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REVIEW

ENL: structure, function, and roles in hematopoiesis and acute myeloid leukemia

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Abstract
ENL/MLLT1 is a distinctive member of the KMT2 family based on its structural homology. ENL is a histone acetylation reader and a critical component of the super elongation complex. ENL plays pivotal roles in the regulation of chromatin remodelling and gene expression of many important proto-oncogenes, such as *Myc*, *Hox* genes, via histone acetylation. Novel insights of the key role of the YEATS domain of ENL in the transcriptional control of leukemogenic gene expression has emerged from whole genome Crisp-cas9 studies in acute myeloid leukemia (AML). In this review, we have summarized what is currently known about the structure and function of the ENL molecule. We described the ENL's role in normal hematopoiesis, and leukemogenesis. We have also outlined the detailed molecular mechanisms underlying the regulation of target gene expression by ENL, as well as its major interacting partners and complexes involved. Finally, we discuss the emerging knowledge of different approaches for the validation of ENL as a therapeutic target and the development of small-molecule inhibitors disrupting the YEATS reader pocket of ENL protein, which holds great promise for the treatment of AML. This review will not only provide a fundamental understanding of the structure and function of ENL and update on the roles of ENL in AML, but also the development of new therapeutic strategies.

Keywords ENL/MLLT1 · Histone acetylation · Hematopoiesis · Acute myeloid leukemia (AML) · MLL · Epigenetic therapy

Introduction

Acute myeloid leukemia (AML) is a disease commonly found in older people with the average age at presentation of 67 years [1, 2]. However, older patients are often not eligible for bone-marrow transplantation and are less tolerant to intensive chemotherapy. Hence, the prognosis of AML disease remains dismal [3, 4]. Mutations or chromosomal rearrangements involving transcription factors, tumor suppressor genes, oncogenes, and epigenetic modifiers are fundamental to AML leukemogenesis. These genetic and epigenetic abnormalities are not only characteristics of AML, but are also implicated as therapeutic targets and prognostic indicators [5–7].

The mixed-lineage leukemia (MLL) gene on chromosome 11q23 is one of the most frequently rearranged genes characterized in leukemia [8, 9]. **E**leven–**n**ineteen **l**eukemia (ENL, also named *MLLT1*: myeloid/lymphoid or **m**ixed-**l**ineage **l**eukemia; **t**ranslocated to, **1**) are a common fusion partner gene to MLL (MLL–ENL) resulted from t(11;19)(q23;p13.3) translocation. MLL–ENL positive AML is an aggressive disease with poor outcome [10]. ENL protein typically resides in large protein complexes such as super elongation complex (SEC) [11]. ENL protein functions as a histone acetylation reader. It is also an RNA polymerase II (RNA Pol II) elongation factor and a homologous nuclear protein that promotes transcription during the elongation

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stage [12]. Recurrent mutations within the highly conserved YEATS domain of ENL has been identified in children with Wilms tumor, suggesting that deregulation at the elongation stage resulting in cancer pathogenesis [13]. Notably, emerging data from two research groups independently unveiled the functional importance of ENL gene in the pathogenesis of AML [14, 15].

In this review, we describe the ENL gene and protein structure and examine the function of ENL in histone acetylation, translation, and elongation. We summarize the current knowledge on ENL in normal hematopoiesis and leukemogenesis. We also discuss ENL's essential targeted genes and activated pathways, particularly in AML. Finally, we review the potential approaches in developing novel anti-AML therapies either by targeting ENL or disrupting interaction with its partners.

Structural insights into ENL gene and protein

ENL gene

The orthologs of human ENL gene are found in 281 organisms and the ENL gene is conserved in chimpanzee, rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog. The human ENL gene shares close nucleotide sequence homology with zebrafish (75%) and mouse (85%), suggesting that ENL is likely to have fundamentally critical biological functions. The human ENL gene (NM_005934) is located on chromosome 19p13.3, containing 12 exons and 4530 bps in length (Fig. 1a).

ENL protein structure

The human ENL protein (NP_005925.2) consists of 559 amino acids and has a molecular mass of 62 kDa (Fig. 1b). It contains a hydrophobic N-terminal YEATS domain and C-terminal ANC1 (nuclear anchorage protein 1) homology domain (AHD) [16–18]. The name YEATS was derived from five proteins (**Y**af9, **E**NL, **A**F9, **T**af14, and **S**as5) [19], which were first found to contain this domain. The ENL YEATS domain comprises of about 75 amino acids [20]. The characteristic YEATS domain is highly conserved from human to chimpanzee, mouse, rat, and frog (Fig. 1b). The AHD domain is responsible for binding with other factors involved in transcriptional activation, such as DOT1L (disruptor of telomeric silencing 1-like), AF4, and CBX8 [18]. A two-amphipathic α-helix is formed by 84-amino acid on the C-terminus of ENL, which is crucial in providing protein–protein interaction [11, 21]. The C-terminal also fuses to the Gal4 DNA binding domain and results in transactivation [21]. The amino terminus (N-terminus) of ENL protein is conserved and it contains transactivation (TA) domain [21]. The transactivation domain is conserved with nuclear anchorage protein 1 (ANC1), a stoichiometric component of two yeast basal transcription complexes (TFIIF and TFIID) and the SWI/SNF chromatin remodelling complex [21].

Various MLL–ENL deletion mutants were constructed to identify the region on ENL that are essential for the function of the MLL–ENL fusion [22]. MLL–ENL constructs in the absence of amino acids 430–475 at N-terminus of ENL continue to possess the ability to stimulate cell proliferation, whereas the deletion of C-terminus on ENL causes the fusion protein to lose its effect completely [22]. The deletion or mutation of either of the two helical structures on ENL also results in the loss of transformation function in MLL–ENL [22]. This provides evidence that the C-terminal and the two helical structure of ENL are critical and essential for transactivation.

ENL functions

YEATS domains are acyl-lysine readers that are capable of recognizing repeats of short-chain histone acylation, such as acetylation, propionylation, butyrylation, and crotonylation [23]. The ENL YEATS domain has a role in transcriptional regulation and histone binding [12]. Compared to acetylation, the YEATS domains favor binding to crotonylation with an increase of two-to-sevenfold binding affinity [23], this could offer insights into the functions of proteins with YEATS domains in epigenetic regulation. YEATS domains also have a functional reader pocket which has an "open-end" feature that allows the YEATS domain to bind to a wider range of acyl chain extensions from two-carbon acetyl to four-carbon crotonyl [23].

The YEATS domain binds to a subset of acetylated histone peptide, including H3K27ac, H3K9ac, and H3K18ac to promote oncogenesis. In YEATS domain, the H3K27ac peptide is attached to the acidic surface on the top of the eight-stranded β-sandwich fold, while the acetylamide group of K27ac is sandwiched by the aromatic residues [15]. Recent studies showed that mutated ENL YEATS domain appeared to have a reduced amount of polymerase II on ENL-target genes, leading to the suppression of oncogenic gene expression, therefore, proving its importance in recruiting polymerase II [15, 16].

In vitro pull-down assay revealed that ENL is capable of binding to histones H3 and H1, and the N-terminal YEATS domain is essential in binding to histones [11]. ENL is able to interact with PRC1 (polycomb-repressive complex 1) that is capable of transcriptional suppression [24]. Protein kinase ATM (ataxia telangiectasia mutated) is activated upon DNA DBS (double-strand breaks) which then phosphorylates ENL. Phosphorylated ENL in SEC

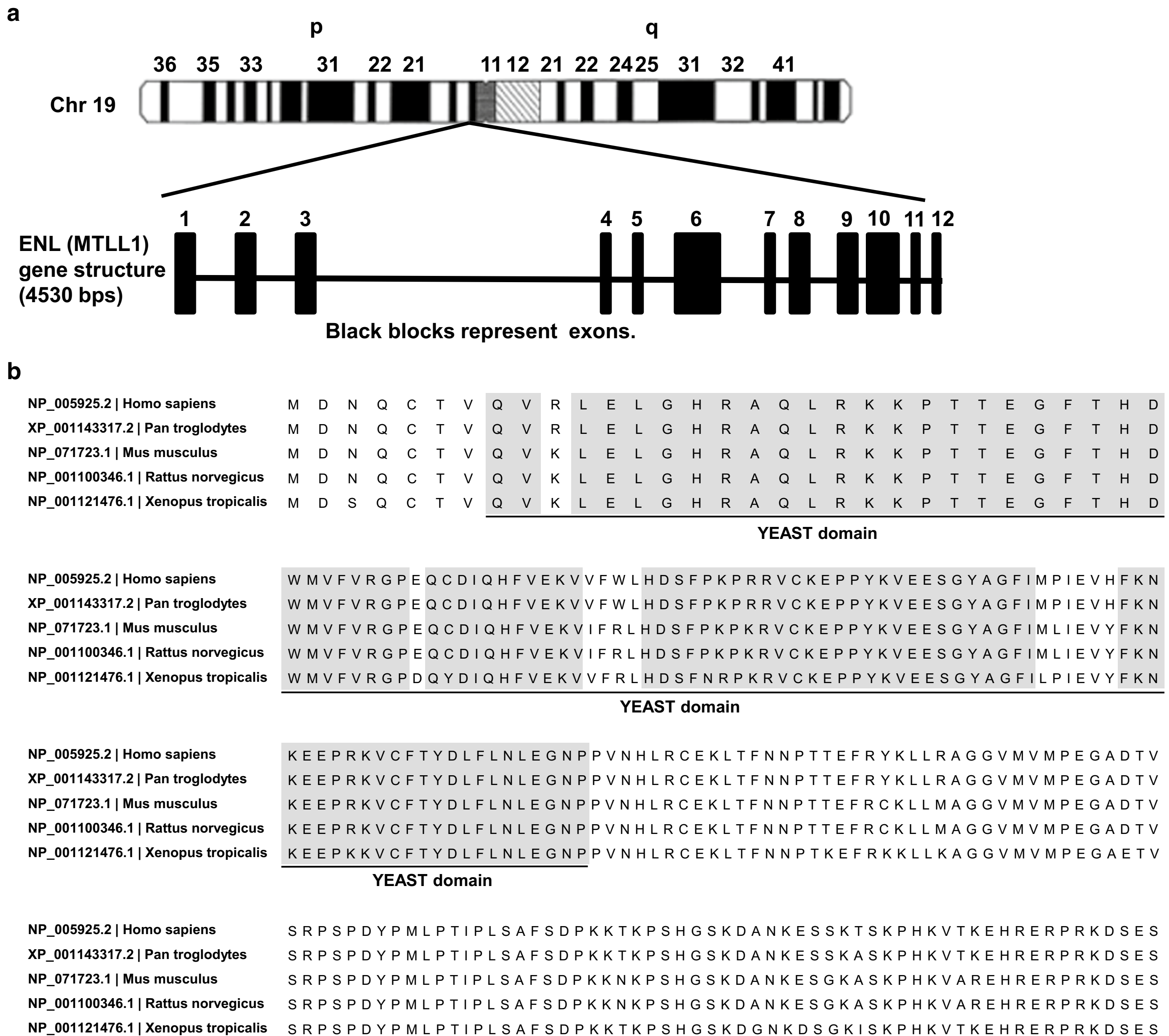

Fig. 1 Genomic structure of ENL gene and amino acid sequences of ENL protein. **a** Schematic of the ENL gene which is located at chromosome 19p13.3 and about 4.5 kb long, containing 12 exons which are represented in black blocks. **b** Partial amino acid sequence alignment of known and predicted ENL homologues across different species. For NCBI accession numbers, an ''NP_'' prefix suggests a known protein, whereas an ''XP_'' prefix implies a predicted protein based on genetic analysis. Characteristic functional domain, YEATS of ENL is underlined and labelled. Amino acids conserved in all species are shaded

binds to PRC1 components, E3-ubiquitin-ligase BMI1 and RING1B, leading to transcription suppression [18, 24]. Interactions between ENL with SEC and PRC1 result in transcriptional switching of elongation to repression and genotoxic stress for DBS repair near transcriptional sites [18]. H2A also becomes ubiquitinated after recruitment of PRC1 by phosphorylated ENL in SEC at transcriptional elongation sites, causing transcription suppression [18]. The AHD of ENL allows it to change binding partners during post-transcriptional modifications.

In summary, ENL is a chromatin reader which recognizes the acetylation of H3. In addition to being associated with SEC, p-TEFb, ENL forms complex with histone-H3 methyltransferase DOT1L, an H3K79 specific methyltransferase [25]. These results suggest the function of ENL in histone modification and transcriptional elongation, while its loss impedes the initiation of transcriptional elongation.

ENL in normal hematopoiesis

The murine ENL gene shares 86% of similarity in amino acid sequence identity with the human homolog ENL [26]. Furthermore, their genomic organization is identical and exhibits high homology in 5′ regulatory element. Targeted disruption of ENL in mice leads to no homozygous offspring, suggesting an embryonic lethality after ENL knockout. However, mice heterozygous for the targeted ENL mutation displays normal fertility and their whole blood count and peripheral blood film appear normal [26]. Two recent studies using either Crispr–Cas9 or short-hairpin RNA (shRNA) technology to silence murine ENL in LSK (Lin − Sca1 + c-Kit −) hematopoietic stem cell population confirm that loss of ENL has minimal effect on the colony-formation ability, growth, and lineage differentiation of LSK cells [14, 15]. However, it is important to note that we have to wait for the comprehensive results from conditional knockout of ENL in adult hematopoietic compartment, and then, the definitive role of ENL in hematopoiesis can be fully understood.

ENL in the pathogenesis of AML

The t(11;19)(q23;p13.1) chromosomal translocation is one of the common translocation observed in AML [27, 28]. This translocation results in the fusion of *ENL* to the *MLL* (*KMT2A*) gene with consequent expression of an MLL–ENL chimeric protein. The frequency of MLL/ENL fusion is about 3.5% in de novo AML with MLL rearrangement [29]. ENL promotes the pathogenesis of acute leukemia by maintaining deregulated gene expression via fusion with MLL. A full length transforming protein is created when C-terminal 126 amino acids of ENL fuse with the N-terminal domain of MLL [16], including the DNA-binding motifs such as AT hooks and methyltransferase (MT) domains [16, 30]. The deletion of AT hooks and MT domains was found to impact and decrease the transforming ability of MLL–ENL [22]. The gain-of-function MLL–ENL is capable of transforming hematopoietic stem cells (HSCs) in vitro and can specifically stimulate transcriptional elongation [22]. Recent studies using novel genomic engineering technologies, like transcription activator-like effector nucleases (TALEN) and Crispr–Cas9, to knock in MLL–ENL in CD34+ human umbilical cord blood stem cells validate the ability of MLL–ENL for leukemia initiation, serving as first hit [31, 32]. Different transgenic mouse models have been explored to define the role of MLL–ENL in the transformation of AML [33–35]. These mouse models not only have recapitulated characteristics of human mixed-lineage AML, but also provided the biological insights into MLL–ENL-mediated leukemogenesis. On the other hand, in a serial transplantation of conditional MLL–ENL expressing HSCs experimental setting, despite cells gain additional (secondary) mutations, diseased mice are cured by depletion of MLL–ENL expression [36]. These results show that the MLL–ENL fusion gene is the determining factor for the leukemic transformation and confirms the therapeutic value of MLL–ENL fusion gene.

ENL in immunosurveillance

MLL–ENL can also result in the transformation of non-immunogenic hematopoietic stem cells into immunogenic cells and stimulate NK cell-mediated immunosurveillance [37]. In MLL–ENL hematopoietic precursor cells, CD48 is downregulated, while the activating ligands for NKG2D and DNAM-1 are upregulated as compared to their normal counterparts [37]. MLL–ENL fusion protein inhibits the normal T cell development both in vitro and in vivo, probably by interfering with Notch signalling which is crucial for early T-cell differentiation [35], causing lineage reassignment into myeloid tumors [33]. MLL–ENL appears to be induced mostly in immature T cells and results in lineage reassignment to the myeloid pathway. However, if MLL–ENL fusion protein is induced in matured T cells, the neoplastic cells will be presented as T-cell leukemia [33]. There seems to be a lack of B-cell tumor(s) induced by MLL–ENL as the fusion protein is not able to commit to CD19+ B cells [35, 38]. These data suggest that the activity of MLL–ENL fusion protein may be specific for certain cell type or differentiation stage [38].

Target genes of MLL–ENL fusion protein

The structure of MLL–ENL fusion protein is similar to the core-binding factor fusions and retinoic acid fusion proteins, which can impose a blockage on myeloid differentiation [39]. The MLL–ENL fusion protein was thought to be the first genetic event that occurred and that further mutations, such as *c-Myc* mutation, are required to result in a full-blown AML [40]. The proto-oncogene *c-Myc* encodes a nuclear phosphoprotein that is involved in growth control, differentiation, and apoptosis. The abnormal expression of *c-Myc* is frequently associated with cancer and plays a part in hindering myelomonocytic development [40]. The overexpression of Myc protein might help to increase the oncogenic effect of MLL–ENL fusion protein and cause further obstruction in myeloid differentiation [40]. Bromodomain and extra-terminal (BET) family proteins and acetyl-lysine binding factors were also implicated in the transcription control of oncogenic *Myc* in MLL–ENL leukemogenesis [14]. There

seems to be two ways of how MLL–ENL interact with *Myc* to cause an impact, either by sharing a mutual pathway that triggers a group of target genes or by simultaneously activating specific targets that cannot be activated by MLL–ENL oncoprotein or *Myc* alone [40]. However, mutation of Myc protein itself is necessary yet insufficient to cause transformation of haematopoietic cells [40]; thus, it would be essential to identify other potential oncogenes that contribute to MLL–ENL leukemogenesis.

In addition to *c-Myc*, the leukemogenic MLL–ENL fusion protein also activates a large number of its downstream genes contributing to its transformation ability [39–48]. The majority of these downstream targets are transcription factors, which are known to have important roles in hematopoiesis and cancer development [39–48]. For example, a number of HoxA gene family members, including HoxA4 to HoxA11, are sustainably induced by MLL–ENL protein, and defined as "Hox code" [39, 42–44, 46]. Among the HoxA family, HoxA9 is the most studied and validated target in various in vitro and transgenic mouse model. The ectopic expression of HoxA9, together with Meis1, leads to the same phenotype of differentiation block and transformation observed in MLL–ENL-knock-in mouse progenitor cells [39]. A number of human genes in which expression is positively regulated by MLL–ENL fusion protein and their critical roles in MLL–ENL-mediated leukemogenesis are summarized (Table 1).

Cooperative partners and complexes of MLL–ENL fusion protein

Oncogenic MLL–ENL protein also stimulates the elevated expression of receptor tyrosine kinase FLT3 [39]. In consistence, microarray study of primary AML samples revealed that FLT3 is constantly expressed in MLL-associated leukemia [49]. Taken together, FLT3 is likely to play a "second-hit" role in MLL-induced leukemia [50, 51], while differentiation arrest caused by MLL–ENL fusion protein serves as the "first hit". A recent study using Crispr–Cas9 technology to knock in MLL–ENL fusion gene into CD34+ hematopoietic progenitor cells confirmed its ability as "first hit" to initiate leukemia [32]. It seemed that the progression of MLL-rearranged AML is also related with increased Ras GTPases activation [52], where Ras is a potent driver of early T-cell development [35]. Ras and *c-Myc* are oncogenic partners when expressed together collaboratively cause the transformation of primary cells [40]. MLL fusion oncoproteins regulate the expression of *Frat* gene especially *Frat2* to sustain leukemia-associated Rac activity [52]. Increased Wnt signalling and Rac GTPase activation, of both Rac1 and Rac2, are crucial in the development of preleukemic cells to leukemic MLL fusion cells. The loss of MLL–ENL fusion protein directly leads to the downregulation of Rac1 activity as well as *Frat1* and *Frat2* expressions in preleukemic cells [52]. Therapy-targeting Wnt signalling or Rac GTPases could be potentially effective against MLL–ENL oncoprotein in AML.

Upon interacting with MLL and entering the cytoplasm, ENL was found to associate with another fusion protein of MLL, ABI1 (Abl-Interactor 1). Despite their different cellular locations, where ABI1 is located in cytoplasm and ENL in the nucleus, ABI1 is able to interact with ENL either by binding to the ENL C-terminal or to the full length ENL itself [30]. However, it is uncertain if association with ABI1 contributes to MLL–ENL leukemogenesis.

Polycomb-repressive complexes

The polycomb group (PcG) proteins are a family of highly conserved epigenetic transcriptional repressors that were initially discovered in *Drosophila melanogaster* to silence Hox genes [53]. The human PcG proteins assemble a few multi-subunit complexes termed polycomb-repressive complexes (PRCs), as they function to repress some genes critical for embryonic development and cellular differentiation through the regulation of chromatin organization and maintenance of transcriptionally inactive state [54]. Basically, they can be classified into two different complexes: PRC1 and PRC2–PRC4 (PRC2/3/4). The canonical PRC1 complex consists of a Cbx, Pcgf, Ring, and Phc protein, while the core components of PRC2 complex are composed of Ezh2, Eed, and Suz12 [55]. In MLL-rearranged leukemia, ENL, as a subunit of SEC, cooperates with PRCs in the transformation of hematopoietic cells.

ENL has also been discovered to be associated with the PRC1. ENL acts as a scaffold that binds to CBX8, a PRC1 component, and to the elongation machinery, respectively, resulting in enhanced transformation efficiency [56]. It has been reported that in response to DNA double-strand break (DSB), ENL in concert with PRC1 induced a functional switch from transcriptional elongation to transcriptional repression [57]. Mechanistically, DSB leads to the activation of ATM, which phosphorylates ENL at conserved SQ sites in SEC. The phosphorylated ENL increases its binding affinity to BMI1 and RING1B, a heterodimeric E3-ubiquitin-ligase complex in PRC1, resulting in a rapid switch to transcription repression at transcriptional elongation sites [57]. Therefore, these results suggest that ENL plays a safeguard role in maintaining genome integrity under genotoxic condition.

In addition to PRC1, ENL is able to recruit human Polycomb 3 (hPc3) protein via its C-terminal which is involved in chromatin remodelling [11, 16]. Interaction with hPc3 can disrupt the structure and dysregulate the function of a normal Polycomb protein complex in the cell. Changes in the

Table 1 List of genes identified as MLL–ENL targets which are critical for leukemia transformation

Gene symbol	Function cluster	Leading model(s)	Phenotype	Literature
c-Myc	Transcription factor, proto-oncogene	Primary mouse hematopoietic cells	Loss of Myc reverses MLL–ENL-induced differentiation arrest and transformation	[40]
HoxA7, HoxA9	Homeobox transcription factor	Primary mouse hematopoietic cells, MPMP cell lines Human cell lines and primary AML patient samples	A genetic reliance on Hoxa7 and Hoxa9 in MLL–ENL-mediated transformation of myeloid leukemia	[42], [39]
			Promoting multiple histone modifications at HoxA9 gene locus	[46]
			HOXA9 depletion inhibits proliferation and induces apoptosis specifically in MLL-rearranged cells	[43]
Meis1	Homeobox transcription factor	Primary mouse hematopoietic cells, human REH and mouse M1 cell lines	Co-expressing Meis1 and HoxA9 sufficient to substitute for MLL–ENL-mediated differentiation block and transformation	[39]
HoxA4 to HoxA11	Homeobox transcription factor	Primary mouse hematopoietic cells	Aberrantly sustaining the expression of a “Hox code” induced by MLL–ENL may contribute to leukemogenesis	[44]
Mef2c	Transcription activator	Knockout mouse, primary human AML samples	Mef2c deficiency compromises homing and invasiveness of the MLL–ENL leukemic cells	[47]
c-Myb	Transcription factor, proto-oncogene	Primary mouse hematopoietic cells, human AML cell lines and primary samples	Silencing Myb reduces HOXA9 and MEIS1 gene expression, accompanying decrease in global H3K4 methylation, leading to diminish the transformation ability of an MLL–ENL fusion protein.	[45]
Evi-1	Transcription factor, zinc finger protein	Primary mouse hematopoietic cells	MLL–ENL binds onto Evi-1 gene promoter region and activates its expression selectively in the undifferentiated hematopoietic stem cell	[41]
Eya1, Six1	transcription coactivator	Primary mouse hematopoietic cells and human AML cell lines	Eya1 in cooperation with Six1 immortalizes hematopoietic progenitor cells	[48]

expression level of Polycomb proteins are often correlated with aberrant cell cycle and tumorigenesis [16].

p-TEFb and DOT1L complexes

The positive transcription elongation factor b (P-TEFb) is the master modulator of RNA polymerase II during transcriptional elongation [58]. p-TEFb is a heterodimer kinase formed by CDK9 and Cyclin T1 or T2 that targets serine 2 on carboxy-terminal domain (CTD) of RNA Pol II for phosphorylation [59, 60]. There are two distinct groups of downstream targets of MLL–ENL, depending on the binding of ENL to either p-TEFb or DOT1L [59]. The interaction of ENL and P-TEFb targets mainly *Hox* and TALE genes for immortalisation [39], while Hox genes are directly regulated by the fusion protein. *Hox* gene family including *HOXA7*, *HOXA9*, and *Hox* cofactors *MEI1* and *Pbx3* are found consistently upregulated in MLL–ENL. The silencing of RNA Pol II after transcription initiation is induced via phosphorylation of negative elongation factor proteins by P-TEFb [59]. AF4 family protein interacts with ENL and P-TEFb elongation factor in the endogenous complex (AEP) in hematopoietic cells and MLL oncoproteins fused with either AF4 or ENL to occupy MLL-target chromatin [60]. AEP might be a suitable target for therapy against MLL-rearranged leukemia.

Disruptor of telomere silencing 1 (DOT1) of budding yeast and its mammalian homolog, DOT1L (DOT1-Like), are a distinct class of histone lysine methyltransferases, which specifically catalyze methylation of histone 3 lysine 79 (H3K79me1, 2, and 3), primarily linked to actively transcribed genes [61, 62]. DOT1L-containing complex (DotCom) is comprised of multiple subunits, linking ENL, AF9, AF17, and AF10 [63]. Several seminal studies demonstrated that DOT1L is required for the development and maintenance of MLL-rearranged leukemia [64–67]. Association of ENL with DOT1L through the highly conserved hydrophobic C-terminus in cell nucleus is crucial for transformation [25]. The activity of DOT1L is necessary for MLL–ENL fusion cells; it is also essential for clustered *Hox*—homeobox genes transcription [59]. Inhibition of DOT1L is found to downregulate MLL–ENL fusion target genes [14], which include *CDKN1B*, *CDKN2C*, *HOXA9,* and *MEIS1*. ENL co-localised with components of DOT1L through its AHD [24]. Interestingly, ENL is essential and required in MLL-wild-type acute leukemia, while DOT1L is only selectively required in MLL-rearranged leukemia and is not sufficient for transactivating MLL-target genes [14, 60]. This suggests that DOT1L does not have a primary rate-limiting role in MLL-rearranged leukemogenesis.

A mouse study was carried out to investigate the role of the MLL–ENL fusion protein in migration and proliferation, where the leukemic cells were injected into non-conditioned syngeneic mice [68]. Five percent of leukemic cells were found in the mice femurs with little proliferation 3 days after injection, and at day 20, the leukemic cells proliferated and formed clusters on the surface of blood vessels in femoral diaphysis [68]. $B220^+$ B lymphocytes were found proximate to the leukemic cell clusters and there were decreased number of femoral $B220^+IgM^+$ cells. Leukemia only developed after 30 days of injection. MLL–ENL oncoprotein is able to transform mouse or human hematopoietic precursors to leukemic cells; however, the development and proliferation of leukemic cells at different stages might require different bone-marrow environment and could have different effects on surrounding hematopoietic or stromal cells [68].

In vivo, the depletion of ENL causes reduction in leukemia growth and extension in survival [69]. The knocking down of ENL resulted in the downregulation of *HOXA9*, *CDKN1B,* and *MEIS1* [60]. Upregulation of myeloid lineages differentiation and downregulation of leukemic stem cells and *MYC*-associated gene were observed when there is loss of ENL [69]. Depletion of ENL also resulted in reduced occupancy of CDK9, an SEC component, as well as DOT1L-mediated H3K79 on ENL-target genes [15]. These results indicate that ENL modulates the recruitment of Pol II through SEC and DotCom, and in turn regulates gene expression. Some of these cooperative partners and complexes of MLL–ENL are summarized in Fig. 2.

ENL as a promising therapeutic target

The poor 5-year survival rate of AML patients highlights the need to develop novel therapeutic strategies for this type of aggressive blood cancer [70–72]. Recently, two groups provided compelling evidence that ENL is critical for the maintenance of AML and YEATS domain of ENL as a promising intervene target [14, 15]. Two different approaches including structure-based mutagenesis to disrupt the interaction between YEATS domain and histone acetylation or a novel chemical genetic strategy to degrade ENL protein were employed. They converged on the conclusions that targeting YEATS domain by either intervening interaction or reducing ENL protein impairs AML cell survival and reverses the differentiation arrest of AML cells in vitro and in vivo. Mechanistically, the loss of acetyl-lysine binding capacity by YEATS mutant ENL-Y78A led to less occupancy and abundance of Pol II at ENL-target genes, suggesting that the ENL YEATS domain is essential for the development of AML [14, 15]. However, there is no small-molecule inhibitor currently available for specific targeting its fusion partner MLL. An indirect approach for targeting MLL–ENL is possible. Dot1L, a histone H3K79 methyltransferase, directly binds to MLL and is aberrantly recruitment to MLL-regulated target genes, like Hox gene

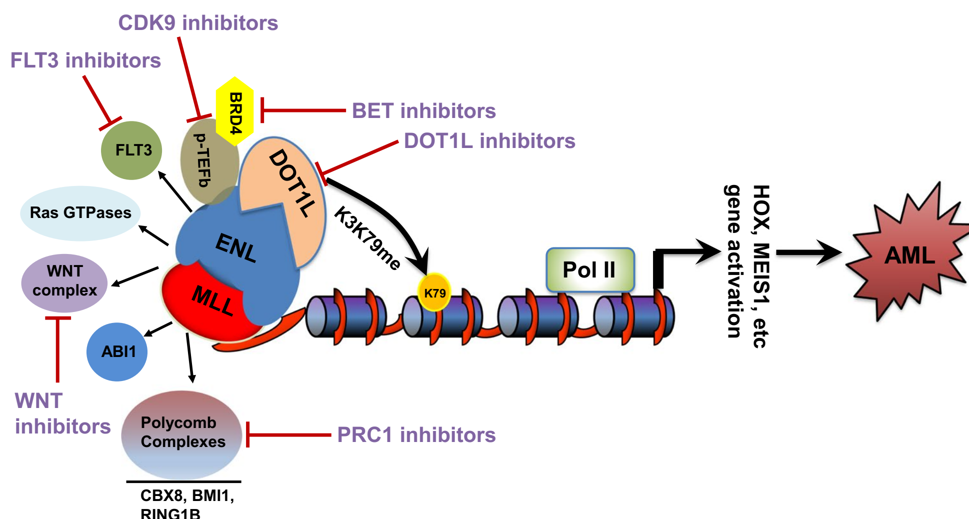

Fig. 2 Key cooperative partners and complexes that are associated with MLL–ENL in leukemia. MLL–ENL contributes to leukemogesis through interaction with some important proteins or regulatory complexes by altering its activity and downstream target genes. For example, MLL–ENL fusion protein recruits DOT1L, a histone methyltransferase, which methylates lysine 79 of histone H3 (H3K79), stimulating RNA Pol II–mediated transcriptional elongation. As the consequence, some key oncogenes, such as HOX gene family, MEIS1, are constitutively activated, leading to the development of AML. A number of small-molecule inhibitors that specifically target some key cooperative partners and complexes of MLL–ENL are highlighted as potential therapeutic interventions

family, resulting constitutive activation of these prominent oncogenes in leukemogenesis. Biochemistry and genetic animal experiments revealed that the enzymatic activity of Dot1L is indispensable for MLL-mediated leukemogenesis. Thus, abrogation of DotL activity has emerged as an effective way to target AML with MLL rearrangements. Pinometostat (EPZ-5676) is a first-in-class small-molecule inhibitor for Dot1L developed by Epizyme (Cambridge, MA) [73]. Pinometostat is safe and modest response was observed. The only two patients who achieved complete remission (CR) are both MLL–ENL positive [73]. From this clinical observation, we reasonably assume that targeting ENL might have certain advantage over its fusion partner MLL as CR was not attained in patients with other MLL rearrangements or MLL–PTD (partial tandem duplication). Further studies are required to validate this assumption. Taken together, these data indicate that similar to DOT1L inhibitor, JQ1, and other bromodomain inhibitors, chromatin-competitive small-molecule inhibitors targeting YEATS domain of ENL protein can be developed as an effective anti-AML therapy (Fig. 2).

There are also several potential approaches that can target MLL–ENL fusion protein or its downstream pathways for anti-leukemia therapies. Immunomodulatory drugs may also be used for preventing leukemic development targeting the NK cell-activating ligands [37]. As the C-terminal of ENL and its helical structure are vital for transactivation, possible therapy could involve drugs targeting the ENL structure to avoid cells transactivation. Blocking the downstream pathways such as Notch and Wnt signalling, as well as effectors, could also be a promising approach. Inhibition of CDK9 or DOT1L activity may be an alternative approach for targeting ENL in leukemia [60]. An increasing number of studies have demonstrated CDK9 as a druggable target for the development of cancer therapeutics [74–76]. CDK9 inhibitors, for example, Atuveciclib (Bayer) and AZD4573 (AstraZeneca), have been investigated in Phase I dose escalation in relapsed/refractory haematologic malignancies (NCT02345382 and NCT03263637, respectively) [77]. It is worthy of note that CDK9 inhibitors do not exhibit selective sensitivity against MLL–ENL positive leukemias. It was found that depletion of ENL sensitises cells towards BRD4 inhibition with BET inhibitor JQ1 [15] (Fig. 2). Although these potential treatments are promising with an anti-AML effect, they have to be carefully validated in clinic. Equally important is to closely monitor patients for any haematopoietic toxicity and other adverse side effects following administration of these inhibitors.

Conclusions

In summary, ENL/MLLT1 is one of the best characterized YEATS domain containing protein in human. ENL is a subunit of SEC and a histone acetylation reader. Thus, it is an important transcription regulator member of the KMT2 family based on its structural homology. ENL selectively recognizes histone lysine acylation and increases the catalytic rate of Pol II transcription by suppressing transient pausing by the polymerase at multiple sites along the DNA. Importantly, ENL mediates multiple functions in transcription

elongation, histone modification, histone variant deposition, and chromatin remodelling. In agreement with its important functions, dysfunction of ENL protein often induced by chromosomal translocations is considered as the initiator of leukemogenesis. As such, the modulation of ENL expression or disruption of ENL protein interactions could be a new, viable approach for epigenetic therapies through the use of either small molecular inhibitors targeting the reading pocket of YEATS domain or targeting its binding partners, such as DOT1L, CDK9 of p-TEFb complex, BRD4, WNT complex, PRC1, and FLT3 (Fig. 2). As drug resistance frequently occurs in monotherapy, the combination of ENL antagonists with chemotherapy or other targeted therapy should be evaluated (Fig. 2). These novel inhibitors will add to our armamentarium to battle leukemia.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

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