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RUNX1/AML1: A Central Player in Hematopoiesis

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Abstract

It has been well established that a number of transcription factors play critical roles in regulating the fate of hematopoietic stem cell populations. One of them is the leukemia-associated transcription factor acute myeloid leukemia 1 (AML1; also known as runt-related transcription factor 1, or RUNX1). This gene was originally cloned from the breakpoint of the t(8;21) reciprocal chromosome translocation and was later recognized as one of the most frequent targets of leukemia-associated gene aberrations. Gene-targeting experiments revealed that transcriptionally active AML1 is essential for the establishment of definitive hematopoiesis. More specifically, this gene functions in the emergence of the hematopoietic progenitor cells from the hemogenic endothelium by budding in the aorta-gonad-mesonephros region, and its expression points to the sites with strong potential for the emergence of hematopoietic stem cells. This review discusses aspects of the biologic properties of AML1 in early hematopoietic development. *Int J Hematol.* 2001;74:252-257.
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Key words: RUNX1; AML1; Hematopoiesis; AGM region; Hemogenic endothelium

1. Introduction

Hematopoietic development is a complex process, which generates 2 waves of discrete cellular populations during embryogenesis. In mice, for example, the first wave of primitive hematopoiesis emerges in the yolk sac at approximately day 7.5 post coitus (embryonic day [E]7.5) and fades out during midgestation. In contrast to this temporal wave, the second wave of hematopoiesis occurs at approximately E9.5 in the fetal liver and involves all 3 hematopoietic lineages of adult-type hematopoiesis, including lymphoid progenitors. The hematopoiesis sites are transferred to bone marrow and spleen prior to birth, and these tissues remain as the major sites for hematopoiesis throughout the life span, so that this wave is referred to as definitive hematopoiesis. Several lines of experimental evidence indicate that definitive hematopoiesis does not originate from hematopoietic stem cells in the yolk sac but has an intraembryonic origin in the so-called aorta-gonad-mesonephros (AGM) region [1], where definitive hematopoietic progenitors directly differentiate by budding from the endothelium of the floor of the great vessels (hemogenic endothelium), including the dorsal aorta and the vitelline and umbilical arteries.

It has been well established that a number of transcription factors play critical roles in regulating the fate determination of hematopoietic stem cell populations [2]. These factors are divided into 2 groups: factors that regulate both primitive and definitive hematopoiesis, including Tal1/SCL and LMO2/rbtn2, and other factors, including RUNX1/AML1 (runt-related transcription factor 1/acute myeloid leukemia 1), CBFβ(PEBP2β), c-Myb, Pu.1, and E2A, whose activities are required for the development of some or all of the hematopoietic lineages of definitive origin. Among the latter group, AML1 is unique in that the disruption of this gene results in the complete loss of definitive hematopoiesis of all lineages. Accumulated evidence on the biochemical and biological characteristics of this molecule are revealing and have contributed to further clarification of how this molecule functions and led to new insights into the mechanism of fate determination of the hematopoietic stem cells of definitive hematopoiesis. This brief review addresses some aspects of the biologic roles played by AML1 in early hematopoietic development, focusing on the findings obtained from murine gene-targeting experiments.

2. RUNX1/AML1 as the Target of Chromosomal Translocation or Gene Aberration in Human Leukemia

AML1 was originally cloned from the breakpoint of chromosome 21 in t(8;21)(q22;q22), which is associated with 40%

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of acute myeloblastic leukemia (AML) of the French-British-American (FAB) classification M2 subtype [3]. *AML1* has subsequently been shown to be one of the most frequent targets of leukemia-associated gene aberrations, including t(3;21), which is found in the blast crisis of chronic myelocytic leukemia [4] and myelodysplastic syndromes [5]; t(12;21), which is observed in pediatric B-lineage acute lymphocytic leukemia [6,7]; and t(16;21) [8], which is associated with secondary leukemias. Interestingly, the dimerizing partner (see below), core-binding factor (CBF)β, has been identified as the target of the leukemia-associated chromosomal abnormalities inv(16)(p13;q22) and t(16;16)(p13;q22) [9]. Altogether, these translocations comprise approximately 25% of AML and approximately 20% of acute lymphoblastic leukemia in pediatric and young adult populations. Moreover, recent studies have revealed that *AML1* is also disrupted by several other rare chromosomal translocations and is inactivated by genomic mutations in occasional cases of adult AML and pedigrees of familial platelet disorder with predisposition to AML (FPD/AML) [10,11], thus further documenting the high prevalence of *AML1* alterations in human leukemia.

3. Biochemical Properties of AML1

AML1 encodes the DNA-binding subunit of the heterodimeric transcription factor complex, known as the core-binding factor or CBF (or polyomavirus enhancer binding protein 2: PEBP2) [12,13]. The 128 amino acids near the N-terminus of AML1 are known as the Runt domain because of their high homology (69% identity at the amino acid level) to the *Drosophila* developmental genes *runt* and *lozenge* and because both the DNA binding and association with CBFβ of AML1 are mediated through this domain [14]. Runt is a nuclear protein that functions in segmentation control, sex determination, and neural development through controlling target-gene transcription [15], whereas Lozenge is a transcriptional regulator functioning in the regulation of photoreceptor cell differentiation [16] and the cell fate determination of hemocytes of fly embryos and larvae [17].

AML1 was suggested to regulate a number of hematopoietic genes, including the *granulocyte-macrophage colony–stimulating factor (CSF)*, *CSF-1 receptor, myeloperoxidase*, *neutrophil elastase*, and the *T-cell antigen receptor* genes, which are mediated through the binding of AML1 to the PEBP2 site TGT/cGGT in their transcriptional *cis*-elements [18,19]. Biochemical experiments have shown that AML1 transactivates the expression of reporter genes that are constructed with the transcriptional *cis*-elements of the genes described above.

Biochemical studies have demonstrated that the “classic” transactivating domain of AML1 resides within its C-terminal portion to the Runt domain. For example, Kanno et al showed that the residues of 291 to 371 are interchangeable with the activation domain of a prototypic transcription factor, the bacterial GAL4 molecule [20]. AML1 has a 5–amino acid stretch, VWRPY, in the C-terminus, which is conserved among all AML1 family genes and was suggested to function as a binding site for the transcription corepressor, the Groucho/Transducin-like Enhancer of split (TLE) [21,22]. It is known that the *Drosophila* Runt represses the transcription of *even skipped* (*EVE*), depending on its association with the Groucho protein through its C-terminal VWRPY motif [23], in addition to its transactivating effect on a number of known target genes. Consistent with this notion, mammalian AML1 has been reported to also function as a transcriptional repressor for the *p21/Waf1/Cip1* gene promoter [24]. Thus, AML1 functions as both a transcription activator and a transcription repressor, in a context-dependent fashion.

4. Biologic Properties of AML1 in Early Hematopoietic Development

4.1. Insights Obtained From Initial Gene-Targeting Experiments

The physiological role of AML1 was identified as a result of gene-targeting experiments. Two lines of *AML1*-deficient mice were generated [25,26]. One targeted exon 4, which corresponds to the middle of the Runt domain, whereas the other targeted exon 5, so that no functional molecule could be produced from these targeted alleles. Consequently, an almost identical phenotype was observed among these mutants, with an almost complete penetration: The mutant mice suffered from complete absence of fetal liver definitive hematopoiesis and died of multiple bleeding in the central nervous system and soft tissues during the midgestational period [25,26]. No hematopoietic elements of definitive origin were observed in tissue sections of the fetal liver or on smear preparations of peripheral blood of the mutant embryos. Their fetal livers and yolk sacs lacked hematopoietic precursors of any definitive cell lineages that could form colonies in vitro. This phenotype clearly indicated that AML1 (or, in other words, the genes regulated by AML1) is essential for the development of definitive hematopoiesis in all cell lineages (Figure 1). In contrast, primitive erythropoiesis in the yolk sac was minimally affected, suggesting that AML1 is not required for this lineage. Furthermore, chimeric mouse analysis using *AML1*$^{-/-}$ embryonic stem (ES) cells, which were sequentially targeted for both *AML1* alleles, revealed that *AML1* is essential for lymphoid lineages, because the mutant ES cells did not contribute to lymphoid tissues in the chimeric mouse. The chimeric mouse analysis also revealed that the defect resulting from *AML1* deficiency was cell-autonomous in that the double-mutated ES cells failed to develop hemato-lymphoid lineages, although a normal microenvironment was provided by the host cells in the chimeric animals [25,27].

This hematopoietic effect mediated by AML1 is completely dependent on the presence of the beta partner of the complex, because the targeted disruption of *CBFB* resulted in a phenotype almost identical to those observed in the *AML1*-deficient mice [27-29]. The association of CBFβ with the Runt domain not only increases the binding affinity of this transcription complex to the DNA site, as stated earlier, but this binding also functions to protect the AML1 molecule from ubiquitination-dependent degradation and results in a prolonged half-life [30]. These effects of CBFβ appear to be necessary to keep the AML1 molecule physiologically functional.

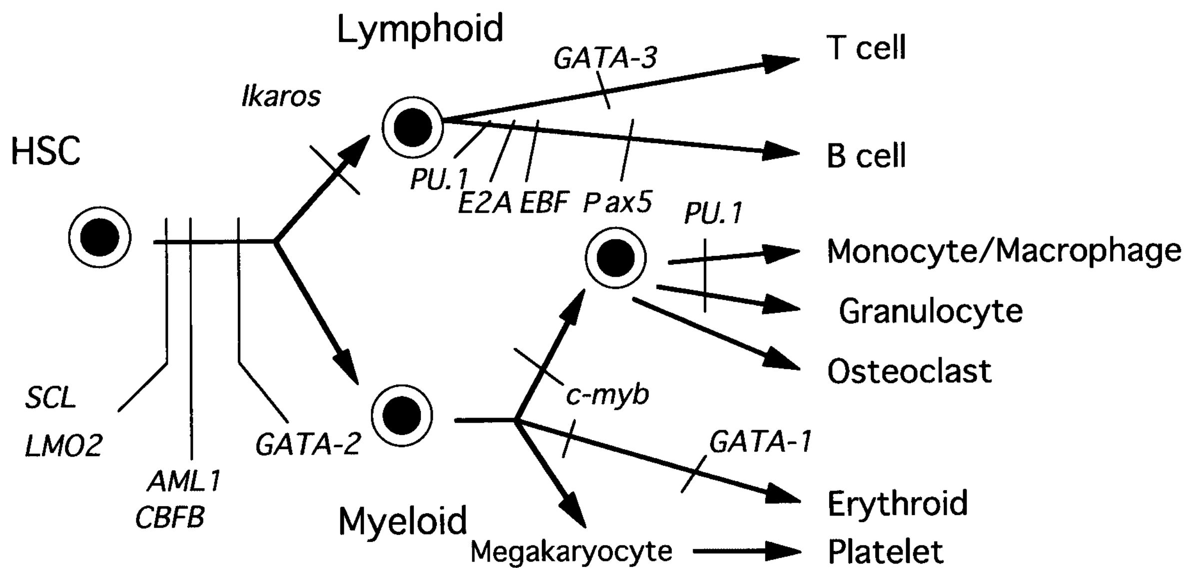

Figure 1. Schematic representation of the affected cell lineages of gene-targeting experiments. Acute myeloid leukemia 1 (AML1) is essential for the development of all lineages of definitive hematopoiesis.

The notion that the hematopoietic phenotype found in *AML1*-deficient mice is due solely to the lack of this gene was formally confirmed by an experiment in which the hematopoietic defect duplicated in vitro using an ES cell differentiation system was successfully rescued by reintroducing *AML1* cDNA from a knockin allele [31]. This experiment also revealed that the activation domain of AML1 is necessary for this biologic activity, whereas the repression subdomain, including the C-terminal VWRPY motif, is not [31], meaning that the biologic significance mediated through this subdomain remains to be clarified.

4.2. Cause of Bleeding: Evidence of a Close Relationship Between Hematopoietic Stem Cells and Angiogenesis

Why *AML1*-deficient mutants develop multiple bleeding has remained an unanswered question for a long time, because simple lack or deficiency of platelets rarely causes fatal bleeding during the gestation period, as documented in some gene-manipulated mouse lines, such as p45/NF-E2–deficient lines [32]. Recently, Takakura et al reported that the P-Sp (para-aortic splanchnopleura; the approximate counterpart of AGM in developmentally earlier embryos) region from E9.5 *AML1*-deficient embryos was not fully competent to develop in vitro angiogenesis in a coculture with the OP-9 feeder-cell layer, in contrast to the control cultures in which angiogenesis was readily supported by the P-Sp culture [33]. It is important to note that the emergence of angiogenesis was induced by the addition of hematopoietic progenitor cells or angiopoietin-1 to the culture of *AML1*-deficient P-Sp, indicating that environmental factors produced by hematopoietic stem cells (HSCs) interact with the endothelial population that is responsible for the angiogenesis. This finding indicates that the bleeding observed in the *AML1*-deficient embryos was caused by the lack of remodelling of the vessels during the midgestation period, secondary to the lack of HSC-derived growth factors that are essential for the normal developmental process of angiogenesis.

4.3. Dose Effect on AML1 Function

The biologic effect of AML1 appears to be influenced by the loss of 1 allele. The fetal livers of *AML1*-heterozygous embryos tend to have fewer progenitors than those from wild-type embryos [26]. Similarly, P-Sp culture experiments showed that fewer hematopoietic precursors were readily observed in *AML1*-heterozygous embryos than those found in wild-type controls [34], further underscoring the notion that so-called haploinsufficiency is present in the AML1 action for early hematopoietic regulation. In other words, a loss of 1 allele, which results in the reduction of the gene products to virtually half, strongly influences the biologic function of AML1. In adult animals, however, no phenotype led by haploinsufficiency has been identified [25,26], so further analysis is needed.

As is often the case with molecules with a haploinsufficient phenotype, the expression of *AML1* appears to be strictly regulated. In contrast to the ubiquitous expression of AML1's functionally associated partner, *CBFB*, the expression of *AML1* is tissue and stage specific [13,35,36]. In addition, "rescue" of the *AML1*-deficient hematopoietic defect has so far been optimally achieved when the *AML1* cDNA with its full-length C-terminus was expressed from an artificial knockin allele, not by simple forced expression of the gene under its heterologous promoters [31]. Thus, the biologic activity of AML1 appears to be dose dependent.

4.4. Further Specification of the Precise Sites Where AML1 is Required

Consistent with the above observations, tracing of *AML1* expression by means of the knocked-in reporter gene marker has been successfully used to further specify the precise sites where AML1 is required at various stages of definitive hematopoietic development. To this end, North et al generated a mouse line with an artificial *AML1* allele to express bacterial β-galactosidase as an AML1 fusion molecule under the influence of authentic *cis*-elements of this gene locus [37]. Analysis of the expression of the β-galactosidase marker in mouse embryos of this line indicates that AML1 starts to be detectable in the extraembryonic mesoderm at E7.5 and can then detected in the precursor cells in the blood island of the yolk sac, after which its expression can be detected in the primitive erythroid cells. As for the embryo proper, the expression signals are observed in the endothelium of the floor of the dorsal aorta, as well as of the vitelline and umbilical arteries [37]. These areas are within the so-called AGM region, where the activity of definitive hematopoietic stem cells is detected.

These areas are known to be the first sites where hematopoietic progenitors of definitive origin emerge. More specifically, the precise histological analyses [38,39] and cell-fate–tracing experiments [40] indicate that hematopoietic cell clusters emerge from a small population of endothelial cells in these sites of the AGM region. This paradigm showing that the definitive HSC is derived from the endothelium, in other words, evidence of the physiological existence of a bipotent progenitor population, or "hemangioblast," was further confirmed by the documentation of experimental evidence [41]. However, it remains unknown whether the entire definitive hematopoietic population is derived from this bipotent progenitor. The possibility that some populations exist that are generated independently from the differentiation pathway described here remains to be tested.

The strong signal for *AML1* expression exactly matches the hemogenic endothelium and attached hematopoietic cell clusters. An important finding is that crossing a β-galactosidase mouse with an *AML1*-deficient background resulted in reduced expression of *AML1* from the sites for the hemogenic endothelium and the disappearance of hematopoietic cell clusters [37]. Disappearance of the hematopoietic clusters attached to the endothelium was also found in an independent mouse line of *AML1*-null genotype [42], thus confirming this phenomenon. These experimental findings clearly indicate that AML1 is specifically required during the process of definitive hematopoiesis at the stage of budding of the hematopoietic cell clusters from large vessels in the AGM region (Figure 2).

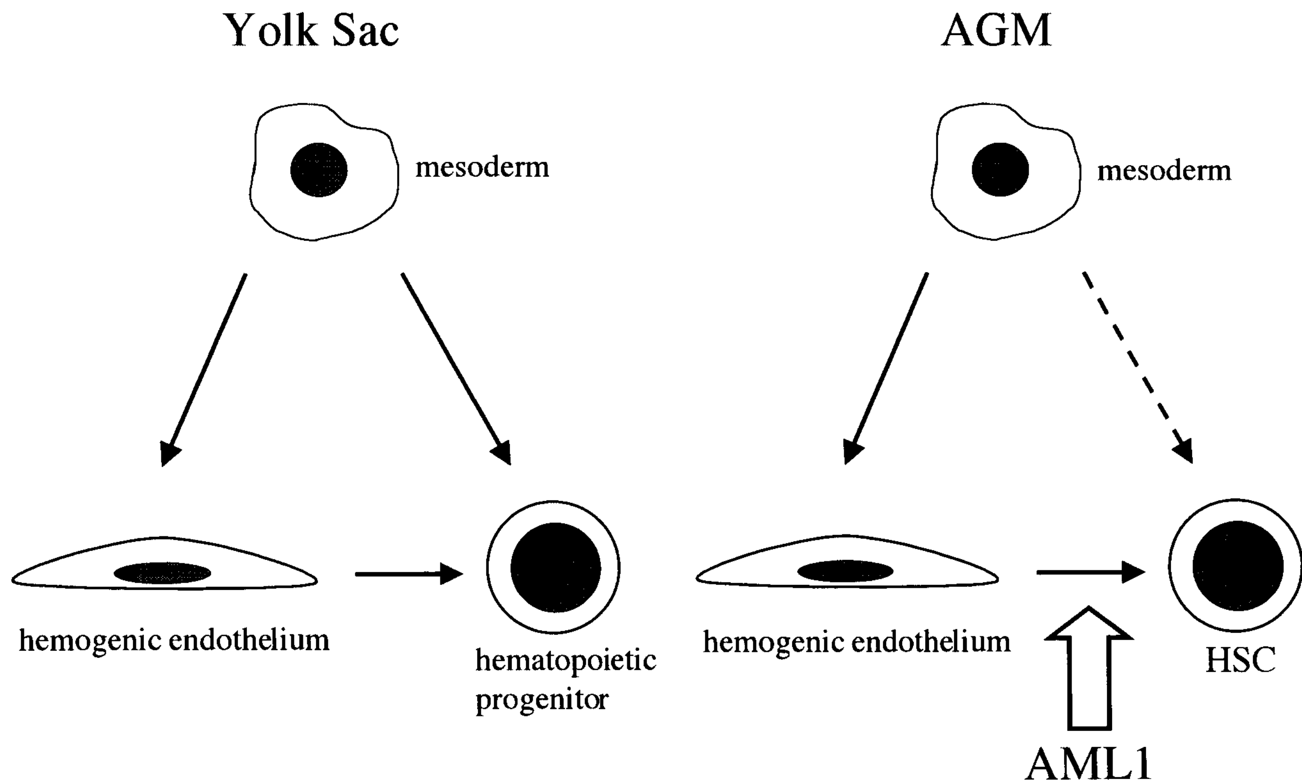

Figure 2. Acute myeloid leukemia 1 (AML1) is required in the step in which the definitive hematopoietic stem cell (HSC) emerges from the hemogenic endothelium of the aorta-gonad-mesonephros (AGM) region.

4.5. Coincidence of AML1 Expression and HSC Production Sites

Within the AGM region during early to midgestation, a panel of HSCs with a hierarchical order is produced. Somewhat committed progenitors, such as CFU-C or CFU-S, emerge at approximately E8 or E9 [43], and the HSCs with in vivo long-term hematopoietic reconstitution ability can be detected at a somewhat later stage, ie, approximately E10 [43]. The latter cells have been recognized by means of transplantation to reconstitute the hematopoiesis of the lethally irradiated adult mouse, resulting in persistence for several months, and are thus considered to belong to the cell population that most fulfills the definition of the HSC: multipotent with self-renewal ability. To assess which hematopoietic progenitors are most closely related to the expression of *AML1*, careful analysis of the sublocalization of the HSC within the AGM region was performed with reference to the AML1–β-galactosidase site [44]. Highly abundant HSCs are detected at the anterior site of the dorsal aorta on E11, followed by spreading of the site into the posterior aorta and urogenital ridges. This geographical characterization of the site and its spreading thereafter correlates with that of the site where strong AML1 expression is detected in the β-galactosidase mouse [44]. Thus, the expression of *AML1* points to the sites with strong potential for the emergence of HSCs. It would be of interest to investigate whether HSCs could be further enriched if, in addition to conventional cell surface antigens, a living marker was used for the generation of the knockin marker mouse.

Cai et al [45] reported that, in contrast to the appearance of the HSCs in E10 within AGM and in E11 within yolk sac in wild-type embryos, the HSCs in the yolk sac appeared 1 day earlier in the heterozygous embryos. In addition, these HSCs retained a high frequency for the positive recipients, implying that HSCs may have been increased in this organ. In contrast, HSC activity decreased in heterozygous mice when they were assessed using explant culture. Again, disruption of 1 allele of AML1 resulted in the alteration of the temporal and spatial patterning of the emergence of the HSCs. Although the mechanisms underlying these phenomena remain to be elucidated, these observations strongly underscore the notion that AML1 functions in cell fate determination, and its level is important.

Little is known about the transcriptional regulation of the *AML1* gene. The 2 distinct promoter regions in the gene locus are known to be differentially activated during early hematopoietic development [46,47]. Initial findings regarding their function suggest that the distal promoter appears to have a closer relationship with the hematopoietic progenitors of definitive origin in that the expression driven by the distal promoter is dominant in the sorted hematopoietic progenitor cell population [46] and that the transcription by this promoter diminishes when active AML1 is disrupted [47], as was the case for the β-galactosidase knockin mouse [37]. Multiple AML1-binding sites are present in the promoter regions [48], suggesting the existence of an autoregulatory mechanism. Characterization of its expression awaits further clarification.

5. Conclusions

Hematopoietic development is closely related to angiogenesis. Most, if not all, definitive HSCs emerge from hemogenic endothelium in the AGM region during the early developmental stages. Molecular genetic experiments have revealed that AML1 is required for the HSCs at this very early stage of hematopoietic development. It is now clear that the levels of AML1 are critical in maintaining a proper hematopoietic process. The available evidence so far suggests that this molecule actively functions in fate determination of HSCs. Thus, the elucidation of the molecular basis of its actions should contribute to further defining the regulatory mechanism of hematopoietic development.

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