Review Article

Role of AML1/Runx1 in the pathogenesis of hematological malignancies

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***AML1/Runx1*, originally identified as a gene located at the breakpoint of the t(8;21) translocation, encodes one of the two subunits forming a heterodimeric transcription factor. AML1 contains a highly evolutionally conserved domain called the Runt domain, responsible for both DNA binding and heterodimerization with the partner protein, CBFβ. AML1 is widely expressed in all hematopoietic lineages, and regulates the expression of a variety of hematopoietic genes. Numerous studies have shown that AML is a critical regulator of hematopoietic development. In addition, AML1 and CBFβ are frequent targets for chromosomal translocation in human leukemia. Translocations lead to the generation of fusion proteins, which play a causative role for the development of leukemia, primarily by inhibiting AML1 function. Point mutations that impair AML1 function are also associated with familial and sporadic leukemias. Loss of AML1 function is thus implicated in a number of leukemias through multiple pathogenic mechanisms. However, AML1-related translocations or haploinsufficiency of AML1 are not immediately leukemogenic in animal models, suggesting that additional genetic events are required for the development of full-blown leukemia. (Cancer Sci 2003; 94: 841–846)**

Hematopoiesis is an exquisitely regulated process that generates terminally differentiated cells in the peripheral blood from immature progenitors in the bone marrow. Mature blood cells consist of a variety of components, such as erythrocytes, granulocytes, lymphocytes, and platelets, the number of which is strictly maintained in spite of the short life of many of these cells. For this tight control, hematopoietic progenitors are required to properly proliferate, differentiate into more mature cells, or undergo apoptosis according to circumstances. Many lines of evidence have indicated that hematopoiesis can be achieved by a finely tuned cooperation of hematopoietic genes in progenitor cells.

Leukemia is a malignant disease that results from the acquisition by hematopoietic progenitors of gene mutations that confer deregulated proliferation, impaired differentiation, and a survival advantage. In humans, specific chromosomal translocations are detected in up to 65% of cases with acute leukemia. Isolation of translocation-related genes has revealed that hematopoietic transcription factors are frequent targets for leukemia-associated gene rearrangements. Among them is the *AML1* (acute myeloid leukemia 1) gene, which was originally identified through its location on human chromosome 21 at the t(8;21)(q22;q22) breakpoint.[1)] Acute myeloid leukemia (AML) can be divided into subgroups (M0–M7) by virtue of morphology and immunophenotype, which are roughly related to maturation stages of myeloid, erythroid, and megakaryocytic lineages. t(8;21) is associated with about 30% of the M2 subtype of AML and is one of the most common translocations in AML, appearing in 18% of AML cases with discernible chromosomal translocations.

Biochemical functions of AML1

AML1 is also called Runx1 (Runt-related protein 1), PEBP2αB (polyoma virus enhancer-binding protein 2αB), or CBFα (core-binding factor α), and is one of the two subunits forming a heteromeric transcription factor. AML1 contains a domain of 128 amino acids that is highly conserved among species (Fig. 1). A region of homology for this domain was first noted with *Drosophila melanogaster* Runt, a DNA-binding protein involved in segmentation, sex determination, and neural development, and hence it was named the Runt domain. The Runt domain of AML1 mediates both DNA binding and heterodimerization with the β subunit, which is called CBFβ or PEBP2β. The consensus sequence for DNA binding was revealed to be PyGPyGGT, which is named the PEBP2 sequence, and the same sequence preference seems to be shared by all the Runt protein family. CBFβ does not directly contact DNA but enhances the DNA-binding ability of AML1 as a heterodimeric complex, and protects it from ubiquitin-mediated proteolysis.

The mammalian Runt protein family consists of three distinct members, AML1/Runx1, AML2/Runx3, and AML3/Runx2. While AML1 is a key regulator of hematopoiesis at several critical steps, AML3 plays an essential role in osteogenesis, haploinsufficiency of which results in the skeletal disorder cleidocranial dyplasia. By contrast, several lines of investigations have unveiled critical functions of AML2 in the control of gastric epithelial cell growth, axonal projection of dorsal root ganglion, and more recently in the late stage of T cell maturation.[2)]

AML1 possesses at least three alternatively spliced isoforms (AML1a, AML1b, and AML1c) that are distinctly expressed in hematopoietic cells. In AML1b and AML1c, carboxy (C)-terminal to the Runt domain lies a region that contains sequences of defined biochemical functions, which are absent in AML1a. Functional antagonism between the isoforms has been reported, in which AML1b can release myeloid cells from the AML1a-mediated differentiation block.[3)] The AML1/CBFβ complex regulates the expression of a large number of hematopoiesis-related genes, including those for myeloperoxidase, the macrophage colony-stimulating factor receptor (M-CSFR), interleukin-3, neutrophil elastase, granzyme B, the T-cell receptors (TCRs), and the B-cell receptors.[4)] The PEBP2 sequence is found in transcriptional *cis*-elements of these genes, to which AML1/CBFβ binds and then initiates transcription (Fig. 2A). However, AML1 seems to be a relatively weak activator of tran-

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scription, due to the fact that DNA binding of AML1/CBFβ on its own is necessary but insufficient to exert full activation of gene transcription. Instead, AML1 functions as an organizing protein that facilitates assembly of transcriptional activation complexes. Indeed, the PEBP2 sequences are frequently adjacent to binding sites for other hematopoietic transcription factors, such as Ets, C/EBPα, and Myb, within several target gene promoters. All of these factors are known to cooperate with AML1 to activate gene transcription. In addition to these *cis*-interactions, AML1 associates with transcriptional cofactors including p300 and CREB-binding protein (CBP), which facilitate transcription by acetylating histones and rendering the promoter structures more accessible.[5] Other coactivators that interact with AML1 include Yes-associated protein (YAP) and ALY, the latter being a protein that activates the TCRα promoter by facilitating assembly of LEF-1, Ets, and AML1.[4] AML1 can also function as a transcriptional repressor, depending on the target gene and the cellular milieu, by recruiting corepressors, such as transducin-like enhancer of split (TLE), the nuclear receptor Ear-2, and mSin3A (Fig 2B).[4, 6] Of these, TLE, a mammalian homolog of *Drosophila* Groucho protein, interacts with AML1 by recognizing its C-terminal VWRPY motif. Similarly, AML1 acts as a transcriptional repressor for *p21/WAF1/CIP1* in NIH3T3 cells,[7] and *CD4* in thymocytes.[2] Although the mechanism that governs whether AML1 acts as an activator or a repressor is not yet fully understood, one possibility is posttranslational modification, including phosphorylation by extracellular signal-regulated kinase that stimulates transcriptional activity of AML1 in response to growth factor stimuli.[8]

AML1 in normal hematopoiesis

During vertebrate embryogenesis, hematopoietic development consists of two distinct waves of discrete cellular components. The first wave of primitive hematopoiesis emerges in the yolk sac at embryonic day (E) 7.5, which is replaced at E9.5 by definitive hematopoiesis, generally described as the second wave. Progenitors for definitive hematopoiesis originate from para-aortic splanchnopleure (P-Sp) at E8.5, and hematopoietic stem cells (HSCs) appear in the aorta-gonad-mesonephros (AGM) at E9.5. These cells subsequently colonize the fetal liver, where they expand and differentiate. After birth, definitive hematopoiesis shifts to the bone marrow and the spleen, where adult hematopoietic cells are generated throughout life. Homozygous disruption of *AML1* in mice results in midgestation embryonic lethality at E12.5 with massive bleeding in the central nervous system, showing a profound defect in definitive hematopoiesis.[9] In contrast, primitive hematopoiesis seems to be relatively spared. Hematopoietic progenitors of any lineage that can produce colonies *in vitro* are absent from the fetal liver of *AML1*-deficient mice, and no hematopoietic stem cell activity is detected in their AGM region.[10] The results establish an essential role for AML1 at the level of definitive hematopoietic progenitors or stem cells. It is noteworthy that mutated *CBFβ* alleles in mice phenocopy these mutations in *AML1*-deficient mice, indicating that CBFβ is essential for AML1 function *in vivo*.[11] Recent studies revealed that AML1 is required for the generation of HSCs from the endothelium of the great vessels (hemogenic endothelium), including the vitelline and umbilical arteries, and the AGM region.[12] On the other hand, it was shown that HSCs in turn promote angiogenesis and vessel remodeling by secreting growth factors, lack of which may cause fatal hemorrhage in *AML1*-deficient mice.[13]

Due to embryonic lethality of *AML1*-deficient mice, little is known about the function of AML1 in adult hematopoiesis, with the exception of thymocyte development. A critical role for AML1 has been proposed on the basis that AML1 is highly expressed in the thymus throughout mouse development and that the target genes for AML1 include those of TCRs. Immature thymocytes lacking CD4 and CD8 differentiate into double-positive (DP) cells within the thymus upon successful rearrangement of TCRs, a step described as β selection. Then they are positively selected to survive and committed either to the CD4 single-positive (SP) or to the CD8 SP cells, which emigrate to peripheral lymphoid tissues. Blockade of AML1 function by the dominant negative form in mice results in impairment at multiple steps of T cell ontogeny, including the transition from the DP to the SP stage, the maturation of SP thymocytes.[14] In contrast, forced expression of AML1 produces enhanced differentiation of thymocytes to the CD8 SP lineage,[15] and attenuates differentiation of naïve T cells to the Th2 subset, the latter presumably being through downregulation of GATA3 transcription factor.[16] Furthermore, investigations using T-cell specific *AML1*-deficient mice demonstrated that AML1 is required for CD4 silencing in immature thymocytes,

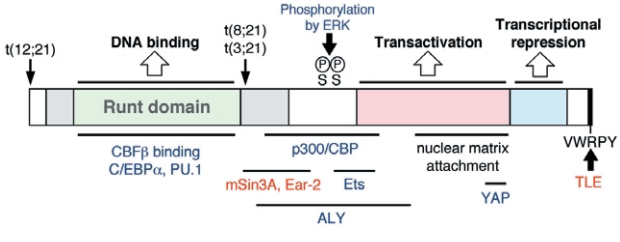

Fig. 1. Functional anatomy of AML1. DNA binding and heterodimerization with CBFβ are mediated by the Runt domain (green). The Runt domain also associates with other DNA-binding proteins. The negative regulatory regions for DNA binding are located adjacent to the Runt domain (gray). Shown are the locations of other sequences for transactivation (red), transcriptional repression (blue), and nuclear matrix attachment, ERK phosphorylation sites, binding regions for coactivators p300/CBP, ALY, YAP, corepressors mSin3A, Ear-2, and TLE. Arrows indicate breakpoints in t(8;21), t(3;21), and t(12;21). Almost all sequences downstream to the Runt domain are lost in t(8;21) and t(3;21), while they are retained in t(12;21). ERK, extracellular signal-regulated kinase; ALY, ally of AML1 and LEF-1.

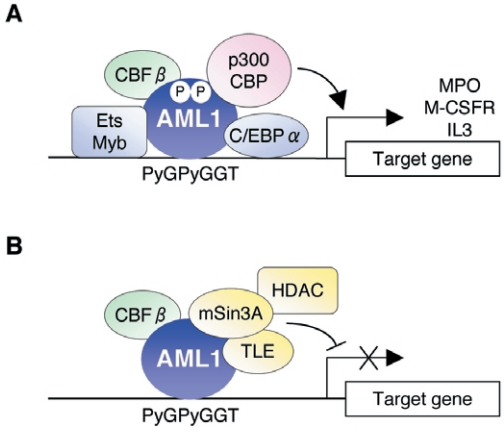

Fig. 2. Transcriptional complex formation by AML1. (A) AML1 binds to the consensus DNA sequence (PEBP2 sequence) present in the *cis*-elements of target genes. The PEBP2 sequence is often adjacent to binding sites for other DNA-binding proteins, including Ets, Myb, and C/EBPα. AML1 regulates gene expression in cooperation with these lineage-specific transcription factors. AML1 mediates transcriptional activation (A) or repression (B) upon recruitment of non-DNA-binding coactivators (p300/CBP) or corepressors (mSin3A, HDAC, and TLE). Whether AML1 acts as either an activator or repressor may be determined by several factors, including phosphorylation of AML1 or relative levels of cofactors.

efficient β selection, and upregulation of CD8 at the transition to the DP stage.[2)]

Chromosomal translocations associated with AML1/CBFβ

Thus far, more than a dozen different chromosomal translocations of human acute leukemia that involve AML1 are known. Notably, the gene encoding *CBFβ* is also disrupted in inv(16)(p13q22) associated with the AML subtype of M4 with eosinophilia, that accounts for 10% of adult cases with AML.[17)] t(8;21) and inv(16) translocations confer a favorable outcome in adults with AML who receive high-dose chemotherapy. t(12;21)(p13;q22) is another common translocation that occurs in 25% of pediatric acute lymphocytic leukemia of the B precursor phenotype.[18)] It defines a distinct subgroup of patients with a favorable prognosis, although this point is controversial. t(3;21)(q26;q22), though a less common translocation, is found in cases with myelodysplastic syndrome or progression of chronic myelogenous leukemia (CML) to blast crisis.[19)]

The molecular consequence of t(8;21), inv(16), t(12;21), and t(3;21) is the generation of AML1-ETO/MTG8, CBFβ-SMMHC, TEL/ETV6-AML1, and AML1-Evi-1 fusion proteins, respectively (Fig. 3). These fusion proteins have the common functional property that they act as a dominant negative inhibitors for the normal *AML1* allele. For example, the AML1-ETO fusion protein retains the ability to bind to the *cis*-elements of the AML1-target genes, but does not initiate gene transcription, which enables the fusion protein to interfere with transcriptional activity of native AML1. Similar *in vitro* data have been obtained for TEL-AML1 and AML1-Evi-1 fusions.[20, 21)] Evidence consistent with this notion includes the observation that AML1-ETO represses the expression of $p14^{ARF}$, that is a target gene for transactivation by AML1.[22)] In addition, AML1-ETO exerts a dominant inhibitory function during hematopoietic development. Heterozygous knock-in mice were generated in which AML1-ETO expression is directed from the endogenous *AML1* promoter.[23)] Although these mice have one fully intact *AML1* allele, they die *in utero* showing a phenotype nearly identical to that of *AML1*-deficient mice. Similar data have been generated for CBFβ-SMMHC, in which heterozygous knock-in of the gene for CBFβ-SMMHC to the *CBFβ* allele recapitulates the *AML1*- and *CBFβ*-knockout phenotypes.[24)] In the following sections, we will go further to focus on the detailed function of each fusion protein in leukemogenesis.

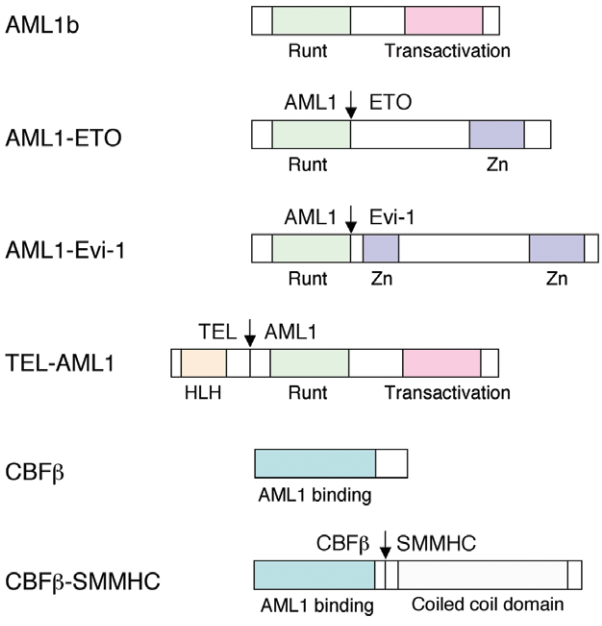

Fig. 3. Structure of fusion proteins generated in AML1-related leukemia. All AML1 fusion proteins retain the Runt domain. In CBFβ-SMMHC, the AML1-binding domain of CBFβ is fused to the coiled-coil domain of SMMHC, which mediates cytoplasmic targeting of the fusion protein. Arrows indicate breakpoints in the fusion genes. Zn, zinc finger domain; HLH, helix-loop-helix domain.

AML1-ETO in t(8;21)

AML1-ETO consists of the amino (N)-terminal half of AML1, including the Runt domain, fused in-frame to all but the first 30 N-terminal amino acids of ETO, a mammalian homolog of *Drosophila* protein Nervy.[25)] ETO is a nuclear protein and exerts its activity by associating with N-CoR, a transcriptional repressor that recruits mSin3A and histone deacetylases (HDACs).[26)] Through multiple binding sites on ETO, mSin3A can also bind to ETO independently of N-CoR. In AML1-ETO, the C-terminal sequences of AML1 for transactivation are replaced with the ETO moiety, which serves to recruit repressor complexes to the fusion protein. The affinity of AML1-ETO for CBFβ is higher than that of native AML1, which would lead to elevated DNA binding to the fusion protein.[27)] Thus, transcriptional repression of AML1 target genes by AML1-ETO seems to be due to aberrant recruitment of repressor complexes (Fig. 4). It is reasonably convincing to suppose that interference with the normal activity of AML1/CBFβ is one of the critical properties of AML1-ETO in leukemogenesis; this idea is supported by several lines of experiments. For example, transduction of AML1-ETO into myeloid cells slows terminal differentiation induced by granulocyte-colony stimulating factor (G-CSF), whereas forced expression of native AML1 can restore differentiation.[5)] However, it should be noted that more is involved than just the dominant inhibitory effect on the functions of AML1-ETO; some newly gained activity may also contribute to leukemogenic potential.[28)] For example, AML1-ETO has been reported to induce M-CSFR expression synergistically with AML1, or to elevate the expression of G-CSF receptor through upregulation of C/EBPε. AML1-ETO inhibits promyelocytic leukemia gene protein (PLZF), which normally represses transcription of genes involved in cell proliferation, C/EBPα, or PU.1, a critical regulator for myeloid differentiation. The analysis of AML1-ETO knock-in mice revealed upregulation of a ubiquitin-specific protease UBP43, which blocks

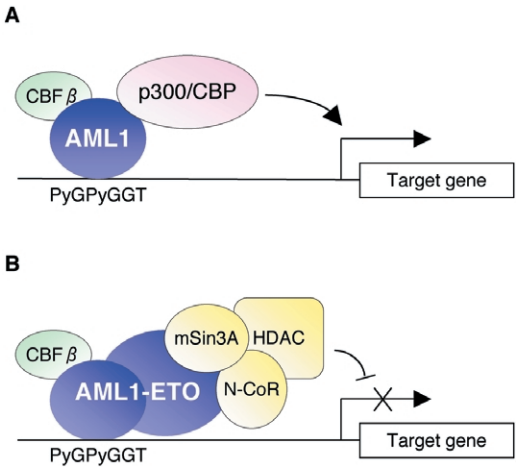

Fig. 4. Repressor recruitment by AML1-ETO. As a consequence of t(8;21), AML1 is interrupted between the Runt domain and the transactivation domain, which fuses to ETO. Although native AML1 activates target gene expression (A), AML1-ETO interferes with AML1 binding to DNA and recruits transcriptional complexes that contain N-CoR, mSin3A, and HDAC (B). These complexes in turn repress the expression of genes normally regulated by the native AML1 complex.

monocytic differentiation when overexpressed on myeloid cells. Differential display analysis using AML1-ETO-expressing cells identified several downstream genes, including a putative posttranscriptional regulator TIS11b. These gains-of-function may contribute to perturbed maturation of HSCs, as discussed below.

Neither of the two types of transgenic mice expressing AML1-ETO has developed leukemia throughout the normal lifespan.[29, 30] Moreover, investigations using a conditional knock-in allele also demonstrated that AML1-ETO on its own does not cause leukemia, but requires additional mutations for the manifestation of a leukemic phenotype.[31] In spite of this, abnormal maturation and proliferation of HSCs or progenitor cells were noted in these animals. Similar hematopoietic derangement was observed in retroviral transduction systems that deliver AML1-ETO exclusively into the hematopoietic cells.[32, 33] Taking these results together, we may conclude that AML1-ETO perturbs the maturation program of hematopoietic progenitors, which is critical for causing leukemia, but has only a restricted capacity for the induction of full-blown leukemia.

CBFβ-SMMHC in inv(16)

inv(16) results in a protein product that fuses the first 165 amino acids of CBFβ to the coiled-coil region of a smooth muscle myosin heavy chain (SMMHC). The *CBFβ-MYH11* fusion gene, which encodes the CBFβ-SMMHC fusion protein, is exclusively associated with a specific AML subtype, M4, that is characterized by eosinophilia. CBFβ-SMMHC can bind to AML1 in place of native CBFβ, and inhibits AML1 function in a dominant-negative manner. In transient transfection experiments, as well as in a knock-in mouse model, this fusion protein was shown to sequester AML1 into cytoskeletal filaments in the cytoplasm, which presumably prevents AML1 from efficiently activating gene transcription in the nucleus.[34] Several studies reported another mechanism that alters the transcriptional properties of AML1. The C-terminal 163 amino acids of the SMMHC region interacts with mSin3A, and HDAC activity was detected with the CBFβ-SMMHC fusion protein.[35] Thus, CBFβ-SMMHC acts as a transcriptional repressor by introducing a repression domain into the CBFβ protein through the SMMHC moiety. As a consequence, the AML1 complex is converted into a constitutive transcriptional repressor. Chimeric mice generated with *CBFβ-MYH11* knock-in embryonic stem (ES) cells did not develop leukemia regardless of the distinct contribution of the knock-in ES cells to HSCs in the bone marrow. Upon treatment with an additional mutagen, however, they suffered from a malignant disease reminiscent of human inv(16) leukemia, indicating that CBFβ-SMMHC can be a prerequisite, but requires additional events for leukemogenesis, as does AML1-ETO.[36]

AML1-Evi-1 in t(3;21)

In the t(3;21) translocation, a breakpoint between the Runt domain and the transactivation domain of AML1 fuses *AML1* with the full-length *Evi-1* gene on the long arm of chromosome 3. *AML1-Evi-1* was primarily identified in the blastic phase of CML,[19] and the fusion protein is envisioned to cause leukemic transformation of hematopoietic cells. *Evi-1* was originally isolated as a gene existing in a common locus of retroviral integration in myeloid tumors in AKXD mice. This gene encodes a 145 kDa nuclear-localized protein that possesses two clusters of zinc finger domain. Although Evi-1 shows a very restricted pattern of expression in normal hematopoietic populations, its frequent transcriptional activation has been documented in a subset of myeloid malignancies, suggesting a critical role of Evi-1 in malignant transformation of hematopoietic cells as a dominant oncogene. Some evidence suggests that Evi-1 works as a negative regulator of gene expression, while the characteristics of Evi-1 as a transcriptional activator have also been described. Evi-1 elevates intracellular AP-1 activity and stimulates the c-*fos* promoter, which contributes to Evi-1-mediated cellular transformation.[37, 38] Forced expression of Evi-1 blocks granulocytic differentiation of myeloid cells induced by G-CSF, and causes a decrease in colony formation of hematopoietic progenitors in response to erythropoietin. Evi-1 antagonizes the growth-inhibitory effects of TGF-β by repressing the transcriptional activity of Smad3,[39] and prevents stress-induced cell death by inhibiting c-Jun N-terminal kinase.[40] Based on these findings, Evi-1 is thought to possess the abilities to promote growth and block differentiation in some types of cells.

Multiple mechanisms have been proposed for leukemogenesis in the t(3;21) translocation. The previous study revealed that AML1-Evi-1 dominantly represses transactivation by intact AML1, thereby leading to blockade of myeloid cell differentiation.[21] The Evi-1 moiety in the fusion protein interacts with C-terminal binding protein (CtBP), a transcriptional corepressor.[41] Thus, the repression may arise through the aberrant recruitment of repressor complexes. In addition, the affinity of AML1-Evi-1 for CBFβ is greater than that of wild-type AML1, as is the case with AML1-ETO, which may also account for the dominant inhibitory effects of the fusion protein.[27] Some properties of Evi-1 are also shared by AML1-Evi-1, including induction of AP-1 activity and repression of TGF-β signaling.[38, 42] These newly acquired functions may also contribute to leukemogenic potential in t(3;21).

TEL-AML1 in t(12;21)

TEL is a ubiquitously expressed protein of 453 amino acids that contains a helix-loop-helix (HLH) oligomerization domain and Ets DNA binding domain, both of which are found in other members of the Ets family of transcription factors. The t(12;21) translocation fuses the 5′ HLH domain of TEL to the N terminus of AML1 to generate TEL-AML1, in which the C-terminal transactivation domain of AML1 is intact, unlike AML1-ETO and AML1-Evi-1.[18] Nonetheless, TEL-AML1 fails to activate transcription but rather inhibits AML1-dependent transactivation of the target genes. This inhibition requires not only the HLH domain of TEL, which is known to recruit HDACs, but also the Runt domain and the mSin3A-interacting domain of AML1.[20] Thus, the fusion protein presumably converts AML1 into an unregulated transcriptional repressor containing mSim3A and HDAC. Repression of TEL function may also be of importance in leukemogenesis, as shown by the frequent deletion of the residual TEL allele in t(12;21).[43] The analysis of syngeneic twins who develop t(12;21) leukemia has shown that *TEL-AML1* gene rearrangement may arise *in utero* but that leukemia develops at different times in life after a long latency period of years, suggesting the necessity of a second mutation for leukemogenesis.[44] This is supported by the finding that transgenic mice, in which TEL-AML1 was expressed in lymphoid tissue, did not develop leukemia over a time period of 24 months.[45]

Gene dosage effects of AML1 in leukemia

Familial platelet disorder with a propensity for AML (FPD/AML) is an autosomal dominant disorder that is characterized by moderate thrombocytopenia and progression to AML with age. Germ-line monoallelic mutations of *AML1* have been identified in FPD/AML.[46] Point mutations in *AML1* have also been identified in about 10% of sporadic myeloid malignancies and are most often associated with the minimally differentiated subtype of AML (AML M0).[47] The majority of the mutations fall in the Runt domain and impair AML1 function through loss of DNA binding. Monoallelic mutations are seen in a variety of leukemias, while biallelic mutations are closely related to AML M0. Complete deletion of *AML1* with a fully functional resid-

ual allele was noted in one pedigree with FPD/AML.[46] Thus, it would appear that loss of one *AML1* allele is sufficient for predisposition to leukemia. Consistently with this, hemizygosity for the mutant *AML1* allele in the mouse embryo is associated with an increase in HSCs and a decrease in committed progenitors, suggesting that haploinsufficiency of AML1 would affect maturation and proliferation of hematopoietic cells.[10] However, other mutations could have a partial dominant inhibitory activity. For example, certain missense mutations in the Runt domain act as dominant-negative inhibitors through sequestration of CBFβ.[47, 48] Families carrying mutations that act in a dominant-negative fashion seem to show a larger proportion of affected individuals who develop leukemia than do families with monoallelic deletions. Furthermore, a significant proportion of cases have mutations in both alleles of *AML1* in sporadic leukemia. Monoallelic mutations are often accompanied by additional karyotypic abnormalities, suggesting the necessity of additional genetic events for leukemogenesis. Available evidence indicate that progression to leukemia is more likely to occur in the cases with lower activity of AML1.

Recent analysis of the *AML1* locus has shown that high-level gene amplification of *AML1* can be detected in some cases with human leukemia. Those cases primarily constitute pediatric ALL, some of which have been confirmed to show an increase in *AML1* transcripts.[47] These observations provide support for the hypothesis that gain of function or increased dosage of AML1 can contribute to leukemogenesis. There are also several lines of experimental evidence that suggest a role of AML1 family members as dominant oncogenes. For example, forced expression of AML1 causes cellular transformation in fibroblasts.[49] T-cell specific overexpression of AML3 in transgenic mice induces lymphoma synergistically with other oncogenes.[50] In addition, the *AML1* locus might be targeted by proviral insertions in myeloid leukemia and T-cell lymphoma in mice.[50] The precise role of increased AML1 dosage in leukemogenesis will be addressed in future investigations.

Perspective

Numerous studies have revealed that AML1 exerts a wealth of diverse effects on leukemogenic processes. In translocation-related leukemias, many of those effects appear to depend upon deregulated transcriptional activation, which would become a potential target for therapeutic intervention. Small molecules that inhibit HDAC activity are one class of candidates that may counteract the effects of the fusion proteins. Although many investigations suggest that additional genetic events are necessary for the induction of overt leukemia, little is known about candidates for second mutations in AML1-related leukemia. Identification of such mutations would also provide an important clue for molecularly targeted therapies. In this regard, establishment of appropriate animal models for AML1-related leukemia will greatly facilitate the development of new therapeutic modalities. In spite of its definite role in hematopoietic ontogeny during embryogenesis, how AML1 contributes to adult hematopoiesis remains to be elucidated. Establishing the full picture of AML1 function in adult hematopoiesis may also serve to throw more light on the mechanisms of leukemogenesis.

Although there are many important papers in this field, we have not been able to mention all of them for reasons of space. We apologize to those people whose papers could not be cited. This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

1. Miyoshi H, Shimizu K, Kozu T, Maseki N, Kaneko Y, Ohki M. t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. *Proc Natl Acad Sci USA* 1991; **88**: 10431–4.
2. Taniuchi I, Osato M, Egawa T, Sunshine MJ, Bae SC, Komori T, Ito Y, Littman DR. Differential requirements for Runx proteins in CD4 repression and epigenetic silencing during T lymphocyte development. *Cell* 2002; **111**: 621–33.
3. Tanaka T, Tanaka K, Ogawa S, Kurokawa M, Mitani K, Nishida J, Shibata Y, Yazaki Y, Hirai H. An acute myeloid leukemia gene, AML1, regulates hemopoietic myeloid cell differentiation and transcriptional activation antagonistically by two alternative spliced forms. *EMBO J* 1995; **14**: 341–50.
4. Perry C, Eldor A, Soreq H. Runx1/AML1 in leukemia: disrupted association with diverse protein partners. *Leuk Res* 2002; **26**: 221–8.
5. Kitabayashi I, Yokoyama A, Shimizu K, Ohki M. Interaction and functional cooperation of the leukemia-associated factors AML1 and p300 in myeloid cell differentiation. *EMBO J* 1998; **17**: 2994–3004.
6. Imai Y, Kurokawa M, Tanaka K, Friedman AD, Ogawa S, Mitani K, Yazaki Y, Hirai H. TLE, the human homolog of groucho, interacts with AML1 and acts as a repressor of AML1-induced transactivation. *Biochem Biophys Res Commun* 1998; **252**: 582–9.
7. Lutterbach B, Westendorf JJ, Linggi B, Isaac S, Seto E, Hiebert SW. A mechanism of repression by acute myeloid leukemia-1, the target of multiple chromosomal translocations in acute leukemia. *J Biol Chem* 2000; **275**: 651–6.
8. Tanaka T, Kurokawa M, Ueki K, Tanaka K, Imai Y, Mitani K, Okazaki K, Sagata N, Yazaki Y, Shibata Y, Kadowaki T, Hirai H. The extracellular signal-regulated kinase pathway phosphorylates AML1, an acute myeloid leukemia gene product, and potentially regulates its transactivation ability. *Mol Cell Biol* 1996; **16**: 3967–9.
9. Okuda T, van Deursen J, Hiebert SW, Grosveld G, Downing JR. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell* 1996; **84**: 321–30.
10. Cai Z, de Bruijn M, Ma X, Dortland B, Luteijn T, Downing RJ, Dzierzak E. Haploinsufficiency of AML1 affects the temporal and spatial generation of hematopoietic stem cells in the mouse embryo. *Immunity* 2000; **13**: 423–31.
11. Wang Q, Stacy T, Miller JD, Lewis AF, Gu TL, Huang X, Bushweller JH, Bories JC, Alt FW, Ryan G, Liu PP, Wynshaw-Boris A, Binder M, Marin-Padilla M, Sharpe AH, Speck NA. The CBFb subunit is essential for CBFa2 (AML1) function *in vivo*. *Cell* 1996; **87**: 697–708.
12. North TE, de Bruijn MF, Stacy T, Talebian L, Lind E, Robin C, Binder M, Dzierzak E, Speck NA. Runx1 expression marks long-term repopulating hematopoietic stem cells in the midgestation mouse embryo. *Immunity* 2002; **16**: 661–72.
13. Takakura N, Watanabe T, Suenobu S, Yamada Y, Noda T, Ito Y, Satake M, Suda T. A role for hematopoietic stem cells in promoting angiogenesis. *Cell* 2000; **102**: 199–209.
14. Hayashi K, Natsume W, Watanabe T, Abe N, Iwai N, Okada H, Ito Y, Asano M, Iwakura Y, Habu S, Takahama Y, Satake M. Diminution of the AML1 transcription factor function causes differential effects on the fates of CD4 and CD8 single-positive T cells. *J Immunol* 2000; **165**: 6816–24.
15. Hayashi K, Abe N, Watanabe T, Obinata M, Ito M, Sato T, Habu S, Satake M. Overexpression of AML1 transcription factor drives thymocytes into the CD8 single-positive lineage. *J Immunol* 2001; **167**: 4957–65.
16. Komine O, Hayashi K, Natsume W, Watanabe T, Seki Y, Seki N, Yagi R, Suzuki W, Tamauchi H, Hozumi K, Habu S, Kubo M, Satake M. The Runx1 transcription factor inhibits the differentiation of naive CD4+ T cells into the Th2 lineage by repressing GATA3 expression. *J Exp Med* 2003; **198**: 51–61.
17. Liu P, Tarle SA, Hajra A, Claxton DF, Marlton P, Freedman M, Siciliano MJ, Collins FS. Fusion between transcription factor CBFb/PEBP2b and a myosin heavy chain in acute myeloid leukemia. *Science* 1993; **261**: 1041–4.
18. Golub TR, Barker GF, Bohlander SK, Hiebert SW, Ward DC, Bray-Ward P, Morgan E, Raimondi SC, Rowley JD, Gilliland DG. Fusion of the TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. *Proc Natl Acad Sci USA* 1995; **92**: 4917–21.
19. Mitani K, Ogawa S, Tanaka T, Miyoshi H, Kurokawa M, Mano H, Yazaki Y, Ohki M, Hirai H. Generation of the AML1-EVI-1 fusion gene in the t(3;21)(q26;q22) causes blastic crisis in chronic myelocytic leukemia. *EMBO J* 1994; **13**: 504–10.
20. Hiebert SW, Sun W, Davis JN, Golub T, Shurtleff S, Buijs A, Downing JR, Grosveld G, Roussell MF, Gilliland DG, Lenny N, Meyers S. The t(12;21) translocation converts AML-1B from an activator to a repressor of transcription. *Mol Cell Biol* 1996; **16**: 1349–55.
21. Tanaka T, Mitani K, Kurokawa M, Ogawa S, Tanaka K, Nishida J, Yazaki Y, Shibata Y, Hirai H. Dual functions of the AML1/Evi-1 chimeric protein in the mechanism of leukemogenesis in t(3;21) leukemias. *Mol Cell Biol* 1995; **15**: 2383–92.
22. Linggi B, Muller-Tidow C, van de Locht L, Hu M, Nip J, Serve H, Berdel

WE, van der Reijden B, Quelle DE, Rowley JD, Cleveland J, Jansen JH, Pandolfi PP, Hiebert SW. The t(8;21) fusion protein, AML1-ETO, specifically represses the transcription of the p14(ARF) tumor suppressor in acute myeloid leukemia. *Nat Med* 2002; **8**: 743–50.
23. Okuda T, Cai Z, Yang S, Lenny N, Lyu CJ, van Deursen JM, Harada H, Downing JR. Expression of a knocked-in AML1-ETO leukemia gene inhibits the establishment of normal definitive hematopoiesis and directly generates dysplastic hematopoietic progenitors. *Blood* 1998; **91**: 3134–43.
24. Castilla LH, Wijmenga C, Wang Q, Stacy T, Speck NA, Eckhaus M, Marin-Padilla M, Collins FS, Wynshaw-Boris A, Liu PP. Failure of embryonic hematopoiesis and lethal hemorrhages in mouse embryos heterozygous for a knocked-in leukemia gene CBFB-MYH11. *Cell* 1996; **87**: 687–96.
25. Miyoshi H, Kozu T, Shimizu K, Enomoto K, Maseki N, Kaneko Y, Kamada N, Ohki M. The t(8;21) translocation in acute myeloid leukemia results in production of an AML1-MTG8 fusion transcript. *EMBO J* 1993; **12**: 2715–21.
26. Gelmetti V, Zhang J, Fanelli M, Minucci S, Pelicci PG, Lazar MA. Aberrant recruitment of the nuclear receptor corepressor-histone deacetylase complex by the acute myeloid leukemia fusion partner ETO. *Mol Cell Biol* 1998; **18**: 7185–91.
27. Tanaka K, Tanaka T, Kurokawa M, Imai Y, Ogawa S, Mitani K, Yazaki Y, Hirai H. The AML1/ETO(MTG8) and AML1/Evi-1 leukemia-associated chimeric oncoproteins accumulate PEBP2b(CBFb) in the nucleus more efficiently than wild-type AML1. *Blood* 1998; **91**: 1688–99.
28. Michaud J, Scott HS, Escher R. AML1 interconnected pathways of leukemogenesis. *Cancer Invest* 2003; **21**: 105–36.
29. Rhoades KL, Hetherington CJ, Harakawa N, Yergeau DA, Zhou L, Liu LQ, Little MT, Tenen DG, Zhang DE. Analysis of the role of AML1-ETO in leukemogenesis, using an inducible transgenic mouse model. *Blood* 2000; **96**: 2108–15.
30. Yuan Y, Zhou L, Miyamoto T, Iwasaki H, Harakawa N, Hetherington CJ, Burel SA, Lagasse E, Weissman IL, Akashi K, Zhang DE. AML1-ETO expression is directly involved in the development of acute myeloid leukemia in the presence of additional mutations. *Proc Natl Acad Sci USA* 2001; **98**: 10398–403.
31. Higuchi M, O'Brien D, Kumaravelu P, Lenny N, Yeoh EJ, Downing JR. Expression of a conditional AML1-ETO oncogene bypasses embryonic lethality and establishes a murine model of human t(8;21) acute myeloid leukemia. *Cancer Cell* 2002; **1**: 63–74.
32. de Guzman CG, Warren AJ, Zhang Z, Gartland L, Erickson P, Drabkin H, Hiebert SW, Klug CA. Hematopoietic stem cell expansion and distinct myeloid developmental abnormalities in a murine model of the AML1-ETO translocation. *Mol Cell Biol* 2002; **22**: 5506–17.
33. Mulloy JC, Cammenga J, MacKenzie KL, Berguido FJ, Moore MA, Nimer SD. The AML1-ETO fusion protein promotes the expansion of human hematopoietic stem cells. *Blood* 2002; **99**: 15–23.
34. Adya N, Stacy T, Speck NA, Liu PP. The leukemic protein core binding factor b (CBFb)-smooth-muscle myosin heavy chain sequesters CBFa2 into cytoskeletal filaments and aggregates. *Mol Cell Biol* 1998; **18**: 7432–43.
35. Durst KL, Lutterbach B, Kummalue T, Friedman AD, Hiebert SW. The inv(16) fusion protein associates with corepressors via a smooth muscle myosin heavy-chain domain. *Mol Cell Biol* 2003; **23**: 607–19.
36. Castilla LH, Garrett L, Adya N, Orlic D, Dutra A, Anderson S, Owens J, Eckhaus M, Bodine D, Liu PP. The fusion gene Cbfb-MYH11 blocks myeloid differentiation and predisposes mice to acute myelomonocytic leukaemia. *Nat Genet* 1999; **23**: 144–6.
37. Tanaka T, Nishida J, Mitani K, Ogawa S, Yazaki Y, Hirai H. Evi-1 raises AP-1 activity and stimulates c-fos promoter transactivation with dependence on the second zinc finger domain. *J Biol Chem* 1994; **269**: 24020–6.
38. Kurokawa M, Ogawa S, Tanaka T, Mitani K, Yazaki Y, Witte ON, Hirai H. The AML1/Evi-1 fusion protein in the t(3;21) translocation exhibits transforming activity on Rat1 fibroblasts with dependence on the Evi-1 sequence. *Oncogene* 1995; **11**: 833–40.
39. Kurokawa M, Mitani K, Irie K, Matsuyama T, Takahashi T, Chiba S, Yazaki Y, Matsumoto K, Hirai H. The oncoprotein Evi-1 represses TGF-b signalling by inhibiting Smad3. *Nature* 1998; **394**: 92–6.
40. Kurokawa M, Mitani K, Yamagata T, Takahashi T, Izutsu K, Ogawa S, Moriguchi T, Nishida E, Yazaki Y, Hirai H. The evi-1 oncoprotein inhibits c-Jun N-terminal kinase and prevents stress-induced cell death. *EMBO J* 2000; **19**: 2958–68.
41. Izutsu K, Kurokawa M, Imai Y, Ichikawa M, Asai T, Maki K, Mitani K, Hirai H. The t(3;21) fusion product, AML1/Evi-1 blocks AML1-induced transactivation by recruiting CtBP. *Oncogene* 2002; **21**: 2695–703.
42. Kurokawa M, Mitani K, Imai Y, Ogawa S, Yazaki Y, Hirai H. The t(3;21) fusion product, AML1/Evi-1, interacts with Smad3 and blocks transforming growth factor-b-mediated growth inhibition of myeloid cells. *Blood* 1998; **92**: 4003–12.
43. Stegmaier K, Takeuchi S, Golub TR, Bohlander SK, Bartram CR, Koeffler HP. Mutational analysis of the candidate tumor suppressor genes TEL and KIP1 in childhood acute lymphoblastic leukemia. *Cancer Res* 1996; **56**: 1413–7.
44. Maia AT, Ford AM, Jalali GR, Harrison CJ, Taylor GM, Eden OB, Greaves MF. Molecular tracking of leukemogenesis in a triplet pregnancy. *Blood* 2001; **98**: 478–82.
45. Andreasson P, Schwaller J, Anastasiadou E, Aster J, Gilliland DG. The expression of ETV6/CBFA2 (TEL/AML1) is not sufficient for the transformation of hematopoietic cell lines *in vitro* or the induction of hematologic disease *in vivo*. *Cancer Genet Cytogenet* 2001; **130**: 93–104.
46. Song WJ, Sullivan MG, Legare RD, Hutchings S, Tan X, Kufrin D, Ratajczak J, Resende IC, Haworth C, Hock R, Loh M, Felix C, Roy DC, Busque L, Kurnit D, Willman C, Gewirtz AM, Speck NA, Bushweller JH, Li FP, Gardiner K, Poncz M, Maris JM, Gilliland DG. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. *Nat Genet* 1999; **23**: 166–75.
47. Roumier C, Fenaux P, Lafage M, Imbert M, Eclache V, Preudhomme C. New mechanisms of AML1 gene alteration in hematological malignancies. *Leukemia* 2003; **17**: 9–16.
48. Imai Y, Kurokawa M, Izutsu K, Hangaishi A, Takeuchi K, Maki K, Ogawa S, Chiba S, Mitani K, Hirai H. Mutations of the AML1 gene in myelodysplastic syndrome and their functional implications in leukemogenesis. *Blood* 2000; **96**: 3154–60.
49. Kurokawa M, Tanaka T, Tanaka K, Ogawa S, Mitani K, Yazaki Y, Hirai H. Overexpression of the AML1 proto-oncoprotein in NIH3T3 cells leads to neoplastic transformation depending on the DNA-binding and transactivational potencies. *Oncogene* 1996; **12**: 883–92.
50. Cameron ER, Blyth K, Hanlon L, Kilbey A, Mackay N, Stewart M, Terry A, Vaillant F, Wotton S, Neil JC. The Runx genes as dominant oncogenes. *Blood Cells Mol Dis* 2003; **30**: 194–200.