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Epigenetic therapies by targeting aberrant histone methylome in AML: molecular mechanisms, current preclinical and clinical development

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Abstract

While the current epigenetic-drug development is still largely restricted to target DNA methylome, emerging evidence indicates that histone methylome is indeed another major epigenetic determinant for gene expression and frequently deregulated in acute myeloid leukaemia (AML). The recent advances in dissecting the molecular regulation and targeting histone methylome in AML together with the success in developing lead compounds specific to key histone methylation modifying enzymes have revealed new opportunities for effective leukaemia treatment. In this article, we will review the emerging functions of histone methyltransferases (HMTs) and histone demethylases (HDMs) in AML, especially *MLL* rearranged leukaemia. We will also examine recent pre-clinical and clinical studies that show significant promises of targeting these histone methylation modifying enzymes for AML treatment.

Introduction

Although intensive chemotherapy combined with transplantation of haematopoietic stem cells have considerably improved the outcomes in certain subgroups of younger leukaemia patients, acute myeloid leukaemia (AML) as the most common type of acute leukaemia in adults remains highly fatal and around 80% of patients aged over 60 succumb to the disease or the highly toxic treatment regimens [1, 2]. AML is a heterogeneous group of diseases that can be further classified into different subgroups according to their distinctive genetic mutations with variable prognostic significances. In spite of the large arrays of mutations reported in AML, most of them specifically affect transcription factors or key components of epigenetic machinery. Importantly, chimeric fusions that are believed to be the initiating events in translocation leukaemia almost always involve transcription/epigenetic factors [3]. Among them is the *Mixed Lineage Leukaemia Gene (MLL)* that associates with a very poor prognosis and treatment resistant [4]. Similar mutational profiles affecting transcriptional and epigenetic machinery have also been reported in normal karyotype AML (NK-AML), where DNMT3A and NPM are found to be the early events and persist during relapses [5-7], consistently indicating the importance of transcriptional deregulation in AML pathogenesis. In spite of our advance in understanding the genetics of AML, very little has been translated into the clinics and we are still using the same highly toxic and rather ineffective chemotherapies developed over a half-century ago. Therefore, there is an urgent need to identify novel venues for more potent and effective drug development to tackle this formidable disease. While development of small molecule inhibitors to transcription factors remains technically challenging, the recent discoveries of critical function of epigenetic modifying enzymes with structurally rigid motifs and/or catalytic domains in AML pathogenesis have fuelled the enthusiasm to target these intractable oncogenic events. In this review, we will focus on some of the latest pre-clinical and clinical development of epigenetic therapy in AML in particular those involve *MLL* gene rearrangements.

Epigenetic therapies targeting DNA methylation and histone acetylation in AML

The term *epigenetics* refers to alternations of gene expression that are inheritable after cell division without any changes in DNA sequence [8]. In addition to DNA methylation, an increasing number of epigenetic modifications on histones including acetylation, methylation and ubiquitination have been identified and are frequently deregulated in AML [9, 10], resulting in repression of tumour suppressor genes (TSGs) and/or activation of oncogenic pathways [11]. Aberrant DNA methylation and histone acetylation are two most ancient and better-characterized epigenetic changes. DNA methylation, leading to gene silencing, is prevalent in cancers including leukaemia, and has been the target for cancer therapy since the FDA approval of DNA methyltransferase (DNMT) inhibitors (DNMTi), azacytidine and decitabine for the treatment of myelodysplastic syndrome (MDS) and certain AML [12]. Although AML patients aged over 65 years who treated with DNMTi did not show significantly longer overall survival (OS) as compared with conventional care regimen, azacytidine and decitabine displayed safety and better clinical efficacy in patients with unfavourable cytogenetics or myelodysplasia-related changes, indicating that they may be preferable therapies for these "difficult-to-treat" AML population [13, 14]. In addition to DNMTi, a number of pan histone deacetylase (HDAC) inhibitors (HDACi) inducing chromatin remodelling and re-expression of TSGs, are also designed and utilized in AML treatment [15]. While single-agent therapy was reported only having modest clinical activity, combination of HDACi with DNMTi (decitabine, complete remission (CR): 31%) or with Ara-c (cytarabine, CR: 78%, OS: 82 weeks) in clinical trials appeared to be synergistic and profoundly improved responses [16, 17]. While these early endeavours on heterogeneous myeloid malignancies have demonstrated the safety and potential therapeutic values of targeting epigenetic machinery in clinical settings, it also urges the need of better understanding of the epigenetic regulation and exploring novel critical targets for effective AML treatment. To overcome the problems associated with genetic heterogeneity that may in part account for the poor efficacy of DNMTi or HDACi in the clinics, recent studies focusing on systematic analyses of leukaemia carrying chimeric transcription factors or specific mutations affecting histone methylation modifying enzymes provide important insights and novel tractable targets for epigenetic therapies in AML.

The role of histone methyltransferases (HMTs) in AML

Depending on the position and nature of the methylated residues, histone methylation can have positive as well as negative impacts on gene expression [18]. Histone methylation features epigenetic modification in which lysine and arginine residues can be mono-(me1), di-(me2) or even tri-(me3) methylated (for lysine only). In general, methylation of histone 3 lysine 4 (H3K4), lysine 36 (H3K36), lysine 79 (H3K79) as well as asymmetric dimethylation of histone 4 arginine 3 (H4R3) activates gene expression; whereas methylation on other sites like histone 3 lysine 9 (H3K9), lysine 27 (H3K27), histone 4 lysine 20 (H4K20) and symmetric dimethylation of H4R3 associate with transcription repression [18, 19]. H3K4me3 and H3K27me3 that define bivalent marks are predominately mediated by two master

epigenetic regulators, Trithorax group (TrxG) proteins with HRX/MLL as the founding member and Polycomb group (PcG) proteins with EZH1/2 as the catalytic subunits of polycomb repressor complex 2 (PRC2) in mammalian cells [20]. Intriguing, the key components of both TrxG and PcG complexes are frequently mutated in AML.

Investigating the association of chromosome 7q abnormalities in myeloid malignancy has revealed an important role of EZH2 in leukaemogenesis. EZH2 regulates expression of numerous genes critical for stem cell renewal by mediating a H3K27 methylation [21]. EZH2 mutations were found in 9 of 12 patients with chromosome 7q acquired uniparental disomy, and the majority of EZH2 mutations resulted in loss of its H3K27 methyltransferae activity [22], which is in contrast with its gain of function mutation in B-cell lymphoma [23]. Deletion of EZH2 was able to induce a MDS-like disease in a mouse model, suggesting the tumour suppressor function of EZH2 in certain myeloid malignancies [24-26]. On the other hand, loss-of-function mutations of ASXL1, another PcG protein, are usually associated with unfavourable OS and poor CR rate in AML [27]. While its molecular function in leukemic transformation is still unclear, depletion of ASXL1 showed loss of PRC2 mediated H3K27 trimethylation and led to upregulation of *HOXA* genes including *HOXA5* and *HOXA9*. On the contrary, overexpression of ASXL1 resulted in a global increase of H3K27 me2/3 and suppression of *HOXA* genes and cell growth. ASXL1 can interact with EZH2 in human leukemic cells, and loss of ASXL1 resulted in displacement of PRC2 from *HOXA* loci [28]. ASXL1 may also collaborate with BAP1, loss of which led to a MDS-like syndrome in a mouse model, to deubiquitinate H2AK119 at PcG targets [29, 30]. Haematopoietic-specific knockout of ASXL1 profoundly impaired cell differentiation and induced myeloid dysplasia and erythroid dysplasia in knockout mice. Furthermore, transplantation of ASXL1-null LSK cells or bone marrow (BM) cells into recipient mice strikingly caused lethal myelodysplastic disorder [31]. In addition to ASXL1, JARID2 has also been identified as an essential cofactor in promoting PRC2 recruitment to downstream targets. An acquisition of JARID2 mutation showed a positive correlation with disease progress from MDS to AML [32]. Together, these studies reveal the critical role of EZH2 and PRC2 in malignant haematopoiesis.

MLL as a master transcriptional and epigenetic regulator containing a number of functional domains including AT hook and CXXC motifs at the N-terminal and the C-terminal SET domain, which mediates specific H3K4 methylation, is predisposed to abnormal gene rearrangements resulting in a highly aggressive form of leukaemia [33]. As a result of chromosomal translocations, chimeric MLL-fusions resulting from replacement of C-terminal region of MLL including the SET domain by various fusion partners such as AF4/6/9/10, ELL and ENL can form macromolecular complexes through recruitment of a cohort of cofactors including super elongation complex (SEC) (e.g., positive transcription elongation factor b (p-TEFb), MLL fusion partners such as AF4 family and AF9/ENL family), polymerase associated factor complex (PAFc), BRD3/4, MENIN and key histone methyltransferases (HMTs) (e.g.,

DOT1L, PRMT1) to activate gene expression programmes crucial for the transformation [18] (Fig.1A). Identification of key aberrantly recruited HMTs by MLL-fusions provide the first hint of their involvement in human cancer [34].

DOTlL is the only lysine methyltransferase (KMT) known to be responsible for H3K79 methylation in human. Aberrant recruitment of DOT1L specifically associates with an abnormally high level of H3K79me2 on promoters and gene bodies of MLL targets in MLL rearranged leukaemia. The remarkable correlation of H3K79me2 and MLL targets has been referred to as a special epigenetic lesion in MLL leukaemia, implying the essentiality of H3K79 methylation for MLL-driven transcription [35]. Inactivation of DOT1L profoundly suppressed expression of MLL translocation-associated genes (e.g., *HOXA*s and *MEIS1*) and leukaemia development [36-40] (Fig. 1A). Direct fusion of DOT1L to MLL was sufficient to activate transcription of *HOXAs* [39]. Loss of DOT1L resulted in reduction of cell growth, increased differentiation and apoptosis of MLL-AF9 leukaemic cells, indicating its potential as a target for AML therapy [38]. On the other hand, PRMT1 is the founding member of protein arginine methyltransferases (PRMTs) that mediates arginine methylation on both histone (H4R3me2a) and non-histone substrates (e.g., transcription factors and splicing factors). Identification of its essential function in MLL leukaemia had also provided the first evidence of PRMT involvement in human cancer [41]. PRMT1 recruitment is required for a subset of MLL (MLL-EEN and MLL-GAS7) and non-MLL (MOZ-TIF2 and AML1-ETO) leukaemia [41-43]. Its inhibition resulted in specific transcriptional and leukaemic suppression in MLL-rearranged and MOZ-TIF2 leukaemia [42]. Silence of PRMT1with an shRNA approach attenuated the level of H4R3me2a and gene expression of HOXA9 and MEIS1, thus leading suppression of leukaemogenesis of MOZ-TIF2 and MLL-GAS7. More recently, a functional link between PRC2 and MLL leukaemia had also been proposed. EZH2 and EED, two core components of PRC2, had been shown to be required for MLL leukaemia, although the underlying mechanisms remain largely unknown [44, 45]. While these studies highlight the importance of HMTs in MLL leukaemia, emerging evidence also reveals an equally important role of histone demethylases (HDMs) that counteract the functions of HTMs in modulating the epigenetic regulation of gene expression in both normal and cancer settings.

The role of histone demethylases (HDMs) in AML

Protein methyltransferases (including KMTs and PRMTs indicated in the previous section) mediate methylation on specific amino acid residues, which can however be erased by HDMs mostly lysine demethylases (KDMs). Based on their catalytic mechanisms, KDMs can be divided into two major subgroups. The first family including KDM1A and KDM1B is also known as lysine specific demethylase (LSD), consisting of FAD-dependent amine oxidase, which can only remove mono- and di-methyl marks [46]. On the contrary, the second KDM family contains JmjC domain (JMJD), which relies on α-ketoglutarate, Fe(II) and oxygen as cofactors to mediate demethylation of mono-, di- and even tri-methyl-lysine residues [47]. JMJD

demethylases consist of more diverse family members and can be further divided into 7 subfamilies from KDM2 to KDM8 according to their other structurally conserved domains, like PHD and Tudor domains, which may also bear crucial functions in recognizing/reading the histone marks [19].

KDMs can be found differentially expressed in various cancers including leukaemia, and cooperate with transcription factors to activate or repress gene expression. LSD1 is over-expressed in MLL leukaemia and seems to play a crucial role in sustaining the oncogenic transcriptional programmes mediated by MLL-fusions via an unknown mechanism (Fig. 1B). LSD1 suppression by an shRNA approach led to a reduction of mouse MLL leukaemic stem cells (LSCs) [48]. While this study suggests a requirement of H3K4 demethylase for MLL leukaemia, a recent report revealed an opposite role of H3K4 demethylase KDM5B that negatively regulated MLL LSC (Fig. 1C) [49]. In this study, H3K4me2/3 but not H3K79me2 were critical for MLL LSC, and H3K4 methylation levels reduced during differentiation. Suppression of KDM5B significantly promoted disease progression, whereas its overexpression inhibited MLL leukaemia. While the reasons underlying the different results need further investigations, LSD1 on the other hand underpins retinoic acid receptor (RARa)-driven repression of myeloid differentiation associated genes in AML through decreasing the level of H3K4me2 [50]. These results may suggest a more generic role of H3K4 methylation in AML pathogenesis, which may not be specific to MLL leukaemia. Other members of KDMs including KDM2B and JMJD1C also implicate in AML pathogenesis. H3K36me2 demethylase KDM2B that silences p15 expression was sufficient to transform haematopoietic progenitors in vitro, and its depletion significantly impaired HOXA9/MEIS1 driven leukaemogenesis and self-renewal of leukaemic stem cells [51]. H3K9 demethylase JMJD1C was identified as a crucial factor for the maintenance of AML expressing MLL-AF9 in an shRNA functional screen (Fig.1B). Depletion of JMJD1C inhibited cell growth and leukaemogenesis of MLL-AF9 cells by triggering apoptosis [52]. JMJD1C had also been recently implicated in AML1-ETO [53] and HOXA9-mediated leukaemias [54]. JMJD1C was identified as a coactivator in AETFC, a complex formed by AML1-ETO, where JMJD1C maintained low level of H3K9me2, hence enhancing gene expression of AML1-ETO targets. Knockdown of JMJD1C compromised the ability of AML1-ETO to inhibit cell differentiation and impaired colony formation [53]. JMJD1C also interacted with HOXA9 to modulate the downstream genes critical for self-renewal of leukaemic stem cells. Loss of JMJD1C profoundly affected leukaemic transformation driven by HOXA9, indicating yet another KDM family member with a more generic function in AML pathogenesis [54].

Crosstalk between HMTs and HDMs in AML

Although the above reports have directly implicated individual HMT and HDM in AML pathogenesis, their mode of actions and underlying mechanisms remain largely unknown. Recent studies exploring the functional crosstalk between HMTs and HDMs have shed lights into the intricate molecular regulation of aberrant

histone methylome in AML. It has been demonstrated that the chromatin localization of SIRT1, a H3K9 deacetylase, and SUV39H1, a H3K9 methyltransferase, may be disrupted by DOT1L (Fig. 2A). After inhibition of DOT1L, SIRT1 and SUV39H1 bound to MLL targets such as *HOXA7* and *MEIS1* and exerted their function to establish a heterochromatin-like state, in which the level of H3K9me2 but not H3K79me2 was kept considerably high. Deletion of SIRT1 or SUV39H1 significantly desensitised MLL-AF9 leukaemic cells to DOT1L inhibition, whereas pharmacological activation of SIRT1 by SRT1720 strikingly improved the in vivo efficacy of EPZ4777, a DOT1L inhibitor, demonstrating a critical function of this crosstalk in regulating DOT1L inhibitor sensitivity [55]. To search for novel epigenetic regulators that cooperate with PRMT1 in AML pathogenesis, KDM4C was identified to specifically interact with MLL-fusions and MOZ-TIF2 to remove H3K9me3 repressive mark [42]. Together with PRMT1, KDM4C co-regulated the epigenetic programmes for transcriptional deregulation and cellular transformation by increasing the H4R3me2a active mark but attenuating H3K9me3 repressive mark on MLL downstream targets such as *HOXA9* (Fig. 2B). Similar to PRMT1, shRNA-mediated suppression of KDM4C resulted in repression of MLL downstream gene expression programmes, attenuation of leukaemogenesis and a significant improvement of OS in mouse and humanized models, revealing the requirement of the presence of both epigenetic modifying enzymes for the oncogenic functions of MLL-fusions. Since KDM4C binds to MLL N-terminus region, KDM4C is also required for leukaemia induced by other MLL-fusions independent on PRMT1, suggesting a much broader function of KDM4C in maintaining aberrant epigenetic networks in MLL leukaemia. Interestingly, a recent study suggested a potential redundant function among KDM4 family members in AML using a tamoxifen inducible knockout approach [56]. Although characterization of the actual genotype on Kdm4c knockout leukaemic cells was not performed, genetic escape from in vivo deletion of Kdm4 family seems to be a common theme in all the resultant leukaemia with genotyping results, which was in line with the requirement of an unusually high dose of tamoxifen to achieve even in vitro deletion. Nevertheless, these studies consistently indicate critical functions of KDM4 family in acute leukaemogenesis.

The role of KDM in treatment response

In addition to disease progression, KDM has also been implicated in governing treatment response in acute promyelocytic leukaemia (APL) driven by RARa-fusions. APL is the only AML subgroup with a well-established target therapy where All Trans Retinoic Acid (ATRA) can induce transcriptional de-repression and leukaemic differentiation. In spite of success in identifying repressor complexes associated with RARa-fusions, the identity of the activator being recruited by the fusions upon ATRA treatment had remained elusive. To search for such a regulator, PHF8 (KDM7B), a H3K9 demethylase, was found to specifically interact with RARa-fusion proteins to remove H3K9me2 repressive mark upon ATRA treatment [57] (Fig. 3). ATRA treatment results in a conformation change of RARa-fusions, leading to

dissociation of corepressors such as HDAC and PRC2. PHF8 acts as a critical sensor for ATRA treatment response, which is dependent on both the enzymatic activity and the phosphorylation status of two critical serine residues of PHF8 that partly determine its chromatin localization. Genetic or pharmacological activation of PHF8 sensitized ATRA-refractory cells to the treatment whereas its suppression conferred resistance to APL cells. These findings for the first time directly implicate the activity of KDM in governing AML treatment responses, and reveal a novel therapeutic venue to overcome treatment resistant [58]. In addition to PHF8, LSD1 is also involved in the repressive machinery of RAR-fusions (Fig. 3). Inhibition of LSD1 could increase the level of H3K4me2 on the promoters of myeloid-differentiation associated genes and triggered ATRA-therapeutic response in non-APL AML. While ATRA exhibited little effects in non-APL AML, combination of ATRA and the LSD1 inhibitor TCP remarkably initiated cell differentiation of non-APL AML and reduced colony formation and engraftment of AML [50].

Histone methylome as an emerging therapeutic target

Given the critical functions of histone methylome in AML, the first HMT inhibitor targeting DOT1L, EPZ4777 [59] and its second-generation derivative, EPZ5676 [60] have been developed and tested for suppressing MLL leukaemia. Both compounds showed selective inhibitory effects on H3K79 methylation and cells bearing MLL-fusions (Table1). Continuous infusion of DOT1L inhibitors significantly prolonged the OS in murine models with MLL leukaemia [59, 60], leading to the first clinical trial of HMT inhibitors in AML. However, the poor pharmacokinetic characteristics of the DOT1L inhibitors may limit their clinical development [60], and can be partly responsible for the rather modest clinical responses in their early trial results. On the other hand, a PRMT1 inhibitor, AMI-408 could also significantly extend disease latency and OS in mouse models carrying MLL-GAS7 fusion or MOZ-TIF2 [42]. Similarly to DOT1L inhibitors, the efficacy of PRMT1 inhibitors in leukaemia suppression was far inferior to those by genetic or shRNA approaches, indicating the need to improve the pharmacokinetics of these early phase inhibitors. Studies also reported prolonged OS and reduced tumour burden in MLL-AF9 leukaemia model by targeting of a H3K27 methyltransferase EZH2. The EZH2 inhibitor DZNep triggered apoptosis of AML cells through reactivating TXNIP, leading accumulation of reactive oxygen species (ROS) [61]. Depletion of EZH2 or pharmacological inhibition of EZH1/2 by a small molecule UNC1999 up-regulated PRC2 target genes such as p16 and p19 in MLL leukaemic cells [62, 63]. There are also highly effective EZH2 inhibitors such as GSK126 and EPZ5687 for diffuse large B-cell lymphoma [64, 65]. GSK126 and stapled hydrocarbon peptide targeting EZH/EED interaction have been tested in parallel and shown strong suppression of in vitro MLL leukaemia cell growth, although their in vivo efficacy has yet to be demonstrated [66].

Similarly, while very limited *in vivo* data has been shown, a monoamine oxidase (MOA) inhibitor, tranylcypromine (TCP) alone or in combination with ATRA has been used to suppress LSD1 activity in MLL [48] or non-MLL leukaemia in

vitro [50] (Table1), respectively. A TCP derivative, GSK2879552 has entered phase I clinical trials for the treatment of relapsed AML (ClinicalTrials.gov identifier: NCT02177812), however TCP exhibited severe toxicity at efficacious doses in preclinical models, so it is possible that the TCP may result in broad toxicity, especially in central nervous system [67]. Recently, a non-MOA inhibitor SP2509 with similar efficacy but lower general toxicity as compared with TCP was developed (Table1). SP2509 blocked the interaction between LSD1 and the co-repressor CoREST, thus leading to a permissive increase in H3K4me3 on target genes such as p21, p27 and CCAAT/enhancer binding protein. SP2509 was able to effectively suppress colony formation, induced cell differentiation and triggered apoptosis of AML cells with mutant NPM1 but not MLL-fusions [68]. However, while inhibition of LSD1 showed a significant efficacy in a mouse xenograft model, it could only consistently translate into extended OS when it was a combination with PS, a pan-HDAC inhibitor [68]. It is noted that all the above epigenetic targets in particular DOT1L and LSD1 are absolutely essential for normal development and haematopoietic stem cells, which will likely limit the application of effective dose in patients and therefore a combination approach with lower dose may be beneficial. In contrast, KDM4C is largely dispensable for normal development and its complete deletion does not lead to any significant phenotypes in the mouse model. Consistently, an early phase KDM4C inhibitor, SD70 displayed an excellent therapeutic effect on AML expressing MOZ-TIF2 and MLL-fusions [42] (Table 1). Pharmacological inhibition of KDM4 effectively attenuated leukaemogenesis in vivo and extended OS in both mouse and humanized models with primary human MLL leukaemia. Remarkably, SD70 is quite well tolerated and has limited toxicity in these preclinical models, highlighting its therapeutic potentials for AML treatment.

Prospective

Transcriptional deregulation plays the key role in acute leukaemogenesis and probably treatment responses. The emerging functions of various epigenetic modifying enzymes of histone methylome in AML pathogenesis have provided unique opportunities to target this group of dismal disease, in which its treatment regime has not really changed for decades. In addition to the histone mark writers and erasers, it is also possible to target readers that are essential to recognize these specific histone marks for aberrant gene expression and transformation. It has been proposed that WDR5, one of main components of MLL complexes essential for MLL histone methyltransferase activity, recognises H3K4me and presents the K4 side chain for further methylation by MLL [49, 69, 70]. Blocking MLL1-WDR5 interaction by a small molecule inhibitor MM-401 specifically reduced levels of H3K4me at *HOXA* loci, induced myeloid differentiation and triggered apoptosis of mouse MLL-AF9 leukaemic cells [71]. In addition to WRD5 family, significant progress has been made to target bromodomain that recognizes acetyl-lysine marks. One of the best examples is the potential targeting of BRD family in MLL leukaemia. Genetic or pharmacological inhibition of BRD3/4 by I-BET151 (GSK1210151A) or JQ1 led to suppression of

BCL-2, Myc and CDK6 in leukaemic cells, and displayed outstanding efficacy against mouse and human leukaemia cells driven by MLL-fusions *in vitro* and *in vivo* (Table1) [72, 73]. Although rapid development of drug resistant in pre-clinical models in part due to activation of canonical Wnt/b-catenin signalling may pose a threat for effective treatments by BRD inhibitors [74, 75], these studies provide an important proof-of-principle data showing the feasibility of targeting protein-protein interaction involved in epigenetic regulation for leukaemia treatment. Similar principles will likely be applicable to other readers involved in histone methylome such as chromodomain and PHD domain. Future studies in dissecting the molecular regulation of critical histone methylome writers, readers and erasers will open up a promising venue for development of next generation effective AML treatments.

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Figure 1. Roles of HDMs and KDMs in MLL-driven transcription

(A) MLL-fusion complexes are assembled) by recruiting a body of important components (SEC, PAFc, MENIN and LEDGF) to target and facilitate expression of crucial leukaemogenic genes, such as HOXs, MEIS and MEF2C, where HMTs (DOT1L, PRMT1 and MLL) are involved to add active methyl marks (H3K79me2/3, H4R3me2a, H3K4me3), respectively. BRD4, a histone mark reader, is essential for the recruitment of MLL-fusions. (B) In addition to enrichment of active marks, KDMs (e.g., KDM4C, JMJD1C) on the other hand remove repressive marks (H3K9me3) to underpin the active status. Although LSD1 has been suggested to remove H3K4me1/2 marks in MLL leukaemia, its relevance to leukaemia suppression is still largely unknown. (C) KDM5B negatively regulates MLL target genes through demethylation of H3K4me3 active mark. Black arrows indicate methylation, whereas bent red arrows represent demethylation.

Figure 2. Crosstalk between HMTs and HDMs in MLL-driven transcription

(A) When DOT1L is recruited by MLL fusions, it confers H3K79me2 active mark, which may further expel SUV39H1 and SIRT1, hence leading to a reduction in H3K9me2 repressive mark but an increase in H3K9ac activation mark. (B) After binding to MLL fusions and MOZ-TIF2, PRMT1 and KDM4C cooperate to maintain the activation of MLL-driven transcription by conferring a high level of H4R3me2a high but a low level of H3K9me3 repressive mark.

Figure 3. Roles of KDMs in ATRA therapeutic response

PML-RARa forms a repressor complex with RXRa, HDACs and PRCs to inhibit expression of myeloid differentiation associated genes. In non-APL, LSD1 is also recruited to further remove H3K4me2/1, contributing to a more stable repressive status (not shown in figure). ATRA treatment results in a conformational change of PML-RARa, leading to dissociation of HDACs and PRCs, and recruitment of phosphorylated PHF8 to confer transcriptional activation. Activation of PHF8 by okadaic acid (OKA) may sensitise refractory APL cells to ATRA treatment.