## Review Article
# The roles of EZH2 in cell lineage commitment

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**Abstract:** Enhancer of zeste homolog 2 (EZH2), a catalytic component of polycomb repressive complex 2 (PRC2), epigenetically regulates chromatin structure and gene expressions through tri-methylation at histone H3K27 and recruitment of DNA methyltransferases for gene silencing. Despite extensive studies of the role of EZH2 in cancer progression and malignancy, increasing evidence also suggest that EZH2 plays a critical role in stem cells renewal, maintenance, and differentiation into specific cell lineages. Here, we review the updated information regarding how EZH2 contributes to stem cell maintenance, cell lineage determination, including myogenesis, adipogenesis, osteogenesis, neurogenesis, hematopoiesis, lymphopoiesis, epidermal differentiation and hepatogenesis, and how EZH2 is regulated by phosphorylation and microRNAs in these processes.

**Keywords:** EZH2, stem cells, cell lineage, differentiation

## Introduction

The transcriptional status of the genome is tightly regulated by chromosome structure, which is organized by post-translational modifications (PTMs), including methylation, acetylation, phosphorylation, ubiquitination, and ADP-ribosylation on histones [1]. Specifically, chromatin compaction to repress gene transcription is controlled by the polycomb group (PcG) proteins composed of two polycomb repressive complexes (PRCs), PRC1 and PRC2, with distinct functions [2]. Whereas PRC1 recognizes the H3K27me3 through its chromodomain to mediate ubiquitination of H2AK119 and maintain gene repression, PRC2 targets to the gene promoters and tri-methylates histone H3 at lysine 27 (H3K27me3) [3]. The core components of PRC2 are suppressor of zeste-12 (SUZ12), embryonic ectoderm development (EED), and enhancer of zeste homolog 2 (EZH2). Of the PRC2 components, SUZ12 and EED stabilize the complex and are required for the methyltransferase activity of EZH2 [4]. In addition to tri-methylation at histone H3K27 through the SET domain at carboxy-terminal region of EZH2 [5], EZH2 is able to recruit DNA methyltransferases via its amino-terminal region to silence gene expression [6]. Human EZH2 was identified as a homolog of the *Drosophila* enhancer of zeste gene and mapped to chromosome 21q22.2 in 1996 [7]. The role of EZH2 in cancer progression and malignancy has been extensively studied in the last decade [8, 9]. Moreover, growing evidence demonstrates that the PcG proteins are critical and important in the regulation of gene expressions in stem cells maintenance and lineage specification [10]. Here, we focus on the recent progress regarding the roles of EZH2 in cell fate determination of stem cells and its regulatory mechanism.

## EZH2 in stem cell maintenance

Embryonic stem cells (ESCs) are pluripotent cells which differentiate into all cell types in adult organisms. PcG proteins repress the early differentiation marker genes to maintain the pluripotency of ESCs in embryonic stage. At the initiation of cell fate commitment, the early differentiation marker genes are activated by a loss of repressive PRC2-mediated histone H3K27me3 while PcG proteins suppress the late differentiation marker genes for specific
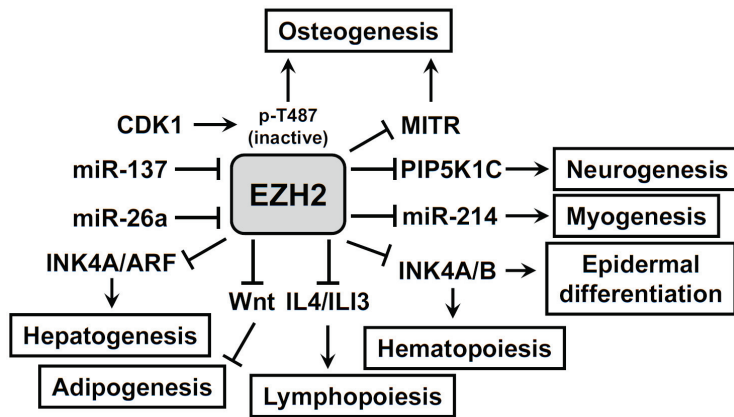
**Figure 1.** A schematic diagram illustrating the role of EZH2 in cell lineage commitment. The role of EZH2-regulated differentiation of stem cells into specific lineages, and the upstream and downstream genes involved in the processes are shown. CDK1, cyclin dependent kinase 1; MITR, myocyte enhancer factor-2 interacting transcriptional repressor; PIP5K1C, type I phosphatidylinositol-4-phosphate 5-kinase-γ; miR, microRNA; INK, cyclin-dependent kinase inhibitor; ARF, alternate open reading frame; IL, *interleukin; Wnt,* wingless-type MMTV integration site family.

cell lineage. Subsequently, a decrease in PcG proteins de-repress the late differentiation marker genes in the terminally differentiated cells in the final stage [11]. Consistent with the early embryonic expression level of PcG proteins, EZH2 is highly expressed in the ESCs and is required for early mouse development [12]. EZH2 is also highly abundant in epidermal progenitor cells but its level decreases upon terminal differentiation [13]. In addition, EZH2 is able to maintain the multipotent identity in adult stem cells, such as hematopoietic stem cells (HSCs) [14], muscle cell precursors (myoblasts) [15], and neural stem cells (NSCs) [16]. Overexpression of EZH2 in HSCs preserves the long-term repopulating potential to prevent HSCs exhaustion in which HSC population is reduced after serial transplantations [17]. Increased EZH2 expression prevents muscle differentiation from myoblasts, and this property is conferred by the histone lysine methyltransferase (HKMT) activity in its SET domain [15]. EZH2 is highly expressed in undifferentiated NSCs, and the expression level of EZH2 is reduced after differentiation into astrocytes while forced expression of EZH2 represses the process [16]. A recent study reports that overexpression of EZH2 in astrocytes induces their de-differentiation toward NSCs. However, these EZH2-induced neural stem cell-like cells lack a differentiation potential, indicating that overexpression of EZH2 alone is insufficient for a complete de-differentiation [18].

### EZH2 in cell fate decision in differentiation

The target genes of PcG proteins have been genome-wide-mapped in murine ESCs [19] and human embryonic lung fibroblast TIG3 cell line [20], showing that numerous development regulators and differentiation-related genes are epigenetically repressed by binding of PcG proteins. EZH2, one of the PcG proteins, has been demonstrated to be involved in differentiation of ESCs or adult stem cells into several cell lineages, including myogenesis, adipogenesis, osteogenesis, neurogenesis, hematopoiesis, lymphopoiesis, epidermal differentiation and hepatogenesis. The roles of EZH2 in cell lineage commitment are illustrated in **Figure 1** and are described below.

*Myogenesis*

The expression of EZH2 is developmentally regulated in skeletal muscle that is inversely correlated to myogenesis. In undifferentiated myoblasts, EZH2 targets to promoter regions of muscle-specific genes by association with the transcriptional regulator, YY1, which contains a zinc finger DNA binding domain. The complex is coupled with the histone deacetylase, HDAC1, to repress transcription of muscle-specific genes. At the onset of transcriptional activation, YY1, EZH2, and HDAC1 dissociate from muscle loci and are replaced with MyoD, SRF, and histone acetyltransferases (HATs) to initiate differentiation into skeletal muscle cells (SMCs) [15]. Moreover, an intronic region containing the microRNA miR-214 is occupied and repressed by EZH2 in undifferentiated myoblasts. Induction of SMC differentiation results in dissociation of EZH2 from chromatin, recruitment of the developmental regulators MyoD and myogenin, and activation of miR-214 transcription. Upon this, miR-214 then negatively regulates EZH2

through a negative feedback mechanism by targeting to its 3'-UTR, leading to accelerated SMC differentiation [21]. Recently, it has been reported that an abnormal expression level of EZH2 is related to muscular disorder. For instance, TNF treatment induces NF-κB expression that suppresses SMC differentiation by recruiting EZH2 and DNMT3b to Notch-1 gene through epigenetic silencing to repress its expression. Thus, in Duchenne muscular dystrophy (DMD), it is likely that elevated level of TNFα from myotubes inhibits the regenerative potential of satellite cells via epigenetic silencing of the Notch-1 signaling [22].

*Adipogenesis and osteogenesis*

There is increasing evidence that shows a reciprocal relationship between adipogenesis and osteogenesis. Numerous molecules have been identified as the modulators in the switch between these two cell lineages. For examples, osteogenic phenotype is dependent on the presence of 1,25 dihydroxyvitamin D3 (1,25(OH)2D3) but is repressed in dexamethasone-treated cells, which shows adipogenic phenotype [23]. The osteoblast homeoprotein, Msx2, promotes osteogenesis and suppresses adipogenesis of multipotent mesenchymal progenitors [24]. Activation of protein kinase A (PKA) enhances peroxisome proliferator-activated receptor gamma 2 (PPARγ2) and lipoprotein lipase (LPL) expressions as in adipogenesis, and inhibits runt-related transcription factor 2 (Runx2) and osteopontin expressions as in osteogenesis by suppressing leptin levels in human mesenchymal stem cells (hMSCs) [25]. In contrast, melatonin enhances Runx2 expression to facilitate osteogenesis and simultaneously suppresses PPARγ expression to inhibit adipogenesis of hMSCs [26]. Besides the molecules mentioned above, recent studies report that the switch between adipogenesis and osteogenesis can be epigenetically regulated by EZH2 as followings: The histone H3K27me3 methyltransferase activity of EZH2 promotes adipogenesis by disrupting the Wnt/β-catenin signaling through direct binding to the promoters of Wnt genes including Wnt1, -6, -10a, and -10b to repress their expression [27]. On the contrary, it has recently been reported that that suppression of methyltransferase activity via phosphorylation of EZH2 at Thr 487 by cyclin dependent kinase 1 (CDK1) results in hMSCs differentiation into osteoblasts [28]. It has also been dem-

onstrated that dissociation of EZH2 from the promoter of myocyte enhancer factor-2 interacting transcriptional repressor (MITR) gene, also named as histone deacetylase 9c (HDAC9c), increases its expression and interaction with PPARγ2 in the nucleus to interrupt PPARγ2 activity and prevent adipogenesis, and thus, promoting hMSC osteogenic differentiation [29].

*Neurogenesis*

A number of developmental regulator genes in murine ESCs are directly occupied and repressed by PcG proteins. Deficiency in the PRC2 component, EED, de-represses PcG target gene expressions and activates neuronal differentiation in murine ESCs [19]. A global decrease in histone H3K27me3 and reduced level of EZH2 occur in retinoic acid-induced neuron differentiation of mouse ESCs [30]. Inactivation of PcG protein by knockout of EED or EZH2 promotes neurogenesis of neural precursor cells (NPCs also known as neural stem cells, NSCs), [31]. EZH2 protein is highly expressed in proliferating NSCs from embryonic mice and its expression decreases after NSCs differentiating into neurons and astrocytes; however, EZH2 level remains high after differentiation into an oligodendrocytic cell lineage. The elevated level of EZH2 starts from oligodendrocyte precursor cells (OPCs) to up to the immature (premyelinating) oligodendrocyte stage, suggesting that a high level of EZH2 in this stage confers OPC proliferation. Overexpression of EZH2 in NSCs increases differentiation into oligodendrocyte but suppresses NSCs differentiation into astrocytes [16]. Forced expression of EZH2 in postnatal mouse astrocytes converts them into proliferating round neurosphere-like clusters, which shows reduced expression of the typical astrocytic genes, GFAP and S100, and increased expression of the NSCs-related genes, nestin, Sox2, musashi, and CD133, indicating that EZH2 is able to guide astrocytes to de-differentiate toward NSCs [18]. In cerebral development, EZH2-null mice show a reduction of the repressive marker, histone H3K27me3, in cortical progenitor cells and results in differentiation both directly to neurons and indirectly via basal progenitor cell genesis in the cerebral cortex [32]. We recently found that EZH2 can negatively regulate intracellular calcium $[Ca^{2+}]$ signaling required for neuron differentiation by repressing type I

phosphatidylinositol-4-phosphate 5-kinase-γ (PIP5K1C) gene. In proliferating hMSCs, EZH2 binds to the PIP5K1C promoter to suppress its transcription and maintains the intracellular $Ca^{2+}$ contents at a basal level. After induction of neuronal differentiation, disassembly of EZH2 protein from the promoter of PIP5K1C increases its expression and synthesis of phosphatidylinositol 4, 5-bisphosphate [PI(4,5) $P_2$], evoking intracellular calcium signaling and advancing neuronal differentiation from hMSCs [33].

*Hematopoiesis and lymphopoiesis*

HSCs can be isolated from bone marrow, peripheral blood, umbilical cord blood [34], and a newly identified source, placenta [35]. HSCs are multipotent and capable of differentiating into multiple hematopoietic lineages, including erythroid (blood cells), myeloid (leukocyte), and lymphoid (lymphocyte) [36]. It is known that the self-renewal capacity and differentiation property of HSCs are modulated by PcG proteins, such as PRC1 component, BMI-1 and the PRC2 component, EZH2 [37]. Serial transplantation impairs the self-renewal and multipotent potentials of HSCs [38]. Overexpression of EZH2 in HSCs completely prevents exhaustion of the long-term repopulating potential of HSCs during repeated serial transplantation [17]. EZH2 selectively controls the INK4A and INK4B but not the ARF genes in the spatial organization of the INK4B-ARF-INK4A locus during HSCs differentiation and cellular senescence [39].

In addition, PcG proteins contribute to regulation of lymphopoiesis of HSCs. The mutually exclusive expression profiles of BMI-1 and EZH2 are observed in the maturation process of peripheral T cells [40]. Histone H3K27me3 methyltransferase activity of EZH2 responds to transcriptional regulation of *interleukins*, IL4 and IL13, expression, which are required for differentiation of naive CD4 T cells toward the T helper 1 (Th1) and T helper 2 (Th2) cells [41]. EZH2 also regulates B cell differentiation and the rearrangement of immunoglobulin heavy chain gene (Igh) during murine B cell development [42]. Inactive somatic mutations of EZH2 have been observed in several myeloid disorders, including chronic myelomono-cytic leukemia (CMML), atypical chronic myeloid leukemia (aCML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasm (MPN) [43].

*Other cell lineages*

In addition to the described cell lineages above, EZH2 is involved in the regulation of epidermal differentiation and hepatogenesis. In epidermal progenitor cells, EZH2 is highly expressed to control proliferation by repressing cyclin-dependent kinase inhibitors, INK4A and INK4B, which suppress cell cycle progression. During epidermal differentiation, decreased level of EZH2 activates INK4A and INK4B expression to reduce cell proliferation and removes histone H3K27me3 to recruit AP1 transcriptional factor to the structural genes that are required for epidermal differentiation [13]. In hepatogenesis, INK4A and ARF are regulated by EZH2 in hepatic progenitor cells. Knockdown of EZH2, rather than BMI-1, significantly impairs the proliferative and self-renewal capability of murine hepatic progenitor cells and promotes the differentiation and terminal maturation of hepatocytes as measured by detection of the metabolic enzyme genes, tyrosine aminotransferase (TAT) and glucose-6-phosphatase (G6P), and periodic acid-Schiff (PAS) staining for intracellular glycogen accumulation. These results demonstrate that EZH2 is critical and essential in proliferative and self-renewal capacity of hepatic progenitor cells and prevents them from differentiation [44].

## Regulation of EZH2

A recent review describes the individual phosphorylation site on EZH2 protein which affects its interactions with chromatin, other PcG members, or non-coding RNAs (ncRNAs) to regulate its functions [45]. In brief, phosphorylation sites that have been identified on EZH2 include: Ser 21 for reducing binding affinity to histone H3 and suppressing HKMT activity [46], Thr 345 (mouse)/Thr 350 (human) for increase of binding to two ncRNAs, HOTAIR and Xist RepA, and recruitment to chromatin [47], Thr 367 (mouse)/Thr 372 (human) for promoting its interaction with YY1 [48], Thr 487 for inhibition of its HKMT activity by disrupting interactions with SUZ12 and EED, and promoting osteogenesis [28], and Thr350 for regulating cancer cell proliferation and migration in prostate cancer cells [49]. Moreover, several microRNAs (miRNAs/ miR) have been identified as the regulators of EZH2. For instance, overexpression of miR-26a post-transcriptionally represses EZH2, and thus, upregulates mRNA expression levels of myoD

and myogenin to facilitate myogenesis [50]. In addition, miR-214 suppresses EZH2 expression through a negative feedback mechanism by targeting its 3'-UTR to promote SMC differentiation, indicating that a regulatory circuit between EZH2 and miR-214 modulates PcG-mediated gene expression during myogenesis [21]. A DNA methyl-CpG-binding protein, MeCP2, epigenetically attenuates specific miRNAs in adult NSCs. For example, a MeCP2-regulated microRNA, miR-137, modulates the proliferation and differentiation of adult NSCs through post-transcriptional repression of EZH2. Expression of miR-137 reduces the level of EZH2 protein, which results in a global decrease of histone H3K27me3 [51]. Together, these findings demonstrate that the crosstalk between EZH2-mediated epigenetic regulation and microRNAs, miR-214 and miR-137, modulates myogenic and neurogenic differentiation, respectively. Another microRNA, miR-101, has been reported to target to EZH2 in various cancer types including prostate cancer [52, 53], bladder transitional cell carcinoma [54], nasopharyngeal carcinoma [55], glioblastoma [56], and non-small cell lung cancer [57]. However, its role in property maintenance and cell fate decision of stem cells will require further investigations.

## Conclusion and perspectives

The PcG proteins are known to epigenetically regulate chromatin structure and stepwise modulate gene expression profiles to maintain stem cell property or enable stem cell differentiation into specific cell lineages. In this article, we have described the updated information regarding EZH2, a catalytic component of PRC2, in pluri- or multi-potency of stem cells and cell lineage commitment by targeting specific gene sets, as well as the regulation of EZH2. Although much has been known from genome-wide mapping the EZH2-targeted genes in different types of cell lines, it will be more meaningful to extend these studies to homogeneous cell populations from specific developmental stages and tissues. Additionally, little is known about the EZH2-interacting proteins between undifferentiated and differentiated stem cells and in specific cell lineages. Proteomics approaches will be useful to genome-widely identify these EZH2-interacting proteins, which will help further identify key modulators of EZH2 in stem cell maintenance and lineage specification. More understanding of the roles of EZH2 in specific cell lineage determination will be beneficial for application of stem cells as therapeutics for injuries or diseases.

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