

REVIEW

Histone methyltransferases and demethylases: regulators in balancing osteogenic and adipogenic differentiation of mesenchymal stem cells

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Mesenchymal stem cells (MSCs) are characterized by their self-renewing capacity and differentiation potential into multiple tissues. Thus, management of the differentiation capacities of MSCs is important for MSC-based regenerative medicine, such as craniofacial bone regeneration, and in new treatments for metabolic bone diseases, such as osteoporosis. In recent years, histone modification has been a growing topic in the field of MSC lineage specification, in which the Su(var)3–9, enhancer-of-zeste, trithorax (SET) domain-containing family and the Jumoni C (JmjC) domain-containing family represent the major histone lysine methyltransferases (KMTs) and histone lysine demethylases (KDMs), respectively. In this review, we summarize the current understanding of the epigenetic mechanisms by which SET domain-containing KMTs and JmjC domain-containing KDMs balance the osteogenic and adipogenic differentiation of MSCs.

International Journal of Oral Science (2015) 7, 197–204; doi:10.1038/ijos.2015.41; published 23 October 2015

Keywords: adipogenesis; histone methylation; histone lysine methyltransferase; histone lysine demethylase; mesenchymal stem cells; osteogenesis

INTRODUCTION

Mesenchymal stem cells (MSCs) are multipotent adult stem cells that can self-renew and differentiate into cells of the mesodermal lineages both *in vitro* and *in vivo*, such as osteocytes, adipocytes, and chondrocytes.^{1–3} Although human MSCs were originally isolated from bone marrow, they have since been found in many other adult tissues.^{4–5} In addition to their differentiation capability and easy access, MSCs can be recruited to sites of tissue damage and be activated to express immunosuppressive molecules and growth factors, thereby regulating immune homeostasis and facilitating tissue repair.^{6–7} Hence, MSCs are considered to be of great promise for regenerative medicine.

The osteogenic and adipogenic differentiation of MSCs has been widely studied.⁸ A balance between these two pathways is critical to maintain homeostasis under physiological conditions, and disruption of this balance leads to bone-related metabolic diseases, such as osteoporosis,^{9–10} which features excessive accumulation of bone marrow adipocytes and a decrease in bone mass.¹¹ Thus, bone repair with MSCs can be facilitated by enhancing osteogenic differentiation and inhibiting adipogenic differentiation. Therefore, understanding of MSC differentiation is critical in the development of new treatments for metabolic bone diseases.

The differentiation of MSCs is a complex process that involves multiple mechanisms. Various signaling pathways, such as Wnt,

Notch, transforming growth factor (TGF)- β , and nuclear factor- κ B (NF κ B), have been demonstrated in the regulation of MSC differentiation.^{12–14} Among these, intricate cross-talk has been identified.^{15–16} In addition, several transcription factors are considered to be essential modulators of MSC differentiation, such as runt-related transcription factor 2 (RUNX2) in osteogenesis and peroxisome proliferator-activated receptor- γ (PPAR- γ) in adipogenesis.^{17–18} In the last decade, growing evidence has shown that epigenetic mechanisms play an important role in regulating MSCs differentiation.^{19–21}

The term “epigenetic mechanism” refers to inheritable changes in gene expression without altering the DNA sequence and includes DNA methylation, histone modification, and small non-coding RNA-associated regulation.²² In eukaryotic cells, chromatin structure is highly dynamic, regulated in large part by histone modifications, such as histone acylation and methylation.²³ During histone methylation, methyl groups are transferred to histone proteins of chromosomes, generally at lysine residues. Specifically, these lysines can be unmodified (me0), mono- (me1), di- (me2), or tri-methylated (me3). The resulting histone methylation states, which contribute to chromatin structural changes, are important in regulating transcription factors’ access to the related gene promoters.²⁴

Recent studies have highlighted histone methylation state as an important modulator in stem cell differentiation.²⁴ Regulation of

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Accepted 3 August 2015

these methylation states is tightly controlled by the opposing activities of histone lysine methyltransferases (KMTs) and histone lysine demethylases (KDMs),²⁵ which are recruited to specific histone lysine residues and are responsible for the establishment and regulation of histone methylation and demethylation, respectively. The Su(var)3–9, enhancer-of-zeste, trithorax (SET) domain-containing family and the Jumonji C (JmjC) domain-containing family represent the major KMTs and KDMs.^{24–25}

In this review, we summarize the current understanding of the epigenetic effects of histone methylation on balancing osteogenic and adipogenic differentiation of MSCs, specifically focusing on the roles of SET domain-containing KMTs and JmjC domain-containing KDMs (Figure 1).

SET DOMAIN-CONTAINING HISTONE METHYLTRANSFERASES

The evolutionarily conserved SET domain functions as the catalytic domain of KMTs by transferring a methyl group from *s*-adenosylmethionine to the ϵ -amine on the side chain of histone lysine residues.²⁵ Aside from DOT1L and the WRAD complex,^{26–27} it is predicted that most KMTs are of a SET domain. These SET domain-containing KMTs seem to be particularly sensitive to post-translational methylation at target lysine sites (Table 1). Although it

has been five decades since histone methylation's discovery,²⁸ its correlation with gene regulation was only established recently.²⁹ SET domain bifurcated 1 (SETDB1) and enhancer of zeste homologue 2 (EZH2) are the most widely studied SET domain-containing KMTs associated with MSC differentiation.

SETDB1

SETDB1, also known as KMT1E/ESET, catalyzes the tri-methylation of histone 3 at lysine 9 (H3K9).³⁰ The SETDB1 protein contains two major functional domains: the C-terminal SET domain catalyzes methylation, while the N-terminal domain is responsible for interaction with other chromatin-modifying enzymes.³¹ Previous studies have shown that SETDB1 is essential for early embryonic development. Indeed, the ubiquitous deletion of the SETDB1 leads to lethality between E3.5 and E5.5, due to defective growth of inner cell mass.³⁰ Recently, growing evidence shows that SETDB1 is critical in the regulation of MSC osteoblastic and adipogenic differentiation.¹⁴

Specifically, SETDB1 is reported to inhibit osteogenic differentiation of MSCs.³² After generating SETDB1-deficient mice driven by Prx1-Cre, Lawson *et al.* found that mesenchymal deletion of SETDB1 led to long bone defects and significant decrease in trabecular bone in both embryos and postnatal mice.^{32–33} These mice also exhibited decreased osteoblasts in long bones, but normal osteoclast formation.

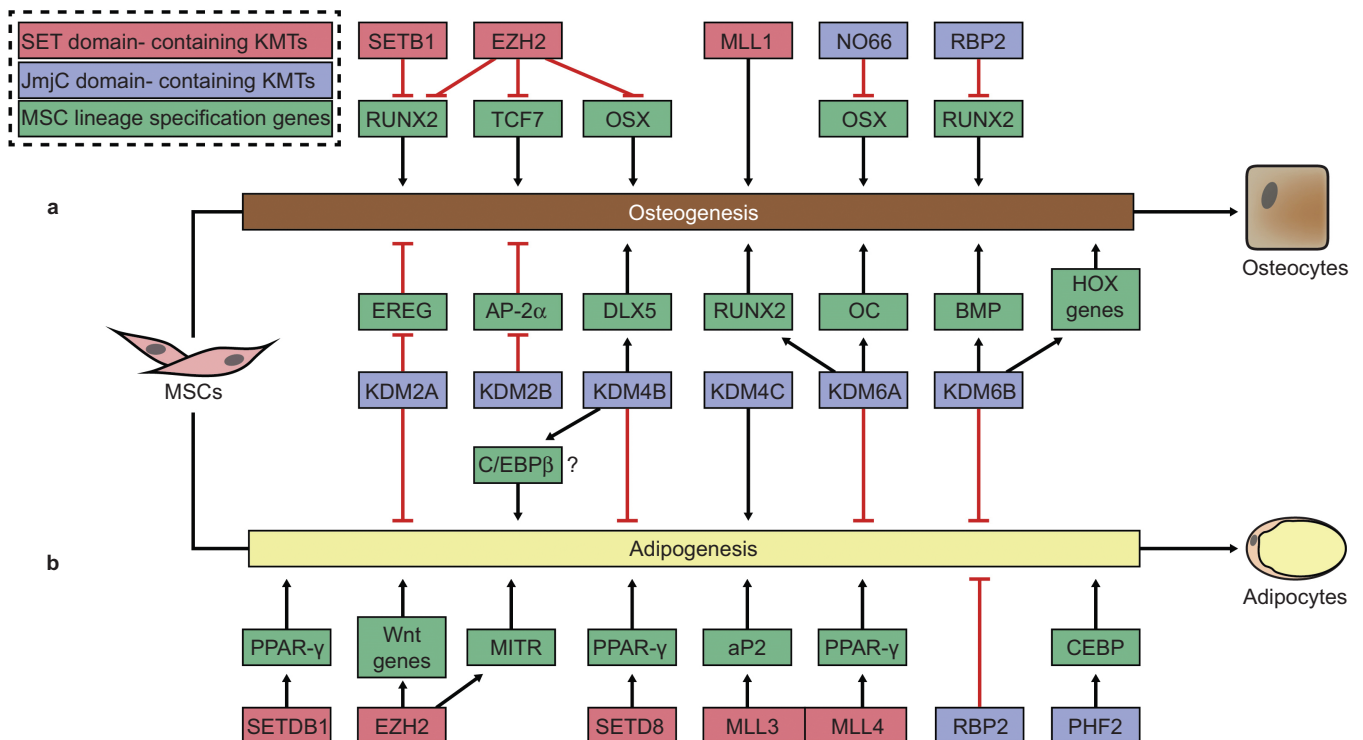


Figure 1 The role of SET domain-containing KMTs and JmjC domain-containing KDMs in osteogenesis and adipogenesis of MSCs. The differentiation of MSC involves a lot of lineage specification genes, including transcription factors and other genes. SET domain-containing KMTs and JmjC domain-containing KDMs regulate the expression levels or transcription activity of the genes to determine the cell-fate. (a) The role of SET domain-containing KMTs and JmjC domain-containing KDMs in osteogenesis of MSCs. (b) The role of SET domain-containing KMTs and JmjC domain-containing KDMs in adipogenesis of MSCs. The related KMTs and KDMs are shown in red and blue boxes, respectively. The red question marker indicates the contradictory reported role of KDM4B in adipogenesis of MSCs. aP2, adipocyte protein 2; AP-2 α , activating enhancer-binding protein 2 α ; BMP, bone morphogenetic protein; C/EBP β , CCAAT/enhancer-binding protein beta; DLX5, distal-less homeobox 5; EREG, epiregulin; EZH2, enhancer of zeste homologue 2; HOX, homeobox; KDM2A/JHM1A, JmjC domain-containing histone demethylase JmjC, Jumonji C; KDM, histone lysine demethylase; MSC, mesenchymal stem cell; MITR, myocyte enhancer factor-2 interacting transcriptional repressor; MLL, myeloid/lymphoid or mixed-lineage leukemia; RBP, retinol-binding protein; OC, osteocalcin; OSX, osterix; PHF2, plant homeodomain finger 2; PPAR- γ , peroxisome proliferator-activated receptor-gamma; RUNX2, runt-related transcription SET, Su(var)3–9, enhancer-of-zeste, trithorax; SETD8, SET domain-containing 8; SETDB1, SET domain bifurcated 1; TCF, transcription factor.

Table 1 The role of SET domain-containing KMTs during osteogenesis and adipogenesis (shaded) of MSCs

KMTs	Specificity	Target	Finding	Reference
SETDB1	H3K9	RUNX2 target genes PPAR- γ target genes	SETDB1 inhibits osteogenesis by reducing transcriptional activity of RUNX2 SETDB1 inhibits adipogenesis by transcriptional activity of PPAR- γ	32,33 35
EZH2	H3K27	RUNX2, TCF7, OC Wnt genes, MITR	EZH2 inhibits osteogenesis by catalyzing tri-methylation of H3K27 in the promoters of RUNX2, TCF7, and OC EZH2 promotes adipogenesis by catalyzing tri-methylation of H3K27 in the promoters of Wnt genes and MITR	42,43 45,46
SETD8	H4K20	PPAR- γ and PPAR- γ target genes	SETD8 promotes adipogenesis by catalyzing mono-methylation of H4K20 in the promoters of PPAR- γ and PPAR- γ target genes	50,51
MLL1	H3K4	Not known	Mice with SET domain mutated MLL1 exhibit skeletal defects	52,53
MLL3, MLL4	H3K4	PPAR- γ , aP2	MLL3 and MLL4 promote adipogenesis by catalyzing methylation of H3K4 in the promoters of PPAR- γ and aP2	54,57

aP2, adipocyte protein 2; EZH2, Enhancer of zeste homologue; KMT, histone lysine methyltransferase; MLL, myeloid/lymphoid or mixed-lineage leukaemia; MITR, myocyte enhancer factor-2 interacting transcriptional repressor; MSC, mesenchymal stem cells; OC, osteocalcin; PPAR, peroxisome proliferator-activated receptor; RUNX2, runt-related transcription factor 2; SET, Su(var)3-9, Enhancer-of-zeste, Trithorax; SETD8, SET domain-containing 8; STDB1, SET domain bifurcated 1; TCF, transcription factor.

These data indicate that SETDB1 is required for differentiation of MSCs into osteoblasts. Moreover, Lawson *et al.* also observed impaired osteoblastic activity in SETDB1-deficient MSCs from bone marrow, with hyperactivity of RUNX2. Consistent with these results, knockdown of SETDB1 also enhances RUNX2-mediated gene transcription *in vitro*, which may be caused by the decrease of H3K9me3 in the promoters of RUNX2 target genes.³³ Although RUNX2 is necessary for osteoblast differentiation, when transgenically over-expressed, it can inhibit osteoblast maturation.³⁴ Collectively, SETDB1 inhibits osteogenic differentiation of MSCs, but it is required for normal skeletal formation due to its ability to repress hyperactive RUNX2-mediated transcription.

SETDB1 also plays a role in regulating adipogenic differentiation of MSCs. SETDB1 is down-regulated by PPAR- γ in rodent models of obesity, such as high-fat feeding mice and the genetically predisposed obese ob/ob mice. A similar down-regulation of SETDB1 is observed in adipogenic differentiation of MSCs *in vitro*, while siRNA-mediated knockdown of SETDB1 promotes adipogenesis with upregulated expression of PPAR- γ , CCAAT/enhancer-binding protein α (C/EBP α), and other adipogenesis-related genes.³⁵

EZH2

EZH2, also known as KMT6A, catalyzes the addition of methyl groups to H3K27.³⁶ This methyltransferase functions in the polycomb repressive complex 2 (PRC2) with suppressor of zeste 12 (SUZ12) and embryonic ectoderm development (EED).³⁷ Notably, SUZ12 and EED are necessary for the catalytic activity of EZH2. Ubiquitous deletion of any of these components causes embryonic death during the post-implantation period.³⁸⁻⁴⁰ For decades, EZH2 has been known to be important for stem cell maintenance and differentiation.^{36,41}

In terms of MSCs differentiation, EZH2 inhibits osteogenesis and promotes adipogenesis. To demonstrate this, suppression of EZH2 by cyclin-dependent kinase 1 (CDK1) results in MSC differentiation to osteoblasts.⁴² Likewise, chemical enzymatic inhibition and siRNA knockdown studies also show that EZH2 is a negative regulator of osteogenesis of MSCs.⁴¹ A genome-wide screen of polycomb target genes demonstrates that EZH2 is present on osteogenesis-related genes in non-differentiated MSCs, such as RUNX2 and transcription factor 7 (TCF7).^{36,42} Mechanistically, EZH2 expression is down-regulated under osteogenic inductive conditions and disassociates from the promoter RUNX2.⁴³ Conversely, a recent study on neural crest cells (NCCs) reported that EZH2 is required for neural crest-derived bone formation. As a result of conditional ablation of EZH2 in NCCs,

the formation of multiple skeletal elements was inhibited and severe craniofacial defects were caused.⁴⁴ Therefore, it is likely that EZH2 has different tissue-specific functions in osteogenesis.

EZH2 methyltransferase activity is required for adipogenesis. Deletion of EZH2 in brown preadipocytes leads to decrease of H3K27me3 on the promoters of Wnt genes, resulting in severe defects in adipogenesis. These differentiation defects could be rescued by ectopic EZH2 and inhibitors of Wnt/ β -catenin signaling. This suggests that EZH2 promotes adipogenesis by repression of Wnt genes.⁴⁵ In addition to Wnt/ β -catenin signaling, myocyte enhancer factor-2 interacting transcriptional repressor (MITR) also prevents adipogenesis by inhibiting the transcriptional activity of PPAR- γ . Acting to promote adipogenesis, EZH2 is present at the MITR promoter, where it inhibits MITR expression in adipocytes. Dissociation of EZH2 from the MITR promoter increases MITR expression and inhibits adipogenesis, while simultaneously enhancing the osteogenic differentiation of MSCs.⁴⁶

Other SET family member genes

SET domain-containing 8 (SETD8), also known as KMT5A, catalyzes mono-methylation of H4K20.⁴⁷ It has been reported to be essential for development and cell cycle progression.⁴⁸ Deletion of SETD8 is lethal in flies⁴⁹ and causes arrested embryonic development during the cleavage stage in mice.⁴⁷ Over-expression of SETD8 by retrovirus enhances adipogenesis, while the siRNA-mediated knockdown of SETD8 impairs adipogenesis.⁵⁰ As a requisite for committed preadipocyte differentiation, SETD8 promotes expression of PPAR- γ and PPAR- γ target genes through H4K20 mono-methylation.⁵¹ Like SETDB1, expression of SETD8 is regulated by PPAR- γ during adipogenesis. Following adipogenic stimuli, PPAR- γ promotes SETD8 expression, which catalyzes H4K20 mono-methylation in the promoters of PPAR- γ and of PPAR- γ target genes to enhance adipogenesis.⁵⁰

Myeloid/lymphoid or mixed-lineage leukaemia 1 (MLL1), also known as KMT2A, catalyzes methylation at H3K4.²⁷ The structure of MLL is complex, including three AT-hook motifs, a DNA methyltransferase homology domain in the amino-terminal half of the protein, plant homeodomain (PHD) region, and a SET domain at carboxyl-terminal.²⁷ Due to the multiple functions of MLL1, deletion of MLL1 in mice has been reported to be lethal, and MLL1 heterozygous mice show retarded growth, hematopoietic abnormalities, and skeletal malformations.⁵² To investigate the specific function of SET domain in MLL1, Terranova *et al.* generated mutated mice, which expressed a SET domain-truncated form of MLL1.⁵³ The mutated mice exhibit bidirectional homeotic transformations of the axial skeleton, and altered transcription of selected target

HOX genes during development.⁵³ The skeletal defects and bone malformations indicate the regulation of osteogenesis by MLL1.

MLL3 and MLL4, the paralogues of MLL1, catalyze methylation at H3K4.^{54–55} Deletion of the catalytic region of MLL3 leads to partial embryonic lethality, growth retardation, and female infertility.^{55–56} Notably, MLL3-null mice have little white adipose tissue, but normal brown adipose tissue (BAT). Compared to wild-type mouse embryonic fibroblasts (MEFs), MEFs isolated from MLL3-null mice show striking defects in adipogenesis. Upon adipogenic induction, MLL3 and MLL4 are recruited to the promoter of adipocyte protein 2 (aP2), an adipogenic marker gene, in a time-dependent manner.⁵⁴ In addition, MLL3 and MLL4 can form a complex with Pax transactivation domain-interacting protein (PTIP). PTIP-deficient MEFs, white preadipocytes, and brown preadipocytes all exhibit impaired adipogenic potential, with decreased enrichment of PTIP and MLL4 on the PPAR- γ promoter.⁵⁷ Collectively, MLL3 and MLL4 facilitate adipogenesis through their KMT activity to promote PPAR- γ and aP2 expression.

In summary, there is increasing evidence showing that SET domain-containing KMTs are essential for MSC differentiation. Major methylation sites mediated by SET domain-containing KMTs are located in the tail histones H3 and H4, such as H3K4, H3K9, H3K27, H3K36, and H4K20.⁵⁸ In general, methylation at H3K4 and H3K36 is associated with transcriptional activation, while methylation at H3K9 and H3K27 is related to transcriptional repression. The role of mono-methylation at H4K20 could be either transcriptional activation or repression.^{25,58} Here, we summarize several SET domain-containing KMTs that integrate upstream stimulation and regulate osteogenesis and adipogenesis of MSCs by methylating the promoter of lineage-specific genes (Table 1). Although additional KMTs have been identified in association with osteogenesis and adipogenesis,^{46,59–60} further investigation to explore their underlying mechanism is necessary.

JmjC DOMAIN-CONTAINING KDMs

Unlike KMTs, KDMs remove methyl groups from histone lysine residues. This reversibility of KMTs was first discovered in 2004.⁶¹ To date, more than 30 histone demethylases have been identified, and they are classified into two classes: the lysine-specific demethylase family and the JmjC family. JmjC domain-containing proteins represent the largest class of potential histone demethylases and act by catalyzing the removal of mono-, di-, and tri-methyl residues *via* a 2OG-Fe(II)-dependent dioxygenase reaction.^{62–63} On the basis of emerging findings, JmjC domain-containing KDMs (Table 2) have been recognized and shown to play crucial roles in transcription regulation, stem cell differentiation, and animal development.^{62,64–65}

KDM2 cluster

The KDM2 cluster, also known as JmjC domain-containing histone demethylase 1 (JHDM1) subfamily, catalyzes the removal of trimethyl marks at H3K4, as well as mono- and di-methyl marks at H3K36.^{66–68} The KDM2 subfamily is comprised of two member genes: KDM2A (JHDM1A/FBXL11) and KDM2B (JHDM1B/FBXL10). KDM2A has shown to be important for proliferation of MSCs, and KDM2B has also been demonstrated to regulate cell proliferation and senescence.⁶⁹ KDM2B-deficient mice show increased apoptosis in the neuroepithelium and mesenchyme at E9.5 and exhibit a failure of cranial neural tube closure.⁷⁰ More recently, KDM2A and KDM2B were reported to regulate MSC differentiation by associating with the BCOR (BCL-6 co-repressor) complex.^{68,71–72}

While BCOR mutation is lethal in males, BCOR mutation in females causes oculofaciocardiodental (OFCD) syndrome, which is characterized by canine teeth with extremely long roots, congenital cataracts, craniofacial defects, and congenital heart disease.⁷¹ KDM2B together with the BCOR complex was found to be recruited to the promoter of activating enhancer-binding protein 2 α (AP-2 α), which is required for osteogenesis. Consequently, KDM2B represses AP-2 α expression by removing the H3K4me3 and H3K36me2 marks in the promoter.⁷¹ This mechanism serves as an explanation for the extremely long roots of canine teeth found in OFCD patients. Like KDM2B, KDM2A is also found to interact with BCOR and inhibit odontogenic differentiation of human stem cells from apical papilla (SCAPs), a type of dental MSCs.⁶⁸ In addition, KDM2A and the BCOR complex are reported to inhibit osteogenesis by increasing histone H3K4/36 methylation in the epiregulin (EREG) promoter, thereby repressing EREG transcription. EREG is required for the expression of osteogenic-related genes, such as osterix (OSX) and distal-less homeobox 5 (DLX5).⁷²

Although Dong *et al.* reported that the depletion of KDM2A in SCAPs enhances adipogenic differentiation *in vitro*,⁶⁸ the effect of KDM2A on adipogenic differentiation is still not fully understood. Additional efforts, especially *in vivo* studies, are needed to explore the role of KDM2 member genes in adipogenesis.

KDM4 cluster

The KDM4 cluster, also known as the Jumonji domain-containing 2 subfamily, catalyzes the removal of di- and tri-methyl marks at H3K9 and H3K36.^{73–74} Five functional KDM4 member genes (KDM4A-E) have been identified in the human genome, of which KDM4A-C are broadly expressed in human tissues, while KDM4D and KDM4E are specifically enriched in the human testes.^{73,75} Partly because they have redundant roles, KDM4C or KDM4D knockout mice are viable without gross abnormalities.^{75–76} However, conditional deletion of KDM4B was recently reported to delay mammary gland development in mice,⁷⁷ while KDM4A is required for skeletal muscle differentiation and neural crest specification.^{78–79} These findings indicate the roles of KDM4 member genes on transcription regulation, stem cell differentiation, and embryonic development.

More recently, KDM4B was reported to enhance osteogenic differentiation of human MSCs.¹⁹ In the bone marrow of aged mice, KDM4B expression is reduced, accompanying decreased osteogenesis. Knockdown of KDM4B by shRNA inhibits osteogenic differentiation in human bone marrow-derived MSCs, while over-expression of KDM4B enhances osteogenesis. After subcutaneous transplantation of MSCs with an HA scaffold, KDM4B is also required for MSC-mediated bone formation *in vivo*. Mechanistically, KDM4B was found to remove the H3K9me3 marks in the promoter of DLX5, activating its expression during osteogenesis. The DLX5 gene plays a critical role in osteoblast differentiation through controlling OSX expression.⁸⁰

The role of KDM4 member genes in adipogenesis is still controversial. Ye *et al.* reported that the knockdown of KDM4B by shRNA enhanced adipogenic differentiation of MSCs and increased the expression of PPAR- γ and CD36 (also known as fatty acid translocase).⁸⁰ These results indicate that KDM4B inhibits adipocyte lineage specification from MSCs. However, KDM4B was also found to act as a co-factor of C/EBP β to promote differentiation of preadipocytes.⁸¹ After KDM4B interacts with C/EBP β , they are recruited to the promoters of the target genes of C/EBP β , such as cell division cycle 45 homolog, mini-chromosome maintenance complex component 3, GINS complex subunit 1, and cell division cycle 25 homolog c. By removing the H3K9me3 marks,

Table 2 The role of JmjC domain-containing KDMs during osteogenesis and adipogenesis (shaded) of MSCs

KMTs	Specificity	Target	Finding	Reference
KDM2A	H3K4me3, H3K36me1/2	EREG	KDM2A inhibits osteogenesis by catalyzing demethylation of H3K4me3 and H3K36me2 in the promoter of EGER through interaction with BCOR complex	72
		Not known	KDM2A inhibits adipogenesis, while depletion of KDM2A in SCAPs enhances adipogenic differentiation	68
KDM2B	H3K4me3, H3K36me1/2	AP-2 α	KDM2B inhibits osteogenesis by catalyzing demethylation of H3K4me3 and H3K36me2 in the promoter of AP-2 α through interaction with BCOR complex	71
KDM4B	H3K9me2/3, H3K36me2/3	DLX5	KDM4B inhibits osteogenesis by catalyzing demethylation of H3K9me3 in the promoter of DLX5	80
		Not known	KDM4B inhibits adipocyte lineage specification from human bone marrow-derived MSCs	80
		C/EBP β target genes	KDM4B promotes adipogenesis by catalyzing demethylation of H3K9me3 in the promoter of C/EBP β target genes in preadipocytes	81
KDM4C	H3K9me2/3, H3K36me2/3	Not known	KDM4C is required for adipogenesis from preadipocytes, which can be blocked by IDH mutation	82
KDM6A	H3K27me2/3	RUNX2, OC	KDM6A promotes osteogenesis by catalyzing demethylation of H3K27me3 at transcription start sites of RUNX2 and OC	43
		Not known	KDM6A inhibits adipogenesis, while the depletion of KDM6A in human bone marrow-derived MSCs enhances adipogenic differentiation	43
KDM6B	H3K27me2/3	BMP and HOX genes	KDM6B promotes osteogenesis by catalyzing demethylation of H3K27me3 in the promoters of BMP and HOX genes	80
		Not known	KDM6B inhibits adipogenesis, while depletion of KDM6B in human bone marrow-derived MSCs enhances adipogenic differentiation	80
NO66	H3K4me1/2/3, H3K36me2/3	OSX target genes	NO66 inhibits osteogenesis by catalyzing demethylation of H3K4me3 and H3K36me3 in the promoters of OSX target genes, such as BSP	89-91
RBP2	H3K4me1/2/3	RUNX2 target genes	RBP2 inhibits osteogenesis by catalyzing demethylation of H3K4me3 in the promoters of RUNX2 target genes, such as OSX and OC	59
		Not known	RBP2 inhibits adipogenesis from preadipocytes, and depletion of RBP2 in preadipocytes enhances adipogenic differentiation potential	94
PHF2	H3K9me2	C/EBP α	PHF2 with C/EBP α promotes adipogenesis by catalyzing demethylation the H3K9me2 in the promoters of adipose genes	98

AP-2 α , activating enhancer binding protein 2 α ; BMP, bone morphogenetic protein; C/EBP, CCAAT/enhancer binding protein; DLX5, distal-less homeobox 5; EREG, epiregulin; HOX, homeobox; JmjC, Jumonji C; KDM, histone lysine demethylase; KMT, histone lysine methyltransferase; MSC, mesenchymal stem cell; OC, osteocalcin; OSX, osterix; RBP, retinol binding protein; RUNX2, runt-related transcription factor 2.

KDM4B facilitates C/EBP β target gene expression.⁸¹ It appears that the conflicting effects are caused by opposing functions of KDM4B at different stages of adipogenesis.

In addition, KDM4C is reported to be required for adipogenesis. Specifically, the inhibition of KDM4C induced by isocitrate dehydrogenase (IDH) mutation or 2-hydroxyglutarate (the product of mutated IDH) is sufficient to block differentiation of preadipocytes.⁸²

KDM6 cluster

The KDM6 cluster catalyzes the removal of di- and tri-methyl marks at H3K27^{64,83} and contains three member genes: KDM6A (UTX),

KDM6B (JMJD3), and UTY. KDM6A and KDM6B were the first to be identified as H3K26me2/me3 demethylases, followed by UTY, which has only recently been found to have overlapping redundancy with KDM6A in embryonic development.⁸⁴ While ubiquitous deletion of KDM6B is lethal,⁸⁵ approximately 25% KDM6A knockout mice survive to adulthood, partly due to the redundancy of UTY.⁸⁴ Based on mice and cell culture models, KDM6A and KDM4B have been demonstrated to be critical in the regulation of stem cells, including M2 macrophage differentiation, muscle differentiation, and neuronal stem cell differentiation.⁸⁵⁻⁸⁷ Recent studies also found that KDM6A and KDM6B play a role in MSC lineage specification.

Both KDM6A and KDM6B promote osteogenic differentiation of MSCs. Hemming *et al.* reported that transcription levels of KDM6A are down-regulated during the process of osteogenesis of human bone marrow MSCs.⁴³ Additionally, siRNA-mediated knockdown of KDM6A inhibits osteogenesis. Conversely, retroviral-mediated over-expression of KDM6A enhances the potential to differentiate into mineral forming osteoblasts and ectopic bone formation *in vivo*.⁴³ This over-expression of KDM6A results in a decrease in H3K27me3 at both RUNX2 and osteocalcin (OC) transcription start sites, which subsequently increases the expression of RUNX2 and OC.⁴³

In addition, KDM6B was shown to be required for osteogenic differentiation of MSCs.^{80,88} Upon osteogenic induction, KDM6B is recruited to the promoters of bone morphogenetic protein (BMP) and homeobox (HOX) genes, each of which promote the osteogenic differentiation of MSCs. When MSCs are depleted of KDM6B, they demonstrate impaired osteogenic differentiation and increased adipogenic differentiation *in vitro*, as well as decreased bone formation after subcutaneous transplantation with a scaffold.⁸⁰ More recently, KDM6B was found to facilitate odontogenic differentiation of dental MSCs by removing the H3K27me3 mark from the BMP promoter.⁸⁸

KDM6A and KDM6B are both reported to inhibit adipogenic differentiation of MSCs. Under adipogenic differentiation conditions, enforced over-expression of KDM6A results in decreased mRNA level of PPAR- γ , C/EBP α , and adiposin. Supporting these findings, functional studies show that the over-expression of KDM6A in MSCs decreases potential to form lipid *in vitro*. Conversely, knockdown of KDM6A shows an increase in lipid formation⁴³ and depletion of KDM6B facilitates adipogenesis and promotes PPAR- γ and CD36 expression. In aged mice, bone marrow MSCs exhibit excessive adipogenic differentiation, which supports evidence of elevated H3K27me3 and decreased KDM6B.⁸⁰ These findings indicate a negative role of KDM6A and KDM6B in adipogenesis. However, the mechanism for KDM6A and KDM6B regulation of adipogenic differentiation is still not clear.

Other related JmjC family member genes

NO66, another JmjC domain-containing protein, catalyzes the removal of mono-, di-, and tri-methyl marks at H3K4, as well as di- and trimethyl marks at H3K36.⁸⁹ NO66 has been found in all the developing bones, such as the E15.5 vertebrae and mandible and E18.5 femur, tibia, and fibula, indicating that NO66 plays a role in osteogenesis.⁸⁹ Depletion of NO66 in preosteoblasts accelerates their differentiation and maturation, and knockdown of NO66 significantly increases the expression levels of osterix (OSX)-dependent matrix-forming genes.⁸⁹ Mechanistically, NO66 interacts with OSX under osteogenic inductive conditions to inhibit OSX transcriptional activity.^{89–90} Both NO66 and OSX can be found present in the promoter of bone sialoprotein (BSP), resulting in demethylation of H3K4me3 and H3K36me3 and repression of BSP expression. A structural study revealed that the hinge domain-dependent oligomerization of NO66 is essential for the interaction with OSX.⁹⁰ However, in OSX-deficient preosteoblasts, NO66 occupancy is increased in the chromatin of the BSP gene.⁹¹ Hence, it is clear that while NO66 may be recruited to the promoters by different mechanisms, NO66 contributes to the repression of OSX target genes and inhibits osteogenesis.

Retinol-binding protein 2 (RBP2), also known as KDM5A, catalyzes the removal of mono-, di-, and tri-methyl marks at H3K4.^{92–93} Deletion of the JmjC domain of RBR-2 (*Caenorhabditis elegans* RBP2 homolog) in *C. elegans* demonstrates that it is important for vulva development.⁹² During mouse embryonic stem cell differentiation, RBP2 is also found to regulate HOX gene expression levels, which are related to develop-

ment and osteogenesis. However, mice with RBP2 deficiency are viable and grossly normal, except for behavioral abnormalities when held upside down.⁹³ RBP2 was originally found to repress osteogenesis in Soas-2 cells (a human osteogenic sarcoma cell line),⁹⁴ and has subsequently been reported to inhibit osteogenesis in adipose-derived MSCs by repression of RUNX2-mediated transcriptional activity.⁵⁹ Depletion of RBP2 results in increased expression levels of osteogenic associated genes, promotes osteogenesis *in vitro*, and increases bone formation after subcutaneous transplantation *in vivo*. Moreover, the increased osteogenesis can be abolished by the knockdown of RUNX2.⁹⁵ In addition, RBP2 is shown to inhibit adipogenesis from preadipocytes, and depletion of RBP2 in preadipocytes enhances lipid formation *in vitro*.⁹⁴ Other studies show that RBP2 seems to exert inhibitory effects during both osteogenic and adipogenic differentiation.^{95–96}

Plant homeodomain finger 2 (PHF2), also known as JHDM1E, catalyzes the removal of di-methyl marks at H3K9.²⁰ However, the enzymatic activity of PHF2 depends on phosphorylation mediated by protein kinase A (PKA).⁹⁷ About 70% of mice with PHF2 deficiency die within 3 days after birth, while the rest of the mice show a decrease in body weight, indicating that adipogenesis is impaired in these mice. Also, deletion of PHF2 in adipose tissue results in a 50% reduction in WAT, while BAT appears normal. Mechanistically, PHF2 was found to be a co-activator of C/EBP α and recruited to the promoter of C/EBP responsive elements, such as C/EBP α , PPAR- γ , and fatty acid-binding protein 4 (FABP4). Depletion of PHF2 in preadipocytes inhibits adipose gene expression and lipid formation.⁹⁸ Together, PHF2 with C/EBP α promotes adipogenesis through demethylation of the H3K9me2 mark in the promoters of adipose genes.

In summary, these findings have revealed an important role of JmjC family genes in the regulation of the balance between osteogenic differentiation and adipogenic differentiation of MSCs. Unlike KMTs, JmjC family genes control transcriptional activity by removing the methylation marks at the specific histone lysine residues. A summary of the current opinions on regulation of osteogenesis and adipogenesis by various JmjC domain-containing KDMs can be found in Table 2. While some JmjC family genes take part in MSC differentiation at an early stage, such as KDM4B and KDM6B in osteogenesis,⁸⁰ others play roles at a later stage, such as NO66 in osteogenesis⁹¹ and KDM4C in adipogenesis.⁸² However, the mechanism of JmjC family genes in adipogenesis is still not well understood. To further investigate, results based on cell culture models need to be verified by *in vivo* studies.

Outlook

Eukaryotic chromatin is organized in both euchromatin (active) and heterochromatin (inactive) forms. Histone methylation marks are key to define these functional states, particularly in promoter regions, as they affect the physical proximity of lysine residues to each other. However, during MSC differentiation, the cross-talk among the histone methylation marks is still elusive to us, and should be taken into account in future studies. For instance, the euchromatic mark H3K4me3 prevents tri-methylation of H3K9 by SETDB1,⁹⁹ but the heterochromatin mark H3K9me3 prevents mono-methylation of H3K4 by SET7.¹⁰⁰ Notably, the cross-talk among histone methylation marks is not restricted to methylation at lysine residues. More information can be found in previous reviews.^{58,101–103}

Another important question concerns the role of opposing functions of KMTs and KDMs in establishing histone methylation states. It is plausible that both methylation and demethylation occur in the promoters of different lineage-specific genes. For instance, EZH2

and KDM6A, which both target H3K27, act as an epigenetic switch to regulate MSC lineage specification.⁴³ However, the methyltransferase activity of SET domain-containing KMTs seems to be specific to one site, while JmjC domain-containing KDMs exhibit more redundancy and tissue specificity.

Interestingly, KMTs and KDMs may interact with each other. For instance, JMJ was shown to be required for efficient binding of the PRC2 complex.¹⁰⁴ A study of mouse embryonic stem cells revealed that KDM4C could interact with the components of PRC2 and assist Ezh2 to fully repress target genes.¹⁰⁴ In addition, KDM5C, a H3K4 demethylase, may interact with H3K9 methylases, which in turn may couple H3K9 methylation to H3K4 demethylation.⁶⁴ These interactions may also be critical in MSC differentiation, especially in its initial stages.

CONCLUSION

MSCs hold great promise for the treatment of difficult bone defects. However, to employ MSCs for clinical use, we must first understand the mechanisms of MSC differentiation. Histone methylation has recently been identified as a key modulator of MSC lineage specification. Furthermore, epigenetic regulation of MSC differentiation is also critical for bone metabolism. In this review, we summarized the epigenetic mechanisms of histone methylation in relation to osteogenesis and adipogenesis of MSCs (Figure 1). Further investigation is still required to gain a more complete understanding, which will in turn enable the use of MSCs in regenerative medicine of large bone defects and metabolic bone diseases.

ACKNOWLEDGEMENTS

This study was supported by the National Institute of Dental and Craniofacial Research grants, K08DE024603-02, DE019412, and DE01651, and by a grant from 111 Project of MOE, China. Publication of this manuscript is supported by Open Fund of State Key Laboratory of Oral Diseases, Sichuan University.

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