Core-Binding Factor: A Central Player in Hematopoiesis and Leukemia

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Abstract

Consistent chromosomal rearrangements are found in blood cells of hematopoietic tumors. In many cases, these rearrangements disrupt genes whose normal function is required for the proper development of blood cells. Excellent examples are the chromosomal rearrangements t(8;21)(q22;q22), t(12;21)(p13;q22), and inv(16)(p13;q22) that disrupt two of the genes encoding a small family of hematopoietic transcription factors, core-binding factors (CBFs). CBFs consist of a DNA-binding CBFa subunit and a non-DNA-binding CBFB subunit. The t(8;21), associated with de novo acute myeloid leukemias, disrupts the CBFA2 (AML1) gene, which encodes a DNA-binding CBFa subunit. The t(12;21), the most common translocation in pediatric acute lymphocytic leukemias, also disrupts CBFA2. The CBFB gene, which encodes the non-DNA-binding subunit of the CBFs, is disrupted by the inv(16) in de novo acute myeloid leukemias. All chromosomal rearrangements involving the CBFA2 and CBFB genes create chimeric proteins, two of which have been unequivocally demonstrated to function as transdominant negative inhibitors of CBF function.

Both the CBFa2 and CBFB genes are essential for normal hematopoiesis in mice, because homozygous disruption of either gene blocks definitive hematopoiesis. Recent data suggest that CBFa2 and CBFB are required for the emergence of definitive hematopoietic stem cells in the embryo from a putative definitive hemangioblast precursor. The transdominant negative inhibitor of CBF created by the inv(16), when present from the beginning or later stages, blocks the emergence of definitive hematopoietic cells in the embryo. On the other hand, chromosomal translocations involving the CBFA2 and CBFB genes in leukemias block hematopoiesis at later steps. This may reflect a difference in the timing at which translocations are acquired in the leukemias, which presumably is subsequent to emergence of the definitive hematopoietic stem cell. The cumulative data suggest that although the earliest requirement for CBFa2 and CBFB is for emergence of definitive hematopoietic stem cells, both genes are also required at later stages in the differentiation of some hematopoietic lineages.

Introduction

Hematopoiesis is the process by which blood cell populations of all lineages are continuously renewed through differentiation from a common progenitor cell called the pluripotent HSC. Many transcription factors are required for blood cell development, and several, such as Scl, Rbtl2, E2A, and the subjects of this review, CBFa2 (AML1) and CBFB, also play key roles in the dysregulation of hematopoiesis manifested as leukemia.

The Involvement of CBFs in Human Disease

CBFa2 and CBFB are members of a small family of transcription factors known as CBFs or polyomavirus enhancer binding protein 2 (1). CBFs are heterodimeric transcription factors that contain one subunit that binds DNA directly (CBFa) and a second, non-DNA-binding CBFB subunit (Fig. 1; Refs. 2–4). Four genes encode subunits of CBF in humans: three encode CBFa subunits (CBFA1, CBFA2, and CBFA3); and one gene encodes the common CBFB subunit, CBFB (2–7).

CBFs play critical roles in both normal developmental processes and disease. The CBFA1 gene, which encodes a DNA-binding CBFa subunit, is required for bone development. Mice lacking both copies of the CBfa1 gene are completely lacking in bone formation, due to a defect in osteoblast differentiation (8, 9). The human homologue, CBFA1, is implicated in a human genetic disorder, cleidocranial dysplasia, an autosomal dominant trait characterized by moderate skeletal malformations (10). The mutations in cleidocranial dysplasia patients inactivate only one copy of the CBFA1 gene, indicating that the disorder is caused by haploinsufficiency of CBFA1.

The CBfa2 and CBFB genes are required for fetal liver hematopoiesis in mice and play major roles in the etiology of many human leukemias. The human homologue of CBfa2 (CBFA2, more commonly known as AML1) is disrupted by the t(8;21)(q22;q22), t(12;21)(p13;q22), and t(3;21)(q26;q22) in acute myeloid and lymphocytic leukemias and in therapy-related leukemias and myelodysplasias (Fig. 1; Refs. 5 and 11–14). The CBFB gene, which encodes the non-DNA-binding CBFB subunit, is disrupted in acute myeloid leukemias by inv(16)(p13;q22), t(16;16), and del(16)(q22) (15). Together, CBFA2 and CBFB are disrupted in approximately a quarter of all de novo acute leukemias, making them the most frequently disrupted genes in acute human leukemias (16–20).

The chromosomal translocations involving the CBFA2 and CBFB genes generate chimeric proteins that contain all or part of CBFA2 (AML1) or CBFB fused to a segment of a protein encoded on the other chromosome involved in the translocation (Fig. 2). The t(8;21) fuses the N-terminal of CBFA2, including its DNA-binding domain, to a protein called ETO (for eight twenty one), encoded on chromosome 22. The t(3;21) fuses the CBFB subunit to one of three different proteins encoded by genes closely linked on chromosome 3: EAP, MDS1, or EVII. The t(12;21) breaks the CBFA2 gene 5′ to the first coding exon and fuses the N-terminal of a protein in the ets family called TEL to the full-length CBFA2 protein. All of these chimeric CBFA2 proteins contain the CBFB (AML1) DNA-binding domain, and they are believed to deregulate the transcription of CBF target genes in a transdominant manner (21–27).

The inv(16) that disrupts the CBFB gene results in a chimeric protein that contains most of the CBFB protein fused to the COOH-terminal α-helical rod domain from a SMMHC (Fig. 2; Ref. 15). It is thought that the CBFB-SMMHC protein blocks wild-type CBF function by assembling into multimers that sequester CBFs subunits into nonfunctional complexes (28–30).
CBF IN HEMATOPOIESIS AND LEUKEMIA

Fig. 1. The CBF complex and its association with leukemias. CBF binds the sequence PyGPyGGT. The CBFβ subunit forms a 1:1 complex with CBFα and increases the binding affinity of CBFα for DNA (2, 4). Three genes encode CBFα subunits, and one of these genes, the CBFA2 gene, along with the CBFB gene, are the most frequently disrupted genes in acute human leukemias.

CBFA2 and CBFB Are Required for the Emergence of Definitive Hematopoietic Stem Cells

Gene disruption experiments in mice demonstrated that both CBFA2 and CBFB are required for the differentiation of all hematopoietic cells derived from the HSCs. Before describing the hematopoietic defect in CBFA2- and CBFB-deficient mice in more detail, it is worthwhile to briefly review the early steps of hematopoiesis in the developing embryo.

Pluripotent HSCs are the source of all blood cells in the adult. However, paradoxically, they are not the first blood cells to develop in the embryo. The first blood cells, which arise from mesoderm, appear in the extraembryonic yolk sac of mouse embryos at ~8 dpc and are primarily primitive nucleated erythrocytes and small numbers of granulocytes and macrophages (31). This first, transient wave of “primitive” yolk sac hematopoiesis is followed shortly thereafter by the appearance of “definitive” hematopoietic progenitor cells, which are capable of differentiating into all adult blood cell lineages. Definitive hematopoietic cells emerge from several sites in the embryo: from the extraembryonic yolk sac; the omphalomesenteric and umbilical arteries; the embryonic splanchnopleure; and from the AGM region (32–37). The first-long term repopulating HSCs, which are cells capable of reconstituting an intact hematopoietic system when transplanted into a lethally irradiated adult mouse, appear at 10.0 dpc in the AGM region (36, 37). After their emergence, definitive hema-

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**Fig. 2.** CBFα2 (AML1) and CBFβ fusion proteins. A, chimeric proteins resulting from chromosomal translocations involving the CBFA2 (AML1) gene. Black rectangle, the DNA-binding domain. Sequences derived from fusion partners are indicated by the stippled, dashed, and lined rectangles. White or black regions represent sequences derived from the CBFA2 (AML1) protein. B, the CBFβ protein and the chimeric CBFβ-SMMHC protein formed by the inv(16). Black rectangle, the sequences responsible for heterodimerizing with CBFA. Stippled region, SMMHC sequences.
topoietic progenitors and long-term repopulating HSCs migrate to the fetal liver, where they further expand and differentiate into definitive enucleated erythrocytes and cells of the myeloid lineage.

Mutations in several genes encoding transcription factors, such as Scl, Rbm2, Gata1, and Gata2, have profound effects on the development of both primitive and definitive blood cell lineages (reviewed in Ref. 38). Other genes (Pu.1, Myb, Eklf, Ikaros, Pax5, E2A, and NF-E2) seem to be required for the development of a subset of definitive hematopoietic lineages but not for the development of primitive blood cells in the yolk sac. Mutations in the Cbfa2 and Cbfb genes specifically impair the development of all definitive hematopoietic lineages but not the primitive erythroid lineage (39–43). Cbfa2- and Cbfb-deficient mouse embryonic stem cells are unable to contribute to definitive hematopoietic lineages in chimeric mice, indicating that the hematopoietic block caused by loss of Cbfa2 and Cbfb, at least in part, is cell autonomous (39, 41).

Analyses of Cbfa2- and Cbfb-deficient mice demonstrated a lack of definitive hematopoietic elements in histological sections of fetal livers. Cells isolated from the yolk sac or fetal liver were also unable to differentiate into definitive erythroid, myeloid, or mixed lineage colonies in vitro. These results, plus the inability of Cbfa2- and Cbfb-deficient mouse embryonic stem cells to undergo lymphopoiesis in chimeric mice, placed the requirement for Cbfa2 and Cbfb at the level of a definitive hematopoietic stem cell. However, the precise nature of the hematopoietic stem cell defect in Cbfa2- and Cbfb-deficient mice could not be resolved from these initial studies, because an impairment of fetal liver hematopoiesis could result from one of multiple defects. For example, Cbfa2 and Cbfb could be required for the emergence of definitive hematopoietic progenitors and HSCs from an HSC precursor. Alternatively, HSCs and definitive hematopoietic progenitors could emerge but fail to migrate from their site of origin to the fetal liver, or once in the liver be unable to further expand and differentiate.

To more precisely define the step at which Cbfa2 and Cbfb are required, we have identified cells that express the Cbfa2 gene and determined their fate in animals lacking the functional CBFα2:CBFβ heterodimer (44). We chose to track only Cbfa2 expression, because although both the CBFα2 and CBFβ subunits are required for the activity of this heterodimer in vivo, the Cbfβ gene appears to be expressed ubiquitously (2, 4). Thus, we reasoned that Cbfa2 expression would more accurately identify the cells in which the active heterodimer is found. We find that Cbfa2 is expressed in sites where definitive hematopoietic cells emerge, including the yolk sac, vitelline and umbilical arteries, and in clusters of hematopoietic cells that appear to bud from the endothelium into the lumina of these arteries. In the AGM region where HSCs first emerge, Cbfa2 is expressed in endothelial cells and in the paraaortic mesenchyme of the dorsal aorta in a ventral to dorsal gradient. Hematopoietic cells expressing Cbfa2 are also found in the fetal liver by 10.5 dpc.

What happens to cells expressing Cbfa2 in embryos lacking the functional CBFα2:CBFβ heterodimer? We find that Cbfa2 deficient embryos contain almost no definitive hematopoietic progenitors, either in their fetal livers or in circulating blood (44). Furthermore, when we examine the sites where definitive hematopoietic progenitors emerge, such as the vitelline and umbilical arteries, the yolk sac, and the AGM region, we find no evidence of hematopoietic cells budding from hemogenic endothelium at these sites. On the basis of these and previous genetic data, we hypothesize that Cbfa2 is required for the emergence of definitive hematopoietic cells from hemogenic endothelia in the developing embryo.

Chromosomal Translocations and Leukemia

Functional assays, as well as more recent genetic studies in mice, established that chromosomal translocations involving the CBFα2 and CBFβ genes create transdominant negative alleles of these genes (21–27, 45–47). In mice, both Yergeau et al. (26) and Okuda et al. (27) modified the Cbfa2 gene in such a way that the chimeric protein created by the t(8;21) was expressed from the locus. Using a similar strategy, Castillo et al. (47) recreated the inv(16) in mice by introducing a cDNA encoding the CBFβ-SMMHC fusion protein into the Cbfb locus. In all three studies, chimeric mice containing the “knocked in” t(8;21) and inv(16) alleles were generated, but these mice failed to produce offspring heterozygous for the knocked-in alleles. Further analysis established that embryos heterozygous for the

Fig. 3. Hematopoietic blocks associated with loss of CBFα2(AML1)-CBFβ function. Cbfa2 and Cbfb are required for the emergence of definitive HSCs in the embryo. The transdominant inhibitors of CBF (AML1-ETO and CBFβ-SMMHC) are associated with acute myeloid leukemias. TEL-AML1, which has not yet been shown genetically to mimic loss of Cbfa2 or Cbfb function during embryogenesis, is found in pre-B-cell leukemias.
t(8;21) and inv(16) alleles died in midgestation, exhibiting a spectrum of phenotypes very similar to that seen in Cbfa2- and Cbfb-deficient mice, including a severe impairment of fetal liver hematopoiesis. These experiments unequivocally demonstrated that the t(8;21) and inv(16) create transdominant negative alleles of Cbfa2 and Cbfb, that when present from the beginning of embryogenesis, block hematopoiesis at essentially the same point as homozgyous loss of Cbfa2 or Cbfb.

Although it is clear from the genetic data that the t(8;21) and inv(16) create transdominant negative alleles of Cbfa2 and Cbfb, how these alleles contribute to leukaemogenesis remains one of the outstanding questions in the CBF field. The t(8;21) and inv(16) are associated with acute myeloid leukemias in humans, indicating a differentiation block at a much later stage of hematopoiesis than seen in the mouse knock-in models (Fig. 3). Furthermore, conspicuously absent in the chimeric mice heterozygous for the t(8;21) and inv(16) alleles were signs of overt leukaemia. What accounts for these discrepancies?

At this time, we can only speculate on the differences in the behavior of these transdominant negative alleles in the developing embryo and the leukaemia patient, but there are several possibilities to consider. One difference is timing; in the mouse knock-in experiments, the t(8;21) allele and the inv(16) allele were present in all cells in the embryo, from the very beginning of development, and before the emergence of HSCs. In contrast, in the leukaemia patient, the translocations are presumably acquired somatically in a single cell that had differentiated to a more committed stage in the hematopoietic pathway. Whether Cbfa2 and Cbfb are required for both the emergence of the HSC, and the further differentiation of some hematopoietic lineages is not known but is certainly implied by the association of the transdominant negative CBF2A2 and CBFB alleles with leukemias.

The second issue to consider is that of secondary mutations. AMLs are usually diseases of adulthood, and presumably multiple mutations are required to generate a full-blown leukaemia. The activation of other oncogenes, or loss of tumor suppressor genes, may contribute to the ability of cells that have undergone CBF2A2 and CBFB translocations to persist and expand to the leukemic state.

Finally, closer inspection of the t(8;21) and inv(16) knock-in mice suggests that hematopoietic inhibition by the dominant negative CBF2A2 and CBFB alleles is not complete. For example, Yergeau et al. (26) demonstrated that, although no definitive hematopoietic colonies of any kind could be cultured from yolk sacs derived from 10.5 dpc Cbfa2-deficient embryos, macrophage-like colonies differentiated from the yolk sacs of embryos heterozygous for the engineered t(8;21) allele. More recently and dramatically, Okuda et al. (27) reported that in their independently derived t(8;21) knock-in mouse strain, dysplastic progenitors for mixed lineage colonies could be found in the fetal livers of day 11.5–13.5 embryos, and these progenitors had an abnormally high self-renewal capacity. The data imply that some hematopoietic cells in the Okuda et al. (27) t(8;21) knock-in embryo make it past the early block in HSC development but fail to complete their differentiation to mature myeloid and erythroid cells. Perhaps in the patient, these cells would form the target population for secondary mutations, leading to acute leukaemia.

References


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