Core Binding Factor Acute Myeloid Leukemia: New Prognostic Categories and Therapeutic Opportunities

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Core binding factor (CBF) is a heterodimeric protein complex involved in the transcriptional regulation of normal hematopoiesis. Mutations in CBF-encoding genes result in leukemogenic proliferative advantages and impaired differentiation of the hematopoietic progenitors. CBF molecular aberrations are responsible for approximately 20% of all adult acute myeloid leukemia (AML). Although CBF-AMLs are considered to have relatively good prognosis compared to other leukemia subtypes, they are a heterogeneous group of disorders and modern therapy frequently leads to relapse and the associated morbidity and mortality. Improvements in risk stratification and development of targeted therapies are needed for better outcomes. In this review we provide a brief overview of the molecular basis, prognostic categories and the advanced treatment strategies for CBF leukemias.
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MOLECULAR BASIS OF CBF LEUKEMIA

Leukemia is a cancer of the developing blood cells caused by mutations leading to either uncontrolled proliferation (class I) or lack of differentiation (class II) or both. The World Health Organization (WHO) classifies acute myeloid leukemia (AML) into several categories based on underlying genetic alterations to facilitate diagnosis and prognosis.[1] Recurrent genetic alterations are frequently observed in AML patients. Among them t(8;21)(q22;q22) and inv(16)(p13q22)/t(16;16)(p13q22) are the most common and result in generation of corresponding abnormal fusion genes *RUNX1-RUNX1T1* and *CBFB-MYH11,*[2] respectively (Figure 1).

Native RUNX1 and CBFβ form a heterodimeric transcription factor complex core binding factor (CBF) that regulates normal hematopoietic ontogeny. CBF is comprised of an alpha subunit and a beta subunit. There are three alpha subunits (RUNX1-3) and one beta subunit (CBFβ) identified to date. The alpha subunit binds to a consensus DNA sequence TGT/cGGT and the beta subunit stabilizes the interaction between the alpha subunit and DNA but does not interact with DNA independently.[3] Association of CBFβ induces a 40-fold increase in the DNA binding affinity of RUNX1.[4] Therefore both subunits are required for maximum transcriptional efficiency of target genes downstream such as lymphocyte-specific protein tyrosine kinase, granulocyte-macrophage colony-stimulating factor-1 receptor, interleukin-3, and myeloperoxidase[5]. It has been found that RUNX1 also interacts with co-activators p300 and CREB binding protein to mediate transactivation.[6] Fetal mice null for *Runx1* or *Cbfb* die of CNS hemorrhage and lack of fetal liver hematopoiesis on embryonic day 11.5–12.5, demonstrating that CBF is required for definitive hematopoiesis.[7–10]

The fusion gene *CBFB-MYH11* was initially identified in 1993[11] and the corresponding fusion protein CBFβ-SMMHC (smooth muscle myosin heavy chain) was identified in inv(16) patient samples in 1996.[12] CBFβ-SMMHC forms large nuclear aggegregates,[13] sequesters the alpha subunit RUNX1 in the cytoplasm,[14] and arrests differentiation of the inv(16) containing human cell line ME-1.[15] The RUNX1 interacting N-terminal region of CBFβ and the myosin multimerizing C-terminal coiled coil domains of SMMHC direct this sequestration process.[16] CBFβ-SMMHC also prevents the ubiquitin-mediated proteosomal degradation of RUNX1 and generates a stable complex that dominantly inhibits normal CBF function.[17] The t(8;21) was first described in 1973[18] and the *RUNX1-RUNX1T1* fusion gene was identified in 1992.[19] The fusion protein product of t(8;21) is comprised of the DNA binding RUNT homology domain of RUNX1 and most of the RUNX1T1 (ETO) except the first 30 amino acids at the N-terminus.[20] The absence of

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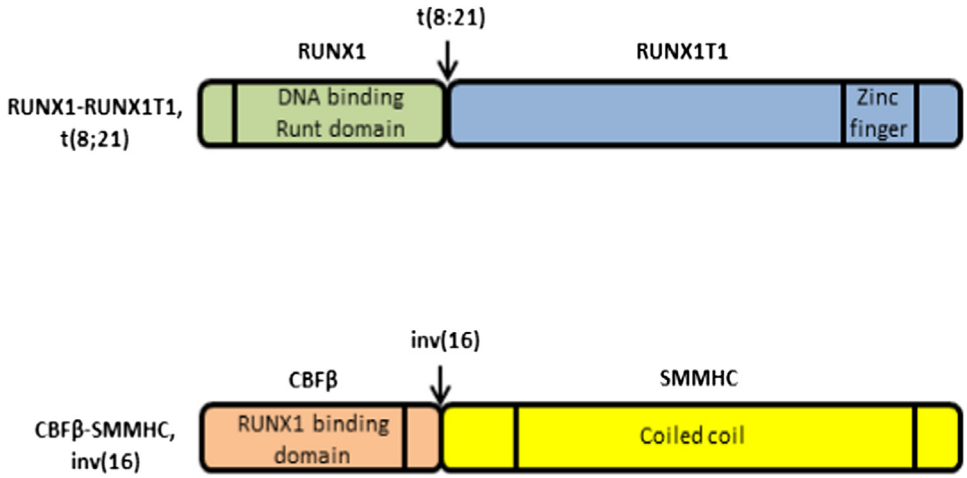

Figure 1. Illustration of fusion protein products from CBF-AML associated mutations and the domains relevant for leukemogenesis. Chromosomal aberrations t(8;21) and inv(16) in CBF- AML produce fusion proteins RUNX1-RUNX1T1 and CBFβ-SMMHC. RUNX1-RUNX1T1 lacks the transactivation domain but retains the DNA binding Runt domain of RUNX1, which is fused to the repressor domain of the RUNX1T1 protein. CBFβ-SMMHC retains the RUNX1 binding domain of CBFβ, which is fused with the coiled coil dimerizing domain of SMMHC.

the C-terminal transactivation domain in the fusion protein RUNX1-RUNX1T1 disrupts normal hematopoiesis in a dominant-negative fashion and therefore specific inactivation of this fusion induces differentiation of the t(8;21)-positive Kasumi-1 cell line.[21] RUNX1-RUNX1T1 also has been shown to silence microRNA-193 resulting in increased leukemogenesis by increasing expression of histone deacetylases (HDAC) and DNA-methytransferase1 (DNMT), and ultimately decreasing PTEN expression.[22] A common potential mechanism of both of these genetic fusion products is the dominant inhibitory effect on native RUNX1 and finally repression of target genes transcription, as mouse embryos heterozygous for *RUNX1-RUNX1T1* or *CBFB-MYH11* have almost identical phenotypes as the *Runx1*$^{-/-}$ or *Cbfb*$^{-/-}$ embryos regarding CNS hemorrhage and hematopoietic defects.[7,8,23,24]

COOPERATING MUTATIONS IN CBF LEUKEMIA

Murine knock-in models have demonstrated that both CBF fusion genes are necessary but not sufficient to cause leukemia and additional mutations are required for the pathogenesis of CBF leukemias.[2,25] Therefore, in preclinical mouse models, mutagenic induction of second mutations are needed for development of AML.[2] In CBF leukemia patients, frequently detected second mutations are NPM1, c-KIT, and FLT3. A study with 300 AML patients (16–60 years of age) showed that 48% of the patients have NPM1 mutations.[26] Another study with 481 AML patients indicated that 20% of the CBF-AML cytogenetic group had FLT3 mutations.[27] On the other hand, KIT mutations have been observed for 6.6%–46.1% of CBF-AML patients.[28] NPM1 plays an important role in ribosomal protein assembly, transport, prevents aggregation of nuclear proteins, and regulates transcriptional activity of p53.[26] Leukemogenesis occurs when cytoplasmic mutant NPM1 inactivates the tumor-suppressor p19Arf in a p53-dependent or -independent manner.[29] Inactivation of nuclear factor (NF)-κB renders CBF-AML with NPM1 mutation more sensitive to chemotherapy.[29] Genetic rearrangements that lead to constitutively active hematopoietic receptor tyrosine kinases (RTK) such as FLT3, c-KIT, JAK2, and RAS family members have been identified in CBF-AML patients.[2] These mutations may be particularly amenable for treatment with specific RTK inhibitors.[2] Haplo-insufficiency of the tumor suppressors TLE1/4 in t(8;21) and overexpression of MN1 in inv(16) have been observed in addition to the epigenetic and post-translational silencing of differentiation-inducing transcription factor CEBPA (CCAAT/enhancer-binding protein alpha) in CBF-AML.[30] There are case reports of rare cooperating mutations such as BCR-ABL[14] and TEL-PDGFRβ[15] fusion proteins in t(8;21) AML.[31] Both are examples of constitutively active tyrosine kinases that provide survival and proliferation advantages to progenitor cells without affecting their differentiation. The synergistic effects of these hyperproliferative phenotypes together with the CBF mutation-associated impaired differentiation lead to the multistep pathogenesis of AML (both class I and class II phenotypes).

PROGNOSIS

Although the CBF genetic rearrangements in AML patients are reported to be associated with relatively favorable prognosis,[32,33] only 40%–60% of adult CBF-AML patients exhibit long-term survival.[28] Additionally all treatment regimens are associated with significant relapse-related morbidity and mortality.[34,35]

Molecularly defined genetic abnormalities are important prognostic factors in AML and important for patient management.[36] A study with 201 adults with de novo