

Regulation of the C/EBP α signaling pathway in acute myeloid leukemia (Review)

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Abstract. The transcription factor CCAAT/enhancer binding protein α (C/EBP α), as a critical regulator of myeloid development, directs granulocyte and monocyte differentiation. Various mechanisms have been identified to explain how C/EBP α functions in patients with acute myeloid leukemia (AML). C/EBP α expression is suppressed as a result of common leukemia-associated genetic and epigenetic alterations such as AML1-ETO, RAR α -PLZF or gene promoter methylation. Recent data have shown that ubiquitination modification also contributes to its downregulation. In addition, 10-15% of patients with AML in an intermediate cytogenetic risk subgroup were characterized by mutations of the C/EBP α gene. As a transcription factor, C/EBP α can translocate into the nucleus and further regulate a variety of genes directly or indirectly, which are all key factors for cell differentiation. This review summarizes recent reports concerning the dysregulation of C/EBP α expression at various levels in human AML. The currently available data are persuasive evidence suggesting that impaired abnormal C/EBP α expression contributes to the development of AML, and restoration of C/EBP α expression as well as its function represents a promising target for novel therapeutic strategies in AML.

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1. Introduction

Acute myeloid leukemia (AML) is characterized by uncontrolled proliferation of myeloid progenitors that exhibit a severe block in their ability to differentiate into mature granulocytes or macrophages (1). The transcription factor CCAAT/enhancer binding protein α (C/EBP α) is a lineage-specific transcription factor in the hematopoietic system and is required for the formation of committed myeloid progenitors from multipotent precursor cells by coupling the direct transcriptional activation of myeloid-specific genes with the arrest of cell proliferation (2).

C/EBP α is specifically expressed in granulocytes, monocytes and eosinophils (3), although it is also found in hepatocytes, adipocytes and type II pneumocytes (4). Previous studies have illustrated the function of C/EBP α in hematopoiesis by promoting granulocyte and monocyte differentiation (2,5,6). Studies have reported that C/EBP α expression is detectable at a low level in the hematopoietic stem cell (HSC) population, and its expression increases as these cells develop into the common myeloid progenitor (CMP) and subsequently the granulocyte-monocyte progenitor (GMP), while conditional C/EBP α deficiency in adult mice blocked the transition from CMP to GMP, resulting in reduced formation of both granulocytes and monocytes (7). Non-conditional targeted disruption of C/EBP α was found to result in a selective block in early granulocyte maturation, and these mice died at birth due to severe hypoglycemia (8). Moreover, knock-in mice with a targeted mutation in the C/EBP α basic region, which led to its dysfunction, predisposed the mice to a myeloproliferative disorder (9). However, when expressed in 32Dcl3

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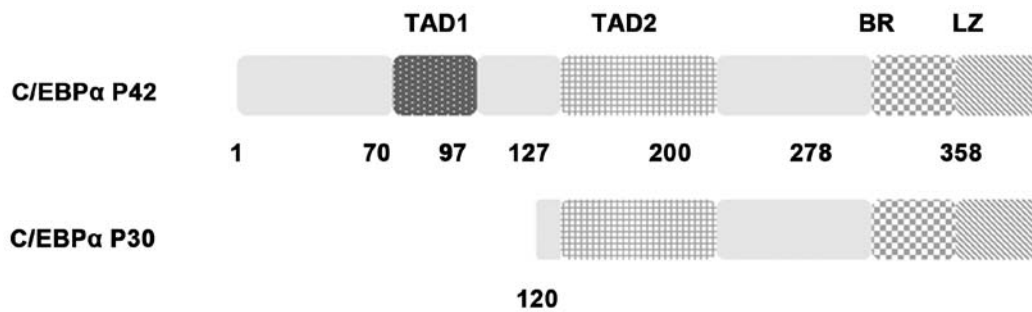


Figure 1. Structure of the two isoforms of C/EBP α . C/EBP α , CCAAT/enhancer binding protein α .

cells, representative of granulocytic progenitors, exogenous C/EBP α promoted granulopoiesis (10). These studies suggest that C/EBP α is a critical regulator of myeloid development. In fact, growing evidence suggests that the function of C/EBP α is critically altered in subsets of AML patients based on various factors.

In this review, we summarized the fundamental role of C/EBP α in myeloid differentiation and the recently identified mechanisms of its activity.

2. Function of C/EBP α in myeloid differentiation

C/EBP α is a member of the basic leucine zipper (bZIP) transcription factor family, of which several members are also expressed in the myeloid lineage (e.g., C/EBP β and C/EBP ϵ) (2). In mammals, C/EBP α is a lineage-specific transcription factor that is required for the formation of committed myeloid progenitors from multipotent precursor cells. The C/EBP α molecule contains transactivation domains (TADs) at its N-terminus and a DNA-binding and dimerization bZIP structure at its C-terminus. Furthermore, C/EBP α is an intronless gene whose mRNA can be translated from two different AUG codons giving rise to two distinct isoforms (p42 and p30). p30 lacks two N-terminal transactivation domains that are only present on p42 (11) (Fig. 1). Unless otherwise indicated, C/EBP α represents the p42 isoform in the present review.

To date, numerous studies have been reported regarding the function of C/EBP α in AML. Firstly, genomic mutations have been detected in the C/EBP α gene in ~5-14% of AML patients (12,13). Among these mutations, N-terminal frame-shift mutations prematurely truncate the full-length p42 form while preserving the p30 form, with the latter inhibiting the remaining wild-type C/EBP α p42 protein in a dominant-negative manner (12). In addition, C-terminal in-frame insertions or deletions were found to disrupt the basic zipper region, thus critically affecting DNA binding (12,14), which was further illustrated by reports that the critical basic region residues of the C/EBP α protein-DNA interaction were identified by an X-ray structure assay (14). Although there is a predominant C/EBP α mutation pattern in AML patients, the majority of AML patients always have more than one C/EBP α mutation (13,15). In addition, reduced expression could disrupt the function of C/EBP α for normal hematopoiesis (16). A detailed study showed that deletion of the C/EBP α gene led to arrest at the CMP to GMP transition, thereby causing reduced formation of both granulocytes and monocytes (7). In addition,

studies have reported that C/EBP α heterodimerizes with AP-1 proteins (C/EBP α :c-Jun or C/EBP α :c-Fos) for preference in directing monoopoiesis (17), whereas C/EBP α homodimers cooperate with NF- κ B p50 to promote granulopoiesis (18). Regulation of macrophage and neutrophil cell fates may also be altered by the relative PU.1:C/EBP α ratio (19).

3. Regulation of the C/EBP α signaling pathway

As a critical factor involved in myeloid differentiation, the function of C/EBP α must be tightly regulated to maintain the differentiation homeostasis. Detailed study of the regulation of C/EBP α could also highlight the understanding of the pathological mechanisms of AML.

Regulators targeting C/EBP α in myeloid differentiation and leukemogenesis. Many researches have focused on the factors regulating C/EBP α expression and its functions in leukemic diseases from different aspects, such as transcriptional repression by fusion genes, ubiquitination modification and epigenetic regulation.

Among the variant translocations in AML, the t(11:17) translocation is the most frequent, and renders resistance to all-*trans* retinoic acid (ATRA) treatment (20). After translocation, RAR α is fused to PLZF to produce two fusion proteins, promyelocytic leukemia zinc finger-retinoic acid receptor α (PLZF-RAR α) and RAR α -PLZF, both of which participate in leukemia development (21). Among them, RAR α -PLZF recruits HDAC1 and causes histone H3 deacetylation at C/EBP α target loci, thereby decreasing the expression of C/EBP α (Fig. 2). In line with this result, HDAC inhibitors were found to restore C/EBP α expression to a modest extent (22). In addition to RAR α -PLZF, C/EBP α expression could also be downregulated through direct transcriptional repression by the fusion oncoprotein AML1-ETO (23). These findings provide molecular evidence for a mechanism through which fusion proteins act as modifier oncogenes that subvert differentiation in the granulocytic lineage by inhibiting the activity of C/EBP α .

Epigenetic modification is envisioned as an important epigenetic mechanism that regulates the expression of myeloid-specific genes in the hematopoietic system during leukemogenesis (24). Hypermethylation of the C/EBP α promoter was first reported preferentially in AML-M2 patients (25). The methylation status of the C/EBP α gene in chronic myeloid leukemia (CML) patients was also investigated, and the data suggested that

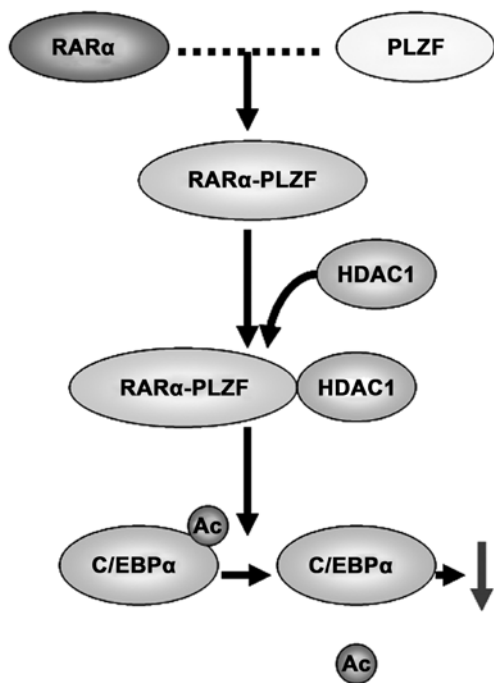


Figure 2. PLZF-RAR α negatively regulates C/EBP α expression. The fusion protein, PLZF-RAR α , recruits HDAC1 and causes histone H3 deacetylation at the C/EBP α promoter, which further decreases the expression of C/EBP α . PLZF-RAR α , promyelocytic leukemia zinc finger-retinoic acid receptor α ; C/EBP α , CCAAT/enhancer binding protein α .

aberrant methylation in the CpG island of the C/EBP α gene promoter could be a common event in CML (26). The role of EZH2 in hematopoietic development and leukemia is still controversial. Some groups suggest that EZH2 acts as a tumor suppressor in the myeloid lineage (27). However, it was also reported that ectopic expression of EZH2 causes a block in myeloid differentiation (28). A recent study found that EZH2 functions as an oncogene to block differentiation through suppressing C/EBP α expression. Notably, C/EBP α was found to be downregulated by EZH2 through methylation modification of its promoter in MA9-induced leukemia (29). Consistent with the above data, HDAC inhibitors restore C/EBP α target gene expression (22). A highly significant association was found between the frequency of C/EBP α gene epigenic modification and myeloid leukemia, while the role of C/EBP α methylation/deacetylation in the development, progression and prognosis in myeloid leukemia warrants further research.

Ubiquitination is an essential posttranslational modification for the modulation of C/EBP α activity. E3 ligases specifically targeting lysine 48 (K48)-linked ubiquitination of C/EBP α could promote the degradation of C/EBP α through the proteasome and thus terminate the downstream signaling transduction. Trib1 and Trib2 are two members of the Tribbles family that function as adapters to recruit E3 ubiquitin ligases and enhance ubiquitylation of its target protein. Consequently, Trib1 and Trib2 induce C/EBP α degradation and inhibit its function (30). E3 ubiquitin ligases, constitutively photomorphogenic 1 (COP1) (31) and E6-associated protein (E6AP) (32), were found to promote the degradation of C/EBP α by promoting its K48-linked polyubiquitination, thereby blocking myeloid differentiation of hematopoietic cells

for tumorigenesis. Notably, during this process, COP1, which contains a COP1-binding motif, is recruited by Trib1 and is essential for downregulation of C/EBP α expression (31) (Fig. 3). However, whether any deubiquitinating enzymes exist to specifically remove K48-linked ubiquitination of C/EBP α and stabilize its expression or whether E3 ligases exist to induce K63-linked polyubiquitination to active C/EBP α function warrant further investigation. The answer to this question may help to elucidate the complexities of modulation of C/EBP α activity through ubiquitination/deubiquitination.

Downstream regulators targeting C/EBP α in myeloid differentiation and leukemogenesis. In addition to its important functions as a key target gene, several studies have demonstrated that C/EBP α may participate in leukemogenesis through regulation of a number of genes directly or indirectly.

To date, several genes have been identified as being directly regulated by C/EBP α in myeloid leukemia; for example, Sox4, which is critical for normal differentiation and expansion of the lymphoid and myeloid lineages (33,34). In normal hematopoiesis, C/EBP α expression was found to be increased over the course of lineage commitment and then suppressed SOX4 expression through binding to its promoter (35). Importantly, leukemic transformation by C/EBP α mutation was partially reversed by Sox4 knockdown (36).

FMS-like tyrosine kinase-3 (FLT3) is a membrane-bound tyrosine kinase receptor. The interaction between the receptor FLT3 and its ligand FL led to crucial signaling during the early stages of the commitment of hematopoietic stem cells (37). Mutation or overexpression of the FLT3 gene enhanced the survival and expansion in a variety of leukemias and was associated with an unfavorable clinical outcome for AML patients (38). Kindler *et al* demonstrated the binding of C/EBP α in human AML on the FLT3 locus, and defined FLT3 as a direct downstream effector of C/EBP α . Furthermore, they demonstrated that bi-allelic C/EBP α mutations may reduce FLT3-mediated leukemogenic signals (37), which suggests that regulation of Flt3 expression could depend on strict C/EBP α activity thresholds in AML.

In addition, glycolytic enzyme hexokinase 3 (HK3) has been defined as a glycolytic enzyme most frequently expressed in myeloid cells and represents the dominant hexokinase in granulocytes accounting for most hexokinase activity (39). By comparing gene profiles in two cohorts of C/EBP α wild-type and mutant AML patients, C/EBP α was found to directly regulate HK3 by binding to its promoter (40). Furthermore, activation of HK3 transcription was found to be dependent on C/EBP α during all-*trans* retinoic acid (ATRA)-mediated neutrophil differentiation of APL cells (41).

As a leukocyte-specific gene, CORO1A has been linked to the inhibition of neutrophil apoptosis, with significantly lower CORO1A mRNA expression in C/EBP α -mutated AML (42,43). Recently, C/EBP α was also demonstrated as a direct transcriptional regulator of CORO1A in APL and C/EBP α -mutated AML patients (44).

In addition to the above-mentioned downstream effectors of C/EBP α -p42, there are still several potential genes regulated by C/EBP α -p30, such as PIN1 and Trib2. PIN1 appears to be important in tumorigenesis since it was found to be overexpressed in many types of cancers (45,46).

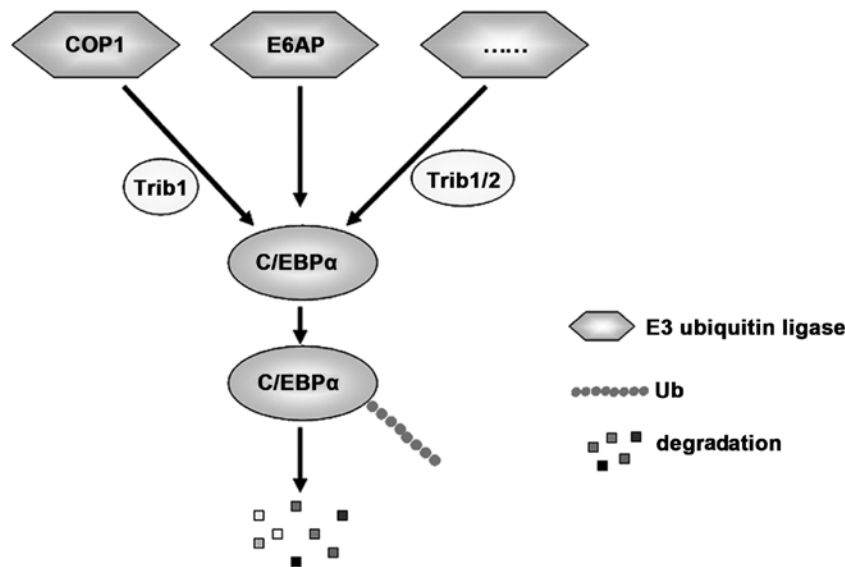


Figure 3. Regulation of C/EBP α by ubiquitin. E3 ubiquitin ligases, such as COP1 and E6AP, mediate C/EBP α proteasomal degradation, which further blocks myeloid differentiation. During this process, E3 ubiquitin ligases are recruited by Trib1 and Trib2. C/EBP α , CCAAT/enhancer binding protein α ; COP1, constitutively photomorphogenic 1; E6AP, E6-associated protein.

Evidence for the role of PIN1 in leukemia includes the fact that it inhibits the ubiquitination of c-Jun, which further blocks granulocyte differentiation (46,47). PIN1 was detected as a target of C/EBP α -p30 in AML, as C/EBP α -p30 recruited the transcription factor E2F1 in the PIN1 promoter to elevate its expression (48). In addition to the role of Trib2 acting as an upstream effector of C/EBP α by mediating its proteasomal degradation, recent research also revealed that Trib2 can also form a feedback regulatory loop with C/EBP α . In normal myeloid progenitor cells, C/EBP α -p42 was found to bind to the Trib2 promoter and inhibit Trib2 activation. Conversely, C/EBP α -p30 activated the Trib2 promoter in preleukemic cells resulting in elevated Trib2 expression, ultimately contributing to the degradation of C/EBP α -p42 and uncontrolled proliferation in AML (49). That is, the exact role of Trib2 depends on the activity of C/EBP α -p30 or C/EBP α -p42 in its specific context. However, the mechanism of the switch between these two isoforms (C/EBP α -p30 or C/EBP α -p42) is still unknown.

In addition to binding to the promoter of several genes to participate directly in leukemogenesis, C/EBP α also indirectly regulates certain genes over the course. For example, transcription factor krüppel-like factor 5 (KLF5), an essential factor for granulocytic differentiation, was found to have a low level in AML (41,50). A study reported that KLF5 is indirectly regulated by C/EBP α , with its activation dependent on C/EBP α during ATRA-mediated neutrophil differentiation in APL cells (41).

DAPK2 is a proapoptotic protein that is mainly expressed in hematopoietic tissue. In addition to participating in different cell death pathways (51,52), Rizzi *et al* (53) and Fang *et al* (54) found a specific function for DAPK2 as an enhancer of neutrophil and erythroid differentiation. Other studies further confirmed that DAPK2 in myeloid cells is dependent on C/EBP α during granulocytic differentiation and this process seems to be indirect (55). Moreover, C/EBP α interferes with E2F1 transactivation of the c-Myc promoter in AML (56),

which may influence proliferation and differentiation in HL-60 cells through VEGF (57) (Fig. 4).

A number of new molecular genetic abnormalities have been identified in AML in the last few decade. Further studies are needed to analyze the interrelation between them involving C/EBP α , and a pivotal co-target gene would bear clinical significance.

Collaborating factors with C/EBP α . As mentioned earlier, C/EBP α -p30 directly activates Trib2 (49) and PIN1 (48) expression, by cooperating with E2F1 in AML. In addition, C/EBP α cooperates with several other proteins in the myeloid lineage, such as Hoxa9/Meis1. Homeobox A9 (HOXA9) is a homeodomain-containing transcription factor that plays a key role in HSC expansion and is commonly deregulated in human acute leukemias (58). Overexpression of HOXA9 always exists along with its cofactor meis homeobox 1 (MEIS1) in the pattern of Hoxa9/Meis1 in AML. Recent studies suggest that C/EBP α acts as a pioneer transcription factor in Hoxa9/Meis1-mediated leukemogenesis through regulating its target genes, *Cdkn2a/b* (59) and *Sox4* (60).

Previous studies have also demonstrated that C/EBP α interacts with its different binding partners, including TBP and TFIIB (basal transcription initiation factors) (61), the SWI/SNF complexes (chromatin remodeling complexes) (62), Rb (tumor-suppressor protein) (63), Cdk2 and Cdk4 (cyclin-dependent kinases), p21 (cyclin-dependent kinase inhibitor) (64), GABP α (cell cycle regulator and transcription factor) (65). All of these may play a crucial role during the process of leukemogenesis.

Small molecules targeting C/EBP α . In search for small molecules that are able to reverse the low expression of the C/EBP α signature, a connectivity map was applied. This analysis predicted positive connectivity between the C/EBP α activation signature and histone deacetylase inhibitors. The results showed that histone deacetylase inhibitors reactivated the

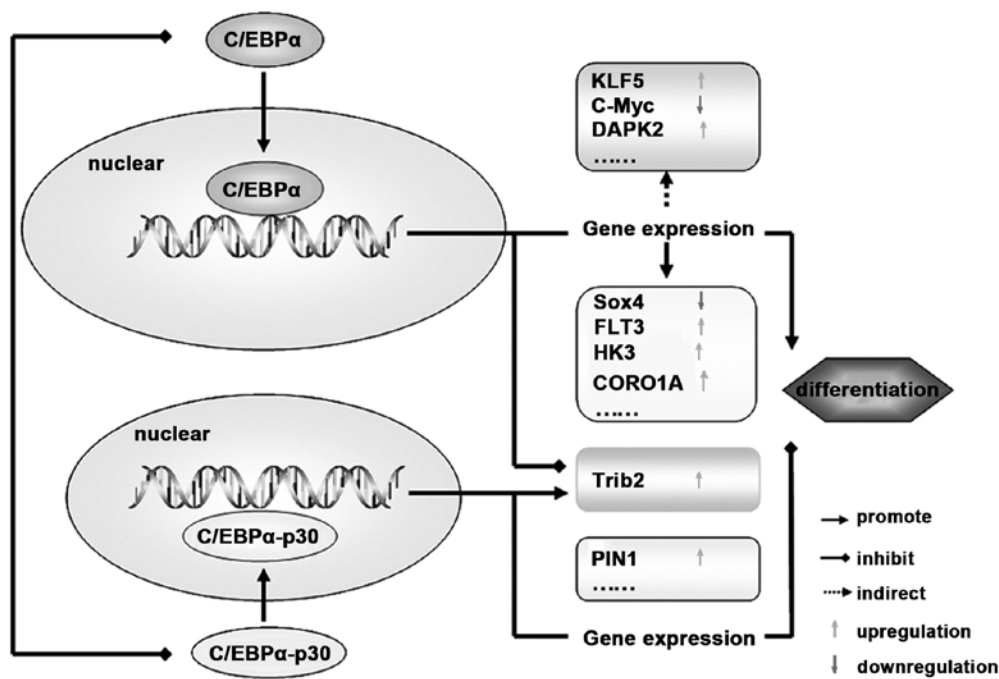


Figure 4. C/EBP α functions through regulation of its target genes. After its translocation into the nucleus, C/EBP α -p42 promotes cell differentiation by regulating the gene expression directly (such as Sox4, FLT3, HK3 and CORO1A) or indirectly (such as KLF5, DAPK2 and C-myc). In addition, C/EBP α -p30 isoform blocks cell differentiation by targeting PIN1 and Trib2. Notably, Trib2 can be regulated by both C/EBP α -p42 and C/EBP α -p30 isoforms, and the switch mechanisms between them need further studies. C/EBP α , CCAAT/enhancer binding protein α ; FLT3, FMS-like tyrosine kinase-3; HK3, hexokinase 3; KLF5, krüppel-like factor 5.

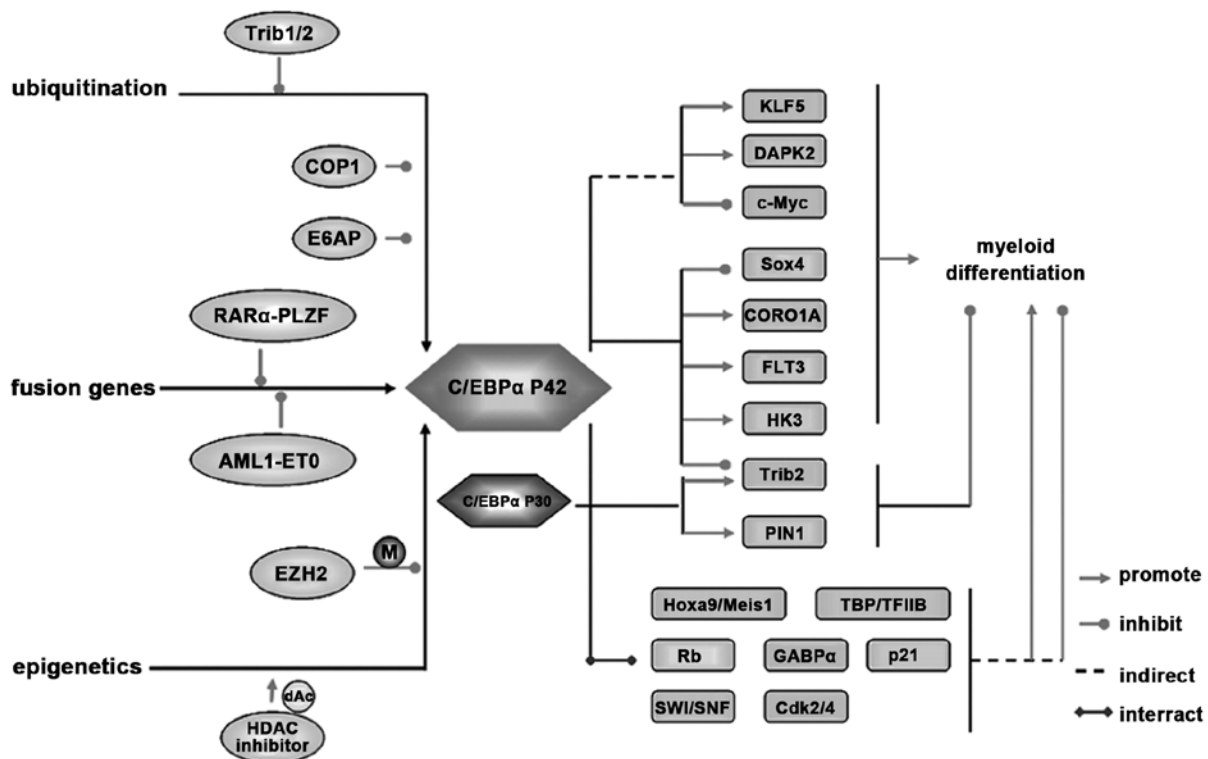


Figure 5. Schematic representation of the mechanism of C/EBP α functions during myeloid differentiation. C/EBP α , CCAAT/enhancer binding protein α .

expression of the C/EBP α signature and promoted granulocytic differentiation of primary samples from the C/EBP α dysfunctional subset harboring biallelic C/EBP α mutations (66), which indicated that HDAC inhibitors could represent a promising

therapeutic approach in this particular subtype of AML (67,68). Cytarabine (or Ara-C) is a pyrimidine antagonist, which interferes with DNA synthesis and is used in upfront and salvage regimens for AML (69). To improve the cytotoxic activity of

Ara-C treatment, various novel drug combinations have been explored (70,71). Recently, it was demonstrated that miR-181a could sensitize a chemotherapy-resistant HL60 cell line to Ara-C treatment (72). Zhao *et al* found that the C/EBP α -p30 isoform could bind to the miR-181a-1 promoter to upregulate its expression. Furthermore, lenalidomide, a drug approved for myelodysplastic syndromes and multiple myeloma, sensitized leukemic cells to cytarabine (Ara-C) chemotherapy by enhancing translation of the C/EBP α -p30 isoform as well as miR-181a levels (73). Additionally, Ko *et al* found that the methylation status of let-7a-3 was inversely correlated with the methylation status in the distal promoter region of C/EBP α in AML (74).

Therefore, it may be promising to design various small molecules targeting the C/EBP α signaling pathway for the treatment of AML.

4. Conclusion

In this review, we summarized the regulatory mechanisms and the functional targets of C/EBP α in AML (Fig. 5). A more detailed molecular analysis of C/EBP α will ultimately highlight a number of new oncogenes that may supplement the prognostic information obtained by conventional karyotyping. Furthermore, targeted therapies should interfere with C/EBP α initiating or cooperating proteins, to improve the treatment of AML. Moreover, as growing evidence implicates aberrant C/EBP α activity in a variety of diseases, including solid tumors and rheumatoid arthritis, small molecular compounds specific for C/EBP α may provide potential strategies for the therapeutic intervention of a variety of diseases.

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