggesting an alternative fusion form, i.e., promoter swapping, in which the downstream partner gene retains intact CDS while its transcriptional regulatory elements are replaced by those of the upstream gene, potentially altering transcriptional regulation.[10] Comparative transcriptome sequencing of *RPLP1* and *PML* versus normal controls (data not shown) showed no significant differences in expression.

In APL, abnormal promyelocytes typically display cytoplasm enriched with azurophilic granules, which manifest as two principal morphological variants. The hypergranular type is distinguished by coarse, densely packed azurophilic granules that may fuse, whereas the hypogranular variant presents with uniformly fine yet densely distributed granules. Notably, a subset of APL cases demonstrates markedly diminished or entirely absent cytoplasmic granulation, including cases carrying the *PLZF-RARA* fusion gene. A diagnostically challenging subset exhibits myeloblast-like morphology, characterized by virtually agranular cytoplasm, variable CD34 expression, and, in some instances, negative MPO staining. These morphological features suggest a potential origin from an intermediate cellular stage bridging myeloblasts and promyelocytes, thereby displaying hybrid characteristics of both developmental states.[2]

In the present case, the leukemic cells exhibited hypogranular morphology. Flow cytometry revealed partial CD34 positivity, a finding commonly associated with the hypogranular variant, while CD36 positivity can occasionally be seen in the hypergranular form. Currently, the molecular pathology of hypogranular APL beyond RARA rearrangement remains poorly characterized. Typically, the hypergranular variant is CD34-negative; in this case, partial CD34 positivity suggests an earlier differentiation stage than that of typical hypergranular APL. The truncated *RARA* from the *RARA* fusion is considered the molecular basis for blocked differentiation in leukemic promyelocytes. As *TBC1D2B::PML* does not involve *RARA*, whether it contributes to this earlier differentiation stage warrants further exploration.

At initial diagnosis, the patient presented with marked hyperleukocytosis. However, he declined treatment, which subsequently accelerated disease progression.

In summary, we identified additional *PML* and *RARA* rearrangements beyond the classic *PML::RARA* fusion in a case of hypogranular APL. These findings may contribute to elucidating the molecular mechanisms underlying the disease.

Ethics Committee Approval: The study protocol was approved by the Medical Ethics Committee of the Affiliated Hospital of Qingdao University (approval number: QYFY WZLL 28785, date: 11.07.2024).

Informed Consent: Written informed consent was obtained from the patient.

Authorship Contributions: Design- Z.S., H.Z.; Data Collection and/or Processing- L.S., Y.L.; Analysis and/or Interpretation- X.Y.; Writing- Z.S., W.W.

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

Supplementary Table: https://www.balkanmedicaljournal.org/img/files/Supple%20Table%202026.2025-12-103.pdf

Supplementary Figure: https://balkanmedicaljournal.org/img/files/Supple-Figure-2026.2025-12-103.pdf

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