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Review Article

EZH2: a pivotal regulator in controlling cell differentiation

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Abstract: Epigenetic regulation plays an important role in stem cell self-renewal, maintenance and lineage differentiation. The epigenetic profiles of stem cells are related to their transcriptional signature. Enhancer of Zeste homlog 2 (EZH2), a catalytic subunit of epigenetic regulator Polycomb repressive complex 2 (PRC2), has been shown to be a key regulator in controlling cellular differentiation. EZH2 is a histone methyltransferase that not only methylates histone H3 on Lys 27 (H3K27me3) but also interacts with and recruits DNA methyltransferases to methylate CpG at certain EZH2 target genes to establish firm repressive chromatin structures, contributing to tumor progression and the regulation of development and lineage commitment both in embryonic stem cells (ESCs) and adult stem cells. In addition to its well-recognized epigenetic gene silencing function, EZH2 also directly methylates nonhistone targets such as the cardiac transcription factor, GATA4, resulting in attenuated GATA4 transcriptional activity and gene repression. This review addresses recent progress toward the understanding of the biological functions and regulatory mechanisms of EZH2 and its targets as well as their roles in stem cell maintenance and cell differentiation.

Keywords: EZH2, polycomb repressive complex, embryonic stem cells, adult stem cells, chromatin modification, methylation

Introduction

All cells in an individual organism possess fundamentally identical genomes; nonetheless, they can function differently in a multicellular organism, highlighting the significance of epigenetic regulatory machinery in controlling developmental cells. During development, cells proceed from totipotency to pluripotency, and then terminal differentiation. Stem cells have the capability to self-renew and generate daughter cells that are able to differentiate into different lineages. Embryonic stem cells (ESCs) are pluripotent and can become all cell types in adult organism [1]. In adult, stem cells also exist in many types of tissues throughout life and may play crucial roles in tissue regeneration and repair. Lineage-restricted adult stem cells are only able to differentiate into limited cell types. For example, hematopoietic stem cells can generate blood cells and mesenchymal stem cells give rise to bone and fat cells, among others [2, 3]. The above-mentioned cell fate determination and development are closely orchestrated by genetic and epigenetic programs to ensure the specific developmental commitments are being faithfully propagated to the progeny cells [4-6]. Epigenetic regulation includes DNA methylation [7], histone modifications [8, 9], incorporation of histone variants, and non-coding RNAs [10, 11]. Genome-wide studies of the epigenetic regulator Polycomb group (PcG) protein targets in stem cells and differentiated cells in different organisms have revealed unique epigenetic profiles that repress developmental genes to prevent premature gene expression in stem cells. Yet, these genes are activated during stem cell differentiation [12]. These epigenetic mechanisms reflect the unique transcriptional status of the genes and provide the specific gene-expression signature during cell differentiation.

Epigenetic regulation by PcG proteins

Accumulating evidence shows that PcG proteins play a critical role in controlling tissue development and maintenance. They were first identified in *Drosophila* as essential proteins required for segmentation to maintain the appropriate spatial patterns of homeotic box gene expression during development and adulthood [13-16]. PcG proteins are able to form chromatin remodeling complexes and are classified into Polycomb repressive complex 1 (PRC1) and 2 (PRC2) in mammals. The constituents of these complexes may be cell context-dependent and differ among organisms, but their core subunits are conserved from *Drosophila* to mammals [16-18]. The major components of the PRC1 complex contain the RING1, BIM1, CBX, and PHC subunits [19, 20]. RING1 possesses E3 ubiquitin ligase activity that monoubiquitylates Lys 119 of histone H2A (H2AK119ub1) to establish a repressive chromatin structure [21]. The core subunits of PRC2 consist of embryonic ectoderm development (EED), suppressor of Zeste 12 (SUZ12), retinoblastoma suppressor associated protein 46/48 (RbAp46/48), and the histone methyltransferase, EZH2, which catalyzes the trimethylation of histone H3 on Lys 27 (H3K27me3) to generate another epigenetic silencing mark [13, 22-25]. On the other hand, Trithorax group (TrxG) proteins antagonize PcG-mediated gene silencing function and activate gene expression by modifying chromatin structure via trimethylation of histone H3 Lys 4 (H3K4me3) [26]. Recent studies have revealed that the PcG proteins may suppress gene expression by maintaining chromatin compaction at the *Hox* loci in ESCs independently of the PRC1 enzymatic activity whereas chromatin decompaction and activation of *Hox* gene clusters are observed during ESC differentiation [27]. Moreover, PcG proteins also maintain repressive chromatin structure by DNA looping with DNA methylation [28], which may block the accessibility of DNA to transcription factors. The exact functions and mechanisms responsible for these high-order chromatin organizations still remain to be elucidated.

EZH2 function and PRC2 recruitment

EZH2 contains a SET domain that catalyzes histone H3 trimethylation on Lys 27 (H3K27me3). Subsequently, the H3K27me3 mark is recognized and bound by the PRC1 subunit, CBX, then the catalytic subunit of PRC1, RING1, monoubiquitylates Lys 119 of histone H2A (H2AK119ub1) to prevent RNA polymerase II-dependent transcriptional elongation and repress gene transcription. Without EZH2 activity, PcG-mediated repression cannot be established and therefore fails to recruit PRC1 to chromatin [13, 17, 29-33]. Although the sequential recruitment model of PRC1/2 is widely accepted, there are reports showing that PRC1/H2AK119ub1-mediated gene repression can occur independently of PRC2/H3K27me3 in mouse embryonic stem cells (mESCs) and differentiated cells [34-36]. Moreover, Tavares et al. recently discovered that PRC2/H3K27me3 is not required for the recruitment of RYBP-PRC1 complex and RYBP-PRC1-mediated H2AK119ub1 on PcG target loci and inactive X chromosome [37]. They utilized PRC2-null mESCs to demonstrate that RYBP-PRC1 complex, consisting of RING1, YY1 binding protein (RYBP), and PRC1 catalytic subunits, mediates H2AK119ub1 without H3K27me3 at a similar level compared to wild type mESCs [37]. Therefore, the recruitment of PRC1 may occur either through PRC2/H3K27me3-dependent or -independent chromatin modifications in ESCs.

The di- and trimethylation activity of PRC2 on H3K27 are also associated with facultative chromatin, a subdivision of heterochromatin that is modulated in a development-specific manner [38]. A study indicated that EZH2 physically interacts with and recruits DNA methyltransferases, DNMT1, DNMT3A, and DNMT3B, to methylate CpG and establish stable repressive chromatin structures [39]. However, other reports indicated that EZH2 is not necessary for the maintenance of promoter DNA methylation once gene promoters are heavily DNA hypermethylated [40, 41] suggesting that the mechanisms for recruitment of DNA methyltransferase by EZH2 to control DNA methylation appear to be much more sophisticated than previously thought and remain to be investigated.

In addition to its well-recognized epigenetic gene silencing function, EZH2 also directly methylates non-histone targets, such as cardiac transcription factor GATA4, resulting in attenuated GATA4 transcriptional activity and gene repression [42]. Global proteomic profiling of phosphopeptides showed that EZH2 is highly phosphorylated [43], but how these phosphorylation sites of EZH2 affect its function is still

obscure. Until recently, several reports have shown that kinases such as AKT, CDK1, and CDK2 [43], directly phosphorylate EZH2 at different residues to reduce its association with histone H3 and other PcG proteins, EED and SUZ12, and increase its interaction with YY1 and the non-coding RNA (ncRNA), HOTAIR. These findings suggest that EZH2 also regulates gene expression during the cell cycle, tissue regeneration, and lineage commitment of stem cells [44-48].

EZH2-mediated H3K27 methylation is also involved in X chromosome inactivation (Xi), a process in which one of the two chromosomes in the female cell is transcriptionally silenced, to generate transcriptionally inactive heterochromatin. Enriched EZH2 and EED were found on the Xi coated by the X inactive-specific transcript (XIST) RNA, an ncRNA transcript, to initiate and maintain an inactive state throughout succeeding cell divisions [49, 50]. Studies in cancer have indicated that deregulation of EZH2 contributes to a variety of tumor development and progression, including breast, lung, prostate, pancreatic, and ovarian cancers as well as glioma, lymphoma, and sarcoma [51].

The core components of PRC1 and PRC2 cannot bind to specific DNA sequences alone; they need other factors to recognize specific DNA sequences. In *Drosophila*, the DNA target sequences of PcG complexes, called the Polycomb response elements (PREs), have been identified. *Drosophila* DNA-binding proteins like Dsp1, Zeste, GAF, PHO, and Grainy head that recognize PREs are known to recruit PcG proteins to the PREs [52-55]. Recent studies also showed two DNA segments in controlling developmental genes, indicating that such PREs are also found in vertebrates [56]. In human ESCs, a potential 1.8-kb PRE containing YY1 (Yin and Yang 1 protein; a mammalian orthologue of the *Drosophil*a PRE DNA–binding protein PHO) binding sites that are DNA targets of PcG complexes was identified between *HoxD11* and *HoxD12* [57]. These findings suggest that YY1 plays a crucial role in PcG complexes recruitment. Interestingly, however, a genome-wide study showed that the binding site for PcG proteins do not clearly overlap with the binding sites for YY1 [58]. More studies will be required to clarify whether transcription factors have an impact on the recruitment of PRC2 in mammals. Another DNA-binding protein, JARID2, was discovered by a genome-wide location analysis to bind to PcG target genes and is essential for PcG proteins recruitment to target genes, ESCs differentiation, and normal development [59].

Apart from DNA-binding factors, the regulation of PcG chromatin engagement is also modulated by long ncRNAs in mammalian cells [15, 60]. For instance, HOTAIR has been shown to promote PRC2 recruitment to the *HoxD* locus in *trans* through interaction with SUZ12 [61]. Moreover, XIST is also necessary to recruit the EED-EZH2 complex to facilitate H3K27 methylation and initiate X chromosome transcriptional silencing [50]. Likewise, a 91-kb KCNQ1OT1 long ncRNA acts in *cis* to recruit PRC2 to the genes in the *Kcnq1* (a potassium voltage-gated channel) domain in a specific lineage-dependent manner to establish transcriptional repression [61-63]. Taken together, these findings suggest that ncRNAs play important roles in PRC2 recruitment.

The roles of EZH2 in stem cells

Notably, PRC2 is a vital mediator in the maintenance of pluripotency in both ESCs and somatic stem cells. Loss-of-function PcG mutations result in early embryonic lethality in mice [18, 64-67]. Mice lacking EZH2 die at early postimplantation stage (~E7.5-E8.5), and this severe embryonic lethal phenotype is similar to mice without other PRC2 core subunits such as SUZ12 and EED [18, 66, 68]. It has been difficult to understand the role of PcG proteins in mammalian development due to early embryonic lethality; thus, conditional knockout models have been used to elucidate the critical role of PcG proteins in specific lineages. For instance, *Ezh2* null ESCs generated from conditional knockout mice exhibit defective mesoendoderm differentiation [68]. In the following sections, we will discuss the roles of EZH2 in coordinating cell differentiation in ESCs, mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), skeletal muscle stem cells (SMSCs) and other tissue-specific stem cells (**Figure 1**).

Embryonic stem cells

ESCs are derived from a transient population of pluripotent cells in the inner mass (ICM) of preimplantation mouse or human blastocysts; they are able to self-renew and maintain the ability to differentiate into any cell type *in vivo* and *in vi-*

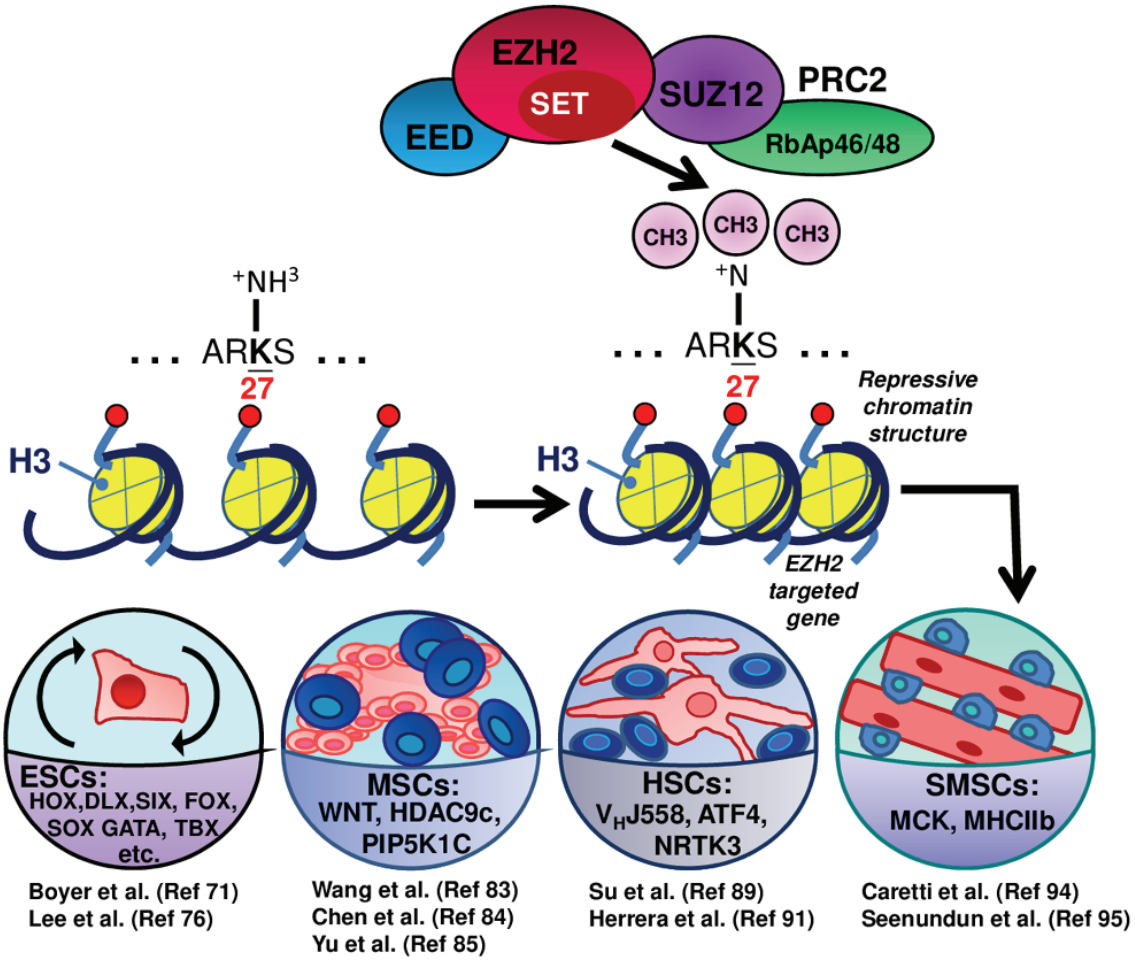

Figure 1. Schematic diagram of the epigenetic regulator Polycomb repressive complex 2 (PRC2)-mediated methylation of histone H3 at Lys 27 (H3K27me3) to establish repressive chromatin marks and alter chromatin structures, contributing to the regulation of development and lineage commitment both in embryonic stem cells (ESCs) and adult stem cells. The polycomb group complex target genes involved in stem cell maintenance and differentiation are shown. ESCs: embryonic stem cells; MSCs: mesenchymal stem cells; HSCs: hematopoietic stem cells; SMSCs: skeletal muscle stem cells; HOX: homeobox; DLX: distal-less homeobox; SIX: six homeobox; FOX: forkhead box; SOX: SRY box; GATA: GATA binding protein; TBX: T-box; WNT: wingless; HDAC9c: histone deacetylase 9c; PIP5K1C: phosphatidylinositol-4-phosphate-5 kinase 1 C; ATF4: activating transcription factor 4; NRTK3: neurotrophic tyrosine kinase receptor 3; MCK: muscle creatine kinase; MHCIIb: myosin heavy chain IIb.

tro [69, 70]. PcG proteins suppress numerous groups of developmental genes like the *Hox* clusters and genes encoding the homeodomain-containing transcriptional factors, such as members of the *Dlx*, *Irx, Lhx, Pou, Pas*, and *Six* gene families [71]. Additionally, promoters of members of the FOX, SOX, GATA, and TBS transcription factors are targeted by PcG complexes to modulate the process of development and progression of diseases [71]. During ESC differentiation *in vitro*, these genes are selectively activated. Moreover, PcG-mediated repression is critical in response to extracellular cues for differentiation capability but does not affect self-renewal of pluripotent ESCs [72]. Importantly, both repressive H3K27me3 and activating H3K4me3 epigenetic marks, called bivalent domains, occupy the PcG-targeted genes in ESCs to maintain ESCs in an undifferentiated state and respond to developmental stimuli [73]. Stalled RNA polymerase II colocalizes with the bivalent chromatin structure marks and prepares to enter elongation process. In addition, PRC1-modulated H2A ubiquitination also plays

a key role in restraining RNA polymerase II in a poised configuration at bivalent genes in ESCs [74], which is consistent with the repression role of PcG for counteracting gene activation.

Ezh2$^{-/-}$ or *Eed*$^{-/-}$ ESCs are severely defective in mesoendodermal lineage commitment [68], and *Suz12*$^{-/-}$ ESCs form embryoid bodies with disorganized structure and abnormal endodermal layer [35]. However, compared with severe differentiation defects resulting from the deletion of PRC2 core components in ESCs, PRC1 inactivation, such as Ring1a/Ring1b double-knockout ESCs, inhibited ESCs proliferation and impaired ESCs maintenance [75]. These findings indicate that PRC1 might be recruited to target genes partially independent of PRC2; this is consistent with the recruitment of PRC1 which can be either through PRC2/H3K27me3-dependent or -independent chromatin modifications in ESCs as mentioned above. There may be other functions of PRC1 involvement in modulation of core transcriptional regulatory circuitry including octamer-binding transcription factor 3/4 (Oct3/4) besides being a downstream effector of PRC2 [75].

A cohort of genes, critical to ESCs pluripotency, are expressed in ESCs, including OCT4, SRY-box 2 (SOX2), and NANOG. These genes are key regulators that direct expression of distinct genes and are crucial for stem cell properties and cell fate determination. Genome-wide ChIP analysis revealed that PcG proteins are co-occupied with most of the repressed targets of OCT4, SOX2, and NANOG transcription factors in human ESCs [76]. This raises a possibility that PcG complexes are recruited to specific targets in ESCs through stem cell-specific DNA-binding factors. In addition, Vire et al. indicated that EZH2 recruits DNA methyltransferase (DNMTs) to their specific targets [39]. A link between PRC2-mediated silencing and DNA methylation in regulation of stem cell maintenance and cell tumorigenesis has also been observed [77-79], demonstrating that established methylation at H3K27 mediated by PRC2 in ESCs affects *de novo* DNA methylation of tumor-specific genes in cancer cells. Although DNA methylation may result in stable gene repression, the silencing effect may permanently suppress differentiation -specific and anti-proliferative genes in stem cells. Consequently, stem cell populations may be desensitized to differentiation cues, leading to everlastingly self-renewal, and subsequently tumor progression.

Mesenchymal stem cells

Human adult mesenchymal stem cells (MSCs) have the ability to self-renew and can be induced to differentiate into multiple lineages such as osteoblasts, adipocytes, chondrocytes, as well as neurons [80-82]. EZH2 has been implicated in MSC differentiation, including osteogenesis, adipogenesis, and neurogenesis [83-85]. Genome-wide EZH2-ChIP-on-chip study showed that EZH2 binds to myocyte enhancer factor-2 interacting transcriptional repressor (MITR) (also known as histone deacetylase 9c or HDAC9c) and represses its expression in adipocytes but not in osteoblasts. Expression of MITR accelerates MSC osteogenesis and inhibits MSC adipogenesis through interaction with PPARγ-2 in the nucleus of osteoblasts, which interrupts PPARγ-2 transcriptional activity, resulting in attenuation of adipogenesis and acceleration of osteogenesis [84].

In osteogenesis, EZH2 is phosphorylated by cyclin-dependent kinase 1 (CDK1) at Thr487, which disrupts EZH2 binding with other PRC2 subunits, SUZ12 and EED [48]. As a result, its methyltransferase activity is inhibited, and MSCs then undergo differentiation into osteoblasts. These findings demonstrated that the regulation of EZH2 by phosphorylation contributes to MSCs lineage commitment. Adipogenesis is inhibited by the activation of Wnt/β-catenin signaling. EZH2 binds directly to the promoters of *Wnt-1*, *-6*, *-10a*, and *-10b* to repress *Wnt* genes and Wnt/β-catenin signaling, which then enhances adipogenesis [83, 86]. All of these findings highlight the importance for EZH2-regulated adipogenic and osteogenic differentiation from MSCs.

A recent study indicated that EZH2 binds to promoter region of phosphatidylinositol-4-phosphate-5 kinase 1 C (*PIP5K1C*) and represses its transcription in MSCs [85]. PIP5K1C is an important regulator involved in inositol 1,4,5-triphosphate (IP_3)-mediated Ca_2^+ signaling after stimulation of G protein-coupled receptor by histamine, and repression of *PIP5K1C* by EZH2 in MSCs reduces Ca_2^+ to a basal level. Knocking down EZH2 de-represses *PIP5K1C* expression and augments the intracellular Ca_2^+ level, thereby enhancing neurogenesis of MSCs. These results suggest that EZH2 suppresses

neuron differentiation through targeting PIP5K1C and negatively modulates the intracellular Ca_2^+ level.

In light of these results showing the multifaceted effects of EZH2 on regulation of MSCs lineage commitment by targeting distinct genes, how EZH2 selectively and specifically exerts repressive function on target genes in MSCs is still unclear. EZH2 might coordinate cell lineage commitment during MSCs differentiation through interaction with other co-factors or response to extracellular or intracellular cues. Further studies will be needed to address this issue.

Hematopoietic stem cells

Hematopoietic stem cells (HSCs) posses the capacity to generate all lineages of blood cells, such as T lymphocytes, B lymphocytes, erythrocytes, platelets, granulocytes, and macrophages. In order to retain hematopoietic homeostasis during lifetime, it is essential to maintain a pool of HSCs. In bone marrow, a small population of multipotent long-term hematopoietic stem cells (LT-HSCs) initiates hematopoiesis and self-renews to sustain the quiescent HSC populations. Through activation by intrinsic and extrinsic mechanisms, LT-HSCs divide into short -term HSCs (ST-HSCs), which give rise to the multipotent progenitors (MPP) to yield an HSC hierarchy that can establish terminally differentiated hematopoietic cells [87, 88]. It has been shown that murine EZH2 plays an important role in B cell development by directing V(D)J recombination, a process in which rearrangement of variable (V), diversity (D), and joining (J) coding segments modulates the assembly of a mature immunoglobulin gene [89]. Deletion of the catalytic domain of murine EZH2 leads to mild deficiency in B cell development, which causes progression of V_H-DJ_H recombination to fail and blocks V_HJ558 recombination. These findings suggest that EZH2 selectively targets V_HJ558 and augments the local methylation of H3K27. Once methylated H3K27 mark is established, the recombination machinery is then directly recruited to the target genes [90].

In addition, EZH2 knock-in (EZH2-KI) mice study showed that enhanced EZH2 expression causes increased number and proliferation of repopulating HSCs. Ezh2-KI mice appear to have a myeloproliferation phenotype that features leukocytosis, myeloid expansion in bone marrow and spleen, and enlargement of the spleen. The hematopoietic stem cell-specific genes, including ecotropic viral-integration site 1 *(Evi-1),* activating transcription factor 4 *(Atf4)* and neurotrophic tyrosine kinase receptor 3 (*Ntrk3)* regulated by EZH2, are aberrantly expressed in hematologic malignancies, suggesting EZH2 exhibits oncogenic role in specific stem cell types [91]. Moreover, in addition to EZH2, BMI1, the subunit of PRC1, also regulates the self-renewal of HSCs [92].

Skeletal muscle stem cells

The activities of muscle regulatory factors (MRFs) MyoD, Myf5, Myf6/MRF4, and myogenin play an important role in myogenic lineage commitment and terminal differentiation. These transcription factors cooperate with sequence-specific co-factors, such as myocyte enhance factor 2 (MEF2) and histone modification enzymes, to promote skeletal muscle differentiation [93]. There is accumulating evidence showing that methylation at H3k27 exerts a critical mark in selective silencing of muscle-specific genes like the muscle creatine kinase (*Mck)* and myosin heavy chain IIb (*MhcIIb*) in undifferentiated myoblasts [94, 95] while MyoD and histone acetylation coordinate the activation of skeletal muscle genes [96]. MyoD, a basic-helix-loop-helix (bHLH) transcription factor, acts as a trans-activator and a key modulator to initiate a myogenic differentiation program. Using ChIP-sequence analysis, Cao et al. observed the induction of MyoD binding to many regulatory regions of genes regulated during skeletal muscle differentiation [96]. MyoD also constitutively binds to a large number of additional sites in myoblasts, but these additional binding sites are not related to the alteration of MyoD-mediated gene expression on the basis of expression array data. Thus, the role of MyoD in broadly regulating chromatin structures in myoblasts remains to be deciphered [96].

In addition to MyoD binding, a number of these genes are suppressed by the PcG machinery in myoblasts as evidenced by the prevalence of EZH2 and H3K27me3 mark at these genes [94]. In order to overcome PcG-mediated gene silencing to ensure that differentiation progresses, several events occur in addition to the induction of MoyD binding. One of which is the recruitment of a homeobox transcription factor

SIX4 along with the histone demethylase UTX (ubiquitously transcribed X chromosome tetratricopeptide repeat protein) to a subset of MyoD targets to demethylate H3K27me3 within the promoter region of muscle-specific gene. Subsequently, UTX proceeds through the coding region of genes to remove the H3K27me3 and therefore RNA polymerase II-dependent transcriptional elongation can progress [95]. Genome-wide studies demonstrated that MEF2, a MADS-box transcription factor, interacts with MyoD to bind to the target genes during myogenesis. Once MyoD, MEF2, and SIX4 bind to the target loci, the gene is then activated. There are specific microRNAs that can repress the expression of both EZH2 and YY1, thereby upregulating the PcG protein target gene and enhancing skeletal muscle differentiation [97, 98].

A recent study by Juan et al. using mice with conditional deletion of EZH2 in a paired box 7 (PAX7)-dependent manner in satellite cells (SCs) indicated that EZH2 is required for postnatal muscle growth and adult muscle regeneration but not for fetal muscle development. SCs, a heterogeneous population of committed myogenic progenitors and noncommitted stem cells, are present in adult skeletal muscle in response to injuries or degenerative conditions [99]. Mice with or without the Pax7-dependent *Ezh2* deletion show similar size and number of myofibers at birth. However, mice lacking *Ezh2* have smaller but similar number of myofibers at 1-week after birth, indicating ablation of EZH2 in $PAX7^+$ SCs contributes to a deficiency in postnatal muscle growth. Additionally, they found that Pax7-induced *Ezh2* depletion also derepresses developmental genes which are not physiologically expressed in SCs, such as *Zic-1* (commonly expressed in early somites and the cerebellum), *Agrp1* (commonly expressed in hypothalamic neurons), *Tbx1* (commonly expressed in cardiac progenitors) and collagen *Col22a1* (specifically expressed in chondrocytes). However, derepression of these mixed-lineage genes is scarcely observed in another mouse model with *MyoD*-dependent *Ezh2* ablation in committed skeletal myogenic cells. These results suggest that when Ezh2 is ablated in committed or differentiated myoblasts (myogenic precursors ($MyoD^+$), the selective genomic chromatin structures are already restrained into a silent mode and are insensitive to epigenetic alteration; therefore, the mixed-lineage genes are not up-regulated. Despite the difference in expression of nonmyogenic genes, similar phenotype of aberrant muscle growth was detected in both *Pax*-induced *Ezh2* and *MyoD*-induced *Ezh2* ablation mice, revealing that EZH2 is critical for regulation of SCs homeostasis [99, 100].

Regulation of other tissue-specific stem cells

Apart from the ESCs and adult stem cells mentioned above, EZH2 is also involved in modulating the cell lineage commitment and differentiation of other tissue-specific stem cells. For example, in the epidermis, EZH2 and EZH2-mediated H3K27me3 control the proliferative capacity of basal epidermal progenitors by silencing the Cyclin-dependent kinase 4 inhibitor (*Ink4A-Ink4B*) locus and restrain the differentiation rate by preventing the AP1 transcriptional activator from binding to epidermal differentiation genes. During epidermal differentiation, EZH2 expression decreases, resulting in inhibition of basal cell proliferation through activation of *Ink4A-Ink4B* locus and acceleration of epidermal differentiation through recruitment of AP1 to epidermal differentiation genes [101].

A recent study demonstrated that EZH2 binds directly to and methylates nonhistone targets such as the cardiac transcription factor, GATA4, in the fetal heart. GATA4 is a critical, dosage-sensitive regulator of heart development in both mice and human. Moreover, GATA4 associates with and recruits p300 to specific chromatin loci. Meanwhile, p300 acetylates GATA4, activating its transcriptional activity. In fetal heart, EZH2 methylates GATA4 at Lys 299, which inhibits its acetylation by p300, decreases the recruitment of p300 to chromatin, and attenuates GATA4 transcriptional activity and gene expression. These results indicate that apart from its well-recognized epigenetic gene silencing function, EZH2 also methylates nonhistone targets, thereby repressing gene expression [42].

Conclusion and perspective

Epigenetic regulation plays an important role in stem cell self-renewal, maintenance, and lineage differentiation. PcG protein, especially EZH2, epigenetically modulates chromatin state through methylation of histones and nonhistone targets, recruitment of DNMT, compaction of chromatin, and DNA looping with DNA methyla-

tion in an enzymatic activity dependent- and independent-manner. EZH2 also orchestrates transcriptional signature and gene expression pattern in a stepwise manner to maintain stem cell potency and fine-tune stem cell lineage allocation. However, the exact functions and detailed mechanisms that mediate these high-order chromatin structures require more in-depth investigations. In addition, EZH2 regulates a variety of biological processes by targeting distinct genes. How EZH2 is specifically and selectively recruited to chromatin to repress gene expression is still not fully understood. It is plausible that EZH2 targets to specific chromatin loci through interaction with other co-factors or in response to extracellular or intracellular cues. Delineating the mechanisms by which PcG proteins/EZH2 regulate cell-fate specific genes may advance our understanding toward the development of stem cell-based therapy and regenerative medicine.

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