We report the molecular and cytogenetic characterization of a novel variant of acute promyelocytic leukemia (APL). The bone marrow showed 88% hypergranular promyelocytes, and the karyotype was 47,XY,+22 [5]/46,XY[30]. Fluorescence in situ hybridization (FISH) indicated disruption and deletion of the 5′-end of the RARA gene. Treatment with all-trans retinoic acid, idarubicin, and arsenic trioxide induced cytogenetic complete remission without morphologic evidence of residual leukemia.

**Introduction**

The majority of acute promyelocytic leukemia (APL) cases are characterized by the PML-RARA fusion gene, usually as a consequence of the t(15;17)(q22;q21) translocation.1 As a result, retinoid sensitivity of the retinoic acid receptor α (RARα), which normally functions as a retinoid-inducible transcription factor, is reduced, causing a block in myeloid differentiation.2 Prolonged disease-free survival can be achieved by combining all-trans retinoic acid (ATRA) and chemotherapy.3 Arsenic trioxide (As2O3) is of proven efficacy in ATRA resistant4,5 APL cases.6,7 Nuclear mitotic apparatus (NUMA),8 signal transducer and activator of transcription 5b (STAT5b),9 RARA fusion partners, and PML, NPM, and NUMA are ATRA responsive, whereas PLZF-RARA is ATRA resistant.10,11

This report describes a new variant APL and identifies the RARA gene rearrangement as the fifth variant APL in which the RARA partner gene has been identified and the second known rearrangement of PRKAR1A in a malignant disease. This trial was registered at www.actr.org.au with the Australian Clinical Trials Registry as number 12605000070639. (Blood. 2007;110: 4073-4076)

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cytarabine 100 mg/m²/d × 7 and amsacrine 100 mg/m²/d × 3) were administered, superimposed on continuous ATRA, and were followed by maintenance therapy (ATRA for 2 weeks every 3 months). Eleven months after diagnosis, a full blood count showed mild anemia (99 g/L), normal neutrophils, and marked thrombocytopenia (20 × 10⁹/L), and the marrow showed essentially normal trilineage hemopoiesis. Cytogenetic analysis and interphase FISH were both normal (46,XY[40] and nuc

FISH, using a RARA probe and a BAC probe (RP11–120M18) encompassing the PRKAR1A gene, confirmed the presence of a PRKAR1A-RARA fusion on der(17) (Figure 1D). The simplest explanation would be an insertion of RARA distal to PRKAR1A followed by a deletion removing 3' PRKAR1A, 5' RARA, and any intervening sequences.

Alternative splicing of PRKAR1A explains the presence of 2 PRKAR1A-RARA PCR products observed in the diagnostic sample. The longer transcript results from cryptic splicing of the first 100 bases of PRKAR1A exon 3 to RARA exon 3, producing an in-frame fusion transcript capable of encoding a 495-amino acid Rlx-RARα fusion protein. Translation of the putative open reading frame would generate a protein containing the Rlx protein dimerization domain fused to the same carboxy terminal end of the RARα protein shared by all RARα rearrangements in APL.11 Splicing of PRKAR1A exon 2 to RARA exon 3 generates a shorter out-of-frame fusion transcript possibly encoding a carboxytruncated chimeric Rlx protein.

PKA is a multimeric protein consisting of 2 catalytic subunits complexed with a regulatory subunit dimer.13 Binding of cAMP to the regulatory subunits produces a conformational change that causes dissociation and de-inhibition of the catalytic subunits.14 Type-I regulatory subunits are generally associated with cytosolic isoforms of PKA, whereas type-II regulatory subunits, capable of interacting with A kinase–anchoring proteins, are found in organelle-localized isoforms of PKA.13

PRKAR1A is involved in another gene rearrangement with the RET proto-oncogene in papillary thyroid carcinomas.15,16 Inherited null mutations of PRKAR1A have been reported in Carney complex.17,18 PRKAR1A-inactivating mutations or down-regulation have also been found in some sporadic adrenocortical tumors.19 In our case, fusion of the Rlx dimerization domain to RARα may be involved in dysregulated RARα homodimerization or heterodimerization with RXX and potentially deregulation of PKA through disruption of Rlx.
Mouse models of APL have varying phenotypic features that depend on the RARA fusion partner. It is conceivable that dysregulation of PKA by disruption of RI/H9251 may have contributed to this patient’s atypical APL presentation. The ATRA responsiveness in this case is unknown because multiple agents were used. However, the remarkable clinical response suggests that the fusion gene products were sensitive to either As$_2$O$_3$ or ATRA or to both. An in vitro model of this variant could help clarify this issue, but based on current knowledge of the mechanisms of As$_2$O$_3$ and ATRA in APL, we propose that both agents may be effective.

Figure 2. Molecular analysis of PRKAR1A-RARA fusion transcripts. (A) The diagram shows the exon structure of the human PRKAR1A and RARA mRNAs (GenBank Accessions NM_002734 and NM_000964, respectively), with translated regions indicated by wide rectangles. Exploded views of exons 2 and 3 of PRKAR1A, and exons 3 and 4 of RARA are indicated with the positions of PCR primers used, PRKAR1A-F1 (5’-gaaccatggagtctggc-3’), PRKAR1A-F2 (5’-ggttggagaaggaggag-3’), RARA-R34o1 (5’-gccgggagaagcccttgcag-3’), and RARA-R3 (5’-cagccctcacaggcgctgac-3’). (B) PRKAR1A-RARA RT-PCR analysis using primers PRKAR1A-F1 and RARA-R34o1 resulted in the detection of 2 transcripts (PCR products of 444 bp [base pairs] and 344 bp) in the diagnostic bone marrow (Dx) of the index patient but no products were amplified from either the 11-month remission sample (11 mo), from Meg01 cell line (Meg), used as a negative control, or in a “no template” control (NTC). DNA molecular weight markers (M) were 100 base pair ladder (GE Healthcare, Buckinghamshire, United Kingdom). (C) Reamplification of the reactions shown in panel B with nested PCR primers PRKAR1A-F2 and RARA-R3 (that exclusively amplify fusions between PRKAR1A exon 3 and RARA exon 3) resulted in production of a single 256-bp product in the diagnostic sample. The absence of a product in the remission sample confirmed PRKAR1A-RARA molecular remission. (D) Partial sequence trace of the predominant in-frame PRKAR1A-RARA fusion transcript product showing the junction of the 2 genes as the result of RNA splicing from a cryptic splice donor within PRKAR1A exon 3. (E) Diagram showing the exon structures (narrow rectangles), open reading frames (wide rectangles), and partial amino acid sequence at the fusion junctions of the 2 main PRKAR1A-RARA transcripts present at diagnosis. PRKAR1A-derived exons and sequences are indicated by gray shading. The in-frame fusion (GenBank Accession EF428110) would be capable of encoding a 495–amino acid protein. The shorter frame-shifted fusion transcript (GenBank Accession EF428111) would encode a truncated RI/H9251 protein with 11 carboxy terminal amino acids (in italics) derived from the frame-shifted RARA exon 3.
to pharmacologic doses of ATRA. Second, there may be synergic effects because of the involvement of PRKAR1A in the fusion. Gene expression profiling of the APL-derived NB4 cell line identified PRKAR1A as one of the genes that is up-regulated in response to ATRA treatment. Thus, ATRA treatment could also up-regulate expression of the in-frame PRKAR1A-RARA fusion transcript or the shorter out-of-frame fusion transcript, both of which might encode defective RIα proteins with dominantly-negative effects on normal RIα. The resulting constitutive activation of PKA activity could lead to increased responsiveness to As2O3, which is known to have synergic effects with cAMP in APL.

Although this report describes a single case expressing PRKAR1A-RARA, the nature of the cryptic cytogenetic lesion raises the possibility that other cases of variant APL carrying the same lesion may have been missed, even with RARA-specific FISH probes.

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References

The *PRKAR1A* gene is fused to *RARA* in a new variant acute promyelocytic leukemia

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