Do RARA/PML fusion gene deletions confer resistance to ATRA-based therapy in patients with acute promyelocytic leukemia?

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Acute promyelocytic leukemia (APL) is characterized by the translocation t(15;17)(q22;q21), resulting in the promyelocytic leukemia (PML)-retinoic acid receptor alpha (RARA) fusion protein[1] in 95% cases whereas variant translocations involving *PLZF* (11q23), *NPM* (5q35), *NUMA* (11q13) and *STAT5b* (17q23) account for the rest.[2] Leukemias with PML-RARA translocations respond well to all-*trans* retinoic acid (ATRA) or arsenic trioxide (ATO) therapy[3] whereas those with PLZF-RARA fusions respond poorly. Although primary resistance to ATRA is rare, secondary or acquired resistance is frequently observed in patients treated with ATRA alone or in combination with other chemotherapy regimens. However, molecular abnormalities mediating resistance to ATRA therapy are underexplored. Here, we report two cases of APL with RARA-PML deletions on der(17) or der(15), which displayed clinical evidence of primary and secondary resistance to therapy, respectively.

Patient 1 is a 63-year-old Caucasian male presented with pancytopenia and gastrointestinal bleeding. Morphologic evaluation of the blood smear showed blasts with Auer rods and cytologic features of hypergranular or 'typical' APL. Flow cytometry demonstrated 41% blasts with the phenotype: CD34−, CD117+, CD33+, CD13+, human leukocyte antigen (HLA)-DR−, CD56+, myeloperoxidase (MPO)+ and CD64+. Karyotype analysis revealed 46,XY,t(15;17)(q22;q21)[19] (Figure 1a). Fluorescence *in situ* hybridization (FISH) analysis using PML/RARA dual fusion probes detected an unusual signal pattern in 74.6% cells (one fused, one green, one red signal) instead of the expected pattern (two fused; one on each derivative chromosome, one red, and one green on normal 15 and 17, respectively). Analysis of metaphases allowed mapping of the deleted fusion signal to der(17)t(15;17) (Figure 1d) and showed a residual PML signal on der(17) (Figure 1d), suggesting that the deletion encompassed a major part of the 3′ end of *PML* and the entire 5′ end of *RARA* on der(17)t(15;17). On further examination of metaphase preparations, one (16%) or

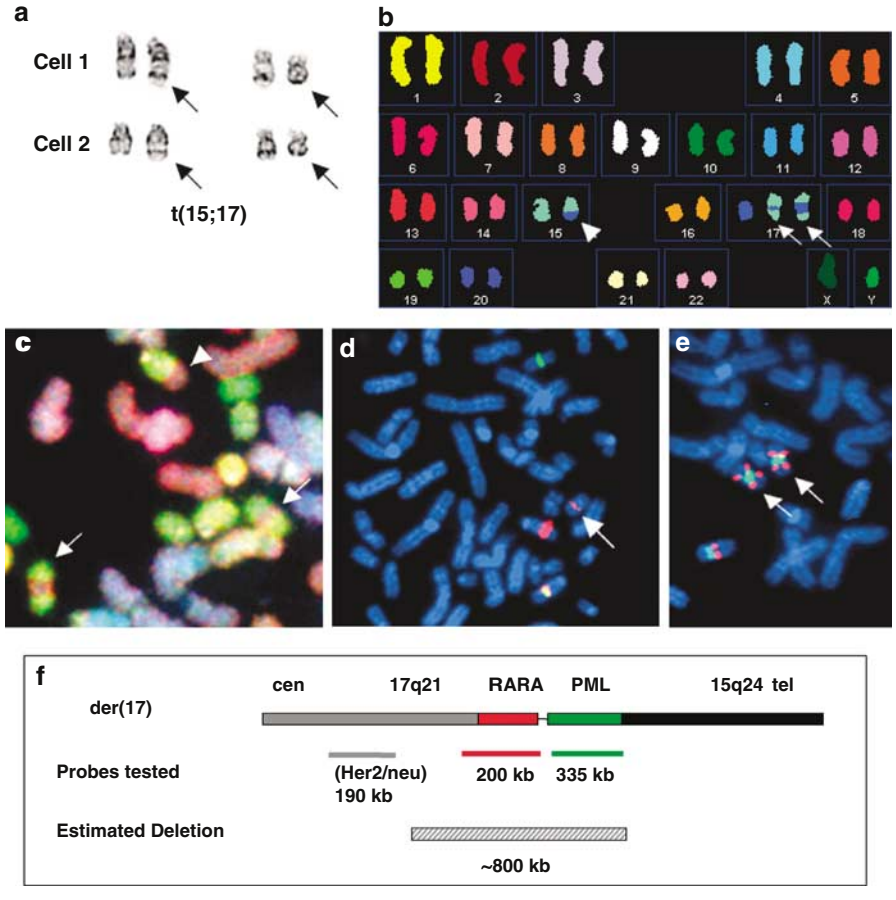

Figure 1 Identification of deletions on der(17) in APL Patient 1. (**a**) Partial karyotype showing t(15;17) (arrows). (**b–c**) Pseudo-colored SKY karyotype (**b**) and partial metaphase of spectral image (**c**) showing t(15;17) and ider(17). Arrowhead indicates der(15) and arrows showing ider(17). (**d**) Detection of deletion on der(17) (arrow) using PML/RARA dual fusion probe. PML labeled with spectrum red and RARA with spectrum green. (**e**) FISH analysis shows the retention of Her-2/neu gene on ider(17) chromosomes (arrows). Green signals indicate centromere 17 and red signals are Her-2/neu. (**f**) Schematic presentation of der(17)t(15;17) showing the estimated size of the deletion (not drawn to scale).

two (36%) copies of ider(17)(q10)t(15;17) were also identified (Figure 1b–c and e). Spectral karyotype (SKY) analysis, performed to rule out other cryptic abnormalities, confirmed the t(15;17)(q22;q21) and ider(17)(q10)t(15;17) (Figure 1b and c).

The patient achieved hematologic remission after treatment with ATRA and idarubicin. However, a bone marrow biopsy 30 days post-therapy showed dysplastic myeloid maturation and flow cytometry detected 2% blasts. Karyotype analysis showed 46,XY,t(15;17)(q22;q21)[6]/46,XY[14] and FISH with PML/RARA probes confirmed t(15;17) in 69.7% cells with a single fusion signal. These findings were consistent with primary resistance to therapy. After re-induction therapy with addition of ATO, the patient is currently in complete hematologic and cytogenetic remission.

Patient 2 is 18-year-old female presented with 2 days of petechiae and intra-cranial hemorrhage. The initial white blood cell count was $38\,000 \times 10^6$/l and platelet count was $15\,000 \times 10^6$/l. Review of the marrow aspirate showed 90% blasts, many with Auer rods, which had morphologic features of 'typical' APL. Flow cytometry detected 80% blasts with the phenotype: CD34−, CD117+, CD33+, CD13+, HLA-DR−, MPO+ and CD64+. Karyotype analysis showed 46,XX,t(15;17)(q22;q21)[5]/47,XX,idem,+8[15]. FISH analysis using PML/RARA dual fusion probes detected a typical signal pattern in 97.1% cells (Figure 2a). During induction treatment (ATRA and daunorubicin 200 mg/m^2), the patient developed ATRA syndrome, and was treated with steroids and dose reduction of ATRA. She achieved morphologic and cytogenetic remission after consolidation treatment with two cycles of ATO and daunorubicin (300 mg/m^2). After 23 months of remission, her leukemia relapsed. The marrow aspirate showed 87% blasts with a phenotype identical to the primary APL. Karyotype analysis demonstrated 46,XX,t(15;17)(q22;q21)[17]/46,XX[3]. FISH analysis using PML/RARA probes revealed 96.3% cells with rearrangement and deletion of PML on der(15) (Figure 2b–d), which was suggestive of a deletion involving the 5′ end of PML gene on der(15). To determine the size of this deletion, we performed FISH utilizing probes flanking both sides of the PML breakpoint. FISH analysis with a 96 kb bacterial artificial chromosome (BAC) clone spanning intron 4 and all of the 3′ end of the PML breakpoint confirmed retention of the 3′ end of the translocated PML on der(17) and a residual signal on der(15), consistent with the PML breakpoint being distal to exon 5 (Figure 2f). Two BAC clones spanning −376 to −107 kb 5′ of the PML gene, showed no evidence of deletions on der(15) (Figure 2e). The size of the deletion was estimated to be 100–150 kb based on loss of the major portion of the 5′ end of PML and retention of signals upstream of the PML gene. These data suggest that PML deletion on der(15) might be responsible with secondary resistance to therapy. After re-induction with

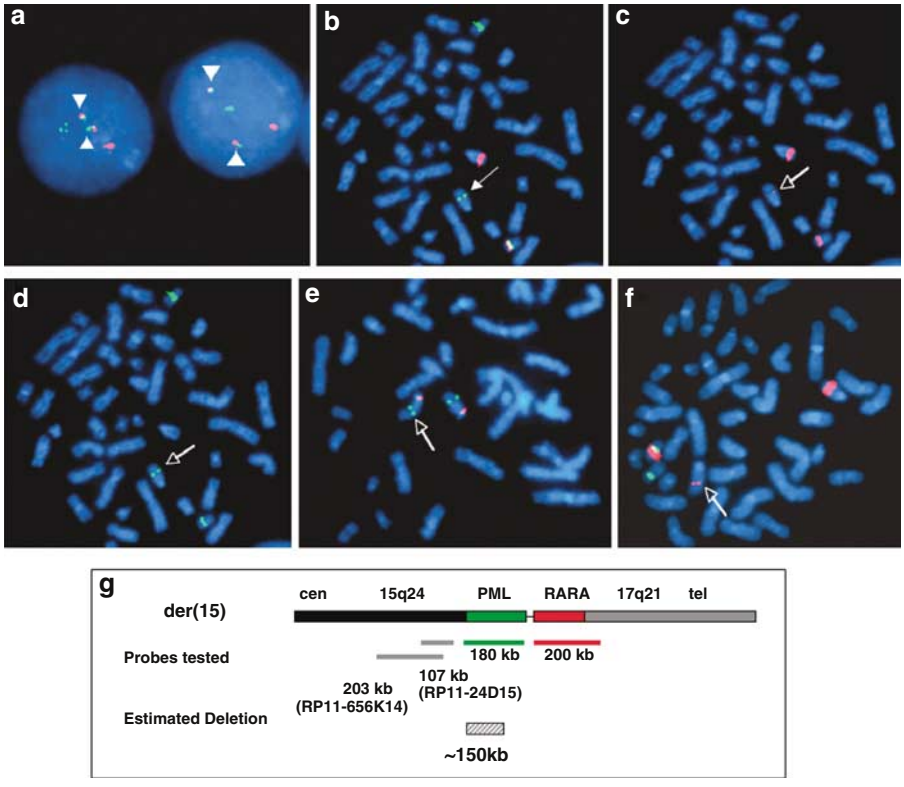

Figure 2 Identification of deletion on der(15) in APL Patient 2. (**a**) Interphase FISH analysis using PML/RARA dual fusion probe shows two fused signals (arrow heads) in blasts from the primary sample. (**b–d**) Metaphase FISH analysis of PML/RARA probe showing one fused signal and deletion of PML on der(15) at relapse (arrow). (**b**) Both probes, (**c**) only PML probe (red) hybridization of the same metaphase shown in (**b**) with deletion of PML region on der(15) indicated by arrow, and (**d**) only RARA probe (green) hybridization of the same metaphase shown in (**b** and **c**) with the retention of translocated RARA on der(15) (arrow). (**e**) Metaphase FISH analysis showing retention of BAC RP11-24D15 on der(15) (arrow) mapped 107 kb upstream of PML (green) and red signals indicate centromere 15. (**f**) FISH analysis of a spectrum red labeled BAC clone (RP11-756N20) spanning intron 4 and the remaining 3′ end of PML showing retention of signals on der(17) (red-green signal) and residual signal on der(15) (red) (arrow). Green signals indicate centromere 17. (**g**) Schematic representation of probes used, showing estimated size of the 5′ end of PML deletion on der(15) (not drawn to scale).

ATO + ATRA, the patient is in complete remission 1 month post-mismatched unrelated donor stem cell transplant.

Submicroscopic deletions associated with chromosomal translocations/inversions have been documented in chronic myeloid leukemia (CML) and leukemias with t(8;21), inv(16)/t(16;16), or mixed lineage leukemia translocations.[4] Deletions in CML patients have been associated with refractory disease and a poor outcome.[5] Chromosomal deletions associated with t(15;17) have not been reported in APL.[4] Similar to CML, submicroscopic deletions in our first case encompassed a large region (~300 kb) 3′ of the *PML* gene and the entire 5′ of the RARA gene including exons 1 and 2, spanning a region of ~250 kb. The Her-2/neu gene, which maps 580 kb proximal to RARA, was retained (Figure 1e, f) suggesting that the deletion breakpoint on der(17) was between 250 and 500 kb centromeric to RARA. To our knowledge, this is the first described case of APL that demonstrated chromosomal deletions in association with a RARA/PML translocation and exhibited primary resistance to ATRA-based therapy. We propose that the genetic deletions on either side of the translocation on 17q might be responsible for the primary resistance. The 300 kb deleted region, distal to PML on der(17q), contains *ISLR*, *STRA6* and *CYP11A1* and loss of one of these genes might account for resistance to therapy. The 400 kb genomic region proximal to 5′ *RARA* on 17q is a gene-rich region, which contains *CDC6*, *WIRE*, *RAPGEFL1*, *CASC3*, *NR1D1*, *THRA*, *THRAP4*, *CSF3*, *PSMD3*, *GSDM1*, *ORMDL3*, *GSDML* and *SPBP2*. Haploinsufficiency of some of these genes may function as tumor suppressors or modifiers. The ider(17) chromosome resulted in *TP53* gene deletion (data not shown), which may further contribute to growth advantage and treatment resistance. Thus, as in CML, large deletions accompanying translocations in APL might impart a proliferative advantage, resistance to cell death, or lead to differentiation arrest.

Primary resistance of APL with t(15;17) to ATRA induction therapy is rare but secondary resistance and relapse of disease after clinical remission has been described. Deletions in the carboxy terminus of *PML* exon 6,[6] mutations of the normal *PML* allele[7] and insertions in *RARA* intron 2[8] have been shown to reduce sensitivity to maturation inducing agents and secondary resistance to therapy. Our second case demonstrated a ~100 kb deletion in the amino terminus of PML, 5′ of exon 6 on der(15). Two genes, *STOML1* (−12 kb) and *LOXL1* (−68 kb), that map immediately upstream are the likely targets of this deletion. Although the function of STOML1 is unknown, LOXL1 is implicated in developmental regulation, senescence, tumor suppression, cell growth control and chemotaxis. Haploinsufficiency or loss of function of these genes may play a role in secondary resistance. Further studies are needed to delineate the frequency of submicroscopic deletions in APL and their molecular and clinical consequence.

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Report on two novel nucleotide exchanges in the *JAK2* pseudokinase domain: D620E and E627E

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The *V617F* point mutation of the *JAK2* gene (located in chromosome 9p24) shows a high frequency in diverse *BCR-ABL*-negative chronic myeloproliferative disorders. A thymidine > guanine substitution at position 1849 leads to a valine to phenylalanine exchange at amino acid 617, which is localized in exon 12. The highest frequency of point mutations of the *JAK2* gene is found in polycythemia vera (80–95% of all cases), which is followed by essential thrombocytosis and idiopathic osteomyelofibrosis (40–55% of all cases). The mutation is further known to occur at low frequency in the so-called unclassifiable MPS (according to the WHO classification), in chronic myelomonocytic leukemia, in myelodysplastic syndrome and in acute myeloid leukemia (AML).[1–5] The cytoplasmic non-receptor Janus kinases regulate tyrosine phosphorylation of