Epigenetics in normal and malignant hematopoiesis: An overview and update 2017

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Global change in the epigenetic landscape is a hallmark of cancer, and the reversible nature of epigenetic aberrations has led to the emergence of the promising field of epigenetic therapy. (1) Hematopoietic neoplasm is one of the major cancer types for which several epigenetic therapies are currently in development. Epigenetic regulation refers to gene regulation achieved by changes in chromatin structure. The basic unit of chromatin, called a nucleosome, consists of an octamer of histone proteins wrapped with DNA. The histone octamer consists of two copies of each of the four core histone proteins: H2A, H2B, H3 and H4. Histone proteins contain a globular C-terminal domain and an unstructured N-terminal tail. The N-terminal histone tails receive a variety of posttranslational modifications, including acetylation, methylation and ubiquitination on specific residues. These histone modifications, together with DNA methylation, regulate gene expression in a highly orchestrated manner without altering the primary DNA sequence. We first summarize the fundamentals of the representative epigenetic modifications (Fig. 1).

Fundamentals of Epigenetic Modifications

DNA methylation. DNA methylation, which is associated with heterochromatin formation and transcriptional repression, occurs at the C-5 carbon of cytosines in DNA to form 5-methylcytosine (5mC) in mammalian cells. (2) There are three major DNA methyltransferases (DNMT) that establish and maintain DNA methylation. DNMT1 plays a key role in maintaining DNA methylation after DNA replication, while DNMT3a and DNMT3b promote genome-wide de novo DNA methylation. DNA demethylation is a dynamic process involving TET and IDH proteins. The TET enzymes, TET1, TET2 and TET3, catalyze the successive oxidation of 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5-fC) and then 5-carboxycytosine (5-caC). These 5mC oxidation products are implicated as intermediates in the conversion of 5mC to unmethylated cytosine, providing a first step in a pathway for active DNA demethylation. TET enzymes need α-ketoglutarate (α-KG) for their catalytic function. α-KG is converted from isocitrate by IDH proteins, IDH1 and IDH2 (Fig. 2).

Histone acetylation. Histone lysine acetylation occurs at several different positions in the histone tail (e.g. H3K9, H3K14 and H3K27), and leads to the open structure of chromatin that allows access of transcriptional factors. Consequently, histone acetylation is associated with active transcription. The processes of acetylation and deacetylation are governed by histone lysine acetyltransferases (KAT) and histone deacetylases (HDAC), respectively. KAT include CREBBP (CBP), EP300 (p300), KAT2B (PCAF), KAT5 (Tip60) and KAT6A (MOZ), while HDAC include HDAC1-11 and SIRT1-7. Bromodomain and extra-terminal (BET) proteins, BRD2, BRD3 and BRD4, recognize and bind to the acetylated histone lysine residues to activate transcription. (3)
Histone methylation. In contrast to histone lysine acetylation, which is generally associated with transcriptional activation, histone lysine methylation leads to transcriptional activation or repression, depending on which residue is modified and the degree of methylation (Fig. 1). Histone lysine methylation is regulated by histone lysine methyltransferases (KMT) and lysine demethylases (KDM) targeting specific lysine in histones. H3K4 methylation, which is tightly associated with gene activation, is mediated by SET domain-containing methyltransferases, such as KMT2A (MLL1) and KMT2D (MLL2). Several enzymes, including SETD2 and WHSC1 (NSD2/MMSET), induce H3K36 methylation. H3K79 is mainly methylated by DOT1L. H3K27 methylation, a well-known mark of silent chromatin, is induced by EZH1 and EZH2. EZH1/2 is part of the Polycomb Repressive Complex 2 (PRC2) containing SUZ12 and EED. An epigenetic factor ASXL1 was also shown to be associated with PRC2 to increase its function. KMT for H3K9 dimethylations/trimethylations include SUV39H1 and EHMT2 (G9a). In addition, the Prdm family proteins MECOM (EVI1) and PRDM16 were identified as H3K9me1-specific KMT. The methylations at H3K27 and H3K9 together constitute the two main silencing mechanisms in mammalian cells.

Several KDM have been shown to have important roles in hematopoiesis. KDM1A (LSD1) removes monomethylated and dimethylated H3K4 and H3K9. KDM2B is a Jumonji (JmjC) domain histone H3K36 di-demethylase. KDM5A is an α-ketoglutarate-dependent JmjC-containing protein, and acts on dimethylated and trimethylated H3K4. KDM6A (UTX) is another JmjC protein and removes H3K27me2/me3.
Histone ubiquitination. In mammals, there are two major complexes formed by Polycomb proteins: Polycomb Repressive Complex 1 (PRC1) and PRC2. As previously described, PRC2 contains three core subunits (EZH1/2, EED and SUZ12), and catalyzes dimethylation and trimethylation of H3K27. Canonical PRC1 contains four core subunits, BMI1 or MEL18, CBX family proteins, PHC, and an E3 ubiquitin ligase RING1 or RNF2. The PRC2-mediated histone methylation triggers recruitment of PRC1 in part due to the ability of the CBX subunit to bind to H3K27me3. The RING1 subunit (RING1 or RNF2) then induces mono-ubiquitination of histone H2A at lysine 119 (H2AK119ub). The H2AK119ub inhibits transcriptional elongation, promotes chromatin compaction, and is associated with transcriptional repression. BAP1, an interacting partner of ASXL1, was identified as a deubiquitinase that removes the ubiquitination on H2AK119.

Genetic Alterations within Epigenetic Modifiers in Hematopoietic Neoplasms

Patient data illuminate the relevance of epigenetic modifications to hematopoietic neoplasms. In this section, we

| Table 1. Epigenetic modifiers dysregulated in hematopoietic neoplasms |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Genes                                 | Activity                  | Genetic alteration | Gain-/Loss-of-function | Diseases                     | Effect of mutations on Transcription |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| DNA methylation                         | DNMT3A          | De novo DNA methylation | Mutation (R882 and others), mostly monoallelic | Loss | AML (20%) | Activation |
| H3K27 KMT, a member of PRC2              |                  |                  |                  |                  |                  |
| EZH2 KMT, a member of PRC2              |                  |                  |                  |                  |                  |
| ASXL1                                  |                  |                  |                  |                  |                  |
| SUZ12                                  |                  |                  |                  |                  |                  |
| EED                                    |                  |                  |                  |                  |                  |
| KMT2A (MLL1)                            |                  |                  |                  |                  |                  |
| KMT2D (MLL2)                            |                  |                  |                  |                  |                  |
| PRDM16                                  |                  |                  |                  |                  |                  |
| MECOM                                  |                  |                  |                  |                  |                  |
| SETD2                                  |                  |                  |                  |                  |                  |
| WHSCL1                                 |                  |                  |                  |                  |                  |
| KDM6A (UTX)                             |                  |                  |                  |                  |                  |
| KDM2B                                  |                  |                  |                  |                  |                  |
| CREBBP (CBP)                            |                  |                  |                  |                  |                  |
| EP300 (p300)                            |                  |                  |                  |                  |                  |

ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; KMT: histone lysine demethylase; KAT, histone lysine methyltransferase; MDS, myelodysplastic syndrome; MM, multiple myeloma; PTD, partial tandem duplication; TBD, to be determined.
DNMT3A. Mutations in DNMT3A have been frequently found across a range of hematopoietic diseases, including acute myeloid leukemia (AML), myelodysplastic syndromes (MDS) and T-cell leukemia/lymphoma. The type of DNMT3A mutation and the frequency of heterozygous versus homozygous mutations vary among different malignancies. In the myeloid neoplasms, R882 residue is the most frequently mutated (60%), and the mutations are typically heterozygous. The R882 mutations are not so predominant (20%) in T-cell leukemia/lymphoma. In addition, the frequency of biallelic involvement mutations are not so predominant (20%) in T-cell leukemia/lymphoma. Thus, IDH1/2 mutations share a common onco- genetic function to TET2 mutations, but they also have distinct leukemogenic effects.

**EZH2 and other PRCC2 genes.** EZH2 plays dual roles in the process of tumor genesis. EZH2 mutations at tyrosine residue 641 have been found in B-cell tumors, including diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL). The EZH2-Y641 mutants drive hypermethylation of H3K27me3, indicating that they are gain-of-function mutations. In addition, overexpression of EZH2 has been reported in natural killer/T-cell (NKT) lymphoma. Conversely, loss-of-function mutations in EZH2 have been detected in MDS and T-cell acute lymphoid leukemia (T-ALL). Mutations in other member of PRCC2, EED and SUZ12, were also found in some cases of MDS and T-ALL. In addition to these mutations in PRCC2 genes, several other oncoproteins were shown to inhibit EZH2 function in MDS cells. For example, ASXL1 mutations promote myeloid transformation through loss of PRCC2-mediated gene repression. Mutations in a splicing factor SRSF2 induce missplicing of EZH2 to promote MDS development. Thus, EZH2 acts as an oncogene in B-cell and NKT lymphomas, while it acts as a tumor suppressor in MDS and T-ALL.

**ASXL1 and ASXL2.** ASXL1 mutations have been frequently found in myeloid neoplasms, including CMLM, MDS and AML, but have rarely been found in lymphoid neoplasms. Most ASXL1 mutations are heterozygous and exist in exon 12 of the gene, generating C-terminally truncated mutations. Whether ASXL1 mutations promote myeloid transformation via a gain-of-function or loss-of-function is under debate. A recent report showed that the mutant ASXL1 proteins are, indeed, expressed in MDS cells, which implies that the mutations are not simple loss-of-function mutations. ASXL1 mutation is also implicated to clonal hematopoiesis, indicating that it is one of the earliest genetic events during the process of myeloid transformation. Mutations in ASXL2, a homologue of ASXL1, were specifically found in AML with the RUNX1–RUNX1T1 (AML-ETO) rearrangement.

**KMT2A (MLL1), KMT2D (MLL2) and KMT2C (MLL3).** Among KMT for H3K4, alterations of KMT2A (MLL1) and KMT2D (MLL2) have been causally implicated in hematopoietic neoplasms. KMT2A gene on chromosome 11q23 is a frequent target of rearrangements. Typically, KMT2A rearrangements result in the loss of the carboxy-terminal methyltransferase domain and an in-frame fusion of the amino-terminal region of KMT2A to 1 of more than 70 known fusion partners. Rearrangements of KMT2A are observed in AML and B-cell acute lymphoid leukemia (B-ALL). The most common partners for the rearrangements are AF9 in AML and AF4 in B-ALL. Many fusion partners of KMT2A interact with DOT1L directly or indirectly in complexes that promote transcriptional elongation. DOT1L is a KAT to induce H3K79 methylation. As a result, KMT2A-fusion proteins gain the ability to induce hypermethylation of H3K79 at the promoter regions of KMT2A target genes. KMT2A
gene is also affected by a partial tandem duplication (PTD) of exons near the 5' end of the KMT2A gene. The KMT2A-PTD has been found in AML and MDS.

KMT2D (MLL2) has recently received considerable attention because it is one of the most frequently mutated genes in DLBCL and FL. (33) KMT2D mutations are predominantly represented by premature stop codons, frameshift insertions or deletions, and splice-site mutations that are predicted to generate truncated proteins lacking part or all of the C-terminal domains. Multiple missense mutations have also been found across the KMT2D gene. KMT2D mutations are found either biallelically or monoallelically. The diverse spectrum of KMT2D mutations suggests that they are the loss-of-function mutations.

In addition, KMT2C (MLL3) was recently shown to be a haploinsufficient 7q tumor suppressor, (34) and a KMT2C gene germ line mutation was found in a pedigree of colorectal cancer and AML. (35)

MECOM (EV1) and PRDM16. Rearrangements around MECOM (EV1) and PRDM16 genes have been detected in a subset of MDS and AML. (36,37) MECOM is located near the chromosome 3q26, and the balanced aberrations involving 3q26 reposition a distal enhancer of GATA2 to ectopically activate MECOM expression. (38,39) PRDM16 is located near the chromosome 14q32, and the reciprocal translocation t(1;3)(q36:g21) induced PRDM16 upregulation. AML with MECOM or PRDM16 rearrangements share many biological features, including micromegakaryocytes, multilineage dysplasia, low myeloperoxidase-expressing blasts and adverse prognosis. (40)

WHSC1 (NSD2/MMSET) and SETD2. Genetic alterations of KMT for H3K36 are also found in hematopoietic neoplasms. The translocation t(4;14) is one of the most common translocations in multiple myeloma (MM). The t(4;14) translocation leads to the generation of IgH-WHSC1 fusion gene to overexpress WHSC1 in MM. (41) The epigenetic role of WHSC1 has been a matter of discussion, but a recent report suggested that the principal activity of WHSC1 is promoting H3K36me2.

SETD2 is a KMT that induces H3K36me3, and a recent study detected recurrent mutations in SETD2 in AML and B-ALL. (42) SETD2 mutations are frequently found, but are not restricted, in KMT2A-rearranged patients. SETD2 mutations were either nonsense or frameshift mutations that truncate the C-terminus domain. One-quarter of the SETD2 mutations are biallelic mutations. The mutation spectrum of SETD2 implies that these mutations are loss-of-function mutations. Consistent with this notion, a global loss of H3K36me3 was observed in leukemic blasts with SETD2 mutations.

KDM6A (UTX) and KDM2B. Mutations and deletions of several KDM have been found in lymphoid neoplasms. KDM6A (UTX) is a known KDM for H3K27 and is located on chromosome X. Mutations and deletion of KDM6A gene were first identified in multiple cancer cell lines including those of MM. (43) A subsequent study found loss-of-function mutations within KDM6A gene in male T-ALL patients. Allelic expression analysis revealed that KDM6A escapes X-inactivation in female T-ALL lymphoblasts and normal T cells. Therefore, inactivation of only one single UTX copy in males will contribute to tumor development, while female cells are protected against single copy loss of UTX. (44) KDM2B is a H3K36 histone demethylase. Mutations and deletion of KDM2B gene were found in approximately 5% of DLBCL. (45)

CREBBP (CBP) and EP300 (p300). The earliest observations linking CREBBP and EP300 to cancer were the identifications of chromosome translocations in AML that disrupt these genes. Those include the t(8;16) and t(8;22) translocations generating KAT6A (MOZ)-CREBBP and KAT6A-EP300 fusions. These translocations appear to increase the KAT activity. (46) Recently, frequent mutations and/or deletions in CREBBP and EP300 genes were identified in DLBCL and FL. (47) These alterations remove or inactivate the KAT coding domain and are usually monoallelic, suggesting that these KATs are haploinsufficient tumor suppressors in B-cell lymphoma. Taken together, it appears that the enhanced KAT activity promotes myeloid leukemogenesis, while the reduced KAT activity contributes to the development of B-cell tumors.

Role of Epigenetic Modifiers in Hematopoiesis: Lessons from Mouse Models

The roles of epigenetic modifiers in hematopoiesis have been investigated using a variety of mouse models. Such studies have revealed new and sometimes unexpected roles of individual epigenetic modifiers during hematopoietic development. In this section, we summarize the physiologic roles of the disease-related epigenetic modifiers in normal and malignant hematopoiesis.

Dnmts (Dmnt1, Dmnt3a, Dmnt3b). Dmnt1 has been shown to be essential for HSC self-renewal. In sharp contrast, Dmnt3a deletion in hematopoietic cells led to expansion of adult HSC upon serial transplantation. (9) The increased self-renewal of Dmnt3a-deficient HSC will explain why loss-of-function mutations of Dnmt3a are frequently detected in a wide range of hematopoietic neoplasms. Furthermore, a recent study showed that Mx1-Cre-mediated Dmnt3a ablation led to the development of myeloproliferative neoplasms (MPN) with myelodysplasia (MDS/MPN), indicating an additional role of Dmnt3a to limit myeloid progenitor expansion in vivo. (48) Dmnt3a-deficient HSC exhibit a global loss of DNA methylation, particularly at the edges of large hypomethylated canyon regions. The hypomethylated regions are enriched for genes associated with HSC self-renewal, such as Hoxa9, Meis1 and Evi1, which resulted in enhanced expression of these stem cell genes. (8) Disruption of Dmnt3b, another de novo DNA methyltransferase, showed only minor effects on HSC. However, combined deletion of Dmnt3a and Dmnt3b resulted in marked expansion of HSC with almost completely blocked hematopoietic differentiation, indicating the functional redundancy of these de novo Dnmts in HSC regulation.

Tet enzymes (Tet1, Tet2, Tet3). Disruption of any of the Tet genes in adult hematopoietic cells leads to increased HSC function as measured by the engrafting potential to recipient mice. (18) Loss of Tet2 resulted in a gradual expansion of myeloid-biased HSC, accompanied with age-associated extramedullary hematopoiesis. Certain strains of Tet2-deficient mice developed a CMML-like disease but with long latency and low penetrance. In contrast to the myeloid-skewed phenotype of Tet2-deficient mice, Tet1-deficiency in hematopoietic cells drove B lymphopoiesis and the late development of a poorly penetrant B-cell lymphoma. Tet3-deficiency in hematopoietic cells did not significantly alter the steady-state hematopoiesis, but augmented the repopulating capacity of HSC. The ablation of Tet1, Tet2 or Tet3 led to a modest decrease in 5hmC levels in bone marrow. Interestingly, mice with combined loss of Tet1 and Tet2 exhibited strikingly decreased incidence and delayed onset of myeloid neoplasms in comparison to Tet2-/− mice, and
instead developed lethal B-cell neoplasms.\(^{50}\) In contrast, double knockout of \(\text{Tet2}\) and \(\text{Tet3}\) in mice induced rapid development of an aggressive myeloid leukemia with almost complete loss of \(\text{ShmC}\) in the bone marrow.\(^{51}\) Of note, combined loss of \(\text{Tet2}\) and \(\text{Tet3}\) resulted in the accumulation of DNA damage and impaired DNA repair, indicating the potential role of \(\text{Tet}\) proteins in the regulation of DNA damage responses. Thus, individual \(\text{Tet}\) proteins have both distinct and redundant functions in HSC and during hematopoietic differentiation.

\textbf{Idh1 and Idh2.} As described previously, the currently available evidence suggests that \(\text{Idh1/2}\) mutations induce \(\text{D2HG}\) production, and the \(\text{D2HG}\)-mediated inhibition of \(\text{Tet}\) enzymes contributes to leukemogenesis. Consistent with this notion, \(\text{Tet2}\) knockout and \(\text{IDH1-R132H}\) knockin mice showed similar phenotypes, including global \(\text{ShmC}\) reduction, altered DNA methylation, impaired hematopoietic differentiation, myeloid skewing and the development of myeloid disorders.\(^{16,22}\) However, in contrast to the \(\text{Tet2}\) knockout mice, \(\text{IDH1-R132H}\) knockin mice have reduced numbers of long-term HSC.\(^{52}\) Mechanistically, it was shown that \(\text{IDH1–R132H}\) downregulates DNA damage sensor ATM by altering histone methylation, leading to impaired DNA repair and reduced HSC self-renewal.

For \(\text{Idh2}\) mutant mice, the expression of \(\text{Idh2-R140Q}\) using a tetracycline-inducible system were generated.\(^{53}\) The \(\text{IDH1-R140Q}\) mice showed normal HSC numbers/functions and lineage differentiation, but exhibited increased extramedullary hematopoiesis. Combined expression of \(\text{Idh2-R140Q}\) together with \(\text{HoxA9/Meis1}\) or \(\text{FLT3}\) mutations in hematopoietic cells produced acute leukemia. Importantly, genetic deinduction of \(\text{Idh2-R140Q}\) in leukemic cells showed profound growth-inhibitory effects, indicating the essential role of mutant \(\text{Idh2}\) in maintaining leukemogenesis.

\textbf{Polycomb genes (Bmi1, Rnf2, Ezh2, Eed, Suz12).} \(\text{Bmi1}\) represents one of the best-characterized \(\text{PRC1}\) members in the regulation of HSC self-renewal.\(^{24,54}\) \(\text{Bmi1-deficient}\) mice showed severe postnatal pancytopenia due to progressive depletion of HSC. Conversely, forced expression of \(\text{Bmi1}\) in HSC increased self-renewal of HSC. \(\text{Bmi1}\) maintains HSC function by repressing the expression of \(\text{p16(Ink4a)}\) and \(\text{p19(Arf)}\) encoded by \(\text{Cdkn2a}\) gene, and also by regulating mitochondrial function and ROS generation. \(\text{Bmi1}\) is also a critical regulator of leukemia stem cells induced by several oncogenes, including MLL-AF9, HoxA9/Meis1 and E2a-Pbx1. \(\text{Rnf2}\), a core \(\text{PRC1}\) member, was shown to restrict the proliferation and differentiation of hematopoietic progenitors by repressing the expression of \(\text{p16(Ink4a)}\) and \(\text{Ccdn2}\).

\(\text{PRC2}\) genes also play important roles in the regulation of hematopoiesis.\(^{24,54}\) \(\text{Ezh2}\) overexpression in HSC enhanced their self-renewal and prevented HSC exhaustion upon serial transplantation. Although \(\text{Ezh2-deficient}\) HSC retained almost normal function,\(^{55}\) combined deletion of \(\text{Ezh1 and Ezh2}\) abolished the repopulating capacity of HSC. \(\text{Ezh2}\) loss in HSC predisposed mice to develop heterogeneous tumors, including MDS, MPN and T-cell leukemia, after the long latency.\(^{56}\) Disruption of \(\text{Ezh2}\) specifically in germinal center (GC) B-cells using \(\text{Cy-Cre}\) resulted in failure to form GC. Conversely, conditional deletion of \(\text{Ezh2-Y641N}\) in GC B-cells induced GC hyperplasia and accelerated lymphomagenesis in cooperation with BCL2.\(^{57}\) These findings clearly indicated the tumor suppressor role of \(\text{Ezh2}\) in myeloid and T-cell tumors, and the oncogenic role of \(\text{Ezh2}\) in B-cell lymphoma. Deletion of \(\text{Eed}\) resulted in the exhaustion of HSC. Decreased genes in \(\text{Eed-deficient}\) HSC are enriched for H3K27me3 targets, including \(\text{Cdkn2a}\), and \(\text{Cdkn2a}\) deletion in \(\text{Eed}\)-knockout mice partially rescued the HSC defect.\(^{55}\) \(\text{Suz12}\) is also required for HSC function and lymphomagenesis.\(^{58}\) Thus, both \(\text{PRC1}\) and \(\text{PRC2}\) members were shown to be involved in the regulation of HSC function and hematopoietic differentiation through the epigenetic control of Polycomb-target genes, such as \(\text{Cdkn2a}\).

\textbf{Axl1 and Bap1.} Hematopoietic-specific deletion of \(\text{Axl1}\) results in progressive, multilineage cytopenia and dysplasia, characteristic features of MDS.\(^{59,60}\) Of note, \(\text{Axl1-deficient}\) HSC exhibited decreased repopulating capacity, which contrasts with the phenotypes of \(\text{Dmnt3a}\) and \(\text{Tet2-deficient}\) HSC that showed enhanced self-renewal. Thus, despite that \(\text{Axl1}\) mutations are one of the earliest mutations presumably occurring in pre-leukemic HSC, \(\text{Axl1-deficiency}\) decreased HSC function. Because \(\text{ASXL1}\) mutations could have some gain-of-function,\(^{30,61}\) whether conditional expression of mutant \(\text{Axl1}\) also decreases HSC function needs to be investigated. \(\text{Axl1}\) deletion and forced expression of \(\text{Axl1}\) mutations induced global reduction of H3K27me3, upregulation of \(\text{Hox}\) genes,\(^{26,27,59}\) and promote development of MDS and AML in combination with \(\text{Tet}\) mutations.\(^{59}\) \(\text{Nrd1}\) mutations,\(^{26,27}\) and \(\text{SETBP1}\) mutations.\(^{62}\) \(\text{Bap1}\) is an \(\text{Axl1}\)-binding partner, and \(\text{Bap1}\) deletion in adult mice also caused MDS-like diseases.\(^{63}\) These findings indicate the important role of \(\text{Axl1/Bap1}\) complex to suppress myeloid transformation.

\textbf{Kmt2a (Mll1), Kmt2d (Mll2) and Kmt2c (Mll3).} \(\text{Kmt2a}\) studies consistently have shown the impaired HSC function in \(\text{Kmt2a-deficient}\) mice. \(\text{Kmt2a}\) regulates multiple HSC genes, including \(\text{Hoxa9, Meis1, Mecom, and Prdm16.}\(^{64}\) Several models for \(\text{KMT2A-rearranged leukemia}\) have been developed using retroviral transduction and mouse knockin strategies.\(^{65}\) \(\text{Retrovirus-mediated transduction of KMT2A-fusion proteins, such as KMT2A (MLL)-AF9 and KMT2A-ENL, can transform not only HSC but also myeloid progenitors to produce AML in vivo.}\) In contrast, \(\text{KMT2A-AF9}\) knockin mice, where oncogene expression is under endogenous regulatory control, efficiently transformed HSC while committed progenitors were transformation-resistant.\(^{66}\) In addition, some stem cell genes, such as \(\text{Mecom},\) were upregulated by \(\text{KMT2A-fusions}\) selectively in HSC but not in progenitors.\(^{66,67}\) These data suggest that cellular origin and oncogene dosage are important for the development of \(\text{KMT2A-rearranged AML.}\) Although few strategies have been successful in recapitulating the B-cell phenotypic characteristic of \(\text{KMT2A-rearranged childhood leukemia,}\) a recent study showed that human \(\text{CD34+ cells transduced with KMT2A fused to murine Af4 developed into a pro-B ALL that recapitulates many features of human disease in immunodeficient mice.}\(^{68}\)

Disruption of \(\text{Kmt2d}\) in mouse B cells using \(\text{Cy-Cre}\) showed that loss of \(\text{Kmt2d}\) led to reduced \(\text{H3K4}\) methylation, enhanced germinal center formation, conferred a B cell-proliferative advantage, and promoted lymphomagenesis in cooperation with \(\text{Bcl2.}\(^{69,70}\)\) These findings clearly indicate that \(\text{Kmt2d}\) acts as a tumor suppressor whose loss facilitates lymphomagenesis by remodeling the epigenetic landscape.

\textbf{Loss of catalytic function of Kmt2c promoted aberrant myelopoiesis}\(^{71}\) which may contribute to the progression of MDS and AML.

\textbf{Mecom (Evil1) and Prdmd16.} \(\text{Mecom and Prdmd16}\) belong to the \(\text{Prdm}\) family, and studies using mouse models have shown that loss of either leads to severe defects of HSC activity.\(^{72,73}\) Interestingly, both \(\text{Mecom}\) and \(\text{Prdmd16}\) were shown to be the
downstream targets of Kmt2a,⁶⁴,⁶⁷ indicating the critical role of Kmt2a–Mecom/Prdm16 axes in HSC.

Kdms [Kdm1a (Lsd1), Kdm2b, Kdm6a (Utx)]. Physiologic functions of Kdms have become an active area of research in the last few years.⁶⁶ Conditional deletion of Kdm1a in HSC resulted in severe pancytopenia, impaired granulocytic and erythroid differentiation, accompanied by the increased H3K4 methylation. Kdm2b deletion in HSC resulted in the reduced HSC activity and defective lymphopoiesis. Conversely, ectopic methylation of Kdm6a (Utx) resulted in severe pancytopenia, impaired granulocytic and erythrocytic progenitors. In contrast, H3K27me3 demethylation by either Kdm6a or Kdm6b (JmjD3) is crucial for the terminal steps of T-cell differentiation.⁶⁷ The preferential impact of Kdm6a/b on late T-cell maturation may be linked to the high frequency of Kdm6a mutations in T-ALL.

Kats [Crebbp (Cbp), Ep300 (p300), Kat6a (Moz)]. Multiple Kats have been shown to play key roles in hematopoiesis.⁴⁶ Studies using Crebbp knockout mice showed that loss of Crebbp led to increased apoptosis, differentiation, and quiescence in HSC. Ep300 is more important for hematopoietic differentiation. Kat6a (Moz) has been shown to be a critical regulator in the generation and development of HSC and progenitors. It should be noted that these Kats appear to regulate hematopoiesis partly through acetylation of non-histone proteins, such as c-Myb, Gfi1 and Foxp3.⁴⁶

Therapies Targeting Epigenetic Modifications

Epigenetic aberrations are potentially reversible and can be restored by epigenetic therapies. In this section, we describe drugs targeting aberrant epigenetic modifications in hematopoietic neoplasms that are in various stages of preclinical and clinical development (Fig. 3).

DNMT inhibitors. The two hypomethylating agents, azacitidine and decitabine, are currently approved for the treatment of several specific forms of MDS and AML.⁷⁷ These drugs are considered to inhibit activity of DNMT, to reverse aberrant DNA hypermethylation and to restore expression of previously silenced genes. However, mechanisms of action of these drugs are not fully understood, and there are currently no established biomarkers that predict the response to these drugs. A recent study reported that mutations in DNMT3A and TET2 genes might predict better responsiveness to treatment with the hypomethylating drugs in MDS.⁷⁸,⁷⁹ Given that DNMT3A or TET2 mutations are supposed to be associated with hypomethylation or hypermethylation of DNA, it is not clear why the inhibitors showed efficacy for both types of MDS. Another study reported that somatic mutations did not predict responses to dacitabine in CMML patients, but instead identified differentially methylated regions of DNA that distinguished responders from non-responders.⁸⁰ Identification of clinically useful biomarkers to predict response to these drugs will be an important future challenge.

IDH mutant inhibitors. Inhibitors for IDH1 and IDH2 mutants have been developed and are already being tested, either as single agents or in combination, in early clinical trials.⁷⁷ These drugs, such as AG-120 for the mutated IDH1 and AG-221 for the mutated IDH2, are selective inhibitors targeting only mutated IDH proteins but not wild-type IDH1/2. Therefore, in theory, these inhibitors will be particularly effective on IDH-mutated leukemia with minimal side effects.

HDAC inhibitors. HDAC inhibitors are the drugs that interfere with the function of histone deacetylase. The most successful clinical application of HDAC inhibitors has been the use of them against T-cell lymphoma.⁸¹ HDAC inhibitors also showed some therapeutic effects on MDS, AML, Hodgkin lymphoma and multiple myeloma.⁷⁷ However, responses to HDAC inhibitors as a single agent in AML or MDS appear to be modest. Clinical studies using HDAC inhibitors in combination with other agents are ongoing. Again, how or why some types of hematopoietic tumors are sensitive to HDAC inhibitors remains unclear.

BET inhibitors. BET proteins are epigenetic readers that recognize the acetylated lysine residues in histone proteins. BET inhibitors reversibly bind the bromodomain of BET to disrupt protein–protein interaction among BET proteins, acetylated histones and transcription factors. Preclinical studies have shown the therapeutic efficacy of the BET inhibitors in various hematopoietic neoplasms, particularly in AML, multiple myeloma and some lymphomas.⁸² Future research should include the development of combination therapies with other drugs, and the identification of biomarkers to predict the response.

DOT1L inhibitors. KMT2A fusion proteins were shown to recruit DOT1L to the promoter regions of KMT2A-target genes. Several small molecules targeting DOT1L have been developed, and showed substantial efficacy against KMT2A-rearranged leukemia in preclinical models.⁸³ Thus, the DOT1L inhibitors are potential options for treatment of KMT2A-rearranged leukemia and are currently under clinical investigation. DOT1L inhibitors may also be effective in
treated other types of hematopoietic neoplasms, such as AML with DNMT3A mutations. Furthermore, it was recently shown that combined pharmacological inhibition of DOT1L and menin-KMT2A had profound anti-leukemic activity against NPM1-mutated AML.

**EZH2 inhibitors.** EZH2 have been attractive drug targets for cancer therapy. Recent findings of the frequent gain-of-function mutations in EZH2 gene indicate that EZH2 represent an ideal therapeutic target in B-cell lymphoma. Indeed, early success has been achieved using EZH2 inhibitors for treatment of lymphomas bearing EZH2 mutations. Given that Polycomb genes establish crosstalk with numerous epigenetic regulators in various types of malignant stem cells, EZH2 inhibitors can potentially be applied to a broader spectrum of hematopoietic neoplasms.

**Other epigenetic inhibitors.** Inhibition of histone demethylases has also substantial potential to reset the aberrant regulation of gene expression in hematopoietic neoplasms. For example, recent studies highlight a potential application of LSD1 inhibition to treat AML. Only a few compounds have been developed as KAT (e.g. CREBBP and EP300) inhibitors. Some of them showed the growth-inhibitory efficacy in solid tumors, but the therapeutic effects of the KAT inhibitors have not been extensively studied in hematopoietic neoplasms. Interestingly, a study showed that pharmacological inhibition of Ep300 was able to abrogate the suppressive functions of Treg cells, and thereby increased tumor immunity. Targeting EP300 could, therefore, be a new approach for cancer immunotherapy.

**Overview and Concluding Remarks**

Despite the tremendous progress that has been achieved, we are still at an early stage in understanding the complex epigenetic regulation in hematopoiesis. This review aims to integrate accumulating knowledge regarding epigenetic (dys)regulation in normal and malignant hematopoiesis. Interestingly, from the epigenetic viewpoint, myeloid neoplasms are very similar to T-cell neoplasms, whereas B-cell lymphomas show a distinct pattern of mutation in epigenetic genes. For example, mutations in DNMT3A and TET2 are frequently found in both myeloid and T-cell tumors, but rarely found in B-cell tumors. Loss-of-function mutations of EZH2 contribute to the development of myeloid and T-cell tumors, while gain-of-function mutations of EZH2 promote B-cell tumors. The resemblance between myeloid and T-cell tumors with respect to epigenetic mutations cannot be easily explained by the classic model of binary split differentiation between lymphoid and myeloid lineages. However, several lines of evidence suggest that the separation of the B- and T-cell lymphoid lineages might occur prior to the loss of myeloid potential in the early stages of hematopoiesis. In addition, these observations suggest that myeloid and T-cell neoplasms will be responsive to similar epigenetic therapies, while distinct approaches may be required to treat B-cell lymphomas.

Of note, the mutational spectrum also indicates that the epigenetic mutations related to active transcription are more associated with myeloid/T-cell tumors, whereas those that repress transcription are associated with B-cell tumors. A good example is, again, EZH2. Gain-of-function mutations of EZH2 in B-cell tumors have been shown to enhance H3K27 methylation and repress transcription. Conversely, loss-of-function mutations of EZH2 in myeloid and T-cell tumors have been shown to reduce H3K27 methylation and activate transcription. In addition, rearrangements of CREBBP and EP300 in AML were considered to increase histone acetylation and enhance transcription. In contrast to these rearrangements, loss-of-function mutations of CREBBP and EP300, which are supposed to decrease histone acetylation and transcription, are quite prevalent in B-cell tumors. As summarized in Table 1, the list of epigenetic mutations suggests that myeloid and T-cell tumors tend to be transcriptionally “active,” while B-cell tumors appear to be transcriptionally “inactive” (Fig. 4). These data may imply that epigenetic mutations found in hematopoietic neoplasms act as general amplifiers or repressors of any given transcriptional state, instead of regulating the expression of specific target genes. This hypothesis could explain why attempts to identify target genes of each epigenetic modifier have been fraught with difficulty and failure.

Many questions remain to be answered. Biochemically, DNMT3A and TET2 have opposite activity to increase or decrease DNA methylation, respectively. Curiously, however, mutation of either DNMT3A or TET2 leads to similar hematopoietic neoplasms, including AML, MDS and T-cell tumors. Moreover, mutations of DNMT3A and TET2 can be found in the same malignant clones, indicating the cooperation between these mutations to promote tumor development. Similarly, IDH mutations, which are thought to promote leukemogenesis by inhibiting TET2 function, coexist frequently with DNMT3A mutations in AML and T-cell tumors. Future research should elucidate the actual role of these epigenetic modifiers in hematopoiesis. Mechanisms of actions of the “epigenetic therapies” warrant further investigation. Earlier studies suggest that the epigenetic drugs reverse aberrant DNA and/or
histone modifications, thereby restoring expression of previously silenced tumor suppressor genes. However, accumulating evidence clearly indicates that this concept is not sufficient to fully explain the therapeutic effects of these drugs. Recently, two studies highlighted another mode of action of demethylating agents in solid tumors. Sensing new rational approaches to the use of such agents in immunotherapy, and, indeed, it was shown that inhibition of DNA methylation could sensitize a murine model of melanoma to anti-CTLA4 immune checkpoint therapy. Potential cross-talk between epigenetic therapies and immunotherapies will be an interesting area of future research.

Disclosure Statement

The authors have no conflict of interest to declare.

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