Disordered epigenetic regulation in MLL-related leukemia

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Abstract Leukemias bearing rearrangements of chromosome 11q23 are of particular interest due to their unique clinical and biological characteristics. 11q23 abnormalities occur in up to 70 % of infant leukemias, and about 10 % of adult acute myelogenous leukemias (AML). Two major rearrangements of the *MLL* gene are found in MLL-related leukemia. The most common of these is balanced translocations in which the N-terminal portion of MLL is fused to the C-terminus of the translocation partner. To date, nearly 100 different chromosome bands have been described in rearrangements involving MLL, and more than 70 known fusion partners of MLL have been cloned and characterized at the molecular level. Another major aberration of the *MLL* gene creates a repeat within the N-terminal MLL resulting in an internal partial tandem duplication (PTD). As a consequence, an extra amino-terminus is added in-frame to full-length MLL, resulting in leukemogenic MLL-PTD. MLL-PTD occurs predominantly in myeloid dysplasia syndromes, secondary AML (s-AML), and de novo AML. The presence of an MLL rearrangement generally confers a poor prognosis. MLL fusions and MLL-PTD are transcriptional regulators that take control of targets normally controlled by MLL, with the clustered HOX homeobox genes as prominent examples. Several epigenetic regulators that modify DNA or histones have been implicated in MLL fusion driven leukemogenesis, including DNA methylation, histone acetylation, and histone methylation. Recently, the histone methyltransferase DOT1L, the bromodomain and extra-terminal (BET) family member BRD4, and the MLL-interacting protein Menin have emerged as important mediators of MLL fusion-mediated leukemic transformation. The clinical development of targeted inhibitors of these epigenetic regulators has heralded promise for the treatment of MLL fusion leukemia. Although the biological function and molecular mechanism for MLL-PTD remains largely unknown, based on the primary protein structure of MLL-PTD and the knowledge gained so far from MLL fusions, newly developed inhibitors of epigenetic regulators could potentially also prove effective in the treatment of MLL-PTD related leukemias.

Keywords MLL-related leukemia · Epigenetics · Therapeutics · Histone modification

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Introduction

Leukemias bearing rearrangements of chromosome 11q23 are of particular interest, due to unique clinical and biological characteristics. 11q23 abnormalities occur in up to

70 % of infant leukemia [1], and about 10 % of adult acute myelogenous leukemia (AML), and many cases of secondary acute leukemia that arise following therapy with topoisomerase inhibitors, such as etoposide [2]. Two major disruptions of *MLL* gene are found in MLL-related leukemia. The most common of these is balanced translocations in which the genomic sequences encoding the N-terminal portion of MLL are fused to sequences encoding the C-terminus of another translocation partner. To date, nearly 100 different chromosome bands have been described in rearrangements involving 11q23 and more than 70 fusion genes have been cloned and characterized at the molecular level [3]. The most frequent translocations generate the MLL–AF6, MLL–AF9, MLL–ENLs, MLL–AF10, and the MLL–AF17 fusion proteins in AML, and the MLL–AF4, MLL–LAF4, MLL–AF5q31, and MLL–ENLl fusion proteins in B-ALL and, in rare cases, T-ALL [4]. Another aberration of the *MLL* gene creates a short repeat within the N-terminal MLL coding sequence resulting in an internal partial tandem duplication (PTD). As a consequence, an extra amino-terminus is added in-frame to full-length MLL, resulting in leukemogenic MLL-PTD. MLL-PTD occurs predominantly in myeloid dysplasia syndromes (MDS), s-AML and also de novo AML. The presence of an MLL rearrangement, either MLL fusion or MLL-PTD, generally confers a poor prognosis. MLL fusions and MLL-PTD are transcriptional regulators that take control of targets normally controlled by MLL with the clustered HOX homeobox genes as prominent examples. Recently, important roles for several epigenetic regulators under both normal cellular conditions and transformed conditions are emerging and shedding light on the biology and mechanisms of MLL rearrangement-induced leukemia. These studies presented potential new targets for rational drug development, which will facilitate improved therapies and patient outcomes in the future.

MLL and its role in normal hematopoiesis

MLL is a functional ortholog of the *Drosophila trithorax* (*trx*) protein, which is involved in maintaining epigenetic transcriptional memory at *homeobox* (*Hox*) gene loci. The SET domain of MLL has histone methyltransferase activity [5] and MLL forms a multicomponent complex that specifically methylates lysine 4 on histone H3 (H3K4), a modification typically associated with transcriptionally active regions of chromatin. Targeted homozygous disruption of *MLL* in mice was embryonic lethal at day 10.5–16, depending on the particular knockout allele [6, 7]. Mll-deficient mice exhibit defective yolk sac and fetal liver hematopoiesis [8, 9], and *Hox* gene expression was initiated but not maintained in these mice [10]. Animals carrying a single normal *Mll* allele are phenotypically abnormal, with mild anemia and thrombocytopenia [8, 9], and studies using chimeric mice reconstituted with *Mll* deficient or hemizygous embryonic stem cells suggest that *Mll* is essential for hematopoietic stem cell (HSC) development itself, or for the transition of HSC to multipotent progenitors [11].

The product of *MLL* gene is a large protein of 3,969 amino acid residues, and contains several conserved domains, including DNA-binding AT hooks, a cysteine-rich CxxC domain with homology to DNA methyltransferases, plant homeodomain (PHD) finger motifs, a Bromo domain (BD), a transactivation domain (TAD), a nuclear receptor interaction motif (NR box), a WDR5 interaction or Win motif, and a C-terminal SET domain, which is responsible for MLL's histone methyltransferase activity [12]. The full-length MLL protein is cleaved by taspase, an aspartic protease, into two fragments MLL-N and MLL-C, which are both core components of MLL complex [13–15]. A growing body of evidence has revealed MLL to be a member of a large multi-protein complex that contains proteins involved in chromatin modification/remodeling. At the amino-terminal end of MLL, the Menin-binging motif (MBM) mediates the interaction with Menin, which is the product of the tumor suppressor gene MEN1 [16]. Menin and MLL form an interaction surface for LEDGF (PSIP1, p75) and LEDGF forms a contact with chromatin through its PWWP domain. MLL–Menin–LEDGE complex is critical for the proper targeting of MLL or MLL fusion proteins to specific target genes [17]. Recently, another important complex, the Polymerase Associated Factor complex (PAFc), has been found to interact with CxxC-RD2 region in MLL [18, 19]. PAFc plays important roles in a wide range of biological processes, including the initiation, elongation, and termination of gene transcription, cell cycle regulation, mRNA processing, H2B monoubiquitination (cooperating with BRE1/RAD6 complex), H3K4 methylation and H3K79 methylation (cooperating with DOT1L) [20]. PAFc–MLL interaction is critical for transcriptional activation of MLL and MLL fusions, and is essential for MLL fusion-mediated leukemogenesis [18].

Within MLL-C subunit lies a TAD domain that recruits the histone acetyltransferase (HAT) cAMP response element–binding protein (CREB)-binding protein (CBP). The CBP and its homolog p300 are general transcriptional co-activators that contain histone and transcription factor acetylation activities [21]. Another protein association with MLL-C is the histone H4 lysine 16 specific acetyltransferase MOF that loosens up chromatin by histone charge neutralization [22]. MLL-C also associates with a core complex of proteins, including WDR5, RBBP5, ASH2L, and DPY30. These cofactors are necessary for MLL methyltransferase activity [23, 24]. At the C-terminus of

MLL-C is a SET domain, which is responsible for the H3K4 methyltransferase activity of MLL (Fig. 1). H3K4 methylation is primarily localized in the promoter and regulatory regions of genes. Some transcription factors, such as Menin, LEDGF, HCF1/2, E2F, NFE2, p53, and c-Myb, have been shown to interact with MLL family members, which could lead to methylation of H3K4 in a locus- or DNA sequence-specific manner [16, 17, 25–29]. A recent study by our group identified a physical and functional interaction between RUNX1 and MLL, and showed that both are required for maintenance of the H3K4me3 mark at two critical regulatory regions of the RUNX1 target gene PU.1 locus [30].

Disordered epigenetic regulation in MLL fusions

Balanced translocations disrupt *MLL* in a breakpoint cluster region that span exons 8–12, thereby deleting the sequences that are conserved with trx and replacing them with one of over 70 different translocation partners. All MLL fusion proteins retain the amino-terminal portion of MLL containing the Menin interaction domain, AT hooks, and the CxxC domain, thus preserving DNA-binding

activity. Interestingly, some reciprocal *MLL* fusion partners fused out-of-frame to the 3′-*MLL* gene segment identified in a 760 *MLL*-rearranged leukemia cohort, which suggests that truncated MLL itself may also contribute to leukemogenesis [3]. It has been shown that the LEDGF-Menin binding motif and the CxxC domain were absolutely necessary for the proper targeting of MLL fusions to specific target gene loci, such as *HoxA9*, and for transcription upregulation and leukemogenesis [16, 17, 31]. Another interaction maintained in MLL fusion proteins is with PAFc via CxxC domain and RD2 region of MLL. PAFc is a transcriptional activation complex associated with RNA polymerase II and H2B monoubiquitination, which was catalyzed by BRE1/RAD6 complex. It appears that MLL fusion proteins employ both protein-DNA interactions involving the AT hooks and CxxC domain and protein–protein interactions with Menin/LEDGE and PAFc for proper targeting to specific loci [32].

Based on the cellular localization, the translocation partners can be divided into two classes. Most frequent MLL partners, such as AF4, AF9, ENL, AF10 and ELL, are localized in the nucleus, while AF6, AF1p, and SEPT6 are localized in cytosol. The MLL translocation partner genes (TPGs) show different functions involved in
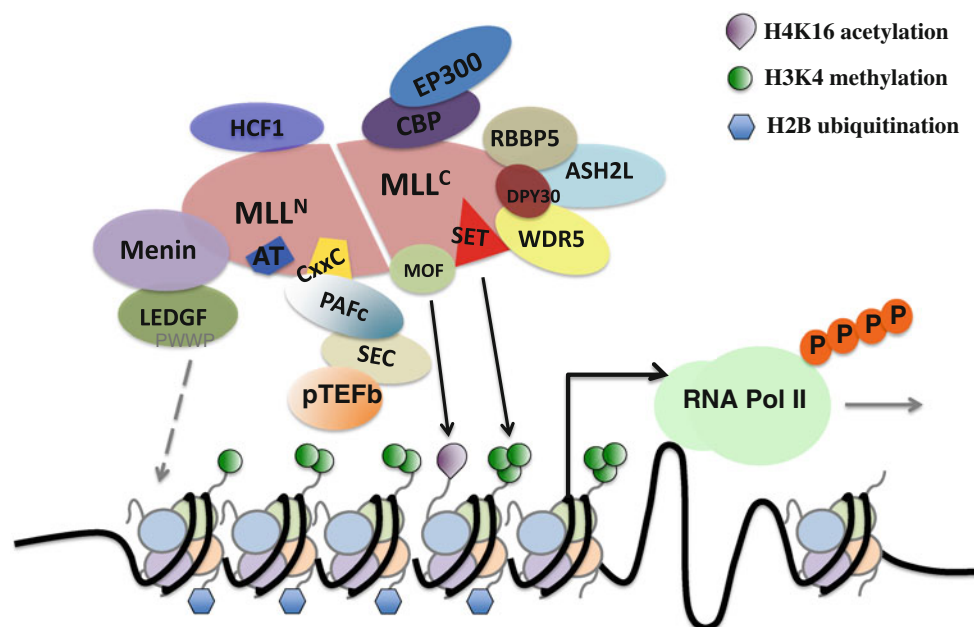
Fig. 1 Wild-type MLL core complex. Wild-type MLL protein is cleaved into MLL^N and MLL^C fragments by Taspase. MLL^N interacts with HCF1, Menin and LEDGF. LEDGF binds to chromatin through its PWWP domain. MLL^N also contains AT hooks and CxxC domain that can bind directly to DNA. The CxxC domain and adjacent RD2 domain of MLL^N interact with Polymerase Associated Factor complex (PAFc), which plays important roles in recruiting Super Elongation Complex (SEC), positive transcription elongation factor b (pTEFb) and other factors. pTEFb releases negative regulation of RNAP II by pTEFb-dependent phosphorylation of RNAP II CTD, priming the target promoter for transcription elongation. PAFc and BRE1/RAD6 complex also catalyzes H2B monoubiquitination. MLL^C fragment contains a SET domain, which is responsible for the H3 lysine 4 methylation activity. A sub-complex WDR5-RBBP5-ASH2L-DPY30 interacts with MLL^C and facilitates SET domain-mediated H3K4 methylation. The C-terminal of MLL protein also contains a transactivation domain (TAD), which recruits CBP/p300 proteins as well as H4 lysine 16 acetyltransferase MOF

transcriptional elongation, histone acetylation, DNA or RNA binding in the nucleus or involved in signaling, and are associated with the membrane or display extracellular localization (Table 1) [3]. A common mechanism for the most frequent *MLL* rearrangements is recruitment of positive transcription elongation factor b (pTEFb). In the presence of pTEFb kinase (CDK9/CCNT1) and TFIIH (CDK7/CCNH), the C-terminal domain-tail of largest subunit of RNA polymerse II (RNA Pol II) is phosphorylated at serine 2 and 5, thus the "promoter-arrested RNA Pol II" is converted into "elongating RNA Pol II" [33]. Emerging evidence shows that the products of *AF4, AF9, ENL, AF10,* and *ELL* participate in the pTEFb-dependent transcriptional activation cycle of RNA Pol II. Normally, AF4 serves as a protein-binding platform for several other proteins involved in the super elongation complex (SEC). pTEFb binds to N-terminal of AF4, whereas ENL and/or AF9 interact with C-terminal of AF4. The assembled AF4 complex recruits DOT1L to facilitate the elongation of RNA Pol II. DOT1L methylates H3K79 to maintain active transcription and/or serve as a transcription memory for target genes loci (Fig. 2).

A recent study has also demonstrated that the recruitment of HDACs to MLL-bound promoters is mediated by the MLL-PHD3-Bromo cassette stabilized by Cyp33 [34]. In MLL-rearranged leukemia, loss of the PHD fingers has been confirmed to be required for the transforming activity [35]. Other MLL fusion proteins that influence histone acetylation are MLL-CBP and MLL-p300, which have been found in therapy-related secondary leukemia [36, 37]. Though the MLL-CBP/p300 function on target chromatin

loci has not been fully investigated, it is reasonable to assume that the permanent recruitment of HAT activity will induce hyperacetylation of chromatin at the target genes and increased transcriptional output, leading to transformation.

Disordered epigenetic regulation in MLL-PTD

Unlike MLL fusions, MLL-PTD has been identified only in AML or MDS. MLL-PTD was first observed in de novo AML with a normal karyotype or trisomy 11 [38]. Cloning of this region revealed a partial duplication within the 5′ region of the *MLL* gene. These duplications consist of an in-frame repetition of MLL exons in a 5′–3′ direction and lead to a potentially translatable sequence [38, 39]. MLL-PTD has been detected in AML and there are only few subtypes of PTD duplications have been found, which are subtype I (Ex9/Ex3 duplication), II (Ex10/Ex3 duplication) and III (Ex11/Ex3 duplication). The frequency of these subtypes in AML is 58, 14, and 28 %, respectively [40].

Two observations reveal potential mechanisms whereby *MLL*-PTD may disturb normal hematopoiesis: (1) repetitive DNA-binding domains (AT hooks and CXXC domain), present in *MLL*-PTD, exhibit transactivation potential in vitro [41]; mechanistically, MLL-PTD may employ more PAFc and PAFc-associated complexes through the duplication of CxxC and RD2 domains to target loci; (2) studies indicate that the other WT MLL allele is silenced by DNA methylation in case of MLL-PTD, while as the WT MLL allele is required for MLL

Table 1 Distribution of major MLL fusion partners in acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) patients with MLL rearrangements; and the cellular functions and localizations of these major translocation partner genes (TPGs)

Translocation partner genes (TPGs)	Leukemia type	Frequency		Cellular function	Localization
		ALL	AML		
AFF1/AF4	ALL, t-ALL	66 %	–	Transcriptional elongation	Nucleus
MLLT3/AF9	AML, t-AML, ALL	8.5 %	30.4 %	Transcriptional elongation	Nucleus
MLLT1/ENL	ALL, AML, t-AML	14.9 %	5.4 %	Transcriptional elongation	Nucleus
MLLT10/AF10	AML, t-AML	2.7 %	14.5 %	Transcriptional elongation	Nucleus
MLLT4/AF6	AML, t-AML, ALL	1.5 %	10.1 %	Signaling and regulation of signaling	Cytosol
ELL	AML, t-AML	–	10.9 %	Transcriptional elongation	Nucleus
EPS15/AF1P	ALL, AML, CML	1.4 %	2.1 %	Endocytosis	Cytosol
MLLT6/AF17	AML	–	2.8 %	Transcriptional elongation	Nucleus
SEPT6	AML	–	2.5 %	Cell cycle, cytokinesis, organization of nucleus cytoskeleton	Nucleus
EP300/P300	t-AML	–	Rare	Histone acetylation	Nucleus
CREBBP/CBP	t-MDS, t-AML, t-ALL	Rare	Rare	Histone acetylation	Nucleus
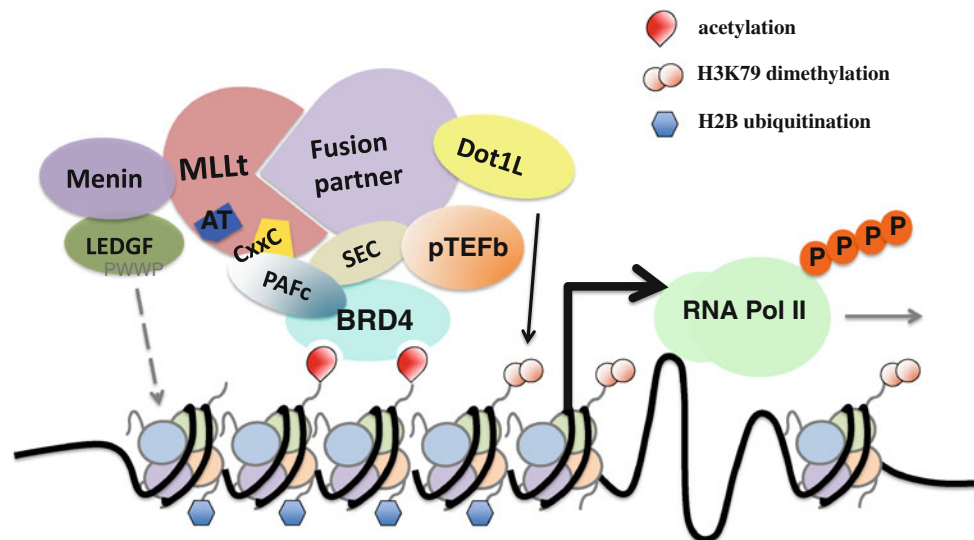
Fig. 2 Protein complexes of major MLL fusions. MLL fusion proteins retain its interaction with Menin, LEDGF and PAFc complex, but lose its PHD fingers region, HCF1 interaction region and SET domain containing MLL^C fragment; therefore, MLL fusions lose the ability to catalyze H3K4 methylation. A subset of major MLL fusions, MLL-AF10, MLL–ENL and MLL-AF9, directly interact with DOT1L, which catalyze the methylation of H3K79. Some other major MLL fusions, including MLL-AF4, MLL-AF5q31 and MLL- ELL1, interact directly with p-TEFb, but indirectly with DOT1L. Most of these major fusion partners directly interact with super elongation complex (*SEC*), together with PAFc, SEC binds bromodomain and extra-terminal (BET) family member BRD4, which is able to bind acetylated histone through its bromodomains

fusion leukemias [42]. The releasing of WT *MLL* gene suppression in *MLL*-PTD positive AML by inhibitors of DNA methyltransferase and histone deacetylase induces AML blast apoptosis [43]. These two findings suggest that *MLL*-PTD pathway(s) of leukemic transformation are different from those resulting from the fusion of *MLL* with partners other than itself.

Murine knock-in models that express MLL-PTD show Hoxa9 but not Meis1 upregulation [44]. Our recent data show that murine MLL-PTD HSPCs exhibit elevated apoptosis at steady state, but become proliferative and resistant to apoptosis when exposed to stresses. The MLL-PTD derived phenotypic short-term (ST)-HSCs/ multipotent progenitors (MPPs) and granulocyte/macrophage progenitors (GMPs) have self-renewal capabilities, rescuing hematopoiesis by giving rise to long-term repopulating cells in recipient mice with an unexpected myeloid differentiation blockade and lymphoid-lineage bias [45]. Hoxa genes have been shown to be important for proliferation and leukemic transformation. However, it is unclear whether they also promote reprogramming or enhanced self-renewal of more differentiated cells like MPP and GMP. Hoxb4 have been shown to promote self-renewal and expansion of immature cells in vitro and in vivo. It has been reported that MLL-PTD AML have upregulated Hoxb genes [46]. Thus, upregulated Hoxa and/or Hoxb genes may contribute to the phenotypes. Further investigation is needed to provide more insights into how MLL-PTD stimulates these altered differentiation and repopulating

properties. MLL is involved in epigenetic regulation of H3K4 methylation. It remains unclear how MLL-PTD affects MLL methyltransferase activity, in which the SET domain is still present in the PTD allele. The understanding of the epigenetic function of MLL-PTD is largely derived from speculations based on the similarity of MLL-PTD protein structure with WT MLL and MLL fusions (Fig. 3). However, data from our group have clearly demonstrated that there is no global change in H3K4me3 and H3K4me2 methylation levels in MLL-PTD heterozygous mice bone marrow cells. Thus, the function of MLL-PTD may be locus-specific. Further studies are needed to gain a better understanding of the details of the molecular mechanism and epigenetic function of MLL-PTD.

Recent trials of epigenetic therapy targeting MLL leukemia

Over the past decade, structurally distinct histone acetyltransferases (HATs), histone deacetylases (HDACs), histone arginine and lysine methyltransferases (HMTs) were identified and linked to gene regulatory complexes. MLL fusion protein complex associates with specific epigenetic modification enzymes and chromatin proteins, which are responsible for its proper targeting to the downstream targets loci and gene activation. These proteins could be served as ideal targets for leukemia therapies.
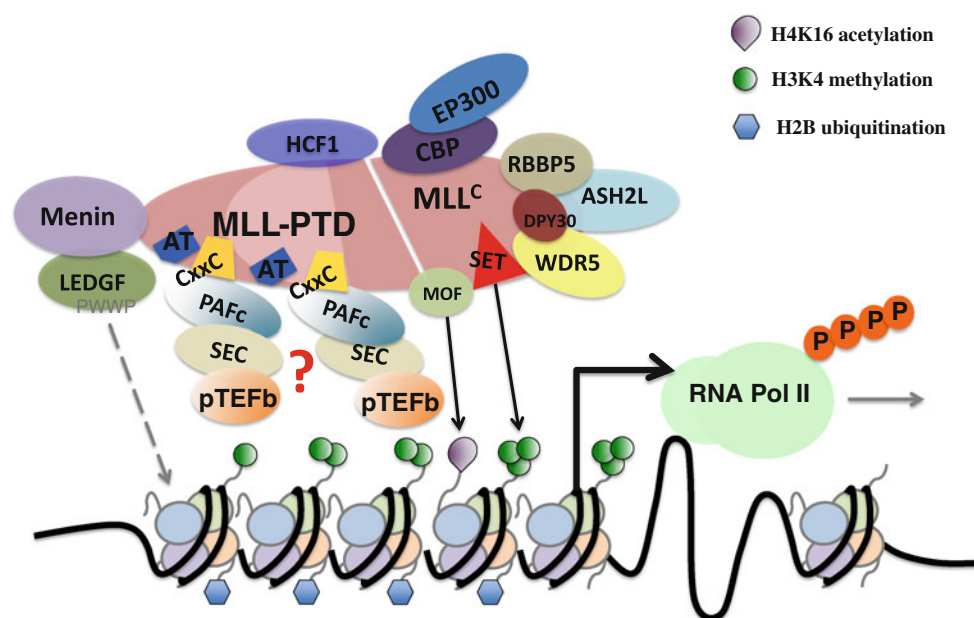
Fig. 3 Hypothetical MLL-PTD complex. MLL-PTD is generated by duplication of the internal exons/introns encoding the N-terminus of wild-type MLL; as a result, this protein has in-frame duplicated AT hooks and CxxC domain of MLL. The PTD regions may recruit two sets of PAFc/SEC/pTEFb complexes and still retain the Menin, LEDGF and HCF1 interaction in the MLL^N, and CBP/p300, MOF and WDR5-RBBP5-ASH2L-DPY30 interactions in the MLL^C as WT MLL. However, this is mostly based on extrapolation from the WT MLL interaction network. Compared WT MLL or MLL fusions, the biological function and molecular mechanism for MLL-PTD remain largely unknown

Although MLL can potentially fuse with many different partners, it has been shown that the most common MLL fusion partners, such as AF4, AF5, AF9, AF10 and ENL exist in a SEC complex that also contained DOT1L [47], and recently studies have shown that MLL-rearranged leukemias, such as MLL–AF4 and MLL–AF9, are dependent on aberrant H3K79 methylation mediated by DOT1L [48]. DOT1L is the only known H3K79 methyltransferase and the only lysine methyltransferase without a SET (Suppressor of variegation, Enhancer of zeste, and Trithorax) domain [49]. H3K79 methylation is associated with transcription elongation, suggesting that the MLL fusion protein enhances expression of leukemogenic target genes through DOT1L-mediated enhanced elongation. The unique characteristics of DOT1L and the important role it plays in MLL leukemia make it an attractive drug target for major MLL-rearranged leukemia. Based on the chemical structures of the S-adenosylmethionine (SAM) substrate, which is a common methyl group donor for HMTs, EPZ004777 is developed to mimics the binding of DOT1L to SAM [50]. Most strikingly, EPZ004777 displays remarkable selectivity for inhibiting DOT1L and has no effect on other HMTs. EPZ004777 could decease H3K79 methylation and block MLL fusion targets expression in vitro, inhibit proliferation, and induce differentiation and apoptosis in cells bearing the MLL gene translocations, such as MLL–AF4 or MLL–AF9 (Fig. 4). EPZ004777 has little effect on non-MLL leukemic cells. The in vivo administration of EPZ004777 has both pharmacodynamics and anti-tumor efficacy in an MLL leukemia mouse xenograft model [51]. These results provide compelling support for DOT1L inhibition as a basis for targeted therapeutics against MLL leukemia. Recently, The Leukemia and Lymphoma Society (LLS), the world's largest voluntary health agency dedicated to blood cancer, has announced that it plans to advance DOT1L as a personalized therapeutic for patients with MLL leukemia. They will support the program through a Phase 1 clinical trial.

A link between the recognition of histone acetylation and MLL leukemia has been discovered, and offers another attractive therapeutic possibility. Members of the bromodomain and extra-terminal domain (BET) family of proteins (including BRD2, BRD3, BRD4 and BRDT) [52] harbor bromodomains, a conserved structural motif that mediates recognition of side-chain acetylated lysine residues. BRD4 and BRD3 have been implicated in human cancer. In addition, BRD4 has recently been identified as a component of a recurrent t (15;19) chromosomal translocation genetically defining the so-called NUT (nuclear protein in testis) midline carcinoma (NMC) [53, 54]. BRD4 binds to transcriptional start sites of genes during the M/G1 transition, influencing mitotic progression [55]. Notably, BRD4 is also a critical mediator of transcriptional elongation, functioning to recruit pTEFb [52, 56]. The fact that BRD4 co-purifies with several components of the PAFc and SEC links BRD4 to MLL-rearranged leukemia. Zuber
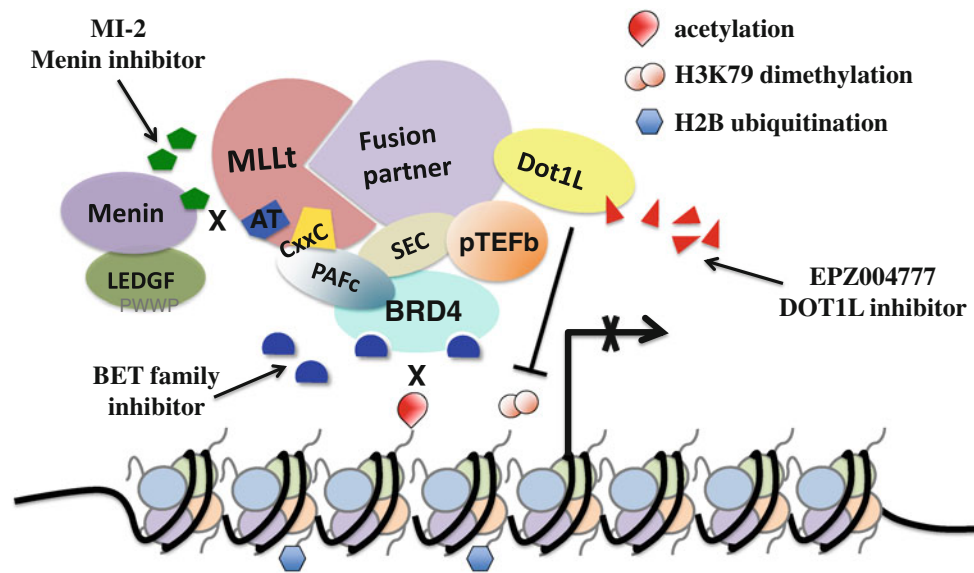
Fig. 4 Epigenetic therapies targeting MLL leukemia. MLL fusion protein complex associates with specific chromatin proteins and epigenetic modification enzymes, which are responsible for its proper targeting to downstream target loci, histone modification and gene activation. These proteins may serve as ideal targets for anti-leukemia therapies. BRD4 interacts with acetylated histones by recognizing two acetylated lysines within a certain distance. Small-molecule inhibitors (JQ1 or I-BET) which bind to BRD4, preventing its interaction with chromatin, lead to repression of downstream targets. DOT1L inhibitor EPZ004777, which mimics the binding of SAM to DOT1L, displays remarkable selectivity for inhibition of DOT1L enzymatic activity and potently inhibits DOT1L to methylate H3K79. EPZ004777 reduces MLL fusion's transcriptional activity and inhibits MLL leukemia development in vitro and in vivo. Small-molecule MI-2, which specifically blocks MLL-Menin interaction, can effectively reverse MLL fusion protein mediated leukemic transformation by down-regulating the expression of MLL fusion target genes required for transformation

et al. [57] identified BRD4 as a unique cancer dependency in MLL-rearranged AML using RNA interference screening. The recent development of direct-acting, competitive inhibitors of BET bromodomains (such as JQ1 and I-BET) has created opportunities for MLL therapy. Researchers identified potent antiproliferative activity in a murine model of MLL–AF9 and mutant NRas (NRasG12D) using BET bromodomain inhibitor JQ1 [57]. JQ1 binds directly to the acetylated lysine (Kac) binding site of BET bromodomains [58], which causes monocytic differentiation and growth arrest in cells with MLL translocations. GSK1210151A (I-BET151), a novel small molecule inhibitor of the BET family, induces early cell cycle arrest and apoptosis against human and murine MLL fusion leukemic cell lines. By displacement of BRD3/4, PAFc and SEC components from chromatin, I-BET151 eventually inhibits MLL target genes transcription (Fig. 4). I-BET151 also has been shown to present significant therapeutic value in the in vivo studies, providing survival benefit in two distinct mouse models of murine MLL–AF9 and human MLL–AF4 leukemias [59].

Although epigenetic enzymes are good targets for therapies, recent evidence has indicated that transcription factors and their co-factors could also be ideal targets for therapies. MLL fusion proteins recruit many transcription complexes through various mechanisms. Even though the details still remain to be elucidated, there is a clear possibility that targeting these interactions as therapeutics may benefit for MLL leukemia therapy [60]. One of the important complexes is Menin–LEDGF factor. Both WT MLL and MLL fusion are recruited to target genes through their direct interaction with Menin and LEDGF. Menin is a tumor suppressor that plays a role in transcriptional activation and directly controls cell growth in endocrine organs. Loss of Menin relieves the differentiation block induced by MLL fusion proteins in transformed leukemic blasts [16]. A Menin–MLL inhibitor, MI-2, was designed as an alternative therapeutic approach to MLL leukemia. MI-2 specifically binds to Menin with nanomolar affinities, and blocks Menin–MLL interaction. Without Menin as the bridge, MLL also loses the interaction with LEDGF and chromatin association. MI-2 induces cell growth arrest, terminal differentiation, and blocks transformation by MLL fusions. MI-2 treatment may downregulate the expression of MLL fusion target genes in particular; MI-2 treatment reduces Menin–MLL–AF9 occupancy on the Hoxa9 locus and impairs Hoxa9 expression [61]. These results suggest that Menin–MLL inhibitors are also feasible targets and are as capable of reversing the leukemogenic activity of MLL fusion proteins as the epigenetic inhibitors (Fig. 4).

Although MLL-PTD related leukemia shows outcomes equally poor as those in MLL fusions, study of the molecular mechanism and targeted therapies for MLL-PTD are far behind those for MLL fusions. Given the similar protein

structure between MLL-PTD and WT MLL, newly developed drugs, such as I-BET or MI2 may also be effective for MLL-PTD leukemia, as SEC/BRD4 and Menin interactions could be critical for MLL-PTD function. Recent evidence has demonstrated a role for histone deacetylase and DNA methyltransferase inhibitors in leukemia mediated by the *MLL*-PTD [62]. In contrast to leukemic cells with *MLL* translocations, blasts with the *MLL*-PTD do not express the wild-type MLL protein from the contralateral unaffected allele. Interestingly, exposure of *MLL*-PTD leukemic cells to histone deacetylase and DNA methyltransferase inhibitors resulted in reversal of the *MLL* wild-type allele silencing. Furthermore, the re-expression of MLL wild-type protein induced selective sensitivity to apoptosis, suggesting that the loss of MLL wild-type function is critical for leukemic transformation induced by MLL-PTD [43]. However, it remains unclear whether the effect of histone deacetylase and DNA methyltransferase inhibitors is specifically mediated through upregulation of wild-type MLL, or through inhibition of MLL-PTD, or another of the pleiotropic effects of these compounds. More efforts will be needed for understanding the MLL-PTD and for finding new therapies for MLL-PTD leukemia.

Concluding remarks

MLL-rearranged leukemias exemplify malignancies with perturbations of the epigenetic regulation. Specific chromatin targeting and modifications that aid in the perpetuation of MLL fusions or MLL-PTD driven oncogenic programs are being defined, which presents novel avenues for therapeutic intervention. Proof-of-concept studies using small-molecule inhibitors targeting the histone methyltransferase DOT1L, the acetyl-histone binding protein bromodomain containing protein 4 (BRD4), or Menin–MLL interaction, have recently been reported, showing potent activity against MLL-rearranged leukemias in preclinical models. It is apparent that intensive efforts will be made toward the further development of small-molecule inhibitors targeting chromatin-associated protein targets in leukemia. These studies may lead to the development of a new generation of targeted therapies in MLL related leukemia.

Despite these emerging and promising results for MLL new therapies, several questions remain to be answered. First, whether all MLL-rearranged leukemias, including MLL-PTD, respond to DOT1L inhibitor or not? Most studies demonstrating the role of chromatin modifications in MLL-rearranged leukemias have been using murine or human cell line models of the most common MLL fusions, such as MLL–AF9, MLL–ENL and MLL–AF4. It is unclear whether leukemic transformation in patients bearing other partner genes, such as MLL–AF6, also involves similar mechanisms, including DOT1L recruitment. There are 70 different translocation partners for MLL and only a few of these involve in SEC complex which interacts with DOT1L. Most of the TPGs are not associated with transcriptional elongation. There are even some reciprocal *MLL* fusion partners fused out-of-frame to the 3′-*MLL* gene segment, which may only produce a truncated MLL fragment instead of MLL fusions. The other major MLL rearrangement, MLL-PTD, apparently does not interact with DOT1L either. Thus, it would be of interest to test whether those leukemia cell lines or patients without SEC component translocations also respond to DOT1L inhibitor. Second, some less common MLL rearrangements, such as MLL–CBP, MLL–extra eleven nineteen protein (EEN) or MLL-PTD, may act in ways that are different from the examples involved in DOT1L. However, Menin and/or iBET inhibitors could show therapeutic efficacy based on their requirement for Menin and PAFc/BRD4 on leukemic transformation, although we could not completely rule out the involvement of SEC/DOT1L in these leukemias. Therefore, it will be also important to test these new drugs on various MLL-rearranged leukemias including MLL-PTD to understand their selectivity to certain type of patients. Third, normal hematopoiesis is notably perturbed following Dot1l deletion [63, 64], but studies suggest MLL fusion driven leukemia displays a higher dependence on DOT1L than normal hematopoietic stem cells. Similar concerns would apply to BRD4 inhibition or Menin inhibition for the general requirement of these factors on gene regulation in normal cells. Studies should focus on the relative dependence of normal versus leukemic cells on these chromatin regulators so that a clinically applicable therapeutic target or window can be identified. These studies may pave the way for the discovery of novel therapies targeting chromatin targeting and modifications altered in MLL-related leukemias.

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