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Mini Review

Epigenetic regulation of hematopoietic stem cells

Shuhei Koide, George R. Wendt and Atsushi Iwama*

Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan

The sequence of DNA is the same in all somatic cells of an organism, despite the variety of cells that exist within the organism. Mammalian blood, for example, contains a number of distinct mature cell types. These mature cells are all derived from hematopoietic stem cells (HSCs). Both HSCs and mature cells have the same genome, but their gene expression is controlled by epigenetic mechanisms such as DNA methylation and histone modification, enabling each cell-type to acquire various forms and functions. Recently, improvements in NGS (next-generation sequencing) technology have allowed extensive epigenetic analysis with small amounts of cells, improving our understanding of the role of epigenetics in stem cells. In this review, we focus on the epigenetic regulation of HSCs.

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*Correspondence should be addressed to:
Atsushi Iwama, Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan. Phone: +81-43-226-2189, Fax: +81-43-226-2191. E-mail: aiwama@faculty.chiba-u.jp

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Introduction

Mature blood cells are produced at a rate of more than 1 million cells per second in an adult human. These cells are all ultimately derived from hematopoietic stem cells (HSCs). HSCs are multipotent stem cells that reside in the bone marrow and can differentiate into all blood cells[1]. Their fate is controlled by epigenetic regulation, defined as the mechanisms that maintain cell-type specific gene expression patterns in daughter cells through cell division independently of the primary DNA sequence. Epigenetic regulation mechanisms include DNA methylation, histone modifications, and various types of RNA-mediated interactions (Fig.1, Table 1). These mechanisms play a key role in cellular memory, the ability of cells to "remember" cell-lineage specific expression patterns induced by transiently expressed factors through subsequent cell divisions[2]. The faithful retention of cellular memory is essential not only for development, but also for the maintenance of cellular homeostasis.

DNA methylation and HSCs

DNA methylation is a potent epigenetic mark that promotes gene silencing. Methylation occurs at the 5-carbon position of cytosines (resulting in 5-methylcytosine or 5-

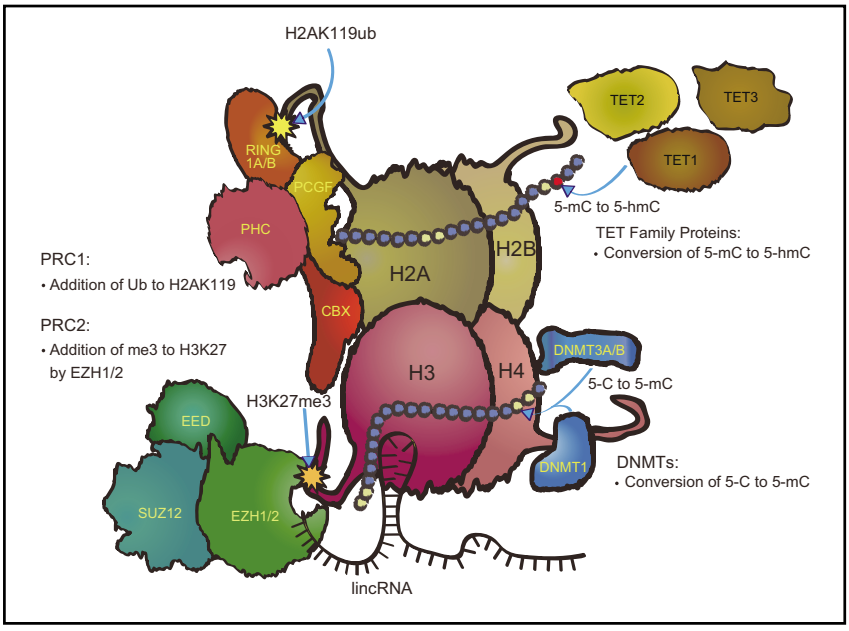

Fig.1 Prevalent examples of epigenetic modifications

In this review, we discuss a range of epigenetic mechanisms, including DNA methylation by DNA methyltransferases, oxidation of methylated DNA by the TET family proteins, modification of histone tails by the Polycomb proteins, and the role that lincRNAs might play in coordinating these epigenetic factors. In this cartoon, the small blue circles indicate unmethylated DNA, the small yellow circles indicate methylated DNA, and small red circles indicate hydroxymethylated DNA. DNA methyltransferases and the TET family proteins are shown modifying their respective targets. Canonical PRC1 and 2 are also shown modifying the histone tails of H2A and H3, respectively. Finally, a lincRNA is shown interacting with EZH2.

mC) found in cytosine-phosphate-guanine dinucleotides (CpGs) through the action of the DNA methyltransferase (DNMT) family of proteins. Genomic CpGs in untranscribed domains such as pericentromeres, introns, and transposons are very highly methylated. Additionally, there are regions of higher CpG density compared to other genomic regions, which form domains called CpG islands. These CpG islands are often found in the promoter region of genes, upstream of the transcription start site. In many cases, transcription status is controlled by the methylation state of CpG islands, with hypomethylation allowing transcription and hypermethylation inhibiting transcription. Methylation patterns undergo rapid changes during fertilization, and primary establishment of these methylation patterns requires the DNA methyltransferase enzymes, DNMT3a and DNMT3b. DNMT1, the most abundant DNA methyltransferase in mammalian cells, is highly expressed during the S phase and faithfully maintains the DNA methylation marks in somatic cells after each replication cycle[3, 4].

Of interest, the DNMT proteins appear to play a unique role in HSCs. Conditional deletion of *Dnmt1* from HSCs results in a loss of differentiation and self-renewal ability during serial transplantations, as well as a tendency for HSCs to be displaced from the niche, suggesting weakened niche interactions. Interestingly, loss of *Dnmt1* appears to affect HSCs and differentiated progenitor cells in a different manner, suggesting a unique role for *Dnmt1* in HSCs[5]. Alternatively, conditional deletion of *Dnmt3a* from HSCs 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 up-regulation of multipotency genes and down-regulation of differentiation factor genes in HSCs[6]. Recently, Beerman and colleagues reported that the DNA methylation patterns in the HSC compartment differ depending on the proliferative history of the cell[7]. Of particular interest, it was found that hypermethylation of Polycomb Repressive Complex 2 targets (a topic addressed later in this review) appears to accompany forced proliferation and aging, suggesting that DNA methylation plays a critical role in regulating the physiological aging of HSCs. Taken together, these findings illustrate the critical and varied roles that DNA methylation plays in HSC maintenance.

TET Family of Proteins

A recent hot topic in epigenetics is the oncogenic proteins of the TET (ten-eleven-translocation) family. TET 1-3 are dioxygenases that catalyze the conversion of 5-mC to 5-hydroxymethylcytosine (5-hmC), which can then be replaced by unmethylated cytosine through further modification and base excision repair pathways[8, 9]. Additionally, it has also been suggested that TET proteins and the 5-hmC marks are capable of acting as an epigenetic mark independent of DNA demethylation in various contexts by as-

Table 1 Epigenetic Writers, Readers, and Erasers

	Writer		*Reader*		*Eraser*	
DNA methylation	DNMT1		MecP2		Tet1/2/3	
	DNMT3A		MBD1/2			
	DNMT3B					
Histone acetylation	***Histone acetyltransferases***		***Bromodomain proteins***		***Histone deacetylases***	
	H2BK5	ATF2,P300	H3K14	ANCCA	H3K4	SIRT1-3,HDAC1/2/3/6/10
	H2BK12	ATF2,P300	H3K18,H4K5K8	BDF1,BRD2/3/4/7,BRDT	H3K9	SIRT1/2/3/7,HDAC1/2/3/6/10
	H2BK15	ATF2,P300	H3K36,H4K20	BRG1,P300	H3K27	HDAC1
	H2BK20	GCN5,P300	H4K16	GCN5		
	H3K9	SRC-1	H3K14	PB-2,PCAF,RSC4		
	H3K14	GCN5,ELP3,ESAL	H3K23	TRIM24		
		HPA2,P300,PCAF				
		SAS2,SAS3,SRC-1				
		TAF1,TIP60				
	H3K18	GCN5,P300				
	H3K27	GCN5				
	H4K5	ATF2,ESAL,HPA2				
		P300,TIP60				
	H4K8	ATF2,ELP3,ESAL				
		GCN5,P300,PCAF				
		TIP60				
	H4K8	ATF2,ELP3,ESAL				
		GCN5,P300,PCAF				
		TIP60				
	H4K12	ESAL,HPA2,P300				
		TIP60				
	H4K16	ATF2,ESAL,GCN5				
		SAS2,TIP60				
Histone methylation	***Lysine methyltransferases***		***Chromodomain***		***Lysine demethylases***	
	H3K9	CLR4,G9A,SETDB1	H3K9me3/2	HP1,CBX1/3/5,CDY	H3K4	LSD1
		SUV39H		CHP1,MPP8,Tip60	H3K9	JMJD1A,JMJD2A,JMJD2C
	H3K4	MLL1-3,SET7/9	H3K27me3/2	Pc	H3K27	JMJD3
	H3K36	NSD1,SET2	3K27/K9me3/2	CBX2/4/6/7/8	H3K36	JMJD2A
	H3K79	DOT1L	***PHD***			
	H3K20	SUV420H1	H3K4me3/2	BPTF,ING1-5,JARID1A		
	H3K27	EZH1,EZH2,G9A		KIAA1718,Lid,MLL1		
				PHF2/8		

Mechanisms of epigenetic regulation include DNA methylation and histone modifications. This table provides a non-inclusive list of the various proteins and complexes that add epigenetic marks (writers), remove them (erasers), and bind them to exert downstream effects (readers).

sociating with O-linked *β-N*-acetylglucosamine transferase (OGT) in ESCs[10] and by associating with the transcriptional repressor MeCP2 in neurons[11].

Of hematological interest, loss-of-function mutations in one or both of the alleles of the *TET2* gene have been reported in numerous types of hematological malignancies. Recent studies have also shown that loss of *Tet2* in HSCs results in a modest expansion of the HSC pool, a competitive advantage in serial transplantation, and a tendency to differentiate into the monocytic lineage. Some heterozygous and homozygous *Tet2*-deficient mice also develop a lethal myeloid malignancy, suggesting that *Tet2* functions

as a tumor suppressor that exhibits haploinsufficiency[12, 13].

Histone Modifications

DNA in eukaryotic cells is spooled around structural proteins known as histones. Histones are composed of a globular domain around which DNA winds and a structurally undefined but highly evolutionarily conserved "tail" domain. These tails are relatively exposed to solvent and are readily accessible to enzymes such as acetyltransferases, methylases, and kinases[14]. Generally, histone acetylation results in activation of transcription[15] whereas the effect of histone methylation differs depending on the target residue in the histone tail.

Polycomb Group Proteins

The polycomb group (PcG) proteins form the polycomb repressive complexes (PRC) 1 and 2 which generally act in transcriptional silencing. 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). Canonical PRC1 contains four core subunits, PCGF (which can include 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 of PRC1 then monoubiquitylates histone H2A at lysine 119 (H2AK119ub1), promoting repression by inhibiting RNA polymerase II-dependent transcriptional elongation and also by promoting chromatin compaction[16]. Though they won't be covered in depth in this review, the trithorax group (TrxG) proteins are generally thought to work opposite of the PcG proteins, turning on gene transcription. For a more detailed review, see Schuettengruber et al[17].

Both the TrxG proteins and the PcG proteins have been shown to be involved in normal and malignant hematopoiesis, in part by regulating the *HOX* genes. PRC2 components appear to function differently at different developmental stages, with Ezh2 being required for fetal hematopoiesis but largely dispensable for BM hematopoiesis[18]. On the other hand, Ezh1 is not required for fetal hematopoiesis but its function is essential for self-renewal and maintenance of HSCs in the BM[19]. Cbx orthologs appear to regulate the function of PRC1 by competing for PRC1 integration. Cbx7 containing PRC1 complexes induce self-renewal in HSCs by repressing the expression of progenitor-specific genes. Incorporation of other Cbx proteins into PRC1 (Cbx2, Cbx4, or Cbx8) results in the repression of HSC-specific genes and the induction of differentiation[20]. Additionally, PRC1 and 2 have been shown to cooperate in the silencing of the *INK4A/ARF* tumor suppressor locus in numerous types of somatic stem cells, including HSCs. The PcG protein BMI1 in particular is critical for the repression of the *INK4A/ARF* locus, as overexpression of Bmi1 enhances the self-renewal ability of HSCs[21] and loss of *Bmi1* results in a depletion of HSCs but concurrent deletion of *Ink4a* and *Arf* rescues the HSC depletion[22].

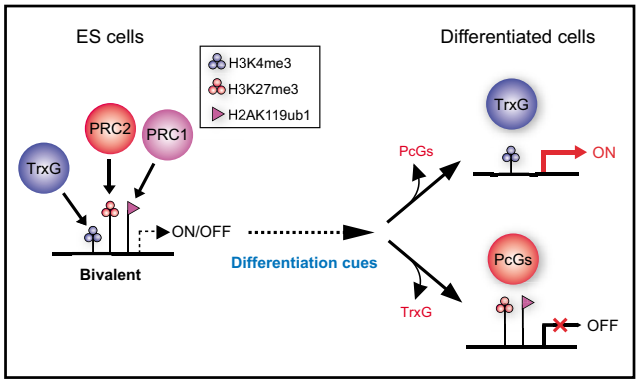

Fig.2 Bivalent domains in embryonic stem cells

The overlap of transcriptional promoting and transcriptional repressing histone marks are referred to as bivalent domains. It is hypothesized that these bivalent domains play an important role in the maintenance of pluripotency in stem cells. Upon receiving differentiation cues, stem cells will tip towards either a transcriptionally active or a transcriptionally repressed state, and the bivalent domain will "resolve".

Bivalent Domains and HSCs

Of note, PRC2 and TrxG proteins have been shown to mark the promoters of developmental regulator genes with bivalent domains consisting of overlapping repressive (H3K27me3) and activating (H3K4me3) histone modifications. This is believed to keep developmental regulators "poised" for activation in embryonic stem (ES) cells. Upon differentiation of ES cells, promoters marked with bivalent domains are resolved into a monovalent state, either active or repressive (Fig.2)[23].

Bivalent domains are also found in adult stem cells, including HSCs[24, 25], although their biological meaning remains unclear. We have previously analyzed HSCs deficient for *Bmi1* in an *Ink4a-Arf*-null genetic background

(*Bmi1*$^{-/-}$*Ink4a-Arf*$^{-/-}$), and found that *Bmi1*$^{-/-}$*Ink4a-Arf*$^{-/-}$ HSCs tend to commit to the B-cell lineage, resulting in a drastic increase in common lymphoid progenitors (CLPs) at the expense of the HSC/multipotent progenitor (MPP) pool size. Loss of *Bmi1* leads to premature expression of B-cell lineage developmental regulator genes, *Ebf1* and *Pax5* in multipotent HSC/MPPs. *Ebf1* and *Pax5* were demonstrated to be transcriptionally repressed by bivalent domains co-regulated by both PRC1 and PRC2 in HSC/MPPs. Thus, *Bmi1* negatively regulates B-cell lineage specification in HSC/MPPs and, by repressing lineage specification, contributes to their multipotency. These findings suggest that PcG proteins maintain HSC multipotency by keeping their differentiation programs poised for activation by repressing a cohort of hematopoietic developmental regulator genes via bivalent domains as they do in ES cells[26].

Long Intergenic Non-Coding RNA and HSCs

Long intergenic non coding RNAs (lincRNAs) are a class of non-coding transcripts that, among other roles, regulate chromatin states and other epigenetic modifications. Often, lincRNAs serve as scaffolds by providing binding surfaces to assemble certain histone-modifying enzymes. For example, *HOTAIR* is a lincRNA transcribed from the *HOXC* locus which interacts with PRC2 in order to silence the *HOXD* genes located on a different chromosome. The 5' domain of *HOTAIR* binds the PRC2 methyltransferase EZH2, while 3' domain of HOTAIR binds to the demethylase LSD1/CoREST/REST complex, promoting H3K27me3 and H3K4 demethylation[27]. A recent study has also shown that the non-coding RNA lincRNA-EPS plays a functional role in regulating erythropoiesis by suppressing *Pycard*-mediated apoptosis, establishing that lincRNAs play a functional role in hematopoiesis[28].

Conclusion

The role of epigenetic regulation in HSCs is gradually becoming clearer. Understanding of the processes regulated by epigenetic mechanisms in HSCs will one day help us to realize the potential of stem cells in regenerative medicine. Indeed, expansion of HSCs by modulating the expression of PcG components such as Bmi1 is already feasible. In addition, several recent reports have shown that noncoding RNAs are critical for the recruitment of transcription factors and epigenetic regulators to their genomic targets, suggesting another potential avenue of epigenetic therapy. By manipulating the epigenetic state, it may one day be possible to control the direction and differentiation of HSCs.

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Conflict of interest

The authors have no conflicting financial interests.

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