Acute promyelocytic leukemia with a cryptic insertion of RARA into PML on chromosome 15 due to uniparental isodisomy: A case report

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Abstract. Acute promyelocytic leukemia is a myeloid disorder that is characterized by the specific t(15;17) variant in ~98% of cases. The typical hypergranular and microgranular or hypogranular types exist, and are frequently associated with disseminated intravascular coagulopathy. Rare cases of promyelocytic leukemia-retinoic acid receptor α (PML-RARA) fusion without the reciprocal RARA-PML have been reported in cytogenetically normal samples. Conversely, fluorescence in situ hybridization (FISH) analysis has revealed a cryptic insertion of the RARA gene into the PML gene on chromosome 15. The current study reports a unique case with a normal karyotype and molecular evidence of the PML-RARA short isoform 3-fusion transcript, with FISH analysis revealing two fusion signals on the two copies of chromosome 15, but absence of the reciprocal on the two copies of chromosome 17. This finding raised the hypothesis of chromosome 15 uniparental isodisomy as consequence of normal chromosome 15 loss and duplication of the rearranged chromosome, as supported by polymorphic loci molecular analysis. The clinical, cytogenetic and molecular characterization of this case are presented and discussed in the present study.

Introduction

The diagnostic hallmark of acute promyelocytic leukemia (APL) is the reciprocal translocation t(15;17)(q24;q21), leading to the disruption of the promyelocytic leukemia (PML) and retinoic acid receptor α (RARA) genes, resulting in PML-RARA and RARA-PML fusion products in ~98% of cases (1-7). The PML-RARA fusion transcript from der(15)t(15;17) serves a key role in leukemogenesis, inhibiting the differentiation and promoting the survival of myeloid precursor cells (8). Three regions of the PML locus are primarily involved in the t(15;17) translocation breakpoint cluster regions (bcrs): intron 6 (bcr1), exon 6 (bcr2) and intron 3 (bcr3), whereas RARA breakpoints always occur in intron 2. As a consequence, there are three possible PML-RARA isoforms, referred to as long (bcr1), variant (bcr2) and short (bcr3) isoforms (9).

Assessment of PML-RARA formation, or variant RARA gene rearrangements by means of conventional karyotyping, fluorescence in situ hybridization (FISH) or reverse transcription-polymerase chain reaction (RT-PCR), is required for the diagnosis of APL (10). In rare cytogenetically normal cases, FISH or molecular methods demonstrate the presence of the PML-RARA fusion gene without the reciprocal RARA-PML, resulting from a submicroscopic insertion of RARA into PML. Since this cryptic insertion has rarely been reported, no prognostic significance has been clearly established (9,11-22); however, a prompt diagnosis and the administration of targeted therapies, including all-trans retinoic acid (ATRA) and arsenic trioxide (ATO), are essential to improve the outcome in these patients (21). Due to the use of contemporary targeted therapy, APL has become a highly curable disease with complete remission rates of >95% and cure rates of >80% (23-27).

To the best of our knowledge, the current case is the first reported with two PML-RARA FISH fusion signals present on the two copies of chromosome 15, as result of a cryptic insertion of RARA into PML and chromosome 15 uniparental isodisomy (iUPD), likely due to loss of the normal chromosome 15 and duplication of the rearranged one (28,29). Written informed consent was obtained from the patient.

Case report

Patient presentation. A 73-year-old female Caucasian patient was admitted to the Humanitas Clinical and Research Center (Milan, Italy) in January 2016 with monocytosis, anemia and
thrombocytopenia incidentally diagnosed during a knee replacement surgery. The patient's medical history revealed β-thalassemia minor trait, obesity, hypertension, mild fasting hyperglycemia and toxic multinodular goiter. In 1982, the patient had undergone a bilateral hysterectomy to remove fibroids, and in 2010 the patient had undergone a cholecystectomy due to cholelithiasis. During the admission, the peripheral blood examination revealed a hemoglobin count of 8.8 g/dl (normal range, 12-16 g/dl) and a platelet count of 4.7x10^9/l (normal range, 13.0-40.0x10^9/l), as well as a white blood cell count of 5.24x10^9/l (normal range, 4.0-10x10^9/l). A peripheral blood cell smear revealed prominent leukocytosis with blast cells accounting for 94% of all nucleated cells, characterized by hypogranular bilobed nuclei (French-American-British classification M3 variant) (30). Peripheral blood flow cytometric analysis revealed positivity for cluster of differentiation (CD) 13, CD33, myeloperoxidase, CD2 and CD9, and a negative result for human leukocyte antigen-antigen D related, CD117, CD15, CD4, CD19, CD14, CD10, CD3 and CD34. The patient was clinically diagnosed with APL. The karyotype, as determined from the peripheral blood, was 46,XX. As Q-LAMP revealed positivity for the PML-RARA transcript, FISH was performed and two fusion signals on the two copies of chromosome 15 were observed.

Treatment. According to the ATRA and idarubicin (AIDA) protocol, the patient was treated with induction chemotherapy and received 45 mg/m² ATRA twice a day and 12 mg/m²/day idarubicin for 3 days (one cycle). The patient developed differentiation syndrome symptoms and disseminated intravascular coagulation with intracranial bleeding. On day 20 post-therapy, the patient was in clinical remission. Molecular analysis using microsatellites and performed on a peripheral blood sample supported the hypothesis of chromosome 15 iUPD.

On day 48 post-therapy, cytogenetic, FISH and RT-PCR analyses were performed, with normal results. The patient received consolidation therapy with 1,000 mg/m²/day cytarabine for 5 days, 5 mg/m²/day idarubicin for 5 days and 45 mg/m² ATRA twice a day for 15 days, and is presently under maintenance therapy. During the clinical course no substantial difference compared with classical APL patients was observed.

Cytogenetic analysis. Cytogenetic analysis was performed on peripheral blood samples incubated for 24 h according to standard procedures (31). A total of 27 spontaneous quinacrine-banded metaphases were analyzed and the karyotype described according to the International System for Human Cytogenetic Nomenclature 2013 criteria (32). Following induction therapy on day 48, the karyotype was obtained from a bone marrow sample following a 24-h incubation period. A total of 25 spontaneous metaphases were analyzed.

FISH analysis. FISH was performed according to the manufacturer's protocol on metaphase and interphase nuclei using the commercially available SureFISH PML-RARA dual-color dual-fusion DNA probe, specific for the PML (15q24; spectrum red) and RARA (17q21; spectrum green) loci (Agilent Technologies, Inc., Santa Clara, CA, USA). A total of 15 metaphases and 100 nuclei were scored for the peripheral blood and the bone marrow samples.

Molecular methods. The presence of the PML-RARA transcript was evaluated using a commercial kit (DiaSorin, Saluggia, Italy) based on the non-PCR quenching loop-mediated isothermal amplification (Q-LAMP) method modified to introduce fluorescent oligonucleotides and a new polymerase with RNA reverse transcription and DNA amplification activity, as previously described by Spinelli et al (33). To investigate the iUPD hypothesis, the analysis of short tandem repeats (STR) present on 15 different alleles comprising the Penta E locus on chromosome 15 was performed on 0.5 ng of DNA at diagnosis and on day 20 post-therapy using a commercial kit (PowerPlex® 16 System; Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol.

Results

Cytogenetics and FISH results. A 46,XX karyotype was observed in the peripheral blood and bone marrow samples. In the peripheral blood sample, interphase FISH revealed normal cells with two red PML and two green RARA signals in 12% of the nuclei, and a variant fusion pattern characterized by two green RARA and two yellow PML-RARA fusion signals in 88% of nuclei. All the metaphase cells analyzed by FISH exhibited two PML/RARA fusion signals, one on each copy of chromosome 15, and two normal RARA signals on the two copies of chromosome 17, consistent with the interphase FISH pattern (Fig. 1). A normal FISH pattern was observed on the bone marrow specimen following induction therapy.

Molecular results. The Q-LAMP assay assessed the presence of the bcr3/short form PML-RARA fusion transcript. The STR analysis of the Penta E locus on chromosome 15 revealed the presence of two peaks of 11 and 12 STR repeats in the remission sample (Fig. 2A) demonstrating that two chromosomes with varying STR numbers were present in the patient's normal cells. Conversely, only the 11 repeat signal was present in the diagnostic sample, representing the APL cells (Fig. 2B). This latter feature is consistent with the presence of only one chromosome 15 or with the presence of two identical copies of chromosome 15 (iUPD).

Discussion

Approximately 9% of APL patients do not harbor the classic t(15;17) translocation; however, certain patients still express the PML-RARA fusion gene. These cases are considered to have a 'cryptic' transcript resulting from sub-microscopic insertions of PML or RARA or more complex rearrangements, thus escaping detection with conventional cytogenetic analysis. In these rare cases, the cryptic transcript is usually detected by RT-PCR. As ATRA and ATO are targeted therapies against the action of the PML-RARA protein, patients who lack the classic translocation but present the fusion product may also benefit from these therapies (14,15,20,21). There have been a number of previous studies describing patients with morphological features of APL and a normal karyotype who are FISH-negative for t(15;17), but RT-PCR-positive for PML-RARA. The treatment with ATRA reveals similar good responses and favorable prognoses compared with
those observed in patients harboring the classic t(15;17) variant (14,15,20,21).

The increasing number of reported cases with cryptic rearrangements supports the requirement for varied integrated diagnostic approaches in order to recognize the presence of fusion products that respond to targeted therapy. In particular, the current case demonstrated the importance of FISH evaluation not only on interphase nuclei, but also on metaphase cells, which allowed the identification of the underlying mechanism leading to the rearrangement. Q-LAMP revealed the presence of the PML-RARA transcript, but only FISH detected the presence of the double insertion of RARA into each copy of the PML gene, supporting the hypothesis of chromosome 15 iUPD, which was then confirmed using microsatellite analysis.

Acquired UPD (aUPD), most frequently segmental, has been reported in 15-20% of patients affected by acute myeloid leukemia, resulting in gene dosage alterations and homozygosity for mutated genes that can provide a proliferative advantage or increased chemoresistance (34,35). Chromosomes 11 and 13 have the highest number of aUPDs, but aUPDs on Xq, 1p, 2p, 2q, 6p, 9p, 17p, 17q, 19q and 21q have also been reported (34,35). To the best of our knowledge, no aUPD has been reported for chromosome 15; however, it is well established that chromosome 15 is imprinted and constitutional UPD causes specific syndromes (28,29).

Numerical somatic UPDs may occur due to mitotic errors, including non-disjunction or loss of a chromosome due to anaphase lag followed by duplication of the remaining chromosome (28,29). The chromosome 15 UPD observed in
the current case may be the result of an insertion of RARA into PML, loss of the normal chromosome 15 and monosomy rescue by duplication of the rearranged one or trisomy consequent to non-disjunction followed by loss of the homolog, as reported in constitutional cases (Fig. 3) (28,29). These two mechanisms may be possible in acute myeloid leukemia, and chromosome 15 trisomy, despite being rare, is more frequently reported (36).

The presence of iUPD in the present case apparently did not affect the good outcome of therapy and the prognosis. However, the consequent loss of heterozygosity for the entire chromosome may have introduced homozygosity for coding polymorphisms with variable functional activity, including those in the drug metabolizing enzymes, non-coding regulatory polymorphisms that result in differential expression of alleles and haploinsufficiency or overexpression of important proteins (37-39). A close follow-up is required for this patient in order to monitor the possible prognostic negative effect due to the iUPD.

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