Core Binding Factor Leukemia

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Contributor: Alessandro Beghini

Acute myeloid leukemia (AML), the most common acute leukemia in adults, is a heterogeneous malignant clonal disorder arising from multipotent hematopoietic progenitor cells characterized by genetic and concerted epigenetic aberrations. Core binding factor-Leukemia (CBFL) is characterized by the recurrent reciprocal translocations t(8;21)(q22;q22) or inv(16)(p13;q22) that, expressing the distinctive RUNX1-RUNX1T1 (also known as Acute myeloid leukemia1-eight twenty-one, AML1-ETO or RUNX1/ETO) or CBFB-MYH11 (also known as CBFβ-SMMHC) translocation product respectively, disrupt the essential hematopoietic function of the CBF. In the past decade, remarkable progress has been achieved in understanding the structure, three-dimensional (3D) chromosomal topology, and disease-inducing genetic and epigenetic abnormalities of the fusion proteins that arise from disruption of the CBF subunit alpha and beta genes. Although CBFLs have a relatively good prognosis compared to other leukemia subtypes, 40–50% of patients still relapse, requiring intensive chemotherapy and allogenic hematopoietic cell transplantation (alloHCT).

Keywords: core binding factor leukemia ; AML ; RUNX1 ; RUNX1T1 ; CBFB ; MYH11 ; miRNA ; chromatin remodeling

1. Introduction

The year 2016 coincided with the 25th anniversary of the first cloning of mammalian Runt (Runt domain)-related transcription factor 1 (*RUNX1*) gene, associated with hematologic disorders [1]. *RUNX1* (AML1) is a master transcriptional regulator of adult hematopoiesis also involved in the establishment, maintenance, and functional integrity of hematopoietic stem cells (HSCs) in embryonic and adult blood compartments [2][3][4]. AML1 post-translational modifications help create scaffolds that interact and bind with multiple members recruited to the core binding factor (CBF), promoting or repressing transcription. At about the same time, the gene encoding *CBFB* (CBFβ) was identified as disrupted by the inv(16) in acute myeloid leukemia [5]. Normally, AML1 and CBFβ form a DNA-binding heterodimer required for binding to the consensus sequence, where it recruits lineage-specifying transcription factors to regulate hematopoietic differentiation. As the Runt-related transcription factor (RUNX) gene family plays important roles in tissue-specific gene expression, it is frequently involved in the malignant transformation of the hematopoietic system. Acute leukemias characterized by the presence of t(8;21) or inv(16) are defined core-binding factor Leukemias (CBFLs), since they both alter the CBF transcription factor complex [6]. Approximately 30% and 13–15% of newly diagnosed pediatric and adult AML patients, respectively, are diagnosed as CBFLs [7]. Although the CBFLs are categorized into a favorable-risk group as compared with other subtypes of AML, approximately 30–40% of the patients still relapse and may require allogeneic hematopoietic cell transplantation (HCT) [8][9]. *RUNX1–RUNX1T1* and *CBFB–MYH11* translocations may represent acquired initiating events occurring in hematopoietic progenitors. However, little is known about the molecular mechanisms that drive the generation of the t(8;21) or inv(16), after which leukemia clonally evolves through accumulation of secondary mutations. The hypothesis that Wnt signaling promotes genomic proximity between *RUNX1* and *RUNX1T1* has been recently examined by experiments establishing that Wnt/β-catenin signaling supports *RUNX1* and *RUNX1T1* expression in hematopoietic precursors and provides spatial information, indicating that transcription of these genes is likely occurring into RNA-polymerase-II nuclear factories (RNAPII-Ser5) [10]. These results suggest a Wnt-mediated model in which an upstream molecular mechanism is capable of favoring and guiding the translocation event [11]. The incremental improvements in understanding the genetic and molecular basis of CBFLs and their association with distinct clinical and biological features provide insights into previously unappreciated cooperating pathways [12][13]. At diagnosis, the disease consists of heterogeneous clusters of cells widely differing from one another in terms of additional genetic lesions, besides sharing the specific chromosomal translocations. Cytogenetic abnormalities that alter the function of the CBF are often associated with specific receptor tyrosine kinase (RTK) mutations, suggesting that additional genetic abnormalities have an essential role in CBFL pathogenesis [14][15]. Despite a common molecular alteration involving a component of the CBF transcription complex, AMLs expressing *RUNX1-RUNX1T1* or *CBFB-MYH11* alterations display a remarkably different genome-wide spectrum of cooperating mutations [14]. Recent studies clearly indicate that AMLs with t(8;21)(q22;q22) and AMLs with inv(16)(p13q22) show different biological and clinical characteristics, supporting the notion that they represent two distinct diseases [7][16]. A series of concomitant evidence in the CBFL proved the existence of a preleukemic phase confirmed by a

prolonged latency observed in experimental models between the occurrence of *RUNX1-RUNX1T1* CBF translocation and the development of overt leukemia [17][18], the persistence of CBFL translocations in normal HSC detected from patients in remission [19][20][21], and the maintenance of *RUNX1-RUNX1T1* at diagnosis and at relapses. *NRAS* (neuroblastoma RAS viral oncogene) is the most frequently mutated gene in CBFL, and over 60% of the cases harbor activating mutations in *NRAS*, *KIT* (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog), *FLT3* (FMS-like tyrosine kinase 3), *KRAS* (Kirsten rat sarcoma 2 viral oncogene homolog), *PTPN11* (protein tyrosine phosphatase non receptor type 11), and/or loss-of-function mutations in *NF1* (neurofibromin1) [9][14][15]. Integrated mutational analysis of the genetic and epigenetic changes that are relevant to the pathogenesis of CBFL would be required for a better risk stratification of patients who would benefit from dose-intensified induction chemotherapy or novel targeted therapies. AML1-ETO (eight twenty-one) (RUNX1-RUNX1T1) is the chimeric protein formed as a consequence of the t(8;21) chromosomal rearrangement, which is among the most recurrent cytogenetic rearrangements in de novo AML. The molecular mechanisms through which AML1-ETO fusion protein exerts multiple effects are not fully elucidated, yet all have focused on its strong repressor function. Moreover, several studies documented the multifunctionality of AML1-ETO fusion protein, including impaired differentiation, apoptosis inhibition, and signal activation for cell proliferation. This model might be oversimplified; however, there is convincing evidence supporting the hypothesis that leukemias are induced by cooperation between alterations in protein-coding genes and microRNAs (miRNAs), an entire novel epigenetic targets linked to leukemia development [22]. The consequences of altered expression and epigenetic status of miRNAs in CBF leukemias have been reported by us and other groups, unveiling that microRNAs are extensively integrated into the molecular networks that control leukemic development and progression [23][24][25][26][27][28].

2. Core Binding Factor Complex: A Critical Role in Hematopoietic Stem Cell Fate

The CBF is a transcription factor complex, which consists of a distinct DNA-binding CBFα subunit (*RUNX1*, *2*, or *3*), and its non-DNA-binding heterodimerization partner CBFβ subunit (encoded by the *CBFB* gene). AML1 is a master regulatory protein expressed throughout all hematopoietic lineages. The *RUNX1* and *CBFB* genes are required for hematopoietic stem cells' (HSCs) emergence and formation during definitive HSC development through to their terminal differentiation, and are key regulators of hematopoiesis at several steps [29][30]. The loss of definitive hematopoiesis observed in *Runx1*$^{-/-}$ or *Cbfb*$^{-/-}$ knockout mice and an expanded HSC compartment in conditional *Runx1*-deficient mice highlight their complex interplay in orchestrating the accurate maintenance of hematopoietic stem cell differentiation [29][30][31][32][33][34][35]. The heterodimerization with CBFβ leads to the phosphorylation of RUNX1, which in turn induces p300 (encoded by *EP300*) phosphorylation by homeodomain interacting kinase 2 (HIPK2) in AML1 [36]. By binding to the core-enhancer sequence, AML1/CBFβ complex functions as an organizing element recruiting other DNA-binding proteins, transcription factors, and co-regulators able to activate-or in some cases, repress-the target gene's transcription. Heterodimerization with core-binding factor-β (CBFβ) confers enhanced DNA binding ability, mediated by the Runt domain in AML1. The presence of CBFβ subunit increases the affinity for DNA and, consistent with predictions, shows a significant enhancement (>40-fold enhancement) of Runt domain DNA binding of full-length AML1 (**Figure 1**) [37]. *RUNX1* and *CBFB* are frequent targets of gene rearrangements through chromosomal translocations and mutations that are associated with human leukemias. *RUNX1* is involved in t(8;21)(q22;q22) and t(12;21)(p13;q22) in acute myeloid and lymphocytic leukemias, and *CBFB* is rearranged in acute myeloid leukemias by inv(16)(p13;q22), t(16;16), and del(16)(q22). These cytogenetic alterations lead to the expression of fusion proteins that disrupt the heterodimeric CBF complex signaling with a dominant prevalence.

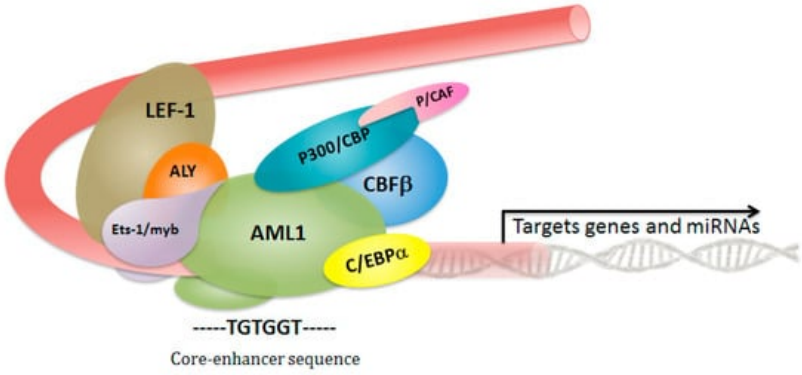

Figure 1. Schematic representation of the core binding factor transcriptional activation complex. DNA binding and heterodimerization with core binding factor-β (CBFβ) are mediated by the Runt domain in Acute myeloid leukemia1 (AML1 or RUNX1). The interaction with CBFβ leads to the phosphorylation of AML1, which in turn induces p300 phosphorylation,

and this is mediated by homeodomain interacting kinase 2 (HIPK2) in AML1. CBP/p300 (CREB binding protein CBP and EP300); C/EBPα (CCAAT/enhancer binding protein alpha); P/CAF (P300/CBP-associated factor); AML1 (acute myeloid leukemia 1 protein); CBFβ (core binding factor subunit beta); LEF1 (lymphoid enhancer-binding factor 1); ALY (Aly/REF export factor); ETS-1 (v-ets erythroblastosis virus E26 oncogene homolog 11).

3. The Genomic Landscape of Core-Binding Factor Acute Myeloid Leukemias

Current treatment guidelines for CBFL with t(8;21) do not take into account heterogeneity in these patients, and thus, all CBFL patients generally receive the same induction and consolidation treatments. Many comprehensive genetic analyses recognize that combination of several genetic alterations is associated with the development of CBFL, and is necessary for a better risk stratification in this leukemia. Although the spectrum of mutations for both CBFL subtypes is similar to the reported signature for AML [38], gene expression and mutation profiling of CBFL identified t(8;21) and inv(16) patients as two distinct subgroups [39], reflecting alternative signals activated in each subtype of CBFL [40]. Moreover, 35% of CBFL patients have two or more mutations in tyrosine kinase (TK) genes coding for pathway effectors (especially *KIT*, *FLT3*, and *RAS* genes); these findings highlight the multiclonality of CBFL. *NRAS* is the most frequently mutated gene in CBFL, more common in *CBFB–MYH11* with a different spectrum of mutations, yet its mutations are not associated with outcome. *KIT* mutations are found in ~40% of CBFL with t(8;21) and ~33% with inv(16); additionally, an enrichment of exon 17 *KIT* mutations has been documented in *RUNX1–RUNX1T1* patients, and are associated with worse outcome [41][42][43][44]. Recent large study created an “International CBF group index for t(8;21)” and validated a new risk scoring system, showing that older age, higher WBC index at diagnosis [45], and KIT D816V/Y mutations were risk factors associated with treatment failure (relapse or death) [46]. These studies strongly support the adverse effect of a *KIT* mutation in the context of CBFL. In addition, a novel finding indicates that pseudodiploidy was also a risk factor in t(8;21). High-risk score patients may benefit from more intensive approaches in the first complete remission (CR1) [46]. Mutations affecting *FLT3*–ITD are present in only 3% of inv(16) AML, whereas they occur in 10% of t(8;21) leukemia patients. In addition to mutations in genes involving TK signaling, alterations in *MGA* (MAX dimerization protein), a negative regulator of MYC signaling, were also recurrently identified in CBFL [47]. Recent results identified CCND2 (cyclin D2) expression as key transmitter of *RUNX–RUNX1T1*-driven AML, promoting cell cycle progression with the cooperation of the transcription factor Activator protein 1 (AP-1), and suggesting new potentially targetable complexes in CBFL [14][48]. Loss-of-function mutations in genes that regulate chromatin-modifying genes (*ASXL1/2*, *EZH2*, *KDM6A*, *BCOR/BCORL1*, *EED*, *SETD2*, *KMT2D*, *KMT2C,* and *CREBBP*) or in genes implicated in the cohesin complex (*RAD21*, *SMC1A*, *SMC3*, *STAG2*) were observed almost exclusively in *RUNX1–RUNX1T1* AML. Cohesin mutations led to a state of increased chromatin accessibility of binding sites for master hematopoietic transcription factors such as AML1 [49]. These findings suggest links between cohesin-mediated alterations in chromatin structure, or chromatin modifiers mutations, and cooperativity with the AML1–ETO fusion oncoprotein, even if cohesin mutations concerned less than 10% of CBFL [15]. CBFL patients with mutations in the above members of the complex, responsible for sister chromatid cohesion during mitosis and DNA repair, lack evidence of aneuploidy or an increase rate of genetic instability without any effect on the outcome. Recently, mutations in *ASXL1* (additional sex combs like 1), *ASXL2* (additional sex combs like 2), *ZBTB7A* (zinc finger and BTB domain conteining 7A), *CCND2*, and *DHX15* (DEAH-box helicase 15) have been frequently identified in *RUNX1–RUNX1T1* but not in *CBFB–MYH11* AML patients [14][50]. *ASXL1* or *ASXL2* truncating mutations, which inhibit myeloid differentiation and induce a myelodysplastic syndrome-like disease in mice [51][52], have been described in ~35% of t(8;21) while are absent in inv(16) AML [15][53][54]. Of interest, chromatin modifier *ASXL1,* as well as cohesin gene mutations, are co-occurring alterations significantly enriched in patients with mutated *RUNX1* AML [55][56]. The nature of cooperating mutations associated with t(8;21)-mediated leukemogenesis is evidenced by additional cytogenetic abnormalities such as trisomy 8 and 4, chromosome 9 deletion, and loss of one of the sex chromosomes [57][58][59]. Increased dosage of the mutated *KIT* (mapped at 4q12) can occur due to trisomy 4, leading to duplication of the mutant *KIT* allele, and suggesting an additional contribution to leukemogenesis [60]. These observations are supported by a higher dosage of N822K *KIT* mutated allele linked to an increased segregation of minichromosomes derived from chromosome 4 that preserve the pericentromeric region containing the *KIT* gene in the t(8;21) positive Kasumi-1 cell line [61]. The most common additional cytogenetic features associated with t(8;21) include loss of either the X or Y chromosome in a disproportionally large number of cases (50–60%), and del(9)(q22) in 15–25% of patients. It has been proposed that haploinsufficiency must be occurring at genes located within shared sequences in the pseudoautosomal regions (PARs) on the X and Y chromosome. A critical event potentially explaining the high incidence of loss of sex chromosomes in t(8;21) may be the loss of *CSF2RA* (colony stimulating factor 2 receptor alpha subunit) gene, encoding for the α subunit of the heterodimeric receptor CSF2 (colony-stimulating factor 2), which control granulopoiesis [62]. However, given that the whole sex chromosome is typically missing and not only the individual *CSF2RA* locus, it is likely that additional haploinsufficient factors on the sex chromosome are

acting to enhance *RUNX1–RUNX1T1*-associated leukemogenesis [63]. Sex chromosome loss was reported as a favorable marker for two-year event-free survival (66.9% vs. 43.0%), and in another study showed a modestly favorable, but not significant, effect on disease-free survival (DFS) [46]. Moreover, this last study found that patients with pseudodiploid karyotypes had worse outcome compared with those with hypodiploidy or hyperdiploidy [46]. In contrast, loss of the Y chromosome showed shorter disease-free survival (DFS) for male patients [64].

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