Favourable outcome in an APL patient with PLZF/RARalpha fusion gene: quantitative real-time RT-PCR confirms molecular responsex

Rare cases of acute promyelocytic leukemia (APL) are associated with a t(11;17) translocation and a PLZF-RARalpha fusion transcript. Because of molecular specificities of the fusion protein, ATRA efficiency is often reduced in these cases. We present herein the case of an 83 year old patient which has been successfully treated by ATRA and Daunorubicin. The described quantitative RT-PCR method allowed successful monitoring and confirmation of the molecular response.

Haematologica 2006; 91:(12)e158-e159

Acute Promyelocytic leukemia (APL) or AML-M3 according to the FAB classification is usually defined by morphological and clinical criteria. Though the majority of APL blasts are defined by heavy azurophilic granules, bundles of Auer rods, and a reniform or bilobed nucleus, 20% of patients display features consistent with a hypogranular or microgranular variant of APL (FAB M3V). APL is associated at the molecular level with the presence of reciprocal translocations involving chromosome 17. The first identified and most frequent translocation is the t(15;17)(q22;q21) translocation generating the chimeric gene PML-RARα. This fusion gene is involved in the APL leukemogenesis and the blockage at the promyelocytic stage of the myeloid stem cell, where RARα and Retinoic Acid (RA) play a key role. It is also the target of the all-trans retinoic acid (ATRA) and arsenic trioxide (AS2O3) sensitivity of APL blasts (see ref 1 for a review). Other cytogenetic variants have been reported: t(11;17)(q23;q21), t(5;17)(q35;q21), t(11;17) (q13;q21) and der (17) (see ref 2 for a review). The RARα gene, located on chromosome 17q21 is always involved, supporting the central role of the x- RARα fusion genes in the pathogenesis of this leukaemia which animal models have confirmed.[2] However, several cellular or clinical characteristics differ according to the fusion partner.[3] The first and most frequently reported translocation is t(11;17)(q23;q21) generating the PLZF-RARα fusion gene.[4] The presence of PLZF-RARα fusion gene has been reported to be associated to cytological and molecular features of APL[5,6] though distinct morphological characteristics are found.[7] Compared to other X-RARα APL, PLZF-RARα cells are resistant to ATRA, both in humans[6,8] and mice models,[9] due to of an increased binding affinity of the chimeric protein for co-repressors.[9] Because PLZF-RARα also confers resistance to chemotherapy and arsenic trioxide (As_2O3) APL patients harbouring this unusual fusion gene have a poor outcome.[2] Thus, these rare PLZF-RARα APL cases require molecular diagnosis for accurate diagnosis and quantitative RT-PCR to monitor efficacy of potential therapeutical approaches.

We report herein the case of an 83 year old patient in whom an AML-M3variant was diagnosed and cytogenetic analysis evidenced the presence of a t(11;17) translocation. A specific RT-PCR, identified a PLZF-RARα transcript (Figure 1a-b). No sign of disseminated intravascular coagulation or abnormal fibrinolysis was observed. Due to age, the patient was treated with ATRA alone (90 mg/day) resulting in a bone marrow blast decrease from 85% to 25% by day 17. At day 20, the patient received a first course of Daunorubicin (60 mg/m^2 on 3 consecutive days) while ATRA was maintained for a further 10 days. A total of three Daunorubicin courses were given at one month interval. Haematological complete remission was obtained at day 74. A conventional maintenance treatment with ATRA, purinethol and methotrexate was initiated for 2 years. At 1.5 year after diagnosis the patient is still in complete haematological remission.

Figure 1. (A) Detection of the PLZF-RARα fusion transcript by RT-PCR. Total RNA extracted from APL blasts and reverse transcribed into cDNA (lane1), water (lane2) and a PLZF-RARα plasmid (lane3) were subjected to amplification with specific PLZF and RARα primers. (B) Sequence of the PCR product at the fusion site between the two genes. (C and D) Standard curve obtained from real-time PCR analysis of serial 10 fold plasmid dilutions. (E) minimal residual disease follow-up by quantitative RT-PCR. CT: chemotherapy, ATRA: All Trans Retinoic Acid.

In order to monitor minimal residual disease in this patient, we developed a real-time quantitative RT-PCR assay (see Table 1 for primers and probe sequences). Total RNA was extracted from bone marrow cells and retro-transcribed as previously described.[10] The PBGD gene was used in order to normalize the PLZF-RARα copy number10 and standard curves were obtained using full length cDNA containing plasmids dilutions (Figure 1c-d). Figure 1e shows the quantitative assessment of normalised PLZF-RARα transcripts in the bone marrow at different time intervals. Despite the decrease of blasts observed in the bone marrow at day 17 of ATRA treatment, the PLZF-RARα copy number did not decrease. This may be attributed to the persistance of differentiated APL blasts as reported for PML-RARα APL cells.[1] A significant decrease in the PLZF-RARα copy number was observed after the first course of Daunorubicin. At the third course the fusion transcript was no longer detectable with a sensitivity of the assay of 10-5. The patient was then in haematological and molecular complete remission.

Thus, this observation confirms that in some cases ATRA may have partial efficacy[6,11] in PLFZ-RARα APLs, at least in reducing the number of APL blasts. The described quantitative RT-PCR method allows successfully monitoring and confirmation of the molecular response obtained with combination therapies such as Daunorubicin.

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Table 1. Primer and probe sequences for RT-PCR amplification of the PLZF-RARα gene

Standard RT-PCR
Forward primer : 5'-CCGTGACCTCTGGCCTCCAC-3'
Reverse primer : 5'- GCTGGGCACTATCTCTTCAG -3'
Quantitative RT-PCR
Forward primer : 5'-GAAGACGTACGGGTGCGAGCTC-3'
Reverse primer : 5'-CTCACAGGCGCTGACCCCATAGT-3'
Probe : 5'-CCAGCCCTCCCTCGCCACCCCCTCTA -3'

Acknowledgements: The authors thank Dr Antonio Parrado for the sequences of PLZF primers.

Key words: quantitative, RT-PCR, APL, PLZF/RAR, t(11;17)
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References

1. Cassinat B, Chomienne C. Biological features of primary APL blasts: their relevance to the understanding of granulopoiesis, leukemogenesis and patient management. Oncogene 2001;20:7154-60.
2. Redner RL. Variations on a theme: the alternate translocations in APL. Leukemia 2002;16:1927-32.
3. Rego EM, Ruggero D, Tribioli C, Cattoretti G, Kogan S, Redner RL et al. Leukemia with distinct phenotypes in transgenic mice expressing PML/RAR alpha, PLZF/RAR alpha or NPM/RAR alpha. Oncogene 2006;25:1974-9.
4. Chen SJ, Zelent A, Tong JH, Yu HQ, Wang ZY, Derre J et al. Rearrangements of the retinoic acid receptor alpha and promyelocytic leukemia zinc finger genes resulting from t(11;17)(q23;q21) in a patient with acute promyelocytic leukemia. J Clin Invest 1993;91:2260-7.
5. Chen Z, Guidez F, Rousselot P, Agadir A, Chen SJ, Wang ZY et al. PLZF-RAR alpha fusion proteins generated from the variant t(11;17)(q23;q21) translocation in acute promyelocytic leukemia inhibit ligand-dependent transactivation of wild-type retinoic acid receptors. Proc Natl Acad Sci USA 1994;91:1178-82.
6. Licht JD, Chomienne C, Goy A, Chen A, Scott AA, Head DR et al. Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukemia associated with translocation (11;17). Blood 1995;85:1083-94.
7. Sainty D, Liso V, Cantu-Rajnoldi A, Head D, Mozziconacci MJ, Arnoulet C et al. new morphologic classification system for acute promyelocytic leukemia distinguishes cases with underlying PLZF/RARA gene rearrangements. Group Francais de Cytogenetique Hematologique, UK Cancer Cytogenetics Group and BIOMED 1 European Community-Concerted Action "Molecular Cytogenetic Diagnosis in Haematological Malignancies. Blood 2000;96:1287-96.
8. Guidez F, Huang W, Tong JH, Dubois C, Balitrand N, Waxman S et al. Poor response to all-trans retinoic acid therapy in a t(11;17) PLZF/RAR alpha patient. Leukemia 1994;8: 312-7.
9. He LZ, Guidez F, Tribioli C, Peruzzi D, Ruthardt M, Zelent A et al. Distinct interactions of PML-RARalpha and PLZF-RARalpha with co-repressors determine differential responses to RA in APL. Nat Genet 1998;18:126-35.
10. Cassinat B, Zassadowski F, Balitrand N, Barbey C, Rain JD, Fenaux P et al. Quantitation of minimal residual disease in acute promyelocytic leukemia patients with t(15;17) translocation using real-time RT-PCR. Leukemia 2000;14:324-8.
11. Petti MC, Fazi F, Gentile M, Diverio D, De Fabritiis P, De Propris MS et al. Complete remission through blast cell differentiation in PLZF/RARalpha-positive acute promyelocytic leukemia: in vitro and in vivo studies. Blood 2002;100:1065-7.