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REVIEW ARTICLE

Super enhancers: Pathogenic roles and potential therapeutic targets for acute myeloid leukemia (AML)

Ziyang Cao [a,b], Yi Shu [c], Jinxia Wang [a,b], Chunxia Wang [a,b], Tienan Feng [a,d], Li Yang [e], Jingbo Shao [f,**], Lin Zou [a,b,c,*]

[a] *Clinical Research Unit, Shanghai Children's Hospital, Shanghai Jiao Tong University, Shanghai 200062, PR China*
[b] *Institute of Pediatric Infection, Immunity, Critical Care Medicine, Shanghai Jiao Tong University School of Medicine, Shanghai 200062, PR China*
[c] *Center for Clinical Molecular Laboratory Medicine of Children's Hospital of Chongqing Medical University, Chongqing 400014, PR China*
[d] *Clinical Research Institute, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, PR China*
[e] *Department of Hematology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, PR China*
[f] *Department of Hematology/Oncology, Shanghai Children's Hospital, Shanghai Jiao Tong University, Shanghai 200062, PR China*

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Abstract Acute myeloid leukemia (AML) is a malignant hematological tumor with disordered oncogenes/tumor suppressor genes and limited treatments. The potent anti-cancer effects of bromodomain and extra-terminal domain (BET) inhibitors, targeting the key component of super enhancers, in early clinical trials on AML patients, implies the critical role of super enhancers in AML. Here, we review the concept and characteristic of super enhancer, and then summarize the current researches about super enhancers in AML pathogenesis, diagnosis and classification, followed by illustrate the potential super enhancer-related targets and drugs, and propose the future directions of super enhancers in AML. This information provides integrated insight into the roles of super enhancers in this disease.

* Corresponding author. Clinical Research Unit, Shanghai Children's Hospital, Shanghai Jiao Tong University, Shanghai 200062, PR China.
** Corresponding author.
E-mail addresses: shaojb@shchildren.com.cn (J. Shao), zoulin@shchildren.com.cn, zoulin74@126.com (L. Zou).
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Introduction

Acute myeloid leukemia (AML) is a highly heterogeneous disease, derived from the malignant clonal proliferation of abnormally differentiated myeloid lineage cells in the hematopoietic system.[1] Accumulating evidence has shown that the molecular characteristics play critical roles in the pathogenesis, classification, and treatment of AML.

It is believed that AML is involved in the oncogenic transformation of haemopoietic stem cells (HSCs), and that cytogenetic abnormalities form the basis of leukemogenesis.[2] DNA methyltransferase 3 alpha (*DNMT3A*) mutations frequently occur in the early stage of AML evolution. One study indicated that *DNMT3A* mutations led to the generation of a clonally expanded pool of HSCs, and further initiated AML leukemogenesis.[3] Recently, whole-exome sequencing and RNA sequencing analysis suggested that several chromosomal structural abnormalities, such as t (8; 21)/RUNX1-RUNX1T1 rearrangements, DNA alterations like isocitrate dehydrogenase 1/2 (*IDH1/2*) mutations, and transcriptional regulatory aberrations leading by the transcriptional activation of oncogenes, may be responsible for the pathogenesis of AML.[4–6]

The diagnosis of AML is based on the morphological assessment of blasts in the bone marrow or peripheral blood, combined with studies of the patient's clinical manifestations, cytogenetics, immunophenotyping, and molecular biology.[7] Previously, the classification system of French-American-British (FAB) was used to divide AML into eight subtypes (M0–M7).[8] At present, the most commonly used classification system is based on a WHO guideline, which identifies six subgroups of AML.[9] Since genetic and molecular abnormalities in AML cells are strong prognostic factors for AML patients, routine cytogenetic tests have also been suggested by the guideline, and have been widely applied to classify the recurrence risk and prognosis for AML for the past 20 years.[10] AML is further classified into three different groups based on the presence of cytogenetic abnormalities and specific molecular characteristics: a favorable group, intermediate group, and adverse group.[8,11]

Although great progress has been made in understanding the pathogenesis, classification, genomic structure, and prognosis of AML, most AML patients still have limited treatment options, mainly depending on traditional standard chemotherapies.[12] The treatments for AML, except acute promyelocytic leukemia (APL, M3), generally include induction therapy, consolidation therapy, and continuation therapy.[9] Recently, targeted therapies and immunotherapies have been explored and developed, including combinations of epigenetic therapy with hypomethylating agents, alone or combination with BCL-2 inhibitors or agents targeting IDH1/2 enzymes, fms-like tyrosine 3 (*FLT3*) mutations, *TP53* mutations, mixed-lineage leukemia (*MLL1*) rearrangement, or with checkpoint inhibitors such as PD1/PD-L1 antibodies, or chimeric antigen receptor (CAR)-T-cell approaches.[1]

Notably, bromodomain and extra-terminal domain (BET) inhibitors, targeting the key component of super enhancers, the bromodomain containing protein (BRD) family, have shown potent anti-cancer effects in early clinical trials in AML patients.[13] In addition, super enhancers have also been shown to play a crucial role in the tumorigenesis of AML, and are being used to identify the disease. In this review, we briefly summarize the characteristics of super enhancers, discuss their roles in the diagnosis/classification/treatment of AML, and explore the possibility of anchoring super enhancers as a novel targeted therapy for this disease.

Super enhancers

The enhancer elements were first defined in the 1980s during studies of the role of SV40 DNA in the transcription enhancement of the rabbit beta-globin gene.[14] Since then, numerous studies have verified enhancers to be a widespread regulatory element that controls the regulation of specific gene expression in various biological processes.[15–17] Besides typical enhancers, non-typical enhancers including super enhancers, stretch enhancers, shadow enhancers, and locus control regions (LCRs) have been reported.[18] In 2013, Young et al identified an exceptionally large enhancer domain associated with gene encoding which they defined as a super enhancer in murine embryonic stem cells (ESCs).[19] After that, extensive studies based on chromatin immunoprecipitation sequencing (ChIP-Seq) and other next-generation sequencing (NGS) technologies have been conducted, and have started uncovering the biogenesis, characteristics, and roles of super enhancers in various cell types and conditions.

All enhancers are defined by the absence of transcription start site (TSS) annotation and low levels of histone H3 lysine 4 methylation (H3K4Me3). However, super enhancers have several unique characteristics: 1) the cluster of the regulatory elements is larger than typical enhancers (super enhancers: ranges from 10 kb to over 60 kb; typical enhancers: 1 kb–4 kb); 2) the number of the super enhancers in cells is fewer than that of typical enhancers (super enhancers: 100–1000, typical enhancers: >10,000); 3) there is extensive histone H3 lysine 27 acetylation (H3K27Ac) and H3K4Me1, as well as the presence of mediator coactivators, EP300 and BRD4, in super enhancers; 4) the binding of transcription factors to super enhancers is much higher than in typical enhancers; 5) the gene expression regulation of super enhancers is much stronger than that of typical enhancers; and 6) super enhancers are more sensitive to the effects of transcriptional inhibitors than typical enhancers.[20–22] Interestingly, transcriptional activators such as BRD4 and mediator complex are reported to form droplets (phase-separated condensates) at super enhancers, gather the transcriptional machine near the super enhancers, and facilitate the compartmentalization of the transcription process.[23,24]

A critical role for super enhancers in promoting oncogene expression and dysregulating signaling pathways has been reported in different types of cancers. For instance, in multiple myeloma, the mediator complex and BRD4 were disproportionately enriched in the super enhancer regions of some key regulators, including *IRF4*, *MYC*, *XBP1*, and *PRDM1/BLIMP-1*.[25] Continued activation of super enhancers in these oncogene regions can lead to tumorigenesis, making persistently activated oncogenes an attractive therapeutic target for these cancers. Second, after

screening 104 small-molecule inhibitors for effects on super enhancers, Jiang et al uncovered and identified several novel oncogenes with the activation of super enhancers in esophageal squamous cell carcinoma (OSCC). They found that P21 (RAC1)-activated kinase 4 (PAK4) was a super enhancer-associated drug target in OSCC.[26] Additionally, cancer types and subtypes have been identified based on the super enhancer landscape in neuroblastoma,[27] with different gene expression profiles identified that were regulated by two different super enhancer-associated transcription factor networks. Subgroup-specific identities have also been identified using super enhancer-associated transcription factors in other tumors, such as esophageal cancer,[28] medulloblastoma,[29] and AML.[30] Thus, super enhancers play critical roles in cancer development, identification, and as targets for therapeutics.

Considering that hematologic malignancies, including acute leukemias, can also be regulated by super enhancers through multiple pathways,[31] we next focus on the functions of super enhancers in regulating tumorigenesis, diagnosis, and therapy in AML.

Roles of super enhancers in AML leukemogenesis

One of the common molecular characteristics of AML is the presence of abnormal transcriptional regulation. Deregulation of the proto-oncogene c-Myc (*MYC*) is considered to result from a series of oncogenic events in AML.[32] In 2013, a cluster of specific super enhancers 1.7 Mb from the *MYC* transcriptional start site was identified at the *MYC* locus. This cluster of distal elements contained focal DNA duplications and was critical for the expression of *MYC*. Notably, BRG1 was required for these lineage-specific super enhancers to maintain the *MYC* oncogenic transcriptional program and promote the development of AML.[33] Moreover, genetic, genomic and biochemical approaches demonstrated the occupancy of BRD4, histone acetyltransferase p300/CBP, and AML hematopoietic transcription factors (PU.1, FLI1, MYB, etc.) at the super enhancer regions of *MYC*. This chromatin-based signaling cascade supports leukemia maintenance in AML.[34] These studies suggest that the regulation of *MYC* expression and leukemogenesis in AML involve this super enhancer.

Besides the deregulation of *MYC*, it has been reported that other super enhancers drive the expression of oncogenes leading to AML leukemogenesis. For example, by applying genomics and genome-engineering approaches in AML patients, one study found that chromosomal translocations of the inv (3)/t (3; 3) fragment in AML could cause dysregulation of GATA binding protein 2 (*GATA2*), a critical hematopoietic stemness factor, and *EVI1*, an oncogenic driver lesion, and thus conferred leukemogenesis. These chromatin rearrangements created a super enhancer for *EVI1* which results in both *EVI1* aberrant overexpression and *GATA2* haploinsufficiency.[35] Furthermore, the binding of PR/SET Domain 16 (PRDM16) to the super enhancer of *PU.1*, a major myeloid regulator, is known to activate *PU.1* expression, resulting in the oncogenic fate conversion of platelets and erythrocytes cells, and transforming those cells into myeloid leukemia stem cells (LSCs).[36] Recently, there was a report showing super enhancer regulatory circuitry of Homeobox A9 (Hoxa9) in AML. Hoxa9 is a critical transcription factor regulating leukemogenesis and Tribbles Pseudokinase 1 (Trib1) functions as a myeloid oncogene in AML.[37] Using a ChIP-seq analysis of Hoxa9, C/EBPα, and H3K27Ac, the study identified the regulation of Hoxa9-driven transcription in leukemogenesis by Trib1: C/EBPα acts as a tumor suppressor through binding and suppressing the function of Hoxa9 at a super enhancer. Trib1 could lead to the release of C/EBPα from the super-enhancer, and Hoxa9 could further initiate oncogene transcription and subsequent myeloid leukemogenesis.[37] The existence of other oncogenic super enhancers active in myeloid development (*PROM1*, *CDK6*, *HOX*, *GFI1*) has also been demonstrated, and these have been shown to be involved in the process of AML leukemogenesis.[38–40]

Therefore, AML cells have been proved to require super enhancers at oncogenes to drive the high expression of oncogenes and initiate leukemogenesis.[41,42] We provide a summary of the main super enhancer-regulatory modules in AML in Figure 1. Based on genetic changes (genomic focal amplification, translocation, etc.) and epigenetic changes (TFs, histone modifications), AML cells develop abnormalities in oncogenic super enhancers, leading to transcriptional deregulation and cancer formation during AML development.

Role of super enhancers in the diagnosis and subtyping of AML

Young's group first analyzed H3K27Ac ChIP-seq data and identified different super enhancer-associated genes in 18 human cancer cells. This research and subsequent studies implied that super enhancers could be used to identify key oncogenes in different cancers.[21] Wang et al also analyzed and reported that super enhancers only appeared in few cell types and were highly cell type-specific using the genome-wide super enhancer landscape of ENCODE (Encyclopedia of DNA Elements), suggesting that super enhancers could be used as a biomarker to classify cell types and cell states.[22]

Super enhancers of the *MYC* region have been identified in many different cancer types, including AML, small cell lung cancer (SCLC), colorectal cancer, and acute lymphoblastic leukemia. In B cell acute lymphoblastic leukemia (B-ALL), STAT5 and IKAROS could recruit the histone acetyltransferase p300 to the super enhancer locus of *MYC* and then promote H3K27Ac at this region. Chromatin hyperacetylation could increase the accessibility of the transcriptional regulatory sites and initiate *MYC* expression.[43] In the colorectal cancer (CRC) cell line HCT-116, TCF4 and H3K27Ac were present at the super enhancer of the *MYC* locus and promoted *MYC* expression.[42] In T cell acute lymphoblastic leukemia (T-ALL) patients, a broad super enhancer region located within the *MYC* transcription starting site +1.47 Mb was identified. This super enhancer could be regulated by NOTCH1.[44] In AML, BRG1 is required for the *MYC* super enhancer, and the *MYC* locus was identified as the location 1.7-Mb distant from the *MYC* transcription initiating site.[33] These results implied that, despite the fact that the super enhancers are all located at

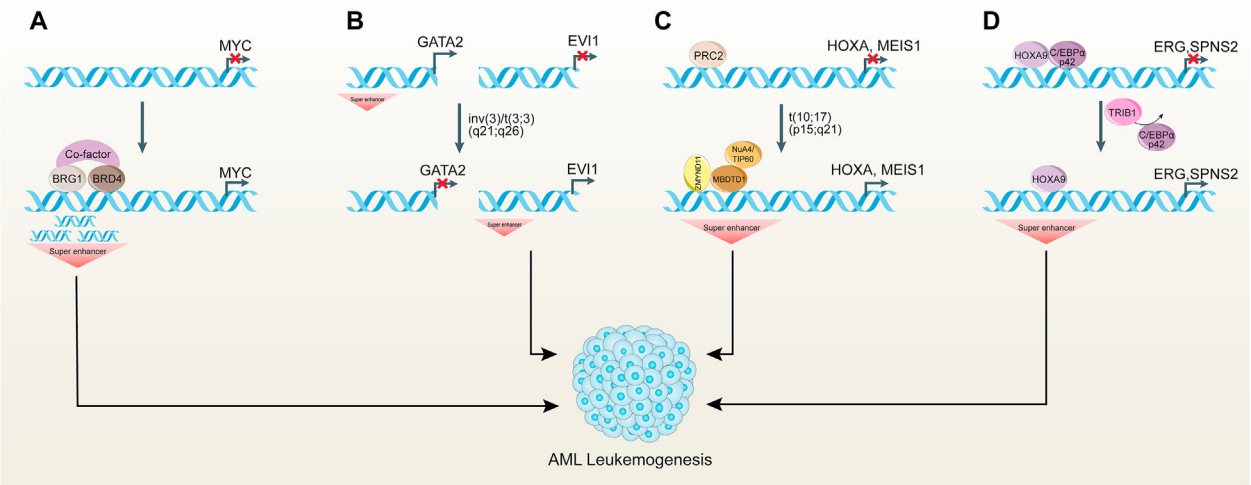

Figure 1 The main super enhancers characterized in AML. **(A)** BRG1, BRD4, and other transcriptional co-factors occupy the focal DNA duplication region of the MYC super enhancer, initiate the transcription of MYC, and promote AML leukemogenesis. **(B)** Chromosome translocation inv (3)/t (3:3) causes super enhancer reallocation of *EVI1*/*GATA2*, activates *EVI1*, and downregulates *GATA2* to initiate leukemogenesis. **(C)** Chromosome translocation t (10; 17) (p15; q21) leads to the formation of fusion gene ZMYND11-MBTD1, which recruits the histone acetyltransferase complex NuA4/Tip60 to the super enhancer of leukemia-promoting genes (*HOXA*, *MEIS1*, *MYB*, *MYC*, and *SOX4*) and results in leukemogenesis. **(D)** TRIB1 releases C/EBPα from the super enhancer to initiate HOXA9-regulated oncogene transcription (*ERG*, *SPNS2*, *RGL1*, and *PIK3CD*) and AML leukemogenesis.

the *MYC* locus, the specific cells can still be identified based on the different regulation modules of the super enhancers.

Of note, the super enhancers varied in some specific AML subtypes. For example, AML patients with the chromosomal translocation t (10; 17) (p15; q21) express an abnormal fusion gene ZMYND11-MBTD1 (ZM). ZM can recruit the histone acetyltransferase complex NuA4/Tip60 into the super enhancer of the leukemia-promoting genes (*HOXA*, *MEIS1*, *MYB*, *MYC*, and *SOX4*) and maintain high expression of these genes.[45] In AML with nucleophosmin (NPM1) mutations, NPM1 mutation increased H3K27Ac at the super enhancer of the *HOXA* and *HOXB* clusters, and increased the expression of these members.[39] AML with inv (3)/t (3; 3) was identified as having a reallocation of super enhancers, which further ectopically activate *EVI1* expression and result in functional *GATA2* haploinsufficiency.[35] APL is a subtype of AML, characterized by the chromosome translocation t (15; 17) and the fusion of the promyelocytic leukemia/retinoic acid receptor-α (PML/RARα) gene. Recently, the PML/RARα fusion gene was reported to activate *GFI1* at its super-enhancer region and maintain APL cells.[40] These findings suggest that super enhancers can be used as an additional tool for AML identification (Fig. 1).

In addition to identifying tumors, super enhancers can also be applied to classify tumors into different subgroups.[46] Using a high-throughput H3K27Ac ChIP platform, one study profiled the super enhancer landscape of 66 AML patients, and identified six subgroups of AML with distinct leukemic cell states. One subtype in this patient cohort harbored a particularly strong super enhancer at the gene locus of retinoic acid receptor alpha (RARA). This subgroup of AML is sensitive to a potent and selective RARα agonist (SY-1425).[47] This finding provides support for a new strategy for target-drug therapy: patients can be classified through the evaluation of super enhancers, and individualized therapy can be recommended based on the super enhancer expression patterns.

In conclusion, super enhancers can help better understand the diagnosis, identification, and classification of AML. In the future, super enhancers may be used as biomarkers for the differential diagnosis and prognostic risk stratification of AML.

Potential therapeutic targets related to super enhancers in AML

Super enhancers can be regulated by various enzymes (acetyltransferases, chromatin remodeling enzymes and/or methyltransferases) and nuclear factors, and thus, they exhibit potential "drug-targetable" properties.[22] Recently, studies have shown that multiple factors can be used as potential targets of tumor-targeted therapy to regulate the activity of super enhancers (Fig. 2).

BET inhibitors (BETi) are competitive inhibitors of BET family proteins, which potently inhibit super enhancer-related gene transcription.[48] Numerous studies have shown that BETi reduce tumor cell proliferation and induce cell death by downregulating the expression of multiple oncogenes. RUNX1 is a master transcription factor that regulates the expression of several genes necessary for hematopoiesis. Studies implied that the *RUNX1* gene has a highly conserved super enhancer (>170 kb), and editing this enhancer using CRISPR/Cas9 repressed *RUNX1* expression, inhibited AML cell growth, and induced cell death in AML cells. Notably, *RUNX1* expression was sharply repressed by treating *RUNX1* mutant AML cells with a BET inhibitor or degrader. This provides novel target therapeutic drugs for *RUNX1* mutant AML.[49] NSD3 is a member of the family of H3K36 methyltransferases, the short isoform of which acts as an

oncoprotein in various cancers. One study revealed that NSD3-short linked BRD4 and CHD8 chromatin remodelers to the super enhancer regions of *MYC*, where they promote the activation of *MYC* expression and maintain the growth of AML cells. Upon treatment with BETi, BRD4-NSD3-CHD8 complexes were released from super-enhancer regions and AML cell proliferation was repressed.[50] The binding of the BET family at the super enhancer loci of several critical oncogenes was also observed, implying that BETis are potent drugs for targeting super enhancers in AML.

CDKs are serine/threonine kinases required for the cell cycle and transcription initiation/elongation.[51] CDK7 is a member of the transcription factor II human (TFIIH) complex and is required for transcription machinery assembly through RNA pol II phosphorylation. Several studies have reported that THZ1, a small molecule inhibitor of CDK7, effectively deregulated super enhancer-related master oncogenic transcription in T-ALL, SCLC, neuroblastoma, and other cancers.[52–54] In AML, one study found that the inhibition of CDK9 led to the selective downregulation of super enhancer-related genes, including *MCL-1*, *MYC*, and *cyclin D1*, and reduced cell survival.[55] Minzel and colleagues developed small molecule pan-CK1α inhibitors, which co-targeted CDK7 and CDK9. In primary mouse AML cells, the CK1α inhibitor specifically reduced the expression of many super enhancer-driven oncogenes, such as *MYC*, *CXCR4*, *IKZF2*, and *CDC16*, and induced apoptosis.[56] Besides CDK7/9, CDK8 and CDK19 are proven negative regulators of super enhancers. A natural product-based drug, Cortistatin A (CA), selectively inhibits CDK8 and CDK19, and exhibited anti-tumor activity *in vitro* and *in vivo*. Mechanically, CA induced significant overexpression of super enhancer-associated tumor suppressor genes (*CEBPA*, *IRF8*, *IRF1*, and *ETV*) in CA-sensitive AML cell lines, and inhibited AML cell proliferation.[57] These observations suggested that targeting the key factors involved in tumor suppressor-related super enhancers may represent a novel therapeutic strategy for AML.

Several classes of drugs targeting enzymes that regulate histone modifications have been developed for cancer therapy, including the histone methylase EZH2 inhibitors and histone deacetylase inhibitors.[58] It is well established that these enzymes participate in super enhancer regulation. For instance, in ZMYND11-MBTD1 fusion AML, the NuA4/TIP60 complex is recruited and enriched at the super enhancer locus of many oncogenes, and it has been suggested that specific KAT5/Tip60 HAT inhibitors might be efficacious in treating ZMYND11-MBTD1-induced AML.[45]

Cytoplasmic/nuclear receptor activators or inhibitors have also been well studied. Orphan nuclear receptor NR4A1 is a functionally redundant tumor suppressor of AML. One study showed that NR4A1 directly bound the super enhancer of the *MYC* locus, removed essential coactivators for *MYC* transcription, and inhibited AML cell proliferation. The authors of that study identified a small molecule inducer of NR4As, dihydroergotamine (DHE). DHE treatment deregulated *MYC* expression through super enhancer inhibition and conferred cell death in AML.[59] Exportin 1 (XPO1) is a nucleocytoplasmic transport protein that participates in the nuclear export of NPM1c (mutated NPM1). NPM1c nuclear export by XPO1 led to super enhancer activation of target genes and maintenance of the leukemic state, while XPO1 inhibition led to the re-localization of NPM1c to the nucleus, decreased the expression of super enhancer-related genes, and prolonged the survival of NPM1-mutated leukemic cells.[39] Recently, through the use of a ChIP-SICAP technique, one study identified poly (ADP-ribose) polymerase 1 (PARP1) as a key factor involved in the

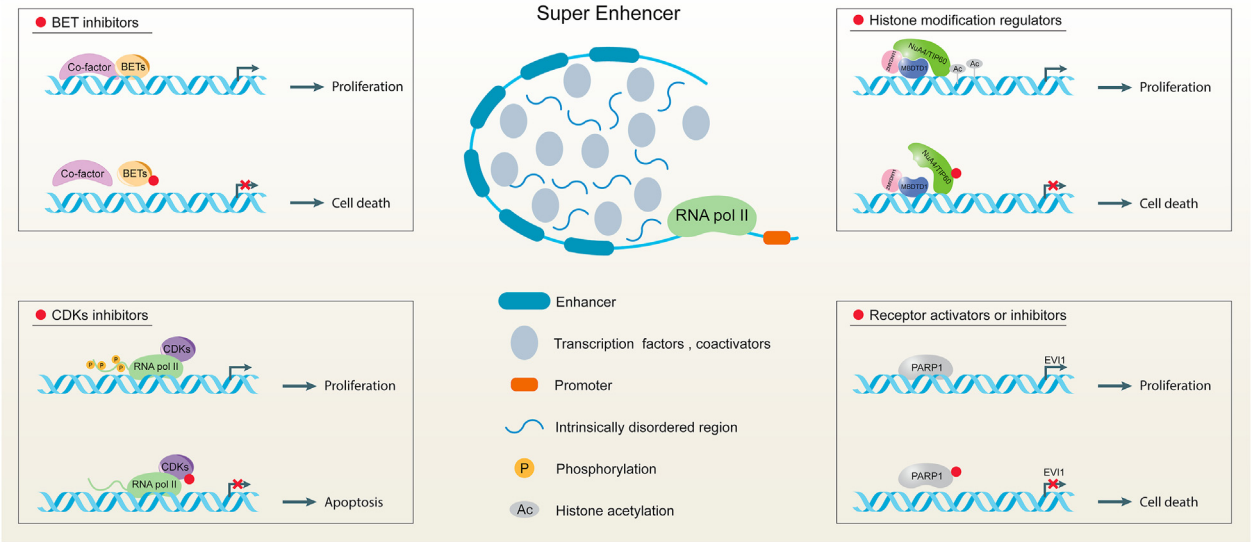

Figure 2 Potential therapeutic targets related to super enhancers. BET inhibitors bind and inhibit BET proteins at super enhancer regions, deregulating target gene expression, and leading to cell death in AML cells. CDK inhibitors, such as CDK7 inhibitors, can depress the phosphorylation of RNA pol II, repress the expression of target genes, and lead to cell apoptosis. Histone modification regulators, such as KAT5/Tip60 inhibitors, lead to histone deacetylation at super enhancer regions, downregulate target gene expression, and promote cell death in ZMYND11-MBTD1-induced AML cells. Receptor activators or inhibitors, such as PARP1 inhibitors, reduce EVI1 expression and decrease cell survival in inv (3)/t (3; 3) AML cells.

oncogenic super enhancer formation in inv (3)/t (3; 3) AML cells. Exposure to a PARP1 inhibitor (PARPi) reduced *EVI1* expression and decreased cell survival in inv (3)/t (3; 3) AML cells, which implied that PARP1 could be a potential target for further studies.[60] Moreover, in AML cells, Myb coordinated with C/EBPβ and histone acetylase p300 at the super enhancers of some myeloid-specific genes. This activation complex led to over-expression of the target genes and maintained the leukemia phenotype. Celastrol was identified as a Myb inhibitor that suppressed C/EBPβ activity and repressed AML cell proliferation in a primary mouse model.[61] All of these studies provide novel strategies that can be used to target super enhancers in AML cells. However, these studies are in the pre-clinical stage, so further studies are needed to confirm the safety and efficacy of these treatments.

Clinical trials of super enhancer-related drugs

BET inhibitors

Clinical trials of BETi drugs have made rapid progress in the last decade since the discovery of JQ1, which is a first-in-class potent and selective inhibitor of BRD4 protein.[62,63] There are or have been several phase 1/2 clinical trials of BET inhibitors for AML (Table 1). For example, a phase 1 dose-escalation study was conducted on the small molecule BET inhibitor OTX015 (MK-8628). OTX015 was given orally at increasing doses to adults with AML who had failed or had a contraindication to standard therapies. Among all the 36 patients, there were three complete remissions, and two patients had partial blast clearance. Common side effects were fatigue and an increase in the serum bilirubin concentration.[64] Another phase 1/2 study was designed to evaluate the safety and preliminary efficacy of GSK525762 in patients with relapsed/refractory (R/R) hematological malignancies. In the phase 1 escalation cohort, 41 R/R AML patients were recruited. Complete responses with incomplete peripheral count recovery were achieved. The most common side effects were dysgeusia, diarrhea, nausea, and elevated bilirubin, which were manageable and reversible.[65] A complete or stable disease was also achieved (66.7%, 20/30) in a phase 1 study of FT-1101 (CC-95775) in patients with R/R AML/MDS.[66] Recently, a phase 1/2 dose-escalation and dose-expansion study of BET inhibitors (INCB054329 and INCB057643) in patients with recurrent and advanced malignancies was reported. Of the 12 AML patients who received INCB057643, one achieved a confirmed complete response, which lasted for 57 days before disease progression occurred.[67] In a study of BET inhibitor ABBV-075 (mivebresib) in patients with R/R AML, the investigational drug was implied to have a tolerable safety profile, with modest efficacy.[68,69] These studies revealed that BET inhibitors have remarkable anti-tumor effects and tolerable adverse events, suggesting that they should be further explored for AML therapy.

CDK inhibitors

There are several CDK inhibitors in clinical trials for AML (Table 1 shows some of these).[72] Alvocidib is a remarkable inhibitor of CDKs with preferential activity against CDK9, 4, and 7, which had shown encouraging findings both in newly diagnosed ($n = 256$) and R/R ($n = 149$) AML patients.[73] Based on the potent anti-tumor effects observed in pre-clinical studies,[56] a phase 1 study was conducted to evaluate the safety and efficiency of BTX-A51, which is a novel inhibitor for CK1α/CDK7/CDK9, in patients with R/R AML or high-risk MDS.[74] This study is still recruiting patients. FLX925 is a dual FLT3 and CDK4/6 inhibitor. A phase 1 clinical trial was designed to assess the safety and efficacy of FLX925 in adult patients with R/R AML. FLX925 showed modest anti-leukemic activity, but without any complete or partial responses observed.[70]

Combination therapies

Combination therapy is one way to overcome drug resistance. In the past few decades, serial studies were conducted to evaluate the efficiency of FLAM (CDKs inhibitor alvocidib, followed by cytarabine and mitoxantrone) in newly diagnosed AML patients. A randomized phase 2 clinical trial revealed significantly higher complete remission rates in the FLAM group than 7 + 3 (cytarabine and daunorubicin) group, despite no differences in overall survival between the two groups.[71] Future studies will likely combine CDK inhibitors or other super enhancer-targeting drugs with conventional chemotherapy in an effort to improve the patient response.

Pre-clinical studies of super enhancer-related drugs

In addition to those clinical trials described above, there have also been pre-clinical studies that showed excellent performance in terms of both safety and effectiveness against AML.

BET inhibitors

Compared to the first-generation inhibitor ABBV-075, a second-generation selective BET inhibitor, ABBV-744, exhibited a higher binding affinity for the BDII bromodomain of BRD4. Experiments in xenograft models of AML demonstrated that the antitumor effects of ABBV-744 were comparable to those of ABBV-075, but with improvements in its bioavailability and tolerability.[75] Moreover, BET inhibitor BI-894999 also showed antitumor effects in pre-clinical studies using AML cell lines, primary patient samples, and mouse xenografts.[76]

CDK inhibitors

Fadraciclib (CYC065) was developed as a novel CDK2/9 inhibitor. Recently, preclinical efficacy studies were conducted in AML cell lines, mouse xenografts, and primary patient samples. Fadraciclib was administered as a single agent or in combination with routine AML chemotherapeutics. The data implied that both monotherapy and combination therapy had preclinical efficacy against AML, related to the gene mutations status of AML.[77,78] Promising

Table 1 The super enhancer-related drugs evaluated in clinical trials for AML.

Target	Agent	Other name	Cancer type(s) evaluated	Combination	Phase	Status
BET	OTX-015	Briabresib	AML, DLBL, ALL, MM	None	1	Reported[64]
	ABBV-075	Mivebresib	AML, MM, NHL	None	1	Reported[68]
				Venetoclax	1	Reported[69]
	ABBV-744	NA	AML	None	1	Terminated
	GSK525762	Molibresib	R/R AML, AML after MDS, new AML	None	1/2	Reported[65]
	PLX2853	NA	R/R AML, MDS	None	1	Recruiting
	TEN-010	RO6870810	R/R AML, MDS	None	1	Completed
	ZEN-3365	NA	Lymphoproliferative Malignancies, R/R AML	None	1	Withdrawn
	JAB-8263	NA	Solid tumor, AML, Myelofibrosis	None	1/2a	Not yet recruiting
	CPI-0610	Pelabresib	Myelofibrosis, AML, MDS	None	1/2	Recruiting
	INCB057643	NA	R/R AML, solid tumors	None or standard-of-care	1/2	Reported[67]
	FT-1101	CC-95775	R/R AML, MDS, NHL	None	1	Reported[66]
				Azacytidine	1	Reported[66]
CDKs	Fadraciclib	CYC065	R/R CLL, AML, MDS	Venetoclax	1	Unknown
	BTX-A51	NA	R/R AML, MDS	None	1	Recruiting
	FLX925	NA	R/R AML	None	1	Reported[70]
	alvocidib	flavopiridol	AML	None or standard-of-care	1-2/3	Reported[71]
	FN-1501	NA	Solid Tumors, R/R AML	None	1	Recruiting
	SEL120	NA	AML, high risk MDS	None	1	Recruiting

Abbreviations: AML, acute myeloid leukemia; DLBL, diffuse large B-cell lymphoma; ALL, acute lymphoblastic leukemia; MM, multiple myeloma; R/R AML, relapsed/refractory AML; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; R/R CLL, relapsed or refractory chronic lymphocytic leukemia.

anti-tumor effects have also been seen in preclinical studies of JSH-009 and SNS-032 (BMS-387032), which specifically inhibit CDK9, and SEL120-34 A, which inhibits CDK8.[55,79,80]

Combination therapies

Most of the studies of combination therapies related to super enhancers have been at the preclinical stage. For instance, in one study, combination therapy with volasertib, a Polo-like kinase (PLK) inhibitor, and BET inhibitor BI-894999, showed strong synergistic anti-tumor effects in *in vitro* and *in vivo* preclinical studies of AML.[81] Studies also demonstrated that the combination of JQ1 and a FLT3 tyrosine kinase inhibitor synergistically induced apoptosis in AML cells expressing FLT3-ITD.[82,83] In addition to the combinations of kinase inhibitors, various studies also combined BET inhibitors with epigenetic regulators as another strategy for AML. Notably, the combination of a BET inhibitor and pan-histone deacetylase inhibitor might also be used in AML or AML cells resistant to FLT3-TKI.[84] Combined treatment with a BET inhibitor and a LSD1/KDM1A inhibitor led to synergistic anti-AML effects both *in vitro* and *in vivo*.[85,86] Moreover, one preclinical study also reported a combination strategy targeting the two key super enhancer regulators (BET and CDK7), which abolished *MYC* expression and exhibited strong synergistic lethality to leukemia cells.[87] The combination of a MDM2 inhibitor and a BETi in AML cell lines, primary patient samples, and mouse xenografts also led to increased cancer cell death, suggesting that the transcriptional inhibition of BRD4 and activation of wild-type TP53 could function together as a potential synthetic therapeutic strategy for AML.[88] These preclinical studies provide paradigms for future clinical trials in AML, and the use of novel combinations of drugs is already being investigated in clinical trials.

BET inhibitor resistance in AML

Based on the clinical and pre-clinical studies described above, BET inhibitors are the most widely developed super enhancer inhibitors for AML. However, resistance to BET inhibitors is a major threat to their effectiveness against cancer.[89] One study conducted a chromatin-focused RNAi screen to explore the mechanisms and find the key factors involved in both primary and acquired BETi resistance in AML. They identified that polycomb repressive complex 2 (PRC2) led to BETi resistance by regulating the transcription of BRD4 target genes (like *MYC*). They also pointed out that the Wnt signaling pathway was a driver of BETi resistance.[90] Another study confirmed that increased Wnt/β-catenin signaling pathway activation might underlie the development of resistance to BET inhibitors in human and mouse AML cells.[91] Wnt signaling pathways are related to numerous other pathways and lead to the development of resistance to a variety of conventional and targeted therapies.[92] The above studies implied that BETi and traditional drugs have similar drug resistance mechanisms and provided new insights that will be useful during the further development of AML therapeutics. Further, the findings of these studies

indicate that epigenetic changes, such as transcriptional reactivation, as well as genetic changes (nucleotide mutations), are closely related to BETi resistance.

Besides the Wnt, various other pathways have also been reported to influence BETi resistance. Using high-throughput drug screening and RNA sequencing analysis, genes involved in NF-κB signaling were identified to be upregulated in BETi-resistant cells. Further experiments revealed that inhibitors of NF-κB signaling improve the response to BET inhibition in both *in vitro* and *in vivo* models.[93] Using genome-scale and cell-based models of spontaneous resistance, one study reported that cell cycle-related factors acted as critical genes that regulating the response and resistance to BETi.[94] It was also reported that the AKT-mTOR pathway was associated with intrinsic BETi resistance, and combining a BETi and a AKT inhibitor could overcome the resistance to the BET inhibitor.[95] The AMPK-autophagy pathway was also identified as being involved in the resistance of AML cells to BET inhibitors.[96]

Ubiquitin-related proteins that regulate the stability of key super enhancer-mediated proteins have also been reported to participate in BET inhibitor resistance. For example, BRD4 interacted with and was de-ubiquitinated by deubiquitinase DUB3. Upregulation of DUB3 stabilized the BRD4 protein level and contributed to BET inhibitors resistance. These results suggest that DUB3 is a potential therapeutic target for overcoming BET inhibitor resistance.[97] The ubiquitin ligase adaptor speckle-type POZ protein (SPOP) was identified as an enzyme that ubiquitinates the BRD2, BRD3, and BRD4 proteins, which led to the degradation of BET proteins. In contrast, SPOP mutants impaired BETs ubiquitination and degradation, and thus led to BET inhibitor resistance.[98] Moreover, an shRNA-based genetic screen was performed and identified another E3 ubiquitin ligase, tripartite motif-containing protein 33 (TRIM33), as a key enzyme participant in BETi resistance. They found that TRIM33 deletion further reduced the BETi-mediated regulation of *MYC*.[99]

In summary, many potential super enhancer-related targets have been discovered, and small molecular inhibitors or activators have been widely explored. Clinical trials have been initiated (or in some cases, completed) for some of these super enhancer-related drugs, with promising results for several agents. Thus, super enhancer-related treatments may soon be available for AML.

Conclusion and perspective

The concept of super enhancers emerged eight years ago. Since then, super enhancers have become a research hotspot in various fields, including oncology. Here, we summarized the functions of super enhancers in the pathogenesis, diagnosis/classification, and targeted therapy of AML. The critical roles of super enhancers in AML have attracted increasing attention in the past few years. However, there is still not enough evidence available to conclude whether DNA mutations in AML are related to super enhancer regions, and whether specific point mutations lead to the activation of super enhancers in AML. Additionally, the clustered DNA regions of super enhancers are so large that the potential targets for gene editing at

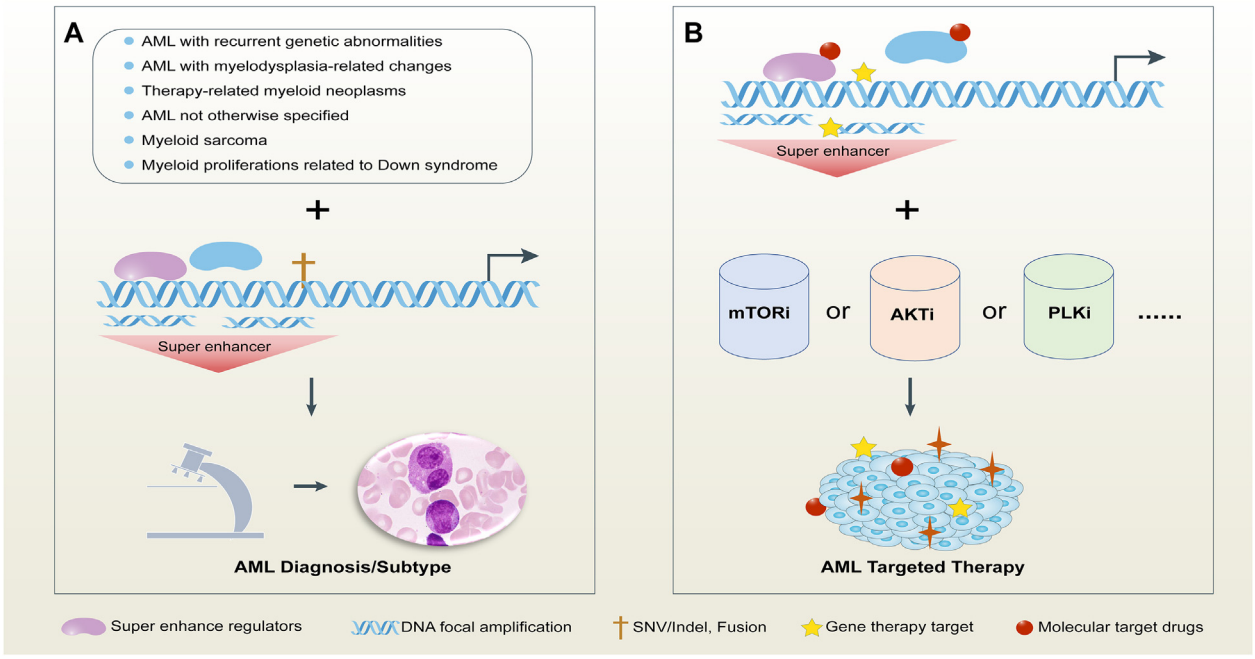

Figure 3 Possible new approaches for the diagnosis and treatment of AML. **(A)** The identification of super enhancers based on the different regulating factors, DNA mutations and chromatin status may provide new biomarkers for the diagnosis and classification of AML. **(B)** Some specific super enhancer-targeting drugs, such as molecular inhibitors or DNA editing approaches, may be useful for the individualized precision treatment of AML; combining super enhancer-related drugs with other agents may be an effective strategy for eradicating AML.

the DNA level are currently unclear. For these reasons, the current super enhancer-related targeted therapies have focused on broad-spectrum anti-super enhancers, such as the small molecular inhibitors of BET and CDK, which are the key proteins involved in super enhancer transcriptional regulation.

Because inhibitors of BET and CDKs may interfere the transcription of numerous proteins, they may lead to significant toxicity and adverse events, so their clinical applications have been limited. Notably, the first-generation CDKs-related drugs showed relatively modest effects, and had obvious toxicity that increased with the dose administered.[70] However, studies to optimize the inhibitors to reduce their side effects and improve their efficacy are still ongoing. More concerning, tumor cells commonly activate more than one parallel pathway, so monotherapy targeting a single signaling pathway leads to drug resistance. We have described various mechanisms of BETi resistance, suggesting areas for further research. Additionally, we have described new ways to define AML subtypes, the transcriptomic characteristics of super enhancers,[100] and whole genomic characteristics that can be used to direct and design critical combination treatments, all of which are topics that should be extensively explored in future studies.

With the development of new technologies in molecular biology, researchers now can integrate different technology platforms including whole genome sequencing (WGS), ChIP-seq, High-throughput chromosome conformation capture (Hi-C), chromosome conformation capture with high-throughput sequencing (4C-seq), and mass spectrometry to precisely explore the DNA sequences or specific regulatory proteins of the super enhancer for individual oncogenes. Furthermore, genome engineering technologies such as CRISPR/Cas9 and TALEN could be applied to edit the specific gene sequence of oncogenic super enhancers, or even trigger targeting agents for specific super enhancers. These new technologies may make it possible to obtain a precision diagnosis of AML and individualize treatment for the disease based on an individual's status for numerous super enhancers.

In perspective, super enhancers may become an integral part of the diagnosis and treatment of AML in the future (Fig. 3). Several specific alterations, including DNA mutations, focal DNA amplification, chromosome translocation, or regulatory protein changes (changes in histone modification-related proteins and chromatin remodeling proteins), in the super enhancer regions of key oncogenes/tumor suppressor genes, can lead to leukemogenesis, and AML can be further identified and classified based on the status of these specific super enhancers. In addition, specific molecular or gene targeted drugs can be used for these super enhancer regions, and using them in combination with other agents is expected to improve the outcome for AML patients in the future.

Author contributions

Conceptualization, L.Z., J.S. and C.W.; Investigation, Z.C. and J.W.; Validation, Z.C. and J.W.; Writing — Original Draft Preparation, Z.C. and L.Z.; Writing — Review & Editing, Z.C., J.W., T.F. and L.Z.; Visualization, Y.S. and L.Y.; Supervision, L.Z., and C.W.; Funding acquisition, L.Z., and Z.C.; All authors have read and agreed to the publication of this version of the manuscript.

Conflict of interests

The authors declare no conflict of interests.

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