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Review Article

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

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Epigenetic regulation in hematopoiesis has been a field of rapid expansion. Genome-wide analyses have revealed, and will continue to identify genetic alterations in epigenetic genes that are present in various types of hematopoietic neoplasms. Development of new mouse models for individual epigenetic modifiers has revealed their novel, sometimes unexpected, functions. In this review, we provide an overview of genetic alterations within epigenetic genes in various types of hematopoietic neoplasms. We then summarize the physiologic roles of these epigenetic modifiers during hematopoiesis, and describe therapeutic approaches targeting the epigenetic modifications. Interestingly, the mutational spectrum of epigenetic genes indicates that myeloid neoplasms are similar to T-cell neoplasms, whereas B-cell lymphomas have distinct features. Furthermore, it appears that the epigenetic mutations related to active transcription are more associated with myeloid/T-cell neoplasms, whereas those that repress transcription are associated with B-cell lymphomas. These observations may imply that the global low-level or high-level transcriptional activity underlies the development of myeloid/T-cell tumors or B-cell tumors, respectively.

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]

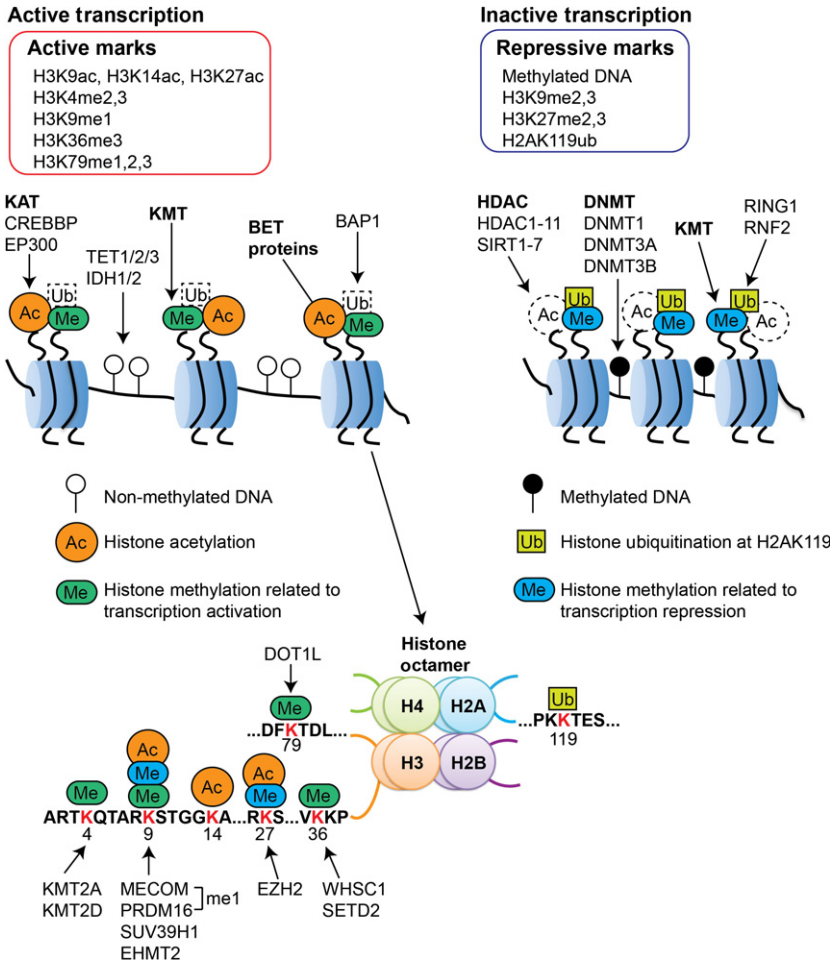

Fig. 1. Epigenetic mechanisms to regulate gene expression. DNA is wrapped around histone protein cores composed of an octamer containing two copies of each core histone: H2A, H2B, H3 and H4. Epigenetic patterns are established by a number of mechanisms, including DNA methylation, histone acetylation, histone methylation and histone ubiquitination. DNA methylation is established and maintained by the DNMT enzymes. IDH and TET proteins promote the early steps of active DNA demethylation (see Fig. 2). Histone acetylation is associated with active transcription, and is induced or removed by histone lysine acetyltransferases (KAT) or histone deacetylases (HDAC), respectively. BET proteins bind to acetylated histone to activate transcription. Histone methylation leads to transcriptional activation or repression depending on which residue is modified and the degree of methylation (see the lower part of this figure). Histone ubiquitination at H2AK119 is associated with transcriptional repression.

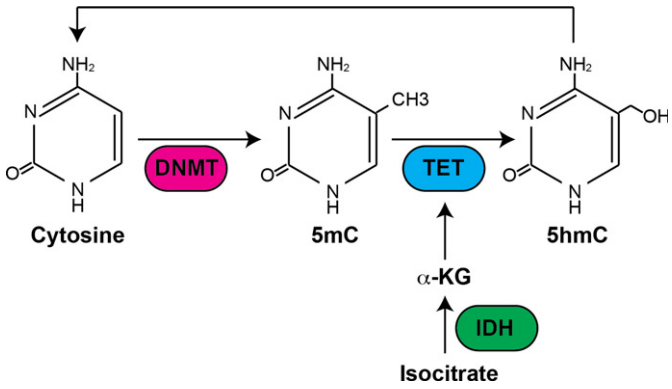

Fig. 2. Regulation of DNA methylation and demethylation. DNMT enzymes methylate the nucleotide cytosine (5-methylcytosine, 5-mC). TET proteins catalyze the oxidation of 5-mC into 5-hydroxymethylcytosine (5-hmC), promoting the demethylation process. IDH proteins promote the conversion from isocitrate to α-KG, which is required for catalytic function of TET enzymes.

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).[4] Methylation of histone H3 lysine 4 (H3K4), H3K36 and H3K79 are associated with transcriptional activation, while dimethylation and trimethylation of H3K9 (H3K9me2, H3K9me3) and H3K27 (H3K27me2, H3K27me3) are associated with transcriptional repression. Monomethylation of H3K9 (H3K9me1) and H3K27 (H3K27me1) are more enriched at active promoters than silent promoters. Histone 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.[5] 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.[6] 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.[7] 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%) MDS (10%)	Activation
			Mutation (R882 and others), many biallelic mutation	Loss	T-cell lymphoma/T-ALL (25%)	
	TET2	Conversion of 5-mC to 5-hmC	Mutation, mostly monoallelic	Loss	AML (20%) MDS (20%) CMML (50%) B-cell lymphoma (5%) T-cell lymphoma/T-ALL (25%)	TBD
	IDH1/IDH2	A cofactor of TET2	R132 (IDH1), R140/R172 (IDH2), monoallelic	Gain	AML (10%) MDS (3%)	TBD
Histone methylation	EZH2	H3K27 KMT, a member of PRC2	Mutations, monoallelic or biallelic	Loss	MDS (5%) T-ALL (10%)	Activation
			Mutation (Y641 and others)	Gain	DLBCL (20%) FL (10%)	Repression
	ASXL1	Associates with PRC1 and PRC2	Mutation, monoallelic	TBD	AML (5%) CMML (45%) MDS (20%)	Activation
	SUZ12	A member of PRC2	Mutation	Loss	MDS/T-ALL (rare)	Activation
	EED	A member of PRC2	Mutation	Loss	MDS/T-ALL (rare)	Activation
	KMT2A (MLL1)	H3K4 KMT	Rearrangements (11q23)	Gain	AML (5%) B-ALL (10%)	Activation
			PTD	Gain	AML/MDS (5%)	Activation
	KMT2D (MLL2)	H3K4 KMT	Mutation, monoallelic or biallelic	Loss	DLBCL (30%) FL (70%)	Repression
	MECOM	H3K9(me1) KMT	Rearrangements (3q26)	Gain	AML/MDS (rare)	TBD
	PRDM16	H3K9(me1) KMT	Rearrangements [t(1;3)]	Gain	AML/MDS (rare)	TBD
	SETD2	H3K36 KMT	Mutation, monoallelic or biallelic	Loss	AML/B-ALL (5%)	Repression
	WHSC1	H3K36 KMT	Rearrangements [t(4;14)]	Gain	t(4:14)MM (30%)	TBD
	KDM6A (UTX)	H3K27 KDM	Mutation/Deletion	Loss	MM T-ALL (15%)	TBD
	KDM2B	H3K36 KDM	Mutation/Deletion	Loss	DLBCL (5%)	TBD
Histone acetylation	CREBBP (CBP)	KAT	Rearrangements	Gain	AML (rare)	Activation
			Mutation/deletion, monoallelic	Loss	DLBCL (15%) FL (40%) relapsed B-ALL (20%)	Repression
	EP300 (p300)	KAT	Rearrangements	Gain	AML (rare)	Activation
			Mutation/deletion, monoallelic	Loss	DLBCL (40%) FL (60%)	Repression

ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; KDM: histone lysine demethylase; KMT, histone lysine methyltransferase; MDS, myelodysplastic syndrome; MM, multiple myeloma; PTD, partial tandem duplication; TBD, to be determined.

summarize genetic alterations within epigenetic modifiers that are frequently present in hematopoietic neoplasms (Table 1).

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.[8,9] 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 is very high, indicating that a more complete loss of DNMT3A function is important for the development of T-cell neoplasms.[8]

Several lines of evidence indicate that *DNMT3A* mutations are one of the earliest events during the course of tumor development. *DNMT3A* mutations have been found at higher variant-allele frequencies than other accompanying mutations in hematopoietic neoplasms.[10] Two independent studies found *DNMT3A* mutations in human HSC purified from AML patients in the absence of other common leukemia-associated mutations.[11,12] Recent extensive analyses of genome sequencing data from individuals without hematological diseases clearly showed that hematopoiesis frequently become clonal with age.[13–15] *DNMT3A* mutations are overwhelmingly the most common mutations in such clonal hematopoiesis. Taken together, *DNMT3A* mutations appear to give a small advantage to HSC that can lead to their dominance over time, which become foundation of the subsequent development of hematopoietic neoplasms. Interestingly, the clonal hematopoiesis is associated with increases in the risk of not only hematopoietic neoplasms but also cardiovascular diseases.

TET2. Recurrent mutations of *TET2* have also been found in a wide range of hematopoietic neoplasms, including AML, MDS, chronic myelomonocytic leukemia (CMML) and T-cell leukemia/lymphoma.[16–18] In addition, TET2 mutations can be found in premalignant HSC in MDS and AML patients,[15] as well as in aged healthy individuals with clonal hematopoiesis.[13–15,19] TET2 mutations are usually heterozygous and are either missense mutations in the C-terminal catalytic domain or nonsense/frameshift mutations in the N-terminal region leading to premature truncation before the catalytic core. Thus, disruption of the catalytic activity of TET2 constitutes a critical background for development of hematopoietic neoplasms. In AML, TET2 mutations are mutually exclusive to IDH1/2 mutations (see below) and WT1 mutations, which probably indicates the presence of IDH1/2-TET2-WT1 pathway to suppress AML.[20,21]

IDH1 and IDH2. Mutations in *IDH1* and *IDH2* are mostly heterozygous and mutually exclusive.[16,22] In IDH1, almost all the disease-associated mutations were found at R132, in the enzyme active site. In IDH2, there are two hot spots for mutation: R140 and R172. IDH2-R140 mutation occurs exclusively in myeloid tumors, whereas the IDH2-R172 mutation is also found in solid tumors, such as glioma. These mutations are found in up to 10% of cytogenetically normal AML. Metabolite profiling studies revealed that the IDH1/2 mutations gain a function to generate a novel oncometabolite D-2-hydroxyglutarate (D2HG) from a-KG. D2HG inhibits the function of α-KG-dependent enzymes such as TET2. Therefore, it is widely believed that IDH mutations induce leukemogenesis mainly by suppressing TET2 function. In line with this notion, mutations in *IDH1/2* and *TET2* are mutually exclusive in AML, and *TET2*-mutated or *IDH*-mutated leukemia cells display an overlapping DNA hypermethylation signature that is associated with decreased 5hmC.[23] However, there are substantial differences between *IDH1/2*-mutated and *TET2*-mutated hematopoietic neoplasms. *TET2* mutations are more common in MDS/CMML than AML, whereas *IDH1/2* mutations are frequently found in AML.[16] Unlike *TET2* mutations, *IDH1/2* mutations are not associated with clonal hematopoiesis in elderly people.[13–15] It should also be noted that *TET2* and *IDH2* mutations are not exclusive but often coexist in T-cell lymphomas.[16] Thus, IDH1/2 mutations share a common oncogenic function to TET2 mutations, but they also have distinct leukemogenic effects.

EZH2 and other PRC2 genes. EZH2 plays dual roles in the process of tumorigenesis.[24] *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 H3K27, indicating that they are gain-of-function mutations. In addition, overexpression of EZH2 has been reported in natural killer/T-cell (NKT) lymphoma.[25] Conversely, loss-of-function mutations in *EZH2* have been detected in MDS and T-cell acute lymphoid leukemia (T-ALL). Mutations in other members of PRC2, *EED* and *SUZ12*, were also found in some cases of MDS and T-ALL. In addition to these mutations in PRC2 genes, several other oncoproteins were shown to inhibit EZH2 function in MDS cells. For example, ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression.[26,27] Mutations in a splicing factor *SRSF2* induce missplicing of EZH2 to promote MDS development.[28] 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 CMML, MDS and AML, but have rarely been found in lymphoid neoplasms.[29] 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.[30] 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* (*AML1-ETO*) rearrangement.[31]

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.[32] 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 (EVI1) and PRDM16. Rearrangements around *MECOM (EVI1)* 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 1p36, and the reciprocal translocation t(1:3)(q36;q21) 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)(p16; q32) 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 KAT 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 (Dnmt1, Dnmt3a, Dnmt3b). Dnmt1 has been shown to be essential for HSC self-renewal. In sharp contrast, *Dnmt3a* deletion in hematopoietic cells led to expansion of adult HSC upon serial transplantation.[9] The increased self-renewal of *Dnmt3a*-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 *Dnmt3a* ablation led to the development of myeloproliferative neoplasms (MPN) with myelodysplasia (MDS/MPN), indicating an additional role of Dnmt3a to limit myeloid progenitor expansion *in vivo*.[48] *Dnmt3a*-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 *Dnmt3b*, another *de novo* DNA methyltransferase, showed only minor effects on HSC. However, combined deletion of *Dnmt3a* and *Dnmt3b* resulted in marked expansion of HSC with almost completely blocked hematopoietic differentiation,[8] 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.[18,49] 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 *Tet2* and *Tet3* in mice induced rapid development of an aggressive myeloid leukemia with almost complete loss of 5hmC in the bone marrow.[51] Of note, combined loss of *Tet2* and *Tet3* resulted in the accumulation of DNA damage and impaired DNA repair, indicating the potential role of Tet proteins in the regulation of DNA damage responses. Thus, individual Tet proteins have both distinct and redundant functions in HSC and during hematopoietic differentiation.

Idh1 and Idh2. As described previously, the currently available evidence suggests that IDH1/2 mutations induce D2HG production, and the D2HG-mediated inhibition of TET enzymes contributes to leukemogenesis. Consistent with this notion, Tet2 knockout and IDH1-R132H knockin mice showed similar phenotypes, including global 5hmC reduction, altered DNA methylation, impaired hematopoietic differentiation, myeloid skewing and the development of myeloid disorders.[16,22] However, in contrast to the Tet2 knockout mice, IDH1-R132H knockin mice have reduced numbers of long-term HSC.[52] Mechanistically, it was shown that IDH1–R132H downregulates DNA damage sensor ATM by altering histone methylation, leading to impaired DNA repair and reduced HSC self-renewal.

For the IDH2 mutations, transgenic mice that express IDH2-R140Q using a tetracycline-inducible system were generated.[53] The IDH-R140Q mice showed normal HSC numbers/functions and lineage differentiation, but exhibited increased extramedullary hematopoiesis. Combined expression of IDH2-R140Q together with HoxA9/Meis1 or FLT3 mutations in hematopoietic cells produced acute leukemia. Importantly, genetic deinduction of IDH2-R140Q in leukemic cells showed profound growth-inhibitory effects, indicating the essential role of mutant IDH2 in maintaining leukemogenesis.

Polycomb genes (Bmi1, Rnf2, Ezh2, Eed, Suz12). Bmi1 represents one of the best-characterized PRC1 members in the regulation of HSC self-renewal.[24,54] *Bmi1*-deficient mice showed severe postnatal pancytopenia due to progressive depletion of HSC. Conversely, forced expression of Bmi1 in HSC increased self-renewal of HSC. Bmi1 maintains HSC function by repressing the expression of p16(Ink4a) and p19(Arf) encoded by *Cdkn2a* gene, and also by regulating mitochondrial function and ROS generation. Bmi1 is also a critical regulator of leukemia stem cells induced by several oncogenes, including MLL-AF9, HoxA9/Meis1 and E2a-Pbx1. Rnf2, a core PRC1 member, was shown to restrict the proliferation and differentiation of hematopoietic progenitors by repressing the expression of p16(Ink4a) and Ccnd2.

PRC2 genes also play important roles in the regulation of hematopoiesis.[24,54] Ezh2 overexpression in HSC enhanced their self-renewal and prevented HSC exhaustion upon serial transplantation. Although *Ezh2*-deficient HSC retained almost normal function,[55] combined deletion of *Ezh1* and *Ezh2* abolished the repopulating capacity of HSC. Ezh2 loss in HSC predisposed mice to develop heterogeneous tumors, including MDS, MPN and T-cell leukemia, after the long latency.[56] Disruption of *Ezh2* specifically in germinal center (GC) B-cells using Cγ-Cre resulted in failure to form GC. Conversely, conditional expression of mutant 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 Ezh2 in myeloid and T-cell tumors, and the oncogenic role of Ezh2 in B-cell lymphoma. Deletion of *Eed* resulted in the exhaustion of HSC. Derepressed genes in *Eed*-deficient HSC are enriched for H3K27me3 targets, including *Cdkn2a*, and *Cdkn2a* deletion in *Eed*-knockout mice partially rescued the HSC defect.[55] Suz12 is also required for HSC function and lymphopoiesis.[58] Thus, both PRC1 and 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 Cdkn2a.

Asxl1 and Bap1. Hematopoietic-specific deletion of *Asxl1* results in progressive, multilineage cytopenia and dysplasia, characteristic features of MDS.[59,60] Of note, *Asxl1*-deficient HSC exhibited decreased repopulating capacity, which contrasts with the phenotypes of *Dnmt3a* and *Tet2*-deficient HSC that showed enhanced self-renewal. Thus, despite that *ASXL1* mutations are one of the earliest mutations presumably occurring in pre-leukemic HSC, *Asxl1*-deficiency decreased HSC function. Because ASXL1 mutations could have some gain-of-function,[30,61] whether conditional expression of mutant *Asxl1* also decreases HSC function needs to be investigated. *Asxl1* deletion and forced expression of Asxl1 mutations induced global reduction of H3K27me3, upregulation of Hox genes,[26,27,59] and promote development of MDS and AML in combination with *Tet* mutations,[59] *Nras* mutations,[26,27] and *SETBP1* mutations.[62] Bap1 is an Asxl1-binding partner, and Bap1 deletion in adult mice also caused MDS-like diseases.[63] These findings indicate the important role of Asxl1/Bap1 complex to suppress myeloid transformation.

Kmt2a (Mll1), Kmt2d (Mll2) and Kmt2c (Mll3). Studies consistently have shown the impaired HSC function in *Kmt2a*-deficient mice. Kmt2a regulates multiple HSC genes, including Hoxa9, Meis1, Mecom and Prdm16.[64] Several models for *KMT2A*-rearranged leukemia have been developed using retroviral transduction and mouse knockin strategies.[65] 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, 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 Mecom, were upregulated by *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 *KMT2A*-rearranged AML. Although few strategies have been successful in recapitulating the B-cell phenotype characteristic of *KMT2A*-rearranged childhood leukemia, a recent study showed that human 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 *Kmt2d* in mouse B cells using Cγ-Cre showed that loss of Kmt2d led to reduced H3K4 methylation, enhanced germinal center formation, conferred a B cell-proliferative advantage, and promoted lymphomagenesis in cooperation with Bcl2.[69,70] These findings clearly indicate that Kmt2d acts as a tumor suppressor whose loss facilitates lymphomagenesis by remodeling the epigenetic landscape.

Loss of catalytic function of Kmt2c promoted aberrant myelopoiesis,[71] which may contribute to the progression of MDS and AML.

Mecom (Evi1) and Prdm16. *Mecom* and *Prdm16* belong to the Prdm family, and studies using mouse models have shown that loss of either leads to severe defects of HSC activity.[72,73] Interestingly, both Mecom and Prdm16 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.[6] 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 expression of Kdm2b in hematopoietic progenitors favors T lymphocyte commitment.[74]

Kdm6a (Utx) is located on the X chromosome. Consequently, female *Kdm6a* knockout mice displayed key features of MDS with chromosomal instability, whereas their male counterparts showed no phenotype.[75] The compensatory effect of a homologue Uty in male mice, which is located on the Y chromosome and has no Kdm activity, indicates the demethylase-independent functions of Kdm6a in HSC and progenitors. In contrast, H3K27me3 demethylation by either Kdm6a or Kdm6b (Jmjd3) is crucial for the terminal steps of T-cell differentiation.[76] 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]

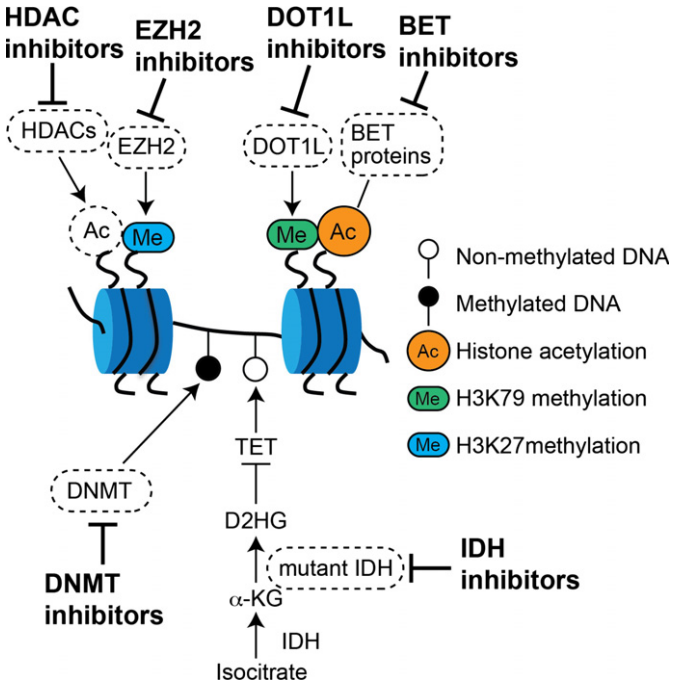

Fig. 3. Ongoing and future therapeutic approaches targeting epigenetic modifiers. Epigenetic modifiers can be potential therapeutic targets. Those include DNMT, mutant IDH proteins, histone deacetylases (HDAC), EZH2 and polycomb complexes, the DOT1L enzyme and BET proteins.

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 dicitabine 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. KTM2A fusion proteins were shown to recruit DOT1L to the promoter regions of KTM2A-target genes. Several small molecules targeting DOT1L have been developed, and showed substantial efficacy against *KTM2A*-rearranged leukemia in preclinical models.[83] Thus, the DOT1L inhibitors are potential options for treatment of *KTM2A*-rearranged leukemia and are currently under clinical investigation. DOT1L inhibitors may also be effective in

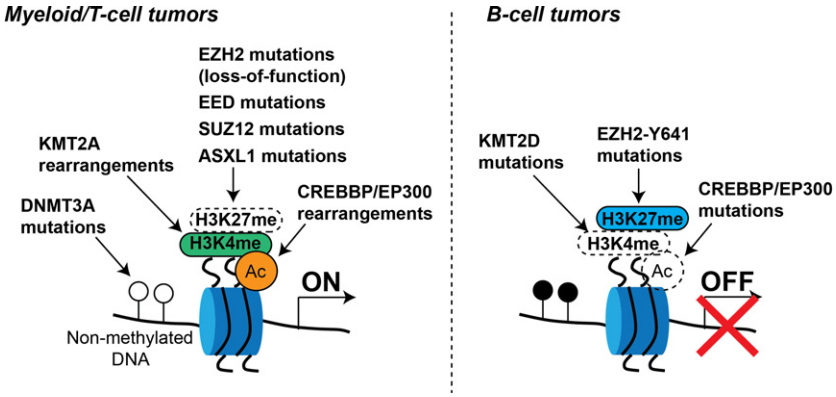

Fig. 4. Epigenetic characteristics in hematopoietic neoplasms. Genetic alterations in myeloid and T-cell tumors are generally associated with "active" transcription, while those in B-cell tumors are more associated with "inactive" transcription. The global low-level or high-level transcriptional activity may underlie the development of myeloid/T-cell tumors or B-cell tumors, respectively.

treating other types of hematopoietic neoplasms, such as AML with *DNMT3A* mutations.[84] Furthermore, it was recently shown that combined pharmacological inhibition of DOT1L and menin-KTM2A had profound anti-leukemic activity against NPM1-mutated AML.[85]

EZH2 inhibitors. EZH2 have been attractive drug targets for cancer therapy.[86] 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.[87] 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.[88] For example, recent studies highlight a potential application of LSD1 inhibition to treat AML.[89] 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.[90] 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.[91,92] 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.[93,94] DNA demethylating agents activated a cellular antiviral program through transcriptional activation of endogenous retroviral sequences. The findings suggested 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.[94] Potential crosstalk 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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