Am J Transl Res 2011;3(3):243-250
www.ajtr.org /ISSN:1943-8141/AJTR1104002

Review Article

The roles of EZH2 in cell lineage commitment

Ruey-Hwang Chou[1,2], Yung-Luen Yu[1,2,3], Mien-Chie Hung[1,3,4]

[1]Center for Molecular Medicine, China Medical University Hospital, Taichung 404, Taiwan; [2]Department of Biotechnology, Asia University, Taichung 413, Taiwan; [3]Graduate Institute of Cancer Biology, China Medical University, Taichung 404, Taiwan; [4]Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA.

Received April 9, 2011; accepted April 15, 2011; Epub April 21, 2011; Published May 15, 2011

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

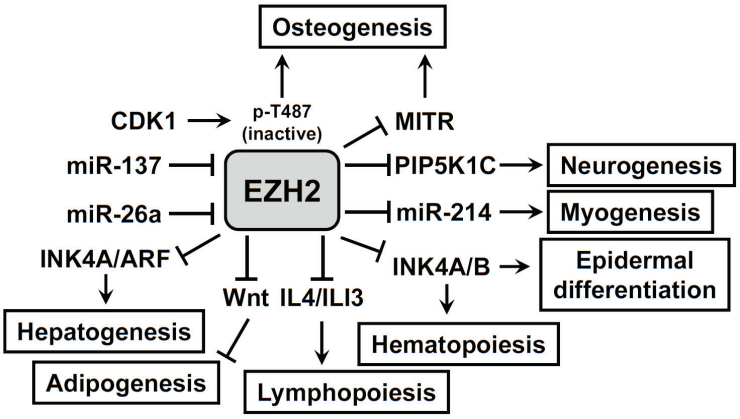

Figure 1. A schematic diagram illustrating the role of EZH2 in cell lineage commitment. The role of EZH2-regulated differentiation of stem cells into specific lineages, and the upstream and downstream genes involved in the processes are shown. CDK1, cyclin dependent kinase 1; MITR, myocyte enhancer factor-2 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 demonstrated 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.

Acknowledgements

We would like to thank Dr. Jennifer L. Hsu at the Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center for helpful discussion and editing this article. This work was supported by grants from the National Institutes of Health (RO1 CA109311 and PO1 CA099031), and The University of Texas MD Anderson-China Medical University and Hospital Sister Institution Fund (to M.-C. H.); NSC99 2320-B-039-030-MY3 (to Y.-L. Y.); and NSC 3111-B-039 and NSC-2623-13-039 (to M.-C. H., and L.-Y. L.).

Please address correspondence to: Mien-Chie Hung, Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, Texas 77030, USA. Tel: 713-792-3668; Fax: 713-794-3270; E-mail: mhung@mdanderson.org; or Yung-Luen Yu, Graduate Institute of Cancer Biology, China Medical University, Taichung 404, Taiwan; Tel: +886-4-22052121 ext. 7933; Fax: +886-4-22333496; E-mail: ylyu@mail.cmu.edu.tw.

References

[1] Munshi A, Shafi G, Aliya N and Jyothy A. Histone modifications dictate specific biological readouts. J Genet Genomics 2009; 36: 75-88.

[2] Schwartz YB and Pirrotta V. Polycomb complexes and epigenetic states. Curr Opin Cell Biol 2008; 20: 266-273.

[3] Sparmann A and van Lohuizen M. Polycomb silencers control cell fate, development and cancer. Nat Rev Cancer 2006; 6: 846-856.

[4] Pasini D, Bracken AP, Jensen MR, Lazzerini Denchi E and Helin K. Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. EMBO J 2004; 23: 4061-4071.

[5] Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS and Zhang Y. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 2002; 298: 1039-1043.

[6] Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, Morey L, Van Eynde A, Bernard D, Vanderwinden JM, Bollen M, Esteller M, Di Croce L, de Launoit Y and Fuks F. The Polycomb group protein EZH2 directly controls DNA methylation. Nature 2006; 439: 871-874.

[7] Chen H, Rossier C and Antonarakis SE. Cloning of a human homolog of the Drosophila enhan-

cer of zeste gene (EZH2) that maps to chromosome 21q22.2. Genomics 1996; 38: 30-37.
[8] Tonini T, D'Andrilli G, Fucito A, Gaspa L and Bagella L. Importance of Ezh2 polycomb protein in tumorigenesis process interfering with the pathway of growth suppressive key elements. J Cell Physiol 2008; 214: 295-300.
[9] Simon JA and Lange CA. Roles of the EZH2 histone methyltransferase in cancer epigenetics. Mutat Res 2008; 647: 21-29.
[10] Rajasekhar VK and Begemann M. Concise review: roles of polycomb group proteins in development and disease: a stem cell perspective. Stem Cells 2007; 25: 2498-2510.
[11] Schuettengruber B and Cavalli G. Recruitment of polycomb group complexes and their role in the dynamic regulation of cell fate choice. Development 2009; 136: 3531-3542.
[12] O'Carroll D, Erhardt S, Pagani M, Barton SC, Surani MA and Jenuwein T. The polycomb-group gene Ezh2 is required for early mouse development. Mol Cell Biol 2001; 21: 4330-4336.
[13] Ezhkova E, Pasolli HA, Parker JS, Stokes N, Su IH, Hannon G, Tarakhovsky A and Fuchs E. Ezh2 orchestrates gene expression for the stepwise differentiation of tissue-specific stem cells. Cell 2009; 136: 1122-1135.
[14] De Haan G and Gerrits A. Epigenetic control of hematopoietic stem cell aging the case of Ezh2. Ann N Y Acad Sci 2007; 1106: 233-239.
[15] Caretti G, Di Padova M, Micales B, Lyons GE and Sartorelli V. The Polycomb Ezh2 methyltransferase regulates muscle gene expression and skeletal muscle differentiation. Genes Dev 2004; 18: 2627-2638.
[16] Sher F, Rossler R, Brouwer N, Balasubramaniyan V, Boddeke E and Copray S. Differentiation of neural stem cells into oligodendrocytes: involvement of the polycomb group protein Ezh2. Stem Cells 2008; 26: 2875-2883.
[17] Kamminga LM, Bystrykh LV, de Boer A, Houwer S, Douma J, Weersing E, Dontje B and de Haan G. The Polycomb group gene Ezh2 prevents hematopoietic stem cell exhaustion. Blood 2006; 107: 2170-2179.
[18] Sher F, Boddeke E and Copray S. Ezh2 expression in astrocytes induces their dedifferentiation toward neural stem cells. Cell Reprogram 2011; 13: 1-6.
[19] Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK, Bell GW, Otte AP, Vidal M, Gifford DK, Young RA and Jaenisch R. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 2006; 441: 349-353.
[20] Bracken AP, Dietrich N, Pasini D, Hansen KH and Helin K. Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. Genes Dev 2006; 20: 1123-1136.
[21] Juan AH, Kumar RM, Marx JG, Young RA and Sartorelli V. Mir-214-dependent regulation of the polycomb protein Ezh2 in skeletal muscle and embryonic stem cells. Mol Cell 2009; 36: 61-74.
[22] Acharyya S, Sharma SM, Cheng AS, Ladner KJ, He W, Kline W, Wang H, Ostrowski MC, Huang TH and Guttridge DC. TNF inhibits Notch-1 in skeletal muscle cells by Ezh2 and DNA methylation mediated repression: implications in duchenne muscular dystrophy. PLoS One 2010; 5: e12479.
[23] Oreffo RO, Virdi AS and Triffitt JT. Modulation of osteogenesis and adipogenesis by human serum in human bone marrow cultures. Eur J Cell Biol 1997; 74: 251-261.
[24] Cheng SL, Shao JS, Charlton-Kachigian N, Loewy AP and Towler DA. MSX2 promotes osteogenesis and suppresses adipogenic differentiation of multipotent mesenchymal progenitors. J Biol Chem 2003; 278: 45969-45977.
[25] Yang DC, Tsay HJ, Lin SY, Chiou SH, Li MJ, Chang TJ and Hung SC. cAMP/PKA regulates osteogenesis, adipogenesis and ratio of RANKL/OPG mRNA expression in mesenchymal stem cells by suppressing leptin. PLoS One 2008; 3: e1540.
[26] Zhang L, Su P, Xu C, Chen C, Liang A, Du K, Peng Y and Huang D. Melatonin inhibits adipogenesis and enhances osteogenesis of human mesenchymal stem cells by suppressing PPARgamma expression and enhancing Runx2 expression. J Pineal Res 2010; 49: 364-372.
[27] Wang L, Jin Q, Lee JE, Su IH and Ge K. Histone H3K27 methyltransferase Ezh2 represses Wnt genes to facilitate adipogenesis. Proc Natl Acad Sci U S A 2010; 107: 7317-7322.
[28] Wei Y, Chen YH, Li LY, Lang J, Yeh SP, Shi B, Yang CC, Yang JY, Lin CY, Lai CC and Hung MC. CDK1-dependent phosphorylation of EZH2 suppresses methylation of H3K27 and promotes osteogenic differentiation of human mesenchymal stem cells. Nat Cell Biol 2011; 13: 87-94.
[29] Chen YH, Yeh FL, Yeh SP, Ma HT, Hung SC, Hung MC and Li LY. MITR is a swith that promotes osteogenesis and inhibits adipogenesis of mesenchymal stem cells by inactivating PPAR{gamma}-2. J Biol Chem 2011; 286:10671-10680.
[30] Lee ER, Murdoch FE and Fritsch MK. High histone acetylation and decreased polycomb repressive complex 2 member levels regulate gene specific transcriptional changes during early embryonic stem cell differentiation induced by retinoic acid. Stem Cells 2007; 25: 2191-2199.
[31] Hirabayashi Y, Suzki N, Tsuboi M, Endo TA, Toyoda T, Shinga J, Koseki H, Vidal M and Gotoh Y. Polycomb limits the neurogenic competence of neural precursor cells to promote astrogenic fate transition. Neuron 2009; 63: 600-613.
[32] Pereira JD, Sansom SN, Smith J, Dobenecker

MW, Tarakhovsky A and Livesey FJ. Ezh2, the histone methyltransferase of PRC2, regulates the balance between self-renewal and differentiation in the cerebral cortex. Proc Natl Acad Sci U S A 2010; 107: 15957-15962.
[33] Yu YL, Chou RH, Chen LT, Shyu WC, Hsieh SC, Wu CS, Zeng HJ, Yeh SP, Yang DM, Hung SC and Hung MC. EZH2 regulates neuronal differentiation of mesenchymal stem cells through PIP5K1C-dependent calcium signaling. J Biol Chem 2011; 286:9657-9667.
[34] Haspel RL and Miller KB. Hematopoietic stem cells: source matters. Curr Stem Cell Res Ther 2008; 3: 229-236.
[35] Lee LK, Ueno M, Van Handel B and Mikkola HK. Placenta as a newly identified source of hematopoietic stem cells. Curr Opin Hematol 2010; 17: 313-318.
[36] Akashi K. Lymphoid lineage fate decision of hematopoietic stem cells. Ann N Y Acad Sci 2009; 1176: 18-25.
[37] Konuma T, Oguro H and Iwama A. Role of the polycomb group proteins in hematopoietic stem cells. Dev Growth Differ 2010; 52: 505-516.
[38] Mauch P and Hellman S. Loss of hematopoietic stem cell self-renewal after bone marrow transplantation. Blood 1989; 74: 872-875.
[39] Kheradmand Kia S, Solaimani Kartalaei P, Farahbakhshian E, Pourfarzad F, von Lindern M and Verrijzer CP. EZH2-dependent chromatin looping controls INK4a and INK4b, but not ARF, during human progenitor cell differentiation and cellular senescence. Epigenetics Chromatin 2009; 2: 16.
[40] Raaphorst FM, Otte AP, van Kemenade FJ, Blokzijl T, Fieret E, Hamer KM, Satijn DP and Meijer CJ. Distinct BMI-1 and EZH2 expression patterns in thymocytes and mature T cells suggest a role for Polycomb genes in human T cell differentiation. J Immunol 2001; 166: 5925-5934.
[41] Koyanagi M, Baguet A, Martens J, Margueron R, Jenuwein T and Bix M. EZH2 and histone 3 trimethyl lysine 27 associated with Il4 and Il13 gene silencing in Th1 cells. J Biol Chem 2005; 280: 31470-31477.
[42] Su IH, Basavaraj A, Krutchinsky AN, Hobert O, Ullrich A, Chait BT and Tarakhovsky A. Ezh2 controls B cell development through histone H3 methylation and Igh rearrangement. Nat Immunol 2003; 4: 124-131.
[43] Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, Waghorn K, Zoi K, Ross FM, Reiter A, Hochhaus A, Drexler HG, Duncombe A, Cervantes F, Oscier D, Boultwood J, Grand FH and Cross NC. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet 2010; 42: 722-726.
[44] Aoki R, Chiba T, Miyagi S, Negishi M, Konuma T, Taniguchi H, Ogawa M, Yokosuka O and Iwama A. The polycomb group gene product Ezh2 regulates proliferation and differentiation of murine hepatic stem/progenitor cells. J Hepatol 2010; 52: 854-863.
[45] Caretti G, Palacios D, Sartorelli V and Puri PL. Phosphoryl-EZH-ion. Cell Stem Cell 2011; 8: 262-265.
[46] Cha TL, Zhou BP, Xia W, Wu Y, Yang CC, Chen CT, Ping B, Otte AP and Hung MC. Akt-mediated phosphorylation of EZH2 suppresses methylation of lysine 27 in histone H3. Science 2005; 310: 306-310.
[47] Kaneko S, Li G, Son J, Xu CF, Margueron R, Neubert TA and Reinberg D. Phosphorylation of the PRC2 component Ezh2 is cell cycle-regulated and up-regulates its binding to ncRNA. Genes Dev 2010; 24: 2615-2620.
[48] Mozzetta C, Consalvi S, Saccone V, Forcales SV, Puri PL and Palacios D. Selective control of Pax7 expression by TNF-activated p38alpha/polycomb repressive complex 2 (PRC2) signaling during muscle satellite cell differentiation. Cell Cycle 2011; 10: 191-198.
[49] Chen S, Bohrer LR, Rai AN, Pan Y, Gan L, Zhou X, Bagchi A, Simon JA and Huang H. Cyclin-dependent kinases regulate epigenetic gene silencing through phosphorylation of EZH2. Nat Cell Biol 2010; 12: 1108-1114.
[50] Wong CF and Tellam RL. MicroRNA-26a targets the histone methyltransferase Enhancer of Zeste homolog 2 during myogenesis. J Biol Chem 2008; 283: 9836-9843.
[51] Szulwach KE, Li X, Smrt RD, Li Y, Luo Y, Lin L, Santistevan NJ, Li W, Zhao X and Jin P. Cross talk between microRNA and epigenetic regulation in adult neurogenesis. J Cell Biol 2010; 189: 127-141.
[52] Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, Laxman B, Cao X, Jing X, Ramnarayanan K, Brenner JC, Yu J, Kim JH, Han B, Tan P, Kumar-Sinha C, Lonigro RJ, Palanisamy N, Maher CA and Chinnaiyan AM. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science 2008; 322: 1695-1699.
[53] Cao P, Deng Z, Wan M, Huang W, Cramer SD, Xu J, Lei M and Sui G. MicroRNA-101 negatively regulates Ezh2 and its expression is modulated by androgen receptor and HIF-1alpha/HIF-1beta. Mol Cancer 2010; 9: 108.
[54] Friedman JM, Liang G, Liu CC, Wolff EM, Tsai YC, Ye W, Zhou X and Jones PA. The putative tumor suppressor microRNA-101 modulates the cancer epigenome by repressing the polycomb group protein EZH2. Cancer Res 2009; 69: 2623-2629.
[55] Alajez NM, Shi W, Hui AB, Bruce J, Lenarduzzi M, Ito E, Yue S, O'Sullivan B and Liu FF. Enhancer of Zeste homolog 2 (EZH2) is overexpressed in recurrent nasopharyngeal carcinoma and is regulated by miR-26a, miR-101, and miR-98. Cell Death Dis 2010; 1: e85.
[56] Smits M, Nilsson J, Mir SE, van der Stoop PM, Hulleman E, Niers JM, de Witt Hamer PC,

Marquez VE, Cloos J, Krichevsky AM, Noske DP, Tannous BA and Wurdinger T. miR-101 is down-regulated in glioblastoma resulting in EZH2-induced proliferation, migration, and angiogenesis. Oncotarget 2010; 1: 710-720.

[57] Zhang JG, Guo JF, Liu DL, Liu Q and Wang JJ. MicroRNA-101 Exerts Tumor-Suppressive Functions in Non-small Cell Lung Cancer through Directly Targeting Enhancer of Zeste Homolog 2. J Thorac Oncol 2011; Epub online Jan 25.