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Epigenetic regulation of hematopoiesis

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Abstract Epigenetic regulation is required not only for development, but also for tissue homeostasis, which is maintained via the self-renewal and differentiation of somatic stem cells. Accumulating evidence suggests that epigenetic regulators play critical roles in the maintenance of both self-renewing hematopoietic stem cells and leukemic stem cells. Recent genome-wide comprehensive analyses have identified mutations in epigenetic regulator genes, including genes whose products modify DNA and histones in hematological malignancies. Among these epigenetic regulators, repressive histone modifications by Polycomb-group complexes have been most fully characterized in hematopoietic stem cells, and are recognized as general regulators of stem cells. Hematopoietic stem cells are controlled by both cell-intrinsic and -extrinsic regulators, including transcription factors, signal transduction pathways, and niche factors. However, there is little insight into the mechanism of how epigenetic regulators act in concert with these factors to ensure blood homeostasis. In this review, we highlight recent findings in epigenetic regulation of hematopoiesis with emphasis on the role of Polycomb-group proteins and DNA-methylation modulators in hematopoietic stem cells and their progeny.

Keywords Polycomb group protein · Histone modification · DNA methylation

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Introduction

Blood is one of the most highly regenerative tissues, with approximately one trillion new cells arising daily in adult human bone marrow (BM). Although adult hematopoietic stem cells (HSCs) remain relatively quiescent, HSCs can enter the cell cycle and either self-renew or differentiate into multipotent progenitors that provide diverse mature blood cells, resulting in a well-known hierarchy of blood cells that maintain homeostasis. HSCs also respond efficiently to stress conditions, such as blood loss, infections, or exposure to cytotoxic agents, via expansion of the HSC and progenitor populations without depletion of the HSC compartment. HSC functions are controlled by both cell-intrinsic and -extrinsic regulators, including transcription factors, signal transduction pathways, and niche factors [1]. However, little is known about how epigenetic regulators act in concert with these factors to ensure blood homeostasis.

Chromatin is composed of nucleosomes, which are the molecular complex of DNA and histone proteins. One nucleosome consists of approximately 147 base pairs of DNA wrapped around a histone octamer. Epigenetics is commonly used to describe the chromatin-based events including DNA methylation, histone modifications, and chromatin structure, which regulate gene expression in a heritable manner. Modifications of DNA and histones can change chromatin structure and serve as specific sites for reader proteins that recruit additional chromatin-modifying proteins and enzymes [2, 3]. Recent advances in this field also indicate that non-coding RNA may play a critical role in epigenetic regulation [4, 5]. Epigenetic regulation allows cells to "remember" their gene expression profiles through subsequent cell divisions without any alterations to their DNA sequences [6].

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Epigenetic regulation is required not only for development, but also for tissue homeostasis, which is maintained via the self-renewal and differentiation of somatic stem cells. Accumulating evidence suggests that epigenetic regulators play critical roles in the maintenance of self-renewing HSCs. This is also true in self-renewing leukemic stem cells (LSCs) [7]. Recent genome-wide comprehensive analyses have identified mutations in epigenetic regulator genes, including genes whose products modify DNA and histones in hematological malignancies (Ref 8 and Omar Abdel-Wahab in this issue) [8]. These findings suggest that epigenetic dysregulation can promote the transformation and maintenance of cancer stem cells.

Among epigenetic regulators, the repressive histone modifications by the Polycomb-group (PcG) complexes have been best characterized in HSCs, and have been recognized as general regulators of stem cells [3]. In this review, we highlight recent findings in the epigenetic regulation of hematopoiesis with an emphasis on the role of PcG proteins and DNA methylation modulators in HSCs and their progeny.

PcG complexes and their functions

PcG genes were initially identified in *Drosophila* as regulators of body segmentation via the repression of homeotic genes, and were subsequently identified in mammals. The PcG gene family comprises a structurally diverse set of proteins that can assemble into multiple chromatin-associated complexes. In mammals, there are two major complexes formed by PcG proteins: Polycomb Repressive Complex (PRC) 1 and 2 [9, 10]. PRC2 contains three core subunits: SUZ12, one of the EED isoforms, and the histone methyltransferase EZH1 or EZH2, which catalyzes di- and tri-methylation of histone H3 at lysine 27 (H3K27me3) [11]. Canonical PRC1 contains four core subunits, BMI1 or MEL18, CBX, PHC, and RING1A or RING1B. Following the recruitment of PRC2 to chromatin, EZH1/2 tri-methylates H3K27 (H3K27me3), which functions to recruit PRC1 in part due to the ability of the CBX subunit of the PRC1 complex to bind to H3K27me3. The RING1 subunit monoubiquitylates histone H2A at lysine 119 (H2AK119ub1) [12, 13]. It has been proposed that the H2AK119ub1 mark promotes repression by inhibiting RNA polymerase II-dependent transcriptional elongation and then promoting chromatin compaction [14, 15]. Biologically, the PRC-dependent repressive H3K27me3 mark is counteracted by the histone H3K4me3 mark mediated by the Trithorax-group (trxG) complex, which includes MLL1 as a core component, resulting in proper target gene expression in a context-dependent manner. Excellent reviews regarding the Trithorax gene family are available elsewhere and also in this issue (Gang Huang) [16, 17].

Several recent reports have indicated that the epigenetic regulation by PRC1 is more complicated than previously thought. For instance, in embryonic stem cells (ESCs), some PcG target genes are repressed by PRC1 even without PRC2, since loss of PRC2 in ES cells does not change the global levels of H2AK119ub1 and the PRC1 component BMI1 is properly recruited to some polycomb target genes [18]. In support of these findings, it has also been shown that PRC1 is recruited to chromatin independently of PRC2 by forming a complex with RYBP instead of CBX. RYBP in PRC1 confers specific and non-overlapping functions onto PRC1 distinct from the classical CBX-containing PRC1, which requires the H3K27me3 mark for its recruitment [19, 20].

Human *Additional Sex Combs Like 1* (*ASXL1*) is one of the homologs of the *Drosophila Additional sex combs* gene, which encodes a chromatin-binding protein that regulates the expression of the *Hox* genes and is required for correct body segmentation. ASXL1 is one of enhancers of *trithorax* and *polycomb* genes that encode proteins required for both gene activation and silencing [21]. Of note, somatic mutations of ASXL1 have recently been reported in myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPN) patients [22]. ASXL1 has been shown to associate with PRC2, but not PRC1, and loss of ASXL1 results in global reduction in PRC2 dependent-H3K27me3 mark [23]. Furthermore, it has been shown that depletion of Asxl1 collaborates with oncogenic N-Ras to promote myeloid leukemogenesis [23]. ASXL1 interacts with BAP1, a deubiquitinase for H2AK119. Bap1 conditional deletion in adult hematopoietic cells results in the formation of an MDS/chronic myelomonocytic leukemia (CMML)-like disease in mice [24]. Although the functional crosstalk between ASXL1 and BAP1 remains unknown, these findings implicate ASXL1 and BAP1 in the epigenetic regulation of hematopoiesis by PcG proteins. The current model of PRC-mediated gene silencing is shown in Fig. 1.

Role of PRC1 genes in hematopoiesis

Over the last decade, many studies utilizing knockout mice deficient for different subunits of PRC1 revealed the critical roles that each PRC1 gene plays in HSCs and their progeny cells [25, 26]. A list of major PRC genes and their functions in hematopoiesis are summarized in Table 1. As described above, PRC1 is now believed to be more diverse than previously thought. In light of this, we must accept that our knowledge of the function of PRC1 in hematopoiesis is still quite limited and requires much more investigation.

Among the PRC1 genes in hematopoiesis, the role of the PcG gene *Bmi1* has been best characterized. Although

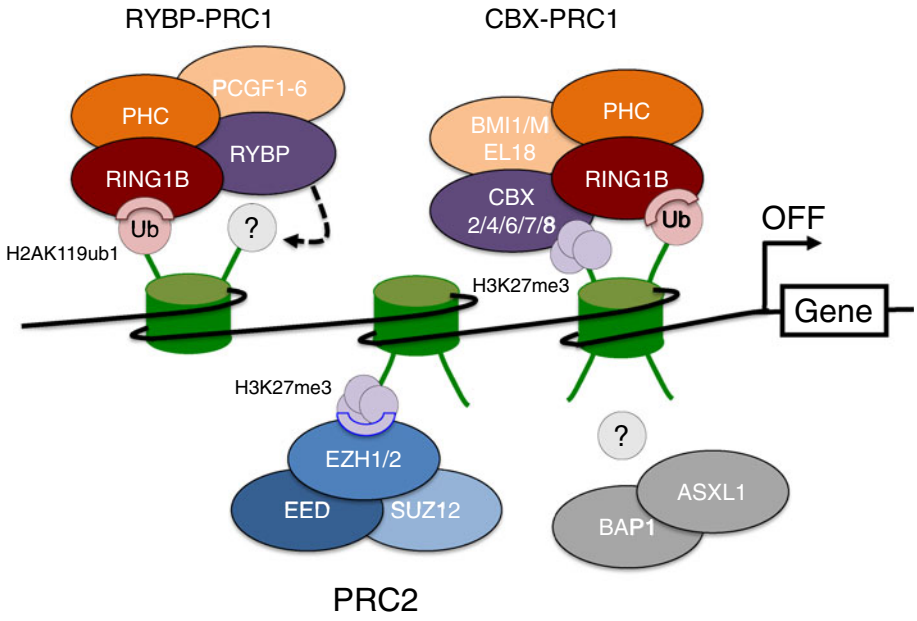

Fig. 1 Epigenetic silencing of PcG complexes. Following recruitment of PRC2 to chromatin, EZH1/2 catalyzes the trimethylation of histone H3 at lysine 27 (H3K27me3). Subsequently, PRC1 binds to H3K27me through CBX subunit, and then RING1B monoubiquitylates histone H2A at lysine 119 (H2AK119ub1). Recently, RYBP1–PRC1 complexes have been identified in ES cells, and these complexes are recruited to their target genes independent of PRC2 and H3K27me3 (recruiting factors are indicated as *gray circles* with a *question mark*). It is unclear how ASXL1 is involved in the BAP1-mediated deubiquitination of H2A and PRC2-dependent H3K27me3 modification

Table 1 List of representative epigenetic genes that regulate murine hematopoiesis and are mutated in human hematological malignancies

Mutant mice	Hematopoietic defects in mutant mice	Types of mutations in humans
DNA methyltransferase		
Dnmt1 hypomorphic mice	Self-renewal defect	
	Enhanced myeloid commitment	
Dnmt3a$^{-/-}$	Enhanced self-renewal	Missense/AML
	Impaired myeloid differentiation	
Dnmt3a$^{-/-}$*Dnmt3b*$^{-/-}$	Self-renewal defect	
5-methylcytosine hydroxylase		
Tet2$^{-/-}$	Enhanced self-renewal	Missense/MPN
PRC1 components		
Bmi1$^{-/-}$	Self-renewal defect	No mutations
		Overexpression in AML
PRC2 components		
Suz12 hypomorphic mice	Enhanced self-renewal	Missense/MDS, AML, MPN
Ezh2$^{-/-}$	Impaired lymphopoiesis	Missense/MDS, AML, MPN
Trithorax gene		
Mll1$^{-/-}$	Self-renewal defect	Translocation/ AML, ALL
Histone acetyltransferase		
Moz$^{-/-}$	Self-renewal defect	Translocation/ AML

AML acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasms

Bmi1-deficient mice do not show defects in fetal liver hematopoiesis, they show severe postnatal pancytopenia due to progressive depletion of HSCs. In the absence of Bmi1, HSCs fail to self-renew in the long term, even though short-term reconstitution capacity is preserved [27, 28]. Bmi1 directly binds to the promoter of the cyclin dependent kinase (CDK) inhibitor gene, $p16^{Ink4a}$ and the tumor suppressor gene, $p19^{Arf}$ together with other PRC1 components and represses their transcription. In *Bmi1*-deficient mice, expression of $p16^{Ink4a}$ and $p19^{Arf}$ is markedly de-repressed in HSCs, restricting the proliferative capacity of HSCs and their progeny. Notably, deletion of both $p16^{Ink4a}$ and $p19^{Arf}$ in *Bmi1*-deficient mice substantially restores the defective self-renewal capacity of HSCs. These findings define $p16^{Ink4a}$ and $p19^{Arf}$ genes as important Bmi1 targets in HSCs [28, 29]. Bmi1 also regulates mitochondrial function by regulating the expression of a number of genes relevant to mitochondrial function and ROS generation. *Bmi1*-deficient cells have impaired mitochondrial function, which causes a marked increase in the intracellular levels of ROS and subsequent activation of DNA damage response [30]. Antioxidant treatment or genetic depletion of Chk2 can rescue impaired proliferation of progenitors and lymphocytes, but not the long-term reconstitution capacity of HSCs in *Bmi1*-deficient mice. Taken together, these reports show that Bmi1 regulates HSC self-renewal, at least in part by repressing the expression of $p16^{Ink4a}$ and $p19^{Arf}$, and by maintaining normal mitochondrial function.

Recently, Bmi1 was also shown to restrict hematopoiesis and antagonizes the development of pathological hematopoiesis in mice. As described above, *Bmi1*$^{-/-}$*Ink4a-Arf*$^{-/-}$

hematopoietic cells reacquire self-renewal capacity [29]. Unexpectedly, however, the loss of Bmi1 augmented reconstituting capacity of BM cells in the *Ink4a/Arf*-null background. Furthermore, *Bmi1*$^{-/-}$*Ink4a-Arf*$^{-/-}$ hematopoietic cells established marked extramedullary hematopoiesis in the spleen and liver and eventually induced lethal myelofibrosis, the most severe chronic MPN. The oncogene *Hmga2*, a direct target of Bmi1, was implicated as one of the responsible genes for the development of lethal myelofibrosis in the absence of Bmi1. These findings indicate that PcG proteins antagonize the development of MPN in the absence of their tumor-suppressor targets, *Ink4a* and *Arf* [31]. Although *Bmi1*-deficiency is self-limiting unless *Ink4a* and *Arf* are deleted first, *INK4A* and *ARF* are frequently inactivated by mutations or transcriptionally repressed by DNA methylation at their promoters in human cancers. These findings suggest that in such situations, tumor cells with impaired PcG function could out-compete cells with normal PcG function since the effects of de-repressed oncogenes, such as *HMGA2*, appear to supersede the effects of de-repressed tumor suppressor genes.

In ESCs, Ring1 proteins repress the expression of developmental regulator genes. Correspondingly, ESCs lacking both Ring1a and Ring1b cannot maintain pluripotency [32]. Deletion of *Ring1b* compromises ESC differentiation but not self-renewal [33], while deletion of *Ring1a* affects neither self-renewal nor differentiation, and only slightly perturbs gene expression [32–34]. These findings suggest redundant and distinct functions of these two paralogs. Mice deficient for *Ring1b* in hematopoietic cells develop a hypocellular BM that paradoxically contains an enlarged, hyperproliferating compartment of immature cells [35]. Ring1b restricts the proliferation of progenitors and stimulates differentiation of their progeny through regulating expression of the cell cycle activator *cyclin D2* and the CDK inhibitor $p16^{Ink4a}$. We must await studies of *Ring1a/Ring1b*-double knockout mice to more fully understand the role of Ring1a and Ring1b in hematopoiesis.

Role of PRC2 genes in hematopoiesis

Contrary to studies of PRC1, there have been relatively few reports detailing the role of PRC2 in HSCs. Heterozygosity for an *Eed* null allele causes myeloproliferative and lymphoproliferative disease in mice [36]. A hypomorphic mutation of *Suz12* and heterozygosity for an *Ezh2* null allele mildly but significantly ameliorate the HSC defects and reduced platelet numbers in mice that lack the thrombopoietin (TPO) receptor [37]. These findings evoke the possibility that PRC2 restricts HSC/progenitor activity. However, it has been reported recently that the complete loss of Eed significantly prolongs survival of leukemic mice and markedly reduces transplantability of leukemic cells into secondary recipients in the MLL-AF9 leukemic fusion protein-induced murine leukemia model [38]. Importantly, *Eed*-deficient MLL-AF9 leukemic cells show complete loss of H3K27me3. These findings clearly indicate that PRC2 is required for the maintenance of the self-renewal capacity of leukemic stem cells. Although the effect of loss of Eed in HSCs has not been reported, these findings also suggest an essential role of PRC2 in HSCs as well.

In contrast, absence of Ezh2 appears not to impair adult hematopoiesis, although it causes severe defects in lymphoid differentiation [39, 40]. In adult BM, the self-renewal capacity of *Ezh2*-deficient HSCs is not compromised and the H3K27me3 is retained at significant levels even in the absence of Ezh2, possibly due to complementation by Ezh1. This is also the case in the MLL-AF9-induced leukemia model in mice. Ezh2 is not strictly required for the self-renewal of MLL-AF9 leukemia, but augments leukemogenicity, in part by reinforcing differentiation blockage [38, 41]. The complementary function of Ezh1 in hematopoiesis, however, has never been tested. Given that the inactivating mutations in EZH2 play critical roles in myeloid malignancies, it is important now to understand how EZH1 compensates for the absence of functional EZH2 in HSCs.

Accumulating evidence has uncovered a broad range of target genes of the PcG proteins that include both growth-promoting and growth-restricting genes. Thus, the PcG proteins fine tune the growth of hematopoietic cells in both a positive and negative manner to maintain hematopoietic homeostasis. The balance between the opposing target genes is critical, and it may be altered depending on the dosage of PcG proteins and concomitant gene mutations in pathological settings. Indeed, inactivating mutations of *EZH2* have been identified in patients with MDS and MPN, showing that PcG genes also have a tumor suppressor function [42, 43]. The deregulated balance between the opposing PcG targets could account for the tumor suppressor function of PRC2 observed in human MDS and MPN. Although loss-of-function mutations in PRC1 genes have never been reported in a clinical setting, mutations in the PRC2 genes have been identified in a growing number of hematological malignancies, including acute lymphoblastic leukemia (ALL) [10].

Effects of forced expression of PcG genes in HSCs

Forced expression of *Bmi1* or *Ezh2* in HSCs augments self-renewal capacity of HSCs and prevents exhaustion of the long-term repopulating potential of HSCs during repeated serial transplantation [44, 45]. Of interest, however,

overexpression of *Bmi1* and *Ezh2* in mice using the conditional knock-in system exhibits different phenotypes in hematopoiesis [46, 47]. Overexpression of *Bmi1* confers resistance to stresses, particularly oxidative stress, onto HSCs, thereby promoting expansion of functional HSCs during ex vivo culture and efficiently protecting HSCs against loss of self-renewal capacity during serial transplantation. However, it does not significantly affect steady state hematopoiesis, including the numbers of HSCs and their progeny. In contrast, *Ezh2* overexpression causes a significant increase in HSCs and myeloid lineage cells in BM. This trend is exacerbated with age and eventually leads to development of severe myeloproliferative disease in mice [47]. These functional differences between the two genes in an overexpression setting are quite intriguing and suggestive of different roles of PRC1 and PRC2 in the regulation of the cell cycle status of HSCs.

Regulation of multipotency of HSCs via bivalent chromatin domains

In ESCs, PRC2 and the TrxG complex mark developmental regulator gene promoters with bivalent domains consisting of overlapping repressive (H3K27me3) and activating (H3K4me3) histone modifications to keep them "poised" for activation [48, 49]. PRC1 also co-regulates the vast majority of developmental regulator gene promoters marked with bivalent domains [50]. Upon differentiation, promoters with bivalent domains are resolved into a monovalent state, either active or repressive. Transcriptional regulation of developmental regulator genes via bivalent domains is essential to maintain ESCs in an undifferentiated pluripotent state [48, 51], and these bivalent domains are also partially shared by HSCs [52, 53]. We previously demonstrated that *Bmi1*-deficient HSCs show accelerated B cell lineage specification. Loss of Bmi1 leads to premature expression of the B cell lineage developmental regulator genes *Ebf1* and *Pax5* and their downstream target genes in HSCs/multipotent progenitors (MPPs), since *Ebf1* and *Pax5* are repressed by bivalent domains in multipotent HSCs/MPPs [54]. These findings suggest that PcG proteins keep differentiation programs poised for activation in HSCs to maintain their multipotency by repressing a cohort of hematopoietic developmental regulator genes via bivalent domains, as they do in ESCs.

DNA methyltransferases and their function in hematopoiesis

DNA methylation of CpG nucleotides is a key epigenetic modification, and CpG-methylation is catalyzed by a family of DNA methyltransferase enzymes: Dnmt1, Dnmt3a, and Dnmt3b [55]. Dnmt3a and Dnmt3b function as de novo methyltransferases, modifying unmethylated DNA, whereas Dnmt1 is thought to function principally by maintaining existing methylated DNA [56, 57]. These factors are essential for embryonic development as their absence results in death in mutant mouse studies. This is also true in hematopoiesis. *Dnmt1*-deficient HSCs show impaired self-renewal and de-repressed expression of myeloerythroid regulators, leading to skewed lineage commitment to the myeloerythroid lineage [58, 59]. While loss of Dnmt3a or Dnmt3b alone does not impair the self-renewal capacity of HSCs, loss of both Dnmt3a and Dnmt3b does, suggesting functional redundancy between Dnmt3a and Dnmt3b [60]. Given that somatic mutations of DNMT3A were recently found in AML and MDS patients [61, 62]; however, hypomorphic DNMT3A is assumed to have some pathological roles in hematological malignancies. In this regard, a recent report of *Dnmt3a*-deficient mice by Goodell's group has provided some clues [63]. *Dnmt3a* expression is highly enriched in long-term HSCs compared to progenitors and differentiated cells. They found that the absence of Dnmt3a progressively impairs the differentiation capacity of HSCs over the course of serial transplantation, and is accompanied by an accumulation of HSCs in the BM. *Dnmt3a*-deficient HSCs showed both increased and decreased methylation at various loci, resulting in an up-regulation of multipotency genes such as *Runx1*, *Gata3*, *Pbx1* and *p21*, and a down-regulation of differentiation factor genes in HSCs, although Dmnt3a loss did not appeared to be sufficient for the development of myeloid malignancies. These findings show that Dnmt3a is a critical epigenetic regulator of HSCs that is required for their efficient differentiation.

Role of TET2 in hematopoiesis

TET family proteins including TET1, TET2 and TET3, have been shown to catalyze the conversion of 5-methylcytosine (5mC) to 5-hydroxymethyl-cytosine (5hmC) [64]. While TET1 is highly expressed in ESCs, TET2 and TET3 are expressed in blood cells, especially in differentiated myeloid cells [65]. Although TET family proteins are thought to activate gene expression via removal of 5mC, Tet1 also functions in transcriptional repression in ESCs [66]. Tet1 binds a significant proportion of polycomb target genes and also associates and colocalizes with the Sin3a co-repressor complex, suggesting a role for Tet1 in gene silencing. In contrast, Tet2 does not associate with the Sin3a complex, and its role in gene silencing remains unknown.

Loss-of-function mutations of TET2 are frequently found in myeloid malignancies such as MDS, MPN, and AML, and also recently have been reported in T cell lymphoma [65, 67]. In agreement with these findings, conditional *Tet2*-knockout mice show expansion and enhanced self-renewal activity of HSCs [67, 68]. *Tet2* loss in hematopoietic cells is sufficient to induce myeloproliferative disorders in mice after a long latency. Although the mechanism by which Tet2 regulates hematopoiesis is not clear, especially since the critical targets of Tet2 are still unknown, Tet2 clearly functions in the restriction of self-renewal and transformation of HSCs. It is assumed that TET3 has a function redundant to TET2, but no mutations have been identified in TET3 in hematological malignancies. Nevertheless, detailed analysis of TET3 will also be important to understand the biological role of hydroxylation of 5mC in hematopoiesis.

Conclusion

We have discussed how hematopoiesis is regulated epigenetically through DNA methylation and histone modifications. However, it is not completely known how DNA methylation and histone modifications are coordinated with each other in hematopoiesis. There are several instructive studies regarding the biological interplay between polycomb-mediated H3K27me3 and DNA methylation during malignant transformation [69]. It is reported that polycomb-mediated H3K27me3 pre-marks genes for de novo methylation in cancer. Polycomb repressive marks are frequently switched into DNA hypermethylation during cancer progression. Although DNA methylation is not necessarily a second event following histone modifications, the epigenetic transition from polycomb-mediated histone modifications to DNA methylation could play a critical role in hematopoiesis. The current era of epigenetic research is giving rise to a new field within HSC biology, and providing novel therapeutic modalities against hematological disorders.

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