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THE PML/RARα FUSION GENE IN THE DIAGNOSIS AND MONITORING OF ACUTE PROMYELOCYTIC LEUKEMIA

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ABSTRACT

The acute promyelocytic leukemia (APL)-specific t(15;17) chromosome abnormality is characterized at the molecular level by rearrangement of the PML and RARα genes, resulting in fusion PML/RARα mRNA and a chimeric protein. Besides its relevance in the pathogenesis of the disease, this hybrid gene represents a specific tumor marker that is rapidly detectable by reverse transcriptase-polymerase chain reaction (RT-PCR) in the RNA extracted from leukemic blasts.

Several studies have highlighted the clinical relevance of PML/RARα detection, which provides a specific diagnosis, prognostic information, and prediction of relapse when monitoring residual disease during the follow-up. In fact, this hybrid gene is detected in 100% of APLs. Rare cases of patients with a morphological diagnosis of FAB M3 AML who lack the specific PML/RARα abnormality have been reported as being unresponsive to differentiation treatment. Finally, all the studies reported so far on PCR monitoring in APL have documented that the identification of small amounts of residual disease at remission strongly predicts impending relapse. Thus, RT-PCR of the hybrid PML/RARα gene is currently performed prospectively as part of cooperative clinical trials aimed at better addressing post-remission treatment in APL.

Key words: acute promyelocytic leukemia, RT-PCR, PML/RARα

Acute promyelocytic leukemia (APL) is a well-defined subset of acute myeloblastic leukemia, type M3 and M3v of the FAB classification, with peculiar biological and clinical features. These features include the frequent association at diagnosis or during the early phase of treatment of a life-threatening coagulopathy, the presence in leukemic blasts of a specific cytogenetic abnormality [the t(15;17) translocation], and the response to differentiating agents.[1-4] Molecular studies have shown that two genes, PML and RARα, on chromosomes 15q24 and 17q21, respectively, are interrupted and rearranged as a result of the translocation, giving rise to hybrid PML/RARα and RARα/PML fusion mRNA products and chimeric proteins. These molecular abnormalities are thought to play a critical role in the pathogenesis of the disease.[4]

Based on the observation that treatment with all-trans retinoic acid (ATRA) can induce differentiation of leukemic blasts both *in vitro* and *in vivo*, as well as clinical-morphological remission in APL patients, most recent trials include ATRA, variably combined with chemotherapy, in the front-line treatment of APL.

In this review, after a brief description of the molecular features of the disease, we will focus on the relevance of detecting the specific PML/RARα fusion gene in the diagnosis, prognosis and disease monitoring of APL.

Molecular genetics of APL

A reciprocal translocation between chromosomes 15 and 17 is found in 70-90% of APL cases by karyotypic analysis, and in 100% of cases using molecular techniques.[5-7]

Interestingly, the t(15;17) is often the only chromosomal abnormality seen in neoplastic metaphases. The breakpoint on chromosome 17 involves the gene coding for the retinoic acid receptor-α (RARα), whereas the breakpoint on chromosome 15 involves a newly characterized

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gene, named PML (for promyelocytic leukemia).[8-13]

RARα, which is a member of the nuclear hormone receptor superfamily, is known to play an important role in normal development and differentiation. Following binding with its natural ligand (retinoic acid, RA), RARα trans-activates target genes by regulating their transcriptional activity.[14,15] Due to the presence of a zinc finger DNA-binding domain, PML is thought to act as a transcription factor. This gene is expressed in hematopoietic cells, particularly those of the granulocytic lineage.[16]

Two fusion genes are formed as a consequence of the reciprocal t(15;17) translocation: the PML/RARα and the RARα/PML on derivatives 15q+ and 17q–, respectively. The PML/RARα hybrid gene is transcriptionally active and expressed in all APL patients, whereas RARα/PML is detectable in only 70% of cases.[17,18] For this reason, PML/RARα is believed to play a major role in the pathogenesis of the disease. Preliminary *in vitro* experiments, in which PML/RARα was expressed in the U937 cell line, have shown that this chimeric gene was able to induce a differentiation block resembling that observed in APL *in vivo*. Interestingly, this maturation arrest was reversed by the addition of high concentrations of ATRA, as is also seen *in vivo* in patients receiving ATRA treatment.[19] In the t(15;17) the breakpoint on chromosome 17q is always located in the second intron of RARα. Due to heterogeneous breakpoints on chromosome 15, different PML portions are retained in the 5' region of PML/RARα, and this produces different fusion gene products in which the 3' RARα portion is always constant.

A detailed characterization study of different PML isoforms in APL patients has shown that the putative DNA binding domain is the only part of the PML gene consistently retained in all cases.[20]

Three different PML breakpoint cluster regions (BCR1, BCR2, BCR3) have been identified. The estimated frequencies of these BCR in the Italian APL population are 58%, 7%, and 35% approximately for BCR1, BCR2 and BCR3, respectively.[20] The clinical significance of these variable PML breakpoints in terms of diagnostic features and prognostic outcome is currently under investigation (see below).

Diagnosis

According to the FAB system, two morphological types may be identified in APL. Besides the more common hypergranular form, a microgranular variant (M3v) type is detectable less frequently. Peculiar features of APL blasts include the presence of granules visible under light microscopy in the typical M3, and only by electron microscopy in M3v, and strong myeloperoxidase and chloroacetate esterase activities.[1-4,21-23] A consistent immunophenotypic pattern (TdT^-, HLA/DR^-, $CD34^-$, $CD7^-$, $CD19^-$, $CD13^+$, $CD33^+$ and $CD9^+$) is reported in several studies, while for M3v, CD2 and CD34 positivity has also been described.[24-28]

Unlike other AMLs, APL is generally characterized at diagnosis by low leukocyte counts and blast infiltration of bone marrow (BM) only. In contrast, M3v is usually hyperleukocytic. Epidemiologically, too, APL differs from the other myeloid leukemias. It occurs most frequently between 15 and 60 years of age, and women are more frequently affected than men. One study reported a prevalence of cases in spring and autumn as compared to winter and summer.[29]

Rapid identification of APL has great relevance in clinical practice. In fact, many patients present with a severe, life-threatening coagulopathy which requires immediate treatment. Moreover, APL responds specifically to ATRA (which seems also effective in controlling the hemorrhagic diathesis). Therefore, the availability of rapid and specific tools for confirming morphological and immunological diagnosis is of paramount importance, particularly in those cases with uncertain morphologic features such as the M3v form.[21-23]

In this context it is important to consider that the t(15;17) genetic lesion not only characterizes all cases of APL, but also is never observed in other leukemic phenotypes. With cytogenetic analysis results can be obtained in 48 hours approximately, but only 70-90% of cases display the t(15;17) translocation. This is probably due either to interpretation problems in evaluating

Table 1. Diagnostic tools in APL.

	Morphology	*Immunologic phenotype*	*Karyotype*	*Molecular biology*
Specificity	good	moderate	very high	very high
Sensitivity	poor	moderate	good	very high*
Time required	< 1 hour	3-4 hours	2-3 days	6-7 hours*
Cost	low	limited	limited	high
Widespread availability	yes	possible	possible	difficult

*by RT-PCR

poor quality metaphases, or to the possible occurrence of microtranslocations. In some cases t(15;17) may be accompanied by other karyotypic changes such as trisomies.[30]

Following the cloning of the genes involved in the specific translocation, it has been possible, by Southern blot or polymerase chain reaction (PCR) techniques, to detect this specific lesion in all patients with M3 and M3v.[6,31] Southern blot hybridization allows specific identification of RARα and/or PML breakpoints, but requires at least 4 days. Reverse transcriptase (RT) PCR amplification of the PML/RARα fusion transcript using specific oligonucleotides is now widely used for both diagnostic and monitoring studies and offers several advantages.[32-36] First, it provides rapid results (6-8 hours in our hands from isolation of mononuclear blasts to PCR evaluation). Secondly, the exact type of breakpoint on the PML gene can be determined. Finally, as will be discussed below in more detail, RT-PCR allows the detection of minimal residual disease (MRD) at high sensitivity levels.

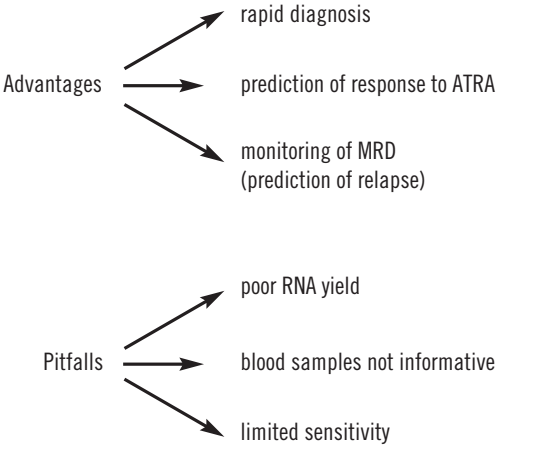
Besides the advantages of RT-PCR in APL, it is worth mentioning some of its pitfalls. Among others, the problems encountered in performing this technique include poor RNA yield and stability, as well as the low expression of the hybrid PML/RARα gene. For these reasons we believe that Southern blot analysis should be recommended for routine laboratories and that the RT-PCR approach is more indicated in a few Centers that have more experience with RNA manipulation and PCR technology.[37] Tables 1 and 2 illustrate a comparison of the various diagnostic tools and the clinical use of RT-PCR in APL.

Prognosis

APL shows a high response rate to induction chemotherapy including anthracyclines. Different from others AMLs, APL is highly responsive to anthracyclines even when they are used as single agents.[38,39] The most fascinating therapeutic peculiarity of APL was revealed in 1988 by a Chinese group who obtained 100% complete remissions using ATRA alone.[40] Such results were reproduced worldwide by several groups.[41-43] In patients treated with ATRA as a single agent this remission is short lived and most ongoing trials now include chemotherapy, either in induction or consolidation phase, in combination with ATRA.

Clinical and experimental evidence indicates that a maturation mechanism (rather than cell lysis) is involved in APL response to ATRA. In fact, complete remission is obtained without causing the bone marrow aplasia characteristi-

Table 2. Advantages and pitfalls of RT-PCR in APL.



CD16 surface markers. Finally, compelling evidence that ATRA is able to induce remission through a differentiation mechanism was reported by our group in 1991.[44] In that study we used a Southern blot approach to describe the same RARα rearrangement in diagnostic blasts and in maturing elements from a patient receiving ATRA.[44] Subsequently, FISH analysis has also been useful in confirming these observations.[42]

RT-PCR studies in APL have several important clinical implications. In addition to diagnosis and disease monitoring, it is important to mention the ability to predict response to ATRA. In fact, only those patients with evidence of a PML/RARα fusion gene in their blasts will respond to ATRA, whereas cases without the specific fusion gene, including those with the rare variant t(11;17), will not.[36,45]

The prognostic role of different PML/RARα transcripts is also being investigated. Huang et al.[46] have reported that patients with a short transcript type (BCR 3) treated with ATRA alone have a more unfavorable prognostic outcome than patients with a long transcript type (BCR1-2). These findings were recently confirmed in another study.[47] Further investigations in larger series are needed in order to establish whether different PML breakpoints are associated with variable diagnostic features and/or prognosis. Finally, the clinical significance of additional karyotypic abnormalities sometimes found in APL has not been evaluated.

Treatment monitoring

Chimeric fusion genes resulting from non random chromosome translocations are specific features of several human hemopoietic tumors. One of the most well-characterized hybrid genes is the BCR/ABL rearrangement detectable in Philadelphia chromosome-positive (Ph^+) leukemias.[48] In the last few years, several other chromosome translocations associated with acute leukemias and resulting in fusion genes have been cloned. These include, among others, the AML1/ETO hybrid found in t(8;21)-positive AML-M2, and the CBFB/MYC chimeric gene which underlies the inv(16) of AML M4 with eosinophilia.[49,50] Similarly to PML/RARα in APL, these hybrid genes are detectable by RT-PCR and are being extensively exploited as tumor markers for the monitoring of treatment outcome.

The evaluation of residual disease is particularly relevant in APL. In fact, even though a very high number of patients achieve complete remission, a significant proportion (45-50%) is reported to relapse within two years.[1-4] Therefore a sensitive method for detecting small amounts of tumor cells in remission may be useful for the identification of those patients needing further treatment.

Clinical studies on PCR monitoring in APL have been reported by various groups. In a preliminary analysis of 35 patients in apparent remission after different treatment modalities (ATRA, chemotherapy, bone marrow transplantation), we provided the first evidence that PCR positivity is correlated with impending relapse.[51] Several other reports including larger series of patients have confirmed these findings.[46,52,53] As for the significance of PCR negativity at remission, it seems that this may be associated, although not invariably, with long-term survival. Patients presumably cured and in remission for 4-12 years have been studied and found to be PCR negative, thus suggesting that PCR negativity should be regarded as our best therapeutic goal at the present time.[54] However, patients who relapse after a negative PCR test have also been reported, albeit infrequently.[46,52,53] This might be explained by the sensitivity level of the method. In fact, compared to the amplification of other chimeric genes (i.e. BCR/ABL in CML), RT-PCR does not allow detection of blast contamination below 10^{-4} in APL.

As far as comparing different treatment modalities is concerned, PCR studies have shown that ATRA alone is unable to eradicate the leukemic clone, whereas chemotherapy result in PCR-negative remission in at least 50-60% of cases.[46,52-54]

Unlike what is observed in APL, the prognostic significance of PCR analysis in other leukemias is unclear. In fact, in BCR/ABL-positive CML and in AML1/ETO-positive AML, cells carrying the chimeric gene are frequently found in long-term survivors.[55,56] Such variabili-

ty in the prognostic value of different fusion genes amplified by PCR may reflect biologic diversity. It may be hypothesized that whereas PML/RARα is a lesion capable of inducing leukemic transformation,[19] the other above mentioned fusion genes may need additional events prior to giving rise to clinically aggressive disease.[57,58]

In light of the prognostic significance of PCR monitoring in APL, we have started a cooperative trial in Italy (GIMEMA/AIEOP-AIDA 0493) in which MRD assessment by RT-PCR is considered in order to choose post-consolidation treatment. In this prospective study the remission BM samples that are required at specific times during the follow-up are analyzed by a central referring laboratory.[59]

In conclusion, PCR evaluation of MRD in APL provides prognostic information which may be used to better address treatment in these patients. Based on this evidence, in the past two years considerable effort has been made to standardize the RT-PCR assay for APL at the international level. The optimization of this methodology for more widespread use and, in particular, its application to clinical studies in which patients are uniformly treated and prospectively monitored may substantially contribute to more adequate therapeutic choices and ultimately increase the cure rate in this disease.

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