THE AML1 GENE: A TRANSCRIPTION FACTOR INVOLVED IN THE PATHOGENESIS OF MYELOID AND LYMPHOID LEUKEMIAS

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ABSTRACT

Background and Objective. The AML1 gene was identified in 1991 by cloning the t(8;21) chromosome translocation associated with FAB M2 acute myeloid leukemia (AML). AML1 encodes a nuclear transcription factor (TF) which shows homology in its 5' part with the Drosophila Melanogaster segmentation gene, runt, and contains a transactivation domain in the carboxyterminal portion. In the t(8;21), AML1 is fused to the ETO (MTG8) gene resulting in a hybrid AML1/ETO mRNA which in turn is translated into a chimeric protein. The objective of this article is to review here the main structural and biological features of AML1 and of its fusion products, with special focus on their clinical correlations and their potential usefulness for prognostic and monitoring studies in human leukemia.

Evidence and Information Sources. The material examined in the present review includes articles and abstracts published in journals covered by the Science Citation Index® and Medline®.

State of Art. The normal AML-1 protein forms the α-subunit of the heterodimeric TF core binding factor (or CBF), whose β-subunit is encoded by the CBFb gene on chromosome 16q22. CBFb is rearranged and fused to the MYH11 gene in the AML M4Eo-associated inv(16) aberration. Thus, the two most common chromosome abnormalities of AML, i.e. t(8;21) and inv(16), affect the two subunits of the same target protein. This suggests that the wild type CBF must exert an important role in the control of myeloid cell growth and/or differentiation. Evidence that AML1 is a pivotal regulator of definitive hematopoiesis has been recently provided by the analysis of AML1 knockout mice. The chromosome region 21q22, where AML1 maps, is involved in several other karyotypic aberrations, such as the t(3;21) translocation associated with a subset of therapy-related myelodysplastic syndrome and AML, and blastic phase of chronic myelogenous leukemia. In this abnormality, three distinct genes EVI1, EAP, MDS1 located on chromosome band 3q26 have been identified which may recombine with AML1. Finally, the recently cloned t(12;21) translocation has been found to involve the TEL gene (coding for a novel TF) on 12p13, and AML1 on 21q22. This alteration, resulting in the production of a TEL/AML1 chimeric protein, is restricted to pediatric B-lineage acute lymphoid leukemia (ALL) where it represents the most frequent molecular defect known to date (up to 25% of cases). Strikingly, the same t(12;21) is identified in only 0.05% of pediatric B-lineage ALL cases analysed by conventional karyotyping. Other relevant characteristics of TEL/AML1-positive ALL are the frequent deletion of the other TEL allele, and the correlation with an excellent prognostic outcome.

Perspectives. It is expected that future studies will provide more detailed information on the leukemogenic effect of AML1 alterations, and better define the prognostic relevance of detecting the hybrid proteins formed by this gene at diagnosis and during remission.

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leukemia (AML), has led to the identification of a novel TF named AML1. In recent years, this gene has been shown to be involved in several other chromosome recombinations seen in either myeloid or lymphoid leukemias (Figure 1).

We will review here the main structural and biological features of AML1 and of its fusion products, focusing on their clinical correlations and their potential usefulness for prognostic and monitoring studies in human leukemia.

### Cloning and characterization of AML1 and AML1/ETO

Two groups have independently contributed the identification of AML1 through strategies including pulsed-field gel electrophoresis (PFGE), fluorescence in situ hybridization (FISH), and generation of YAC clones spanning the 21q22 breakpoint. The AML1 cDNA encodes an opening reading frame of 250 aminoacids, is transcribed from telomere to centromere, and shows homology in its aminoterminal region with the *Drosophila melanogaster* segmentation gene, runt (this region is often referred to as runt homology domain, or RHD). At the 3’ carboxyterminal region, the AML1 gene contains a putative transcription activation domain. Several differently sized cDNA clones of AML1 have been found which vary in the region downstream of the RHD and most likely result from alternative splicings of the same gene. Expression studies have shown that AML1 is detectable in various tissues including hematopoietic cells. Two other genes highly homologous to AML1 have been identified, AML2 and AML3, which map at chromosome bands 1p36 and 6p21, respectively. The AML gene family corresponds to the Cbf/H251 family of murine TF.

AML1 encodes the α subunit of the human core binding factor (CBF), a heterodimeric TF complex formed by two unrelated polypeptides α11 (Figure 2). Interestingly, the CBFα subunit of this TF is encoded by a gene, CBFα, which is rearranged and fused to the MYH11 gene in the inv(16) aberration characteristically associated with FAB M4eos. AML. The CBF binds a core DNA sequence, TGTGGT, present in a number of viral and cellular promoters and enhancers. These latter include the promoter/enhancer regions of T cell receptor genes, G-CSF, GM-CSF, myeloperoxidase, IL5 and IL3. A central part of 118 aminoacids of the 3’ RHD of AML1 participates directly in binding target DNA, while the subunit encoded by CBFβ contributes to increase the binding affinity.

Chromosome 22q breakpoints in the t(8;21) have been consistently mapped within a single intron of approximately 25 Kb, where they truncate the AML1 gene in between its RHD and transactivation domains. In spite of such breakpoint clustering, Southern blot hybridization studies have frequently failed to detect AML1 rearrangements, even in t(8;21)-positive AML cases, probably due to the deletion of DNA fragments complementary to the probes used, or to co-migration of rearranged and germline alleles. As a consequence of the translocation, the RHD of AML1 is conserved and fused in-frame on the der(8) to the ETO (for eight-twenty-one) gene, also named MTG8. This latter, which is retained almost entirely in the hybrid cDNA, is a putative transcription factor containing two zinc finger motifs. The AML1/ETO fusion gene retains the ability to dimerize with the CBFα subunit and to interact with the enhancer core DNA sequence. A scheme illustrating the modular organization of the normal AML1 and ETO proteins and of the AML1/ETO fusion cDNA is shown in Figure 3.

Targeted disruption of the AML1 followed by the analysis of knockout mice have shown absence of...
liver hematopoiesis and hemorrhagic death in AML1–/– transgenic animals, thereby supporting a crucial role of this gene in sustaining definitive hematopoiesis.32,33 Although the leukemogenic mechanisms of AML1 alterations have not yet been elucidated, preliminary evidence indicates that truncation of the gene and/or generation of AML1 fusion proteins may repress myeloid-specific promoters and suppress the transcriptional activation function of the normal AML1.36

Biological and clinical features of AML1/ETO-positive AML

By routine karyotyping, the t(8;21) translocation is detected in 6-8% of all AML cases and in 20-30% of FAB M2 AML.34 Despite the vast majority (> 90%) of t(8;21) are reported as M2 AML, this aberration has also been found, at lower frequencies, in M1 and M4 AML, and in rare cases of myelodysplastic and myeloproliferative syndromes.35 Morphologically, t(8;21) AML blasts display characteristic aspects such as prominent Auer rods, strong myeloperoxidase positivity, hypergranulation and cytoplasmic vacuolization. Marrow eosinophilia is also a frequent feature. The immunophenotypic analysis of t(8;21) AML cells usually disclose CD13/CD33/CD34 and CD56 expression. Interestingly, positive staining for the B-cell associated marker CD19 has been reported in a significant fraction of cases.36

None of the above mentioned morphologic and immunophenotypic characteristics is per se specific for t(8;21) AML; however, their combination strongly suggests the presence of the karyotypic abnormality and should foster cytogenetic and/or molecular search of the translocation. Other biological aspects of t(8;21) AML include the frequent loss of a sex chromosome, the in vitro potential to differentiate into neutrophils and eosinophils after exposure with ILS, and a tendency to form extra-medullary tumors.37

In addition to detecting all cases with the t(8;21), the RT-PCR method has enabled to unravel the presence of the AML1/ETO in some cases with normal karyotype. Interestingly, these cytogenetically negative AML1/ETO-positive AMLs had morphologic features strongly reminiscent of t(8;21) leukemias.41 Presumably, cryptic DNA insertions resulting in micro-translocations account for these false negative cases.

As we will discuss below, the TEL-AML1 gene fusion resulting from the t(12;21) translocation is the most striking example of hidden chromosome abnormality, being detectable in the vast majority of cases only by molecular analysis. Taken together, these data highlight the increasing need for molecular screening of chromosome translocations in human leukemia.

RT-PCR studies of minimal residual disease in AML1/ETO-positive AML

The prognostic role of detecting the presence of hybrid fusion genes at low levels during hematologic remission has been extensively studied by RT-PCR in BCR/ABL-positive leukemias and in PML/RARα-
positive APL. Although several factors (i.e. timing of sampling, therapeutic context, sensitivity of PCR tests) would influence the interpretation of results, it is commonly accepted that this technology offers significant advantages over conventional morphological or immunophenotypic evaluations. In fact, besides the increased sensitivity, these molecular strategies can detect tumor-specific lesions, eliminating any doubt regarding presence of residual malignant cells. In the case of APL, monitoring studies using RT-PCR provide clinically relevant data. In fact, PCR positive tests during remission are strong predictors of subsequent relapse and, conversely, long-term survivors test PCR negative for PML/RARα. Persistence of residual BCR/ABL-positive cells has been reported in a high proportion of CML patients in long-term remission, but these studies were mainly confined to patients treated by allogeneic BMT, i.e. in which a graft-versus-host-leukemia effect may exert a control over small numbers of leukemic cells. In addition, contrary to APL, CML is an indolent disease until its progression to blastic phase. APL, CML is an indolent disease until its progression to blastic phase.

In the past few years, quantitative PCR methods have been developed to detect variations in the amount of chimeric gene transcripts during remission. These techniques are useful in predicting relapse in CML. Most recently, Tobal et al. described the use of a quantitative assay for the amplification of AML1/ETO and suggested that this test might provide relevant prognostic information. However, in this preliminary study, data correlating the detection of increasing amounts of AML1/ETO transcript and occurrence of hematologic relapse were only available for two cases. Thus, further studies are warranted to determine the potential usefulness of quantitative RT-PCR in AML1/ETO positive AML. This implicates the standardization of techniques and sampling timing in various laboratories, as well as the evaluation of MRD results in the context of homogeneous therapeutic trials.

**Involvement of AML1 in the t(3;21) translocation**

The t(3;21) (q26;q22) has been reported as a rare recurring translocation in therapy-related AML or MDS, and in accelerated phase or blast crisis of CML. The aberration is detected as a secondary change in Philadelphia-positive CML, being always associated with myeloid transformation and never with lymphoid blast crisis. Rare cases of de novo AML with the t(3;21) have also been reported. Molecular studies have recently elucidated the genetic events underlying this translocation. At least three distinct genes (named EVI1, EAP and MDS1) which map at 3q26 have been shown to form alternative fusion products with AML1 in the t(3;21). In fact, breakpoints at 3q26 may be scattered over considerable distances and involve one of the three genes spanning a region of at least 400 Kb. As for AML1 breakpoints in the t(3;21), these have been mapped approximately 60 Kb downstream of the t(8;21) breakpoint, and usually occur in intron 5 or 6. The entire coding sequence of EVI1, which codes for a zinc finger DNA-binding protein, is retained and fused to the RHD of AML1 in the t(3;21). EAP, which codes for a ribosomal protein, does not maintain its reading frame in the translocation and translation of AML1/EAP fusion transcript is terminated by a stop codon shortly after the junction. MDS1 is a small gene encoding a protein of 170 aminoacids whose normal function is unknown. As observed in the t(8;21), the RHD of AML1 is truncated from the transactivation domain, and retained in the fusion with EVI1, EAP or MDS. Thus, maintenance of the RHD is a constant feature of all fusion proteins formed in the t(8;21) and t(3;21). The chimeric products of both translocations can act as repressors of the transactivating function of the normal AML1 over myeloid-specific promoters. The fact that chimeric transcripts result-
ing from either the t(8;21) or the (3;21) frequently contain stop codons in the proximity of the 3' end of the RHD suggests that AML1 truncations rather than its fusion to partner genes may play a key role in the leukemogenic mechanism.

The molecular organization of AML1/EVI1, AML1/EAP and AML1/MDS1 is illustrated in Figure 4.

The TEL/AML1 hybrid gene: a hidden chromosome translocation

The TEL (translocation ets-like leukemia) gene was identified following the cloning of the t(5;12) (q33;p13) present in a subset of chronic myelomonocytic leukemia, where it recombines with the platelet-derived growth factor receptor B (PDGFRB) gene.46 By conventional karyotyping, alterations in the 12p12-13 region had been previously described in up to 10% of childhood acute lymphoblastic leukemia (ALL).55 Using more sensitive techniques such as FISH or loss of heterozygosity (LOH) DNA analysis, 12p12-13 deletions had been reported in approximately 25% of pediatric ALL.56

In 1995, two groups cloned the t(12;21) (p13;q22) of ALL and found that the TEL gene is fused in this translocation to AML1.19,20 TEL is a member of the ETS-like family of TF and contains a 5' helix-loop-helix (HLH) domain and a carboxy-terminal DNA-binding region. In the TEL/AML1 hybrid, the translocation fuses the TEL HLH domain to almost the entire AML1 gene including its RHD and transactivation regions (Figure 5). In more than 90% of cases, the fusion gene is expressed as a TEL/AML1 chimeric RNA and is detectable by means of an RT-PCR assay.58-64 Thus, two genes previously known to be involved in translocations specific for myeloid tumors were found to be rearranged in a specific subset of ALL.

The TEL/AML1 abnormality in ALL has been correlated with consistent clinical and biological features. These include: i) young age at presentation, i.e. the vast majority of patients are aged between 2 and 10 years; ii) B-lineage features, with blasts usually staining for TdT, CD10 and CD19, and almost never for T-cell markers; iii) non-hyperdiploid DNA content; and iv) excellent response to chemotherapy and clinical outcome.58-64

According to the reported series, less than 2% TEL/AML1 positive cases have been described in adult ALL, a subset in which 25-30% of patients show the presence of a molecular lesion, the BCR/ABL fusion gene, associated with poor outcome. By contrast, no more than 5% of childhood ALL are BCR/ABL positive. Thus, it is worth noting that adult and pediatric ALL, two diseases undistinguishable on morphologic and immunophenotypic grounds and yet characterized by a remarkably different prognosis, are quite heterogeneous disorders if one looks at their molecular pathogenesis.

Two additional biological features characterizing the TEL/AML1 lesion merit special consideration. Firstly, the t(12;21) is detected in only 0.05% of pediatric ALL by routine karyotyping. Hence, here is an example of cryptic translocation in which molecular analysis is essential. Secondly, several authors have described the loss of the nontranslocated TEL allele in patients with TEL/AML1 rearrangements.20,21 This quite unexpected feature initially pointed to a possible function of TEL as a tumor suppressor gene. Most recent data obtained with FISH analyses rather suggest that TEL deletion might represent an additional lesion acquired by the leukemic cells during disease progression. In fact, the presence of TEL deletion has been reported in leukemic subclones of TEL/AML1 positive ALL at presentation.59,61 Further, in some cases studied sequentially either at diagnosis or at relapse, TEL
deletions were documented at disease recurrence only.61 Whether this secondary event is associated with a more aggressive clinical course in this ALL subset is still unknown.

The recent development of RT-PCR assays for the TEL/AML1 hybrid not only allows to rapidly identify a prognostically relevant marker which is almost undetectable by karyotyping, but also permits to sensitively monitor the response to therapy during the follow-up. However, only few data are currently available on longitudinal MRD studies in TEL/AML1 positive ALL. According to these preliminary reports, the majority of patients achieve a clearance of TEL/AML1 positive cells after 7-8 months of treatment, and PCR positivity detected during hematologic remission is predictive of clinical relapse.63,64 Therefore, PCR positivity during follow-up may dictate aggressive therapeutic approaches.65,66 In the near future a number of studies currently in progress will define the prognostic relevance of PCR monitoring in TEL/AML1 ALL.

Future perspectives

Studies on the AML1 gene have provided new important information on the molecular mechanisms involved in leukemogenesis. Furthermore, novel tumor specific markers are now available to identify previously unknown genetic subsets within the heterogeneous spectrum of AL. This should allow us to better define prognostic AL groups and to perform minimal residual disease studies in a greater number of AL.

The promiscuity of AML1 recombinations, the frequency of gene involvement, and the phenotypic heterogeneity of leukemias bearing AML1 alterations are already impressive. Further studies might still unravel AML1 gene alterations in other leukemia subsets, and/or AML1 rearrangements with other known or as yet uncharacterized genes. Also, additional cryptic translocations might be discovered in other leukemias, hopefully revealing the molecular lesions of the many leukemias currently reported as showing a normal karyotype. The unpredictably vast and intriguing scenario disclosed by investigating AML1 alterations is probably far from being completed.

References


Shurtleff SA, Buijs A, Behm FG, et al. TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pedi- atric ALL and defines a subgroup of patients with an excellent prog- nosis. Leukemia 1995; 9:1985-9.


Shurtleff SA, Buijs A, Behm FG, et al. TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pedi- atric ALL and defines a subgroup of patients with an excellent prog- nosis. Leukemia 1995; 9:1985-9.


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