**Name of Journal:** *World Journal of Stem Cells*

**Manuscript NO:** 89420

**Manuscript Type:** MINIREVIEWS

**How mesenchymal stem cells transform into adipocytes: Overview of the current understanding of adipogenic differentiation**

Liu SS *et al*. MSCs transform into adipocytes

Shan-Shan Liu, Xiang Fang, Xin Wen, Ji-Shan Liu, Miribangvl Alip, Tian Sun, Yuan-Yuan Wang, Hong-Wei Chen

**Shan-Shan Liu, Xin Wen, Miribangvl Alip, Tian Sun, Hong-Wei Chen,** Department of Reumatology and Immunology, Nanjing Drum Tower Hospital, The Affiliated Hospital of Medical School, Nanjing University, Nanjing 210008, Jiangsu Province, China

**Xiang Fang,** Department of Emergency, Nanjing Drum Tower Hospital, The Affiliated Hospital of Medical School, Nanjing University, Nanjing 210008, Jiangsu Province, China

**Ji-Shan Liu,** Key Laboratory for Biomechanics and Mechanobiology of Ministry of Education, School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China

**Yuan-Yuan Wang,** Anhui Key Laboratory of Infection and Immunity, Bengbu Medical College, Bengbu 233000, Anhui Province, China

**Co-first authors:** Shan-Shan Liu and Xiang Fang.

**Author contributions:** Liu SS and Fang X contributed equally to write the paper; Wen X, Liu JS, Alip M, Sun T, and Wang YY provided data; Chen HW designed the review and were responsible for the final proofreading; and all authors have read and approve the final manuscript.

**Supported by** the National Natural Science Foundation of China, No. 82271843 and 31700779; the Key Project supported by Medical Science and Technology Development Foundation, Nanjing Department of Health, No. ZKX20019; and the Natural Science Foundation of Jiangsu Province, No. BK20200137.

**Corresponding author: Hong-Wei Chen, PhD, Associate Professor,** Department of Reumatology and Immunology, Nanjing Drum Tower Hospital, The Affiliated Hospital of Medical School, Nanjing University, No. 321 Zhongshan Road, Nanjing 210008, Jiangsu Province, China. chenhw@nju.edu.cn

**Received:** October 31, 2023

**Revised:** January 15, 2024

**Accepted:** February 18, 2024

**Published online:**

**Abstract**

Mesenchymal stem cells (MSCs) are stem/progenitor cells capable of self-renewal and differentiation into osteoblasts, chondrocytes and adipocytes. The transformation of multipotent MSCs to adipocytes mainly involves two subsequent steps from MSCs to preadipocytes and further preadipocytes into adipocytes, in which the process MSCs are precisely controlled to commit to the adipogenic lineage and then mature into adipocytes. Previous studies have shown that the master transcription factors C/enhancer-binding protein alpha and peroxisome proliferation activator receptor gamma play vital roles in adipogenesis. However, the mechanism underlying the adipogenic differentiation of MSCs is not fully understood. Here, the current knowledge of adipogenic differentiation in MSCs is reviewed, focusing on signaling pathways, noncoding RNAs and epigenetic effects on DNA methylation and acetylation during MSC differentiation. Finally, the relationship between maladipogenic differentiation and diseases is briefly discussed. We hope that this review can broaden and deepen our understanding of how MSCs turn into adipocytes.

**Key Words:** Mesenchymal stem cell; Adipogenic differentiation; Signaling pathway; Noncoding RNA; Epigenetic regulation

Liu SS, Fang X, Wen X, Liu JS, Alip M, Sun T, Wang YY, Chen HW. How mesenchymal stem cells transform into adipocytes: Overview of the current understanding of adipogenic differentiation. *World J Stem Cells* 2024; In press

**Core Tip:** Mesenchymal stem cells (MSCs) are able to differentiate into adipocytes, while the mechanism underlying the adipogenic differentiation of MSCs is not fully understood. Here, we summarize the function of signaling pathways, noncoding RNAs and epigenetic modification in MSC differentiation, and finally discuss the relationship between maladipogenic differentiation and diseases briefly.

**INTRODUCTION**

Mesenchymal stem cells (MSCs) are multipotent stem/progenitor cells capable of self-renewal and differentiation into distinct mesodermal lineages, such as adipocytes, osteoblasts and chondrocytes. The high migratory capacity, excellent expansion potential and reduced immunogenicity of MSCs make them attractive candidates in regenerative medicine[1]. Although initially derived from bone marrow, MSCs can currently be collected from various tissues and organs, including adipose tissue, umbilical cord blood and dental pulp[2]. According to the International Society for Cellular Therapy criteria, MSCs express CD73, CD90, and CD105 but lack CD14, CD11b, CD34, CD45, CD79a or CD19 and HLA-DR expression[3]. In addition, multipotential differentiation remains the hallmark of MSC identity.

Upon differentiation, the transition of MSCs into terminal mesodermal lineages is precisely controlled by certain lineage-specific master regulators. Runx2 is well known to direct MSCs to switch into osteoblasts[4]. Sox9, an early transcription factor, regulates the expression of key genes involved in chondrogenesis[5,6]. For adipogenesis, both CAAT/enhancer-binding protein alpha (C/EBPα)[7] and peroxisome proliferation activator receptor gamma (PPARγ)[8] are vital regulators that favor adipocyte formation.

However, full adipogenic differentiation from MSCs is a long-term complex process in which multipotent MSCs gradually commit to preadipocyte differentiation and eventually differentiate into terminal adipocytes, thus resulting in an adipocytic phenotype. At each step toward adipocytes, the cell fate of MSC derivatives is precisely regulated by signaling pathways and master regulators (*e.g.,* PPARγ and C/EBPα). Moreover, other regulatory elements of noncoding RNAs and epigenetic modifications synergistically play important roles in MSC adipogenesis. Hence, this review summarizes the present knowledge of adipogenesis in MSCs, focusing on adipogenesis regulation by indispensable signaling pathways, noncoding RNAs, methylation and acetylation.

**SIGNALING PATHWAYS**

The lineage commitment of MSCs can be manipulated by employing various chemicals in differentiation media, which modulate key transcription factors during MSC differentiation to track adipogenesis in vitro. Typically, these components include isobutylmethylxanthine (IBMX), indomethacin, dexamethasone (Dex), and insulin. IBMX and Dex are pivotal for initiating adipogenic differentiation. IBMX inhibits phosphodiesterases, leading to an increase in intracellular cAMP levels[9], which subsequently induces changes in transcription factors through protein kinase A activation. Additionally, IBMX directly stimulates the expression of C/EBPβ. Similarly, Dex activates C/EBPδ expression by binding to intracellular glucocorticoid receptors[10]. However for indomethacin, a well-known inhibitor of COX1/2, its adipogenic activity does not stem from COX inhibition but rather from PPARγ activation[11,12]. Insulin enhances glucose uptake for triglyceride synthesis in adipocytes[13].

Under differentiation-inducing circumstances, cellular signals control MSC maturation through the adipocytic route and further promote the differentiation of preadipocytes into mature adipocytes. Preadipocytes are an intermediate state between MSCs and adipocytes. Adipocytes contain fat droplets, while preadipocytes do not necessarily have these structures (Figure 1). Currently, the molecular mechanism involved in the differentiation of preadipocytes into adipocytes is relatively clear, but the principles underlying the transformation of MSCs into preadipocytes are not well understood. Nonetheless, several cytokines and signaling pathways, including the actin, bone morphogenic protein (BMP), and transforming growth factor-beta (TGF-β)/SMAD signaling pathways, are indispensable for MSC adipogenesis.

***Actin and Rho signaling***

Actin, a cytoskeletal protein, is known to play a crucial role in MSC differentiation. It determines cell shape, nuclear shape, cell spreading, and cell stiffness, which eventually affects cell differentiation. MSC lineage commitment is also regulated by actin cytoskeleton-mediated cell type[14], such as a flower shape during adipogenic differentiation and a star shape towards osteogenic transformation in MSCs[15-18]. The actin cytoskeleton regulates the mechanical behavior of cells through its assembly and disassembly. In undifferentiated MSCs, long and thin actin filaments line parallel to the long axis, but in adipogenic differentiation, the actin cytoskeleton reorganizes into a disorganized meshwork surrounding the oil droplet[19]. Notably, zinc finger CCCH-type containing 10 has been proven to be fundamental for adipogenic differentiation by promoting F-actin/mitochondria dynamics to safeguard proper energy metabolism and favor lipid accumulation[20]. The main regulating molecule in the actin cytoskeleton remodeling process is the Rho family of GTPase, including over 20 distinct kinds of Rho family members (RhoA, Rac1, and Cdc42, and *etc.*), which can interact with downstream effector proteins. RhoA mainly regulates the activity of myosin II to generate cellular force and tension in cells. The activation of RhoA is achieved by mechanical stress, and the inhibition of RhoA or its downstream effectors, as well as mammalian diaphanous protein kinase and Rho-associated coiled coil containing protein kinase (ROCK), leads to the reorganization of stress fibers[21]. Several studies have suggested that mechanical stress[22] and chemically induced actin depolymerization[23] favor adipogenesis. The abovementioned kinases (Rho and ROCK) may be regulators of osteoblast differentiation in MSCs[24]. These signaling pathways may play a role not only by changing the cytoskeletal organization of actin but also through the FAK, JNK, and p38 MAPK signaling pathways[25]. Moreover, biomaterials[26] and pathogens[27] induce actin remodeling during MSC differentiation.

***TGF-β/SMAD signaling***

Recently, the TGF-β superfamily has been shown to be crucial in controlling the adipogenesis of MSCs. In order to activate intracellular downstream SMAD family proteins, ligands implicated in TGF-β/SMAD signaling, including activin, inhibin, BMPs, growth differentiation factors (GDFs), TGF-β, Nodal, and others, attach to their cell membrane receptors. TGF-β/SMAD signaling has dual effects on the adipocyte differentiation process, specifically on the adipocyte commitment of MSCs[28]. TGF-β ligands such as TGF-β, myostatin, and GDF11 bind to cell membrane receptors in the TGF-β/SMAD signaling pathway to phosphorylate intracellular downstream SMAD2/3 (R-SMADs), and BMP ligands such as BMP2, BMP4, and BMP7 phosphorylate SMAD1/5/8 (R-SMADs). Activated R-SMADs binding with SMAD4 as a complex translocate into the nucleus to control the expression of target genes. After the genes respond to TGF-β/SMAD signaling, the R-SMAD/SMAD4 complex in the nucleus is depolymerizes and the proteins reenter the cytoplasm. TGF-β/SMAD signaling is adversely regulated by I-SMADs including SMAD6 and SMAD7. Upon transcriptional activation by TGF-β/SMAD signaling, SMAD7 shuttles from the nucleus to the cytoplasm to prevent R-SMAD phosphorylation and SMAD6 competes with SMAD1 to bind to SMAD4[28].

However, other studies have demonstrated that TGF-β signaling promotes the proliferation of MSCs and suppresses the adipocyte commitment of MSCs by inhibiting CEBPα and PPARγ expression. These discrepant results regarding adipocyte commitment may be related to the origins of the bone marrow-derived MSCs (BMSCs) isolated from different species, including mice and humans[29] as MSC origin can influence adipocyte commitment through TGF-β signaling. Notably, various clones isolated from human BMSC lines indeed exhibit different differentiation capacities. A recent study on MSC heterogeneity also suggested that different BAMBI expression levels interfere with the adipogenic capacity of cells[30]. In addition, a recent novel study reported the epigenetic mechanism of adipogenic commitment under TGF-β/SMAD signaling[31].

***BMP signaling***

BMP2/4/7 use SMAD1/5/8 signaling to regulate adipocyte commitment. BMP2, BMP4, and myostatin ligands affect the adipocyte commitment of MSCs. Even the differentiation of adipocyte lineage and brown adipocytes formation in MSCs are directly induced by BMP4 signaling. Both BMP2 and BMP4 signaling activate PPARγ expression to induce adipocyte commitment.

The role of BMP4 signaling has been validated in the commitment process of MSCs. Several studies have indicated that BMP4 can induce the commitment of the pluripotent mouse embryonic fibroblast line C3H10T1/2 to the adipocyte lineage. Upon BMP4 treatment, the expression of the adipocyte markers CEBPα, PPARγ, and adipocyte protein 2 (AP2) was detected in C3H10T1/2 cells, suggesting that these cells can differentiate into adipocytes. When C3H10T1/2 cells pretreated with BMP4 were subcutaneously implanted into thymic mice, they developed into tissue undistinguishable from adipose tissue.

BMP7 also plays an important role in brown adipocyte lineage determination. This signal triggers C3H10T1/2 cells to commit to a brown adipocyte lineage with a significant increases in lipid accumulation and uncoupling protein 1 expression. BMP7 stimulates cell proliferation and differentiation in mouse and human adult MSCs. However, different dosages of BMP seem to result in distinct effects on adipogenesis in mouse BMSCs. Low concentrations of BMP7 stimulated adipocyte differentiation, whereas higher dosages inhibited adipogenesis in mice. In human BMSCs, BMP7 promoted adipogenic differentiation rather than osteogenic or chondrogenic lineage development in high-density micromass culture.

However, the role of BMP2 signaling in adipocyte commitment in MSCs has not been determined. Several studies have shown that BMP2 signaling can induce C3H10T1/2 cells to commit to the adipocyte lineage[32]. Nonetheless, adipogenesis, chondrogenesis, and osteogenesis are plastical. The addition of low-level BMP2 to C3H10T1/2 cells favored adipogenesis[32]. However, treatment with BMP2 enhanced osteoblast commitment and inhibited late adipocyte maturation in human marrow stromal precursors. Mechanistically, similar to BMP4, BMP2 activated the expression and phosphorylation of SMAD1/5/8, which formed a complex with SMAD4. Under these condition, BMP2 suppressed adipogenesis by decreasing the leptin concentration and preventing the formation of cytoplasmic lipid droplets.

**NONCODING RNAs**

Noncoding RNAs, especially microRNAs (miRNAs) and long-chain noncoding RNAs (lncRNAs), also participate in the adipogenic differentiation of MSCs by interfering with signaling pathways and/or transcription factors to regulate adipogenic differentiation. First, miRNAs can positively regulate adipogenesis. miR-135a-5p promotes adipogenesis in human adipose-derived MSCs (ADMSCs) by targeting LATS1 and MOB1B expression, thereby enhancing the HIPPO signaling pathway. During the process of age-related adipogenic differentiation, the levels of both miR-188 and miR-141-3p were markedly greater in aged human BMSCs. Moreover, mice with transgenic overexpression of miR-188 in osterix+ osteoprogenitors had more age-associated bone loss and fat accumulation in the bone marrow than did wild-type mice[33]. However, Periyasamy-Thandavan *et al*[34] reported that human BMSCs treated with miR-141-3p exhibited decreased BMP-2 and RUNX-2 expression and increased C/BEPa2, suggesting the induction of adipocyte lineage differentiation instead of osteogenic differentiation. Interestingly, a recent study combining miRNA chip and RNA-seq data to analyze the correlation between miRNA and mRNA expression profiles during BMSC lipogenic differentiation showed that miR-140-5p may play an important role in regulating its target gene LIFR during adipogenic differentiation[6].

Other miRNAs indeed negatively regulate adipogenic differentiation in MSCs. miR-27b was the first miRNA discovered to function as a negative regulator of adipogenesis in humans[35]. The expression of miR-27b decreased during the adipogenesis of human adipose-derived stem cells (hADSCs). Further binding and luciferase reporter assays demonstrated that miR-27b directly bound to the designated miR-27b response element in the 3’ untranslated region (UTR) of human PPARγ to reduce its expression at the protein level, thus inhibiting adipogenesis. Additionally, the mutual adjustment of miR-27b and lipoprotein lipase expression can effectively regulate the adipogenic differentiation of hASCs[36]. In addition, miR27a, another family member of miR27, is inversely correlated with adipogenic markers such as PPARγ and adiponectin[37]. *In vitro* experiments showed that overexpression of miR-130a increased osteogenic differentiation and attenuated adipogenic differentiation in BMSCs. Furthermore, miR-130a promotes osteoblastic differentiation by negatively regulating Smurf2 expression and suppresses adipogenic differentiation of BMSCs by targeting PPARγ[38].

Interestingly, certain miRNAs can bidirectionally regulate osteogenic and adipogenic differentiation in BMSCs. Li *et al*[39] reported that miR-149-3p expression decreased following adipogenic differentiation but increased after osteogenic differentiation in BMSCs. Further study demonstrated that miR-149-3p manipulated alternative lineage choices between adipocytes and osteoblasts by directly targeting FTO, which is involved in adipogenesis mainly by regulating fat accumulation. Additionally, miR-21 overexpression was found to enhance osteogenic differentiation and inhibit adipogenic differentiation *via* the PI3K/AKT axis in rat BMSCs[40].

Recently, lncRNAs have also been found to be involved in regulating the adipogenic differentiation of MSCs[41]. For example, lncRNA ADINR promotes adipogenesis by binding PAl and recruiting the mll3/4 histone methyltransferase complex. In the process of fat formation, the 4-site trimethylation of the histone H3 lysine residue (H3K4me3) increases, and the H3K27me3 histone modification at the locus of the recombinant human transcription factor CCAAT enhancer binding protein reduces[42]. The lncRNA HOTAIR can affect DNA methylation changes at its binding sites to inhibit hBMSC adipogenic differentiation[43]. Huang *et al*[32] showed that the expression levels of the lncRNAs H19 and miR-675 were significantly downregulated during MSC differentiation into adipocytes, whereas adipogenesis was inhibited if H19 and miR-675 were overexpressed. The expression of another lncRNA from peroxidase, Plnc, increased during the adipose differentiation of MSCs according to microarray analysis. It was confirmed that Plncl enhanced the promoter activity of PPARγ2 by weakening the methylation state of the PPARγ2 promoter. The lncRNA ZFAS1 affects the osteogenic and adipogenic differentiation of mouse BMSCs by sponging miR-499 and upregulating ephrin type-A receptor 5[44]. To date, there are few reports on the inhibition of MSC adipogenic differentiation by lncRNAs[45].

**DNA METHYLATION**

Epigenetic regulation, especially DNA methylation, plays an important role in regulating the differentiation of MSCs into adipocytes[46]. Generally, DNA methylation is carried out by three main types of methyltransferases. DNMT3a/3b catalyze *de novo* DNA methylation, and DNMT1 maintains DNA methylation in somatic cells. Knockdown of the DNA demethylase ALKBH1 was demonstrated to inhibit adipogenic differentiation *via* regulation of HIF-1 signaling in hMSCs[47,48].

Although similar global methylation profiles are normally observed in terminal adipocytes, many differences exist in the expression of DNA methylation genes in MSCs from different tissues. In pigs, the global methylation level was greater in undifferentiated BMSCs than in ADMSCs[49]. The transcription level of the DNMT1 gene increased at the beginning of adipogenesis and then decreased, while the expression levels of the DNMT3a and DNMT3b transcripts increased during differentiation. All the examined MBD genes exhibited similar expression patterns in ADMSCs and BMSCs. However, the transcript abundances of UHRF1 and CBX5 decreased in both systems. The changes in the expression patterns of these genes point to the dynamic nature of DNA methylation during porcine adipogenesis.

Further studies support the notion that tissue source determines the differentiation potential and level of DNA methylation of MSCs. In a study comprehensively characterizing the DNA methylation profiling of osteoblast and adipocyte differentiation, Hou *et al*[50] showed that MSCs from psoriatic derma have a distinguishable promoter methylation profile compared with those from normal derma. Site-specific CpG methylation in the CXCL14 promoter has been confirmed in umbilical cord-derived MSCs[51] and is associated with altered gene expression. Such changes in methylation are evident in LBW infant-derived umbilical cords and may indicate future metabolic compromise through CXCL14. Xu *et al*[52] evaluated the adipogenic differentiation potential of different MSCs and reported that BMSCs had lower adipogenic differentiation potential than ADMSCs. Furthermore, their results suggest that DNA demethylation could be involved, at least partially, in the regulation of Runx2 and PPARγ in ADMSCs and BMSCs.

How does DNA methylation dictate adipocyte differentiation in MSCs with multiple differentiation potentials? In fact, DNA methylation regulates the orientational differentiation balance through particular sequences-transposons, imprinted genes and pluripotency-associated genes. Although Marofi *et al*[53] revealed that methylation of the promoter regions of the Sox9, OCN, and PPARγ2 genes might be one of the main mechanisms adjusting gene expression during the osteoblastic differentiation of MSCs, H3K36me3, catalyzed by the histone methyltransferase SET-domain-containing 2 (SETD2), regulates the lineage commitment of BMSCs. Deletion of Setd2 in mouse BMSCs through conditional Cre expression driven by the Prx1 promoter resulted in bone loss and marrow adiposity. Loss of Setd2 in BMSCs *in vitro* facilitated the differentiation of adipocytes rather than osteoblasts. Furthermore, overexpression of lipopolysaccharide-binding protein partially rescued the lack of osteogenesis and enhanced adipogenesis resulting from the absence of Setd2 in BMSCs. In addition, DNMT3B-mediated DNA methylation of phosphatase and tensin homolog (PTEN) is a key regulator of dental pulp-derived MSC and BMSC lineage commitment. Moreover, the lysine methyltransferase G9a is needed for DNMT3B-mediated PTEN suppression, which activates AKT to promote adipogenesis and inhibit osteogenesis[54].

Zych *et al*[55] determined the effects of these epigenetic mechanisms on adipocyte differentiation in BMSCs and ADSCs using the demethylating agent 5-aza-2’-deoxycytidine (5azadC). The results showed that adipogenic differentiation decreased in a dose-dependent manner concomitant with the downregulation of the expression of the adipocyte genes PPARG and FABP4, and the expression of the antiadipocyte gene GATA2 was induced in the cultures treated with 5azadC. Additionally, the methyltransferase enhancer of zeste homology 2 (EZH2) trimethylates H3K27me3 on chromatin, and this repressive mark is removed by lysine demethylase 6A (KDM6A). Both Ezh2 and Kdm6a were shown to affect the expression of master regulatory genes involved in adipogenesis and osteogenesis and H3K27me3 on the promoters of master regulatory genes. These findings demonstrate an important epigenetic switch centered around H3K27me3, which dictates MSC lineage determination[56]. Furthermore, using methyl-DNA immunoprecipitation (MeDIP) and microarray hybridization, the potential of MSC multidirectional differentiation regulated by DNA methylation through imprinted and pluripotency-associated genes can be predicted. Empolying MeDIP methodology, Choi *et al*[57] reported that the impaired adipogenic differentiation of senescent MSCs at P15 was due to changes in CpG methylation in the LEP promoter.

**ACETYLATION MODIFICATION**

Acetylation and deacetylation are the key cotranslational and posttranslational modifications (PTMs) that integrate metabolic flux and physiological processes within cells, including circadian rhythm, cell cycle progression and energy production[58]. Lysine acetylation is a kind of PTM of proteins, the reactions of which are typically catalyzed by lysine acetyltransferases (KATs). KATs are classified into three families: Gcn5/PCAF (histone KAT KAT2A/2B), p300/CBP (histone KAT KAT3A/3B), and the MYST family[58,59]. Acetylation of the histone H3 N-terminal tail is catalyzed mainly by KAT Gcn5/PCAF as well as p300/CBP, and the H4 tail is predominantly acetylated by the MYST family of KATs. Adipocyte-specific genes undergo selective induction of histone hyperacetylation at their promoter regions, which leads to their upregulation during adipogenesis. Yoo *et al*[60] showed that the level of H3K9 acetylation at the promoters of ADD1/SREBP1c, adiponectin, aP2, C/EBPa and PPARγ was markedly increased after adipogenic differentiation. These results showed that acetylation is fundamentally involved in the regulation of adipogenesis.

***Acetylation***

The master adipogenic transcription factor gene PPARγ is regulated by all three families of KATs. Double knockout of Gcn5/PCAF inhibits the expression of the master adipogenic transcription factor gene PPARγ, thereby preventing adipocyte differentiation[61]. Specifically, Gcn5/PCAF facilitates adipogenesis through the regulation of PPARγ and Prdm16 expression[61]. HIV-1 Tat-interacting protein 60 (Tip60) is a member of the MYST family of KATs that can positively regulate PPARγ transcriptional activity. In mature 3T3-L1 adipocytes, Tip60 interacts with PPARγ and is recruited to PPARγ target genes. Moreover, a reduction in the Tip60 protein can inhibit the differentiation of 3T3-L1 preadipocytes[62]. P300/CBP can regulate glucose and lipid metabolism by acetylating nuclear receptors, such as the bile acid receptor (farnesoid Xactivated receptor)[63], PPARγ, and cytosolic PEPCK-C[64]. Mechanistically, p300/CBP interacts with and enhances the transcriptional activity of PPARγ by acetylating nuclear receptors. Furthermore, p300 acetylates PEPCK-C, inducing its degradation and attenuating gluconeogenesis[64]. Thus, p300/CBP plays an essential role in adipocyte differentiation.

In addition to PPARγ, the acetylation of other genes is involved in adipocyte differentiation. Acetylation of malate dehydrogenase 1 and 2 (MDH1 and MDH2) promotes adipogenic differentiation by activating their enzymatic activity and increasing the intracellular levels of NADPH in 3T3-L1 preadipocytes[65,66]. Following p300 recruitment for lysine acetylation, the gene-repressive activity and function of RIP140 are enhanced as fat accumulates in differentiated adipocytes[67]. Additionally, acetylation of α-tubulin is upregulated during adipogenesis under the control of the KAT MEC-17, SIRT2 and histone deacetylase (HDAC)6, and adipocyte development is dependent on α-tubulin acetylation[68]. Additionally, cavin-1 is acetylated at lysines 291, 293, and 298 (3K) by GCN5 as an KAT to positively regulate lipolysis in 3T3-L1 and zebrafish[69].

***Decetylation***

Deacetylation is mainly mediated by HDACs, including sirtuins, which use NAD+ as a coenzyme. All lysine deacetylases (KDACs) can be divided into four types: Class I KDACs (HDAC1, HDAC2, HDAC3, and HDAC8), class II KDACs (class IIa: HDAC4, HDAC5, HDAC7, and HDAC9; class IIb: HDAC6 and HDAC10), class III KDACs (Sirt1-7), and class IV KDACs (including only one member, HDAC11). HDAC activity is essential for maintaining the preadipocyte pool of the adipogenic lineage. Thus, HDAC inhibition in stem cells has the potential to block preadipocyte generation and thus overall adipogenesis[70]. Adipocyte differentiation is accompanied by decreases in the expression levels of several histone deacetylases, including HDAC1, HDAC2, and HDAC5[71]. Moreover, HDAC1 knockdown promoted adipogenesis in 3T3-L1 cells, and vice versa[60]. HDAC3 has been found to regulate mitochondrial activity and glucose or lipid metabolism in the liver, fat and muscle[72-75]. PPARα-interfered with fatty acid and lipid metabolism, and myocardial lipids accumulated in muscle-specific Hdac3-/- mice receiving a chow diet[72,74]. Furthermore, HDAC3 controls the circadian rhythm of hepatic lipid metabolism[76] and gluconeogenesis[77], which is mediated by the nuclear receptors Rev-erbAα and PPARγ. Finally, HDAC3 can be recruited to the promoter of the PPARγ gene, preventing its expression to regulate adipocyte differentiation in adipose tissue. In addition, high expression levels of HDAC5 and HDAC6 are needed for adequate adipocyte function. In contrast, HDAC9 has been reported to inhibit adipogenesis. In the case of a chronic high-fat diet, proper adipogenic differentiation is impaired, and the expression of the negative regulator of adipogenic HDAC9 is increased. Ablation of HDAC9 in mice can prevent such adverse changes, including weight gain, impaired glucose tolerance, and insulin insensitivity[78-80].

The class III sirtuin-mediated deacetylation reaction couples lysine deacetylation to NAD+ hydrolysis[81]. Many genes related to adipocyte differentiation, such as glucose transporters type 4, AP2 and fatty acid synthase genes, are regulated by Sirt2. This coordinated regulation is attributed to the direct interaction between Sirt2 and acetylation patterns involved in controlling lipogenesis[82]. Sirt2 has also been shown to bind directly to FoxO1 and enhance insulin-stimulated FoxO1 phosphorylation/acetylation and activity[83]. Thus, Sirt2 acts as an important regulator of adipocyte differentiation. SIRT-1 facilitates the deacetylation and interaction of PPARγ and the thermogenic transcription factor PR domain containing zinc finger protein 16 (PRDM-16)[84]. Along with SIRT-1, PRDM-16 regulates the brown fat lineage. Sirt1 also promotes fat mobilization by inhibiting PPARγ in adipocytes[85]. For example, its expression can regulate lipogenesis in 3T3-L1 cells. During the process of adipocyte differentiation, Sirt1 upregulation may promote lipolysis and fat loss. Decreased Sirt1 increases the expression of the adiponectin gene through the FoxO1-C/EBPα transcription complex[85-87].

Interestingly, noncoding RNAs cooperatively interact with KDACs to regulate adipogenic processes. miR-675 can target the 3’ UTRs of HDAC4-6 transcripts, which lead to the deregulation of HDAC4-6 and fat formation. When HDACs are inhibited, the occupancy of H19 and CCCTC binding factor can be reduced, and thus, H1 can be downregulated[88]. The regulation of adipogenesis and gluconeogenesis by KDACs, KATs and noncoding RNAs is summarized in Figure 2.

**ADIPOGENESIS OF MSCs AND DISEASES**

MSCs are believed to exist in every organ in the body. Dysfunction or abnormal differentiation of these cells into adipocytes tends to be associated with various diseases. For example, MSCs from acute graft-*versus*-host disease patients showed reduced adipogenic differentiation in culture[89]. Even under natural physiological conditions, aging can reduce the adipogenic differentiation responses of BMSCs, myeloid-derived suppressor cells, and ASCs, with the most noticeable reduction in adipogenesis occurring in ASCs[90]. Although MSC transplantation has shown beneficial effects in treating autoimmune diseases, the ability of the BAMBIhighMFGE8high MSC subpopulation, which has limited adipogenic differentiation potential, to alleviate SLE is compromised[30].

In contrast, the adipogenic differentiation abilities of MSCs from both polycystic ovary syndrome patients and gestational diabetes mellitus patients were greater than that of MSCs from healthy controls[91,92]. Several studies suggest that pathological conditions affect MSC differentiation. In a hypoperfusion-induced abdominal aortic aneurysm model, perivascular adipose tissue plays important roles in the differentiation of MSCs into adipocytes in response to vascular hypoperfusion[93]. Additionally, abnormal adipogenic differentiation can cause disease. In a glomerulonephritis model, the early beneficial effect of MSCs in preserving damaged glomeruli and maintaining renal function was offset by long-term partial maldifferentiation of intraglomerular MSCs into adipocytes accompanied by glomerular sclerosis[94].

The adipogenic and osteogenic differentiation programs are competitively balanced in MSCs. Many hub or early-responder signaling pathways control the osteogenic and adipogenic fates of MSCs. For example, Wnt signaling upregulates Runx2 expression to promote osteoblast differentiation, which also inhibits PPARγ expression to suppress adipogenic differentiation in BMSCs[95]. In addition, HH signaling and PI3K-Akt are key active pathways involved in the early stages of cell osteogenic differentiation that simultaneously inhibit adipogenesis[96]. A decrease in the balance between the adipogenic and osteogenic potential of MSCs is also often associated with disease occurrence and/or development. In clinical osteoporosis samples, overexpression of miR-10b enhanced osteogenic differentiation and inhibited adipogenic differentiation of hADSCs *in vitro*, which was negatively correlated with the expression of the markers CEBPα, PPARγ and AP2. More recently, the lncRNA NEAT1 was shown to act as a key bone-fat switch in aged BMSCs by orchestrating mitochondrial function and BMSC multipotency[97].

However, the therapeutic potential of MSCs in cancer has been controversial. Some studies have revealed that these compounds can promote cancer pathogenesis, but others have indicated that they have suppressive effects on cancer cells. Hence, additional evidence is needed to understand the role of MSC differentiation in cancer therapy.

**CONCLUSION**

Much encouraging progress has recently been made in understanding how MSCs can differentiate into adipocytes through various signaling pathways, noncoding RNAs, and the epigenetic regulation of phosphorylation, methylation and acetylation. However, there is still a lack of evidence on the importance of gernerating a comprehensive map of adipogenesis in MSCs, especially for the early commitment process from MSCs to preadipocytes. The low efficiency of adipogenic differentiation of MSCs in culture has hampered our understanding of this process. Dissecting the heterogeneity of MSCs will allow us to clearly elucidate the mechanism of adipogenic differentiation. Hopefully, these problems will be addressed with the help of fast-advancing single-cell sequencing techniques, which will shed light on the full path of MSC differentiation into adipocytes, facilitating MSC-based applications in biomedicine.

**REFERENCES**

1 **Wang D**, Li J, Zhang Y, Zhang M, Chen J, Li X, Hu X, Jiang S, Shi S, Sun L. Umbilical cord mesenchymal stem cell transplantation in active and refractory systemic lupus erythematosus: a multicenter clinical study. *Arthritis Res Ther* 2014; **16**: R79 [PMID: 24661633 DOI: 10.1186/ar4520]

2 **Ullah I**, Subbarao RB, Rho GJ. Human mesenchymal stem cells - current trends and future prospective. *Biosci Rep* 2015; **35** [PMID: 25797907 DOI: 10.1042/BSR20150025]

3 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]

4 **Komori T**. Regulation of osteoblast differentiation by transcription factors. *J Cell Biochem* 2006; **99**: 1233-1239 [PMID: 16795049 DOI: 10.1002/jcb.20958]

5 **Wang ZH**, Li XL, He XJ, Wu BJ, Xu M, Chang HM, Zhang XH, Xing Z, Jing XH, Kong DM, Kou XH, Yang YY. Delivery of the Sox9 gene promotes chondrogenic differentiation of human umbilical cord blood-derived mesenchymal stem cells in an in vitro model. *Braz J Med Biol Res* 2014; **47**: 279-286 [PMID: 24652327 DOI: 10.1590/1414-431X20133539]

6 **Yi SW**, Kim HJ, Oh HJ, Shin H, Lee JS, Park JS, Park KH. Gene expression profiling of chondrogenic differentiation by dexamethasone-conjugated polyethyleneimine with SOX trio genes in stem cells. *Stem Cell Res Ther* 2018; **9**: 341 [PMID: 30526665 DOI: 10.1186/s13287-018-0998-7]

7 **Mota de Sá P**, Richard AJ, Hang H, Stephens JM. Transcriptional Regulation of Adipogenesis. *Compr Physiol* 2017; **7**: 635-674 [PMID: 28333384 DOI: 10.1002/cphy.c160022]

8 **Zhuang H**, Zhang X, Zhu C, Tang X, Yu F, Shang GW, Cai X. Molecular Mechanisms of PPAR-γ Governing MSC Osteogenic and Adipogenic Differentiation. *Curr Stem Cell Res Ther* 2016; **11**: 255-264 [PMID: 26027680 DOI: 10.2174/1574888x10666150531173309]

9 **Dey S**, Goswami S, Eisa A, Bhattacharjee R, Brothag C, Kline D, Vijayaraghavan S. Cyclic AMP and glycogen synthase kinase 3 form a regulatory loop in spermatozoa. *J Cell Physiol* 2018; **233**: 7239-7252 [PMID: 29574946 DOI: 10.1002/jcp.26557]

10 **Mitani T**, Takaya T, Harada N, Katayama S, Yamaji R, Nakamura S, Ashida H. Theophylline suppresses interleukin-6 expression by inhibiting glucocorticoid receptor signaling in pre-adipocytes. *Arch Biochem Biophys* 2018; **646**: 98-106 [PMID: 29625124 DOI: 10.1016/j.abb.2018.04.001]

11 **Parra LG**, Erjavec LC, Casali CI, Zerpa Velazquez A, Weber K, Setton-Avruj CP, Fernández Tome MDC. Cytosolic phospholipase A(2) regulates lipid homeostasis under osmotic stress through PPARγ. *FEBS J* 2023 [PMID: 37947039 DOI: 10.1111/febs.16998]

12 **Buelvas N**, Ugarte-Vio I, Asencio-Leal L, Muñoz-Uribe M, Martin-Martin A, Rojas-Fernández A, Jara JA, Tapia JC, Arias ME, López-Muñoz RA. Indomethacin Induces Spermidine/Spermine-N(1)-Acetyltransferase-1 via the Nucleolin-CDK1 Axis and Synergizes with the Polyamine Oxidase Inhibitor Methoctramine in Lung Cancer Cells. *Biomolecules* 2023; **13** [PMID: 37759783 DOI: 10.3390/biom13091383]

13 **Ige S**, Alaoui K, Al-Dibouni A, Dallas ML, Cagampang FR, Sellayah D, Chantler PD, Boateng SY. Leptin-dependent differential remodeling of visceral and pericardial adipose tissue following chronic exercise and psychosocial stress. *FASEB J* 2024; **38**: e23325 [PMID: 38117486 DOI: 10.1096/fj.202300269RRR]

14 **Mishra P**, Martin DC, Androulakis IP, Moghe PV. Fluorescence Imaging of Actin Turnover Parses Early Stem Cell Lineage Divergence and Senescence. *Sci Rep* 2019; **9**: 10377 [PMID: 31316098 DOI: 10.1038/s41598-019-46682-y]

15 **Khan AU**, Qu R, Fan T, Ouyang J, Dai J. A glance on the role of actin in osteogenic and adipogenic differentiation of mesenchymal stem cells. *Stem Cell Res Ther* 2020; **11**: 283 [PMID: 32678016 DOI: 10.1186/s13287-020-01789-2]

16 **Müller P**, Langenbach A, Kaminski A, Rychly J. Modulating the actin cytoskeleton affects mechanically induced signal transduction and differentiation in mesenchymal stem cells. *PLoS One* 2013; **8**: e71283 [PMID: 23923061 DOI: 10.1371/journal.pone.0071283]

17 **Zhao Y**, Sun Q, Wang S, Huo B. Spreading Shape and Area Regulate the Osteogenesis of Mesenchymal Stem Cells. *Tissue Eng Regen Med* 2019; **16**: 573-583 [PMID: 31824820 DOI: 10.1007/s13770-019-00213-y]

18 **Kilian KA**, Bugarija B, Lahn BT, Mrksich M. Geometric cues for directing the differentiation of mesenchymal stem cells. *Proc Natl Acad Sci U S A* 2010; **107**: 4872-4877 [PMID: 20194780 DOI: 10.1073/pnas.0903269107]

19 **Rodríguez JP**, González M, Ríos S, Cambiazo V. Cytoskeletal organization of human mesenchymal stem cells (MSC) changes during their osteogenic differentiation. *J Cell Biochem* 2004; **93**: 721-731 [PMID: 15660416 DOI: 10.1002/jcb.20234]

20 **Audano M**, Pedretti S, Ligorio S, Gualdrini F, Polletti S, Russo M, Ghisletti S, Bean C, Crestani M, Caruso D, De Fabiani E, Mitro N. Zc3h10 regulates adipogenesis by controlling translation and F-actin/mitochondria interaction. *J Cell Biol* 2021; **220** [PMID: 33566069 DOI: 10.1083/jcb.202003173]

21 **Gordon WR**, Zimmerman B, He L, Miles LJ, Huang J, Tiyanont K, McArthur DG, Aster JC, Perrimon N, Loparo JJ, Blacklow SC. Mechanical Allostery: Evidence for a Force Requirement in the Proteolytic Activation of Notch. *Dev Cell* 2015; **33**: 729-736 [PMID: 26051539 DOI: 10.1016/j.devcel.2015.05.004]

22 **Lu J**, Fan Y, Gong X, Zhou X, Yi C, Zhang Y, Pan J. The Lineage Specification of Mesenchymal Stem Cells Is Directed by the Rate of Fluid Shear Stress. *J Cell Physiol* 2016; **231**: 1752-1760 [PMID: 26636289 DOI: 10.1002/jcp.25278]

23 **Samsonraj RM**, Paradise CR, Dudakovic A, Sen B, Nair AA, Dietz AB, Deyle DR, Cool SM, Rubin J, van Wijnen AJ. Validation of Osteogenic Properties of Cytochalasin D by High-Resolution RNA-Sequencing in Mesenchymal Stem Cells Derived from Bone Marrow and Adipose Tissues. *Stem Cells Dev* 2018; **27**: 1136-1145 [PMID: 29882479 DOI: 10.1089/scd.2018.0037]

24 **Prowse PD**, Elliott CG, Hutter J, Hamilton DW. Inhibition of Rac and ROCK signalling influence osteoblast adhesion, differentiation and mineralization on titanium topographies. *PLoS One* 2013; **8**: e58898 [PMID: 23505566 DOI: 10.1371/journal.pone.0058898]

25 **Sun B**, Qu R, Fan T, Yang Y, Jiang X, Khan AU, Zhou Z, Zhang J, Wei K, Ouyang J, Dai J. Actin polymerization state regulates osteogenic differentiation in human adipose-derived stem cells. *Cell Mol Biol Lett* 2021; **26**: 15 [PMID: 33858321 DOI: 10.1186/s11658-021-00259-8]

26 **Di Cio S**, Iskratsch T, Connelly JT, Gautrot JE. Contractile myosin rings and cofilin-mediated actin disassembly orchestrate ECM nanotopography sensing. *Biomaterials* 2020; **232**: 119683 [PMID: 31927180 DOI: 10.1016/j.biomaterials.2019.119683]

27 **Sultana H**, Neelakanta G, Kantor FS, Malawista SE, Fish D, Montgomery RR, Fikrig E. Anaplasma phagocytophilum induces actin phosphorylation to selectively regulate gene transcription in Ixodes scapularis ticks. *J Exp Med* 2010; **207**: 1727-1743 [PMID: 20660616 DOI: 10.1084/jem.20100276]

28 **Li SN**, Wu JF. TGF-β/SMAD signaling regulation of mesenchymal stem cells in adipocyte commitment. *Stem Cell Res Ther* 2020; **11**: 41 [PMID: 31996252 DOI: 10.1186/s13287-020-1552-y]

29 **Elsafadi M**, Manikandan M, Atteya M, Abu Dawud R, Almalki S, Ali Kaimkhani Z, Aldahmash A, Alajez NM, Alfayez M, Kassem M, Mahmood A. SERPINB2 is a novel TGFβ-responsive lineage fate determinant of human bone marrow stromal cells. *Sci Rep* 2017; **7**: 10797 [PMID: 28883483 DOI: 10.1038/s41598-017-10983-x]

30 **Chen H**, Wen X, Liu S, Sun T, Song H, Wang F, Xu J, Zhang Y, Zhao Y, Yu J, Sun L. Dissecting Heterogeneity Reveals a Unique BAMBI(high) MFGE8(high) Subpopulation of Human UC-MSCs. *Adv Sci (Weinh)* 2022; **10**: e2202510 [PMID: 36373720 DOI: 10.1002/advs.202202510]

31 **Elsafadi M**, Shinwari T, Al-Malki S, Manikandan M, Mahmood A, Aldahmash A, Alfayez M, Kassem M, Alajez NM. Convergence of TGFβ and BMP signaling in regulating human bone marrow stromal cell differentiation. *Sci Rep* 2019; **9**: 4977 [PMID: 30899078 DOI: 10.1038/s41598-019-41543-0]

32 **Huang HY**, Hu LL, Song TJ, Li X, He Q, Sun X, Li YM, Lu HJ, Yang PY, Tang QQ. Involvement of cytoskeleton-associated proteins in the commitment of C3H10T1/2 pluripotent stem cells to adipocyte lineage induced by BMP2/4. *Mol Cell Proteomics* 2011; **10**: M110.002691 [PMID: 20713452 DOI: 10.1074/mcp.M110.002691]

33 **Li CJ**, Cheng P, Liang MK, Chen YS, Lu Q, Wang JY, Xia ZY, Zhou HD, Cao X, Xie H, Liao EY, Luo XH. MicroRNA-188 regulates age-related switch between osteoblast and adipocyte differentiation. *J Clin Invest* 2015; **125**: 1509-1522 [PMID: 25751060 DOI: 10.1172/JCI77716]

34 **Periyasamy-Thandavan S**, Burke J, Mendhe B, Kondrikova G, Kolhe R, Hunter M, Isales CM, Hamrick MW, Hill WD, Fulzele S. MicroRNA-141-3p Negatively Modulates SDF-1 Expression in Age-Dependent Pathophysiology of Human and Murine Bone Marrow Stromal Cells. *J Gerontol A Biol Sci Med Sci* 2019; **74**: 1368-1374 [PMID: 31505568 DOI: 10.1093/gerona/gly186]

35 **Karbiener M**, Fischer C, Nowitsch S, Opriessnig P, Papak C, Ailhaud G, Dani C, Amri EZ, Scheideler M. microRNA miR-27b impairs human adipocyte differentiation and targets PPARgamma. *Biochem Biophys Res Commun* 2009; **390**: 247-251 [PMID: 19800867 DOI: 10.1016/j.bbrc.2009.09.098]

36 **Hu X**, Tang J, Hu X, Bao P, Pan J, Chen Z, Xian J. MiR-27b Impairs Adipocyte Differentiation of Human Adipose Tissue-Derived Mesenchymal Stem Cells by Targeting LPL. *Cell Physiol Biochem* 2018; **47**: 545-555 [PMID: 29794473 DOI: 10.1159/000489988]

37 **Kim SY**, Kim AY, Lee HW, Son YH, Lee GY, Lee JW, Lee YS, Kim JB. miR-27a is a negative regulator of adipocyte differentiation via suppressing PPARgamma expression. *Biochem Biophys Res Commun* 2010; **392**: 323-328 [PMID: 20060380 DOI: 10.1016/j.bbrc.2010.01.012]

38 **Lin Z**, He H, Wang M, Liang J. MicroRNA-130a controls bone marrow mesenchymal stem cell differentiation towards the osteoblastic and adipogenic fate. *Cell Prolif* 2019; **52**: e12688 [PMID: 31557368 DOI: 10.1111/cpr.12688]

39 **Li Y**, Yang F, Gao M, Gong R, Jin M, Liu T, Sun Y, Fu Y, Huang Q, Zhang W, Liu S, Yu M, Yan G, Feng C, He M, Zhang L, Ding F, Ma W, Bi Z, Xu C, Yuan Y, Cai B, Yang L. miR-149-3p Regulates the Switch between Adipogenic and Osteogenic Differentiation of BMSCs by Targeting FTO. *Mol Ther Nucleic Acids* 2019; **17**: 590-600 [PMID: 31382190 DOI: 10.1016/j.omtn.2019.06.023]

40 **Wu PY**, Chen W, Huang H, Tang W, Liang J. Morinda officinalis polysaccharide regulates rat bone mesenchymal stem cell osteogenic-adipogenic differentiation in osteoporosis by upregulating miR-21 and activating the PI3K/AKT pathway. *Kaohsiung J Med Sci* 2022; **38**: 675-685 [PMID: 35593324 DOI: 10.1002/kjm2.12544]

41 **Li CJ**, Xiao Y, Yang M, Su T, Sun X, Guo Q, Huang Y, Luo XH. Long noncoding RNA Bmncr regulates mesenchymal stem cell fate during skeletal aging. *J Clin Invest* 2018; **128**: 5251-5266 [PMID: 30352426 DOI: 10.1172/JCI99044]

42 **Zhu E**, Zhang J, Li Y, Yuan H, Zhou J, Wang B. Long noncoding RNA Plnc1 controls adipocyte differentiation by regulating peroxisome proliferator-activated receptor γ. *FASEB J* 2019; **33**: 2396-2408 [PMID: 30277818 DOI: 10.1096/fj.201800739RRR]

43 **Kalwa M**, Hänzelmann S, Otto S, Kuo CC, Franzen J, Joussen S, Fernandez-Rebollo E, Rath B, Koch C, Hofmann A, Lee SH, Teschendorff AE, Denecke B, Lin Q, Widschwendter M, Weinhold E, Costa IG, Wagner W. The lncRNA HOTAIR impacts on mesenchymal stem cells via triple helix formation. *Nucleic Acids Res* 2016; **44**: 10631-10643 [PMID: 27634931 DOI: 10.1093/nar/gkw802]

44 **Wu J**, Lin T, Gao Y, Li X, Yang C, Zhang K, Wang C, Zhou X. Long noncoding RNA ZFAS1 suppresses osteogenic differentiation of bone marrow-derived mesenchymal stem cells by upregulating miR-499-EPHA5 axis. *Mol Cell Endocrinol* 2022; **539**: 111490 [PMID: 34655661 DOI: 10.1016/j.mce.2021.111490]

45 **Hu K**, Jiang W, Sun H, Li Z, Rong G, Yin Z. Long noncoding RNA ZBED3-AS1 induces the differentiation of mesenchymal stem cells and enhances bone regeneration by repressing IL-1β via Wnt/β-catenin signaling pathway. *J Cell Physiol* 2019; **234**: 17863-17875 [PMID: 30919957 DOI: 10.1002/jcp.28416]

46 **Liu J**, Gan L, Ma B, He S, Wu P, Li H, Xiong J. Alterations in chromatin accessibility during osteoblast and adipocyte differentiation in human mesenchymal stem cells. *BMC Med Genomics* 2022; **15**: 17 [PMID: 35101056 DOI: 10.1186/s12920-022-01168-1]

47 **Liu Y**, Chen Y, Wang Y, Jiang S, Lin W, Wu Y, Li Q, Guo Y, Liu W, Yuan Q. DNA demethylase ALKBH1 promotes adipogenic differentiation via regulation of HIF-1 signaling. *J Biol Chem* 2022; **298**: 101499 [PMID: 34922943 DOI: 10.1016/j.jbc.2021.101499]

48 **Cai GP**, Liu YL, Luo LP, Xiao Y, Jiang TJ, Yuan J, Wang M. Alkbh1-mediated DNA N6-methyladenine modification regulates bone marrow mesenchymal stem cell fate during skeletal aging. *Cell Prolif* 2022; **55**: e13178 [PMID: 35018683 DOI: 10.1111/cpr.13178]

49 **Stachecka J**, Lemanska W, Noak M, Szczerbal I. Expression of key genes involved in DNA methylation during in vitro differentiation of porcine mesenchymal stem cells (MSCs) into adipocytes. *Biochem Biophys Res Commun* 2020; **522**: 811-818 [PMID: 31791576 DOI: 10.1016/j.bbrc.2019.11.175]

50 **Hou R**, Yin G, An P, Wang C, Liu R, Yang Y, Yan X, Li J, Li X, Zhang K. DNA methylation of dermal MSCs in psoriasis: identification of epigenetically dysregulated genes. *J Dermatol Sci* 2013; **72**: 103-109 [PMID: 23916410 DOI: 10.1016/j.jdermsci.2013.07.002]

51 **Cheong CY**, Chng K, Lim MK, Amrithraj AI, Joseph R, Sukarieh R, Chee Tan Y, Chan L, Tan JH, Chen L, Pan H, Holbrook JD, Meaney MJ, Seng Chong Y, Gluckman PD, Stünkel W. Alterations to DNA methylation and expression of CXCL14 are associated with suboptimal birth outcomes. *J Hum Genet* 2014; **59**: 504-511 [PMID: 25102097 DOI: 10.1038/jhg.2014.63]

52 **Xu L**, Liu Y, Sun Y, Wang B, Xiong Y, Lin W, Wei Q, Wang H, He W, Wang B, Li G. Tissue source determines the differentiation potentials of mesenchymal stem cells: a comparative study of human mesenchymal stem cells from bone marrow and adipose tissue. *Stem Cell Res Ther* 2017; **8**: 275 [PMID: 29208029 DOI: 10.1186/s13287-017-0716-x]

53 **Marofi F**, Hassanzadeh A, Solali S, Vahedi G, Mousavi Ardehaie R, Salarinasab S, Aliparasti MR, Ghaebi M, Farshdousti Hagh M. Epigenetic mechanisms are behind the regulation of the key genes associated with the osteoblastic differentiation of the mesenchymal stem cells: The role of zoledronic acid on tuning the epigenetic changes. *J Cell Physiol* 2019; **234**: 15108-15122 [PMID: 30652308 DOI: 10.1002/jcp.28152]

54 **Shen WC**, Lai YC, Li LH, Liao K, Lai HC, Kao SY, Wang J, Chuong CM, Hung SC. Methylation and PTEN activation in dental pulp mesenchymal stem cells promotes osteogenesis and reduces oncogenesis. *Nat Commun* 2019; **10**: 2226 [PMID: 31110221 DOI: 10.1038/s41467-019-10197-x]

55 **Zych J**, Stimamiglio MA, Senegaglia AC, Brofman PR, Dallagiovanna B, Goldenberg S, Correa A. The epigenetic modifiers 5-aza-2'-deoxycytidine and trichostatin A influence adipocyte differentiation in human mesenchymal stem cells. *Braz J Med Biol Res* 2013; **46**: 405-416 [PMID: 23797495 DOI: 10.1590/1414-431X20132893]

56 **Hemming S**, Cakouros D, Isenmann S, Cooper L, Menicanin D, Zannettino A, Gronthos S. EZH2 and KDM6A act as an epigenetic switch to regulate mesenchymal stem cell lineage specification. *Stem Cells* 2014; **32**: 802-815 [PMID: 24123378 DOI: 10.1002/stem.1573]

57 **Choi MR**, In YH, Park J, Park T, Jung KH, Chai JC, Chung MK, Lee YS, Chai YG. Genome-scale DNA methylation pattern profiling of human bone marrow mesenchymal stem cells in long-term culture. *Exp Mol Med* 2012; **44**: 503-512 [PMID: 22684242 DOI: 10.3858/emm.2012.44.8.057]

58 **Menzies KJ**, Zhang H, Katsyuba E, Auwerx J. Protein acetylation in metabolism - metabolites and cofactors. *Nat Rev Endocrinol* 2016; **12**: 43-60 [PMID: 26503676 DOI: 10.1038/nrendo.2015.181]

59 **Drazic A**, Myklebust LM, Ree R, Arnesen T. The world of protein acetylation. *Biochim Biophys Acta* 2016; **1864**: 1372-1401 [PMID: 27296530 DOI: 10.1016/j.bbapap.2016.06.007]

60 **Yoo EJ**, Chung JJ, Choe SS, Kim KH, Kim JB. Down-regulation of histone deacetylases stimulates adipocyte differentiation. *J Biol Chem* 2006; **281**: 6608-6615 [PMID: 16407282 DOI: 10.1074/jbc.M508982200]

61 **Jin Q**, Wang C, Kuang X, Feng X, Sartorelli V, Ying H, Ge K, Dent SY. Gcn5 and PCAF regulate PPARγ and Prdm16 expression to facilitate brown adipogenesis. *Mol Cell Biol* 2014; **34**: 3746-3753 [PMID: 25071153 DOI: 10.1128/MCB.00622-14]

62 **van Beekum O**, Brenkman AB, Grøntved L, Hamers N, van den Broek NJ, Berger R, Mandrup S, Kalkhoven E. The adipogenic acetyltransferase Tip60 targets activation function 1 of peroxisome proliferator-activated receptor gamma. *Endocrinology* 2008; **149**: 1840-1849 [PMID: 18096664 DOI: 10.1210/en.2007-0977]

63 **Kemper JK**, Xiao Z, Ponugoti B, Miao J, Fang S, Kanamaluru D, Tsang S, Wu SY, Chiang CM, Veenstra TD. FXR acetylation is normally dynamically regulated by p300 and SIRT1 but constitutively elevated in metabolic disease states. *Cell Metab* 2009; **10**: 392-404 [PMID: 19883617 DOI: 10.1016/j.cmet.2009.09.009]

64 **Jiang W**, Wang S, Xiao M, Lin Y, Zhou L, Lei Q, Xiong Y, Guan KL, Zhao S. Acetylation regulates gluconeogenesis by promoting PEPCK1 degradation via recruiting the UBR5 ubiquitin ligase. *Mol Cell* 2011; **43**: 33-44 [PMID: 21726808 DOI: 10.1016/j.molcel.2011.04.028]

65 **Kim EY**, Han BS, Kim WK, Lee SC, Bae KH. Acceleration of adipogenic differentiation via acetylation of malate dehydrogenase 2. *Biochem Biophys Res Commun* 2013; **441**: 77-82 [PMID: 24134846 DOI: 10.1016/j.bbrc.2013.10.016]

66 **Kim EY**, Kim WK, Kang HJ, Kim JH, Chung SJ, Seo YS, Park SG, Lee SC, Bae KH. Acetylation of malate dehydrogenase 1 promotes adipogenic differentiation via activating its enzymatic activity. *J Lipid Res* 2012; **53**: 1864-1876 [PMID: 22693256 DOI: 10.1194/jlr.M026567]

67 **Ho PC**, Gupta P, Tsui YC, Ha SG, Huq M, Wei LN. Modulation of lysine acetylation-stimulated repressive activity by Erk2-mediated phosphorylation of RIP140 in adipocyte differentiation. *Cell Signal* 2008; **20**: 1911-1919 [PMID: 18655826 DOI: 10.1016/j.cellsig.2008.07.001]

68 **Yang W**, Guo X, Thein S, Xu F, Sugii S, Baas PW, Radda GK, Han W. Regulation of adipogenesis by cytoskeleton remodelling is facilitated by acetyltransferase MEC-17-dependent acetylation of α-tubulin. *Biochem J* 2013; **449**: 605-612 [PMID: 23126280 DOI: 10.1042/BJ20121121]

69 **Zhou SR**, Guo L, Wang X, Liu Y, Peng WQ, Liu Y, Wei XB, Dou X, Ding M, Lei QY, Qian SW, Li X, Tang QQ. Acetylation of Cavin-1 Promotes Lipolysis in White Adipose Tissue. *Mol Cell Biol* 2017; **37** [PMID: 28559430 DOI: 10.1128/MCB.00058-17]

70 **Saidi N**, Ghalavand M, Hashemzadeh MS, Dorostkar R, Mohammadi H, Mahdian-Shakib A. Dynamic changes of epigenetic signatures during chondrogenic and adipogenic differentiation of mesenchymal stem cells. *Biomed Pharmacother* 2017; **89**: 719-731 [PMID: 28273634 DOI: 10.1016/j.biopha.2017.02.093]

71 **Haberland M**, Carrer M, Mokalled MH, Montgomery RL, Olson EN. Redundant control of adipogenesis by histone deacetylases 1 and 2. *J Biol Chem* 2010; **285**: 14663-14670 [PMID: 20190228 DOI: 10.1074/jbc.M109.081679]

72 **Sun Z**, Singh N, Mullican SE, Everett LJ, Li L, Yuan L, Liu X, Epstein JA, Lazar MA. Diet-induced lethality due to deletion of the Hdac3 gene in heart and skeletal muscle. *J Biol Chem* 2011; **286**: 33301-33309 [PMID: 21808063 DOI: 10.1074/jbc.M111.277707]

73 **Fajas L**, Egler V, Reiter R, Hansen J, Kristiansen K, Debril MB, Miard S, Auwerx J. The retinoblastoma-histone deacetylase 3 complex inhibits PPARgamma and adipocyte differentiation. *Dev Cell* 2002; **3**: 903-910 [PMID: 12479814 DOI: 10.1016/s1534-5807(02)00360-x]

74 **Montgomery RL**, Potthoff MJ, Haberland M, Qi X, Matsuzaki S, Humphries KM, Richardson JA, Bassel-Duby R, Olson EN. Maintenance of cardiac energy metabolism by histone deacetylase 3 in mice. *J Clin Invest* 2008; **118**: 3588-3597 [PMID: 18830415 DOI: 10.1172/JCI35847]

75 **Grégoire S**, Xiao L, Nie J, Zhang X, Xu M, Li J, Wong J, Seto E, Yang XJ. Histone deacetylase 3 interacts with and deacetylates myocyte enhancer factor 2. *Mol Cell Biol* 2007; **27**: 1280-1295 [PMID: 17158926 DOI: 10.1128/MCB.00882-06]

76 **Feng D**, Liu T, Sun Z, Bugge A, Mullican SE, Alenghat T, Liu XS, Lazar MA. A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism. *Science* 2011; **331**: 1315-1319 [PMID: 21393543 DOI: 10.1126/science.1198125]

77 **Sun Z**, Miller RA, Patel RT, Chen J, Dhir R, Wang H, Zhang D, Graham MJ, Unterman TG, Shulman GI, Sztalryd C, Bennett MJ, Ahima RS, Birnbaum MJ, Lazar MA. Hepatic Hdac3 promotes gluconeogenesis by repressing lipid synthesis and sequestration. *Nat Med* 2012; **18**: 934-942 [PMID: 22561686 DOI: 10.1038/nm.2744]

78 **Chatterjee TK**, Basford JE, Knoll E, Tong WS, Blanco V, Blomkalns AL, Rudich S, Lentsch AB, Hui DY, Weintraub NL. HDAC9 knockout mice are protected from adipose tissue dysfunction and systemic metabolic disease during high-fat feeding. *Diabetes* 2014; **63**: 176-187 [PMID: 24101673 DOI: 10.2337/db13-1148]

79 **Chatterjee TK**, Idelman G, Blanco V, Blomkalns AL, Piegore MG Jr, Weintraub DS, Kumar S, Rajsheker S, Manka D, Rudich SM, Tang Y, Hui DY, Bassel-Duby R, Olson EN, Lingrel JB, Ho SM, Weintraub NL. Histone deacetylase 9 is a negative regulator of adipogenic differentiation. *J Biol Chem* 2011; **286**: 27836-27847 [PMID: 21680747 DOI: 10.1074/jbc.M111.262964]

80 **Farmer SR**. Transcriptional control of adipocyte formation. *Cell Metab* 2006; **4**: 263-273 [PMID: 17011499 DOI: 10.1016/j.cmet.2006.07.001]

81 **Klein MA**, Denu JM. Biological and catalytic functions of sirtuin 6 as targets for small-molecule modulators. *J Biol Chem* 2020; **295**: 11021-11041 [PMID: 32518153 DOI: 10.1074/jbc.REV120.011438]

82 **Wang F**, Tong Q. SIRT2 suppresses adipocyte differentiation by deacetylating FOXO1 and enhancing FOXO1's repressive interaction with PPARgamma. *Mol Biol Cell* 2009; **20**: 801-808 [PMID: 19037106 DOI: 10.1091/mbc.E08-06-0647]

83 **Jing E**, Gesta S, Kahn CR. SIRT2 regulates adipocyte differentiation through FoxO1 acetylation/deacetylation. *Cell Metab* 2007; **6**: 105-114 [PMID: 17681146 DOI: 10.1016/j.cmet.2007.07.003]

84 **Baskaran P**, Krishnan V, Fettel K, Gao P, Zhu Z, Ren J, Thyagarajan B. TRPV1 activation counters diet-induced obesity through sirtuin-1 activation and PRDM-16 deacetylation in brown adipose tissue. *Int J Obes (Lond)* 2017; **41**: 739-749 [PMID: 28104916 DOI: 10.1038/ijo.2017.16]

85 **Qiang L**, Wang L, Kon N, Zhao W, Lee S, Zhang Y, Rosenbaum M, Zhao Y, Gu W, Farmer SR, Accili D. Brown remodeling of white adipose tissue by SirT1-dependent deacetylation of Pparγ. *Cell* 2012; **150**: 620-632 [PMID: 22863012 DOI: 10.1016/j.cell.2012.06.027]

86 **Puri N**, Sodhi K, Haarstad M, Kim DH, Bohinc S, Foglio E, Favero G, Abraham NG. Heme induced oxidative stress attenuates sirtuin1 and enhances adipogenesis in mesenchymal stem cells and mouse pre-adipocytes. *J Cell Biochem* 2012; **113**: 1926-1935 [PMID: 22234917 DOI: 10.1002/jcb.24061]

87 **Qu P**, Wang L, Min Y, McKennett L, Keller JR, Lin PC. Vav1 Regulates Mesenchymal Stem Cell Differentiation Decision Between Adipocyte and Chondrocyte via Sirt1. *Stem Cells* 2016; **34**: 1934-1946 [PMID: 26990002 DOI: 10.1002/stem.2365]

88 **Xiao T**, Liu L, Li H, Sun Y, Luo H, Li T, Wang S, Dalton S, Zhao RC, Chen R. Long Noncoding RNA ADINR Regulates Adipogenesis by Transcriptionally Activating C/EBPα. *Stem Cell Reports* 2015; **5**: 856-865 [PMID: 26489893 DOI: 10.1016/j.stemcr.2015.09.007]

89 **Ding L**, Ning HM, Li PL, Yan HM, Han DM, Zheng XL, Liu J, Zhu L, Xue M, Mao N, Guo ZK, Zhu H, Wang HX. Tumor necrosis factor α in aGVHD patients contributed to the impairment of recipient bone marrow MSC stemness and deficiency of their hematopoiesis-promotion capacity. *Stem Cell Res Ther* 2020; **11**: 119 [PMID: 32183881 DOI: 10.1186/s13287-020-01615-9]

90 **Beane OS**, Fonseca VC, Cooper LL, Koren G, Darling EM. Impact of aging on the regenerative properties of bone marrow-, muscle-, and adipose-derived mesenchymal stem/stromal cells. *PLoS One* 2014; **9**: e115963 [PMID: 25541697 DOI: 10.1371/journal.pone.0115963]

91 **Fisch SC**, Nikou AF, Wright EA, Phan JD, Leung KL, Grogan TR, Abbott DH, Chazenbalk GD, Dumesic DA. Precocious subcutaneous abdominal stem cell development to adipocytes in normal-weight women with polycystic ovary syndrome. *Fertil Steril* 2018; **110**: 1367-1376 [PMID: 30503136 DOI: 10.1016/j.fertnstert.2018.08.042]

92 **Chen L**, Merkhan MM, Forsyth NR, Wu P. Chorionic and amniotic membrane-derived stem cells have distinct, and gestational diabetes mellitus independent, proliferative, differentiation, and immunomodulatory capacities. *Stem Cell Res* 2019; **40**: 101537 [PMID: 31422237 DOI: 10.1016/j.scr.2019.101537]

93 **Kugo H**, Moriyama T, Zaima N. The role of perivascular adipose tissue in the appearance of ectopic adipocytes in the abdominal aortic aneurysmal wall. *Adipocyte* 2019; **8**: 229-239 [PMID: 31250691 DOI: 10.1080/21623945.2019.1636625]

94 **Kunter U**, Rong S, Boor P, Eitner F, Müller-Newen G, Djuric Z, van Roeyen CR, Konieczny A, Ostendorf T, Villa L, Milovanceva-Popovska M, Kerjaschki D, Floege J. Mesenchymal stem cells prevent progressive experimental renal failure but maldifferentiate into glomerular adipocytes. *J Am Soc Nephrol* 2007; **18**: 1754-1764 [PMID: 17460140 DOI: 10.1681/ASN.2007010044]

95 **Dhinekaran A**, Lakshmi M, Graceline H, Dey A, Adhikari S, Ramalingam S, Ramachandran I, Bisgin A, Boga I, Pathak S, Banerjee A. Regulation of mesenchymal stem cell differentiation by key cell signaling pathways. In: Pathak S, Banerjee A. Stem Cells and Signaling Pathways. Amsterdam: Elsevier, 2024: 1-25

96 **Wu J**, Cai P, Lu Z, Zhang Z, He X, Zhu B, Zheng L, Zhao J. Identification of potential specific biomarkers and key signaling pathways between osteogenic and adipogenic differentiation of hBMSCs for osteoporosis therapy. *J Orthop Surg Res* 2020; **15**: 437 [PMID: 32967719 DOI: 10.1186/s13018-020-01965-3]

97 **Zhang H**, Xu R, Li B, Xin Z, Ling Z, Zhu W, Li X, Zhang P, Fu Y, Chen J, Liu L, Cheng J, Jiang H. LncRNA NEAT1 controls the lineage fates of BMSCs during skeletal aging by impairing mitochondrial function and pluripotency maintenance. *Cell Death Differ* 2022; **29**: 351-365 [PMID: 34497381 DOI: 10.1038/s41418-021-00858-0]

**Footnotes**

**Conflict-of-interest statement:** All the authors report no relevant conflicts of interest for this article.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review started:** October 31, 2023

**First decision:** December 19, 2023

**Article in press:**

**Specialty type:** Cell and tissue engineering

**Country/Territory of origin:** China

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Muzes G, Hungary; Silva-Junior AJD, Brazi **S-Editor:** Wang JJ **L-Editor:** A **P-Editor:**

**Figure Legends**



**Figure 1 Morphological changes in differentiating mouse thymic mesenchymal stem cells.** Schematic illustration of the adipogenic differentiation protocol and cellular morphological changes that occurred during the differentiation process 33 consecutive days after induction. Typically, mesenchymal stem cell preadipocyte commitment occurred in the first days (days 15), followed by the differentiation of preadipocytes into mature adipocytes with increasing lipid droplets. MSC: Mesenchymal stem cell.



**Figure 2 Regulation of adipogenesis and gluconeogenesis by lysine deacetylases, acetyltransferases and noncoding RNAs.** Lysine deacetylases (KDACs) and acetyltransferases (KATs) are important regulators of adipocyte differentiation and gluconeogenesis. Peroxisome proliferation activator receptor gamma is acetylated by Gcn5/PCAF, p300/CBP and Tip60 but deacetylated by Sirt1. In addition, Gcn5/PCAF is also regulated Prdm16 expression to influence adipogenesis. Histone deacetylases (HDACs) 1, 2, 3, 5 and 9 redundantly regulate adipogenesis. Moreover, noncoding RNAs cooperatively interact with KDACs to regulate the adipogenic process. H19/miR-675 can inhibit HDAC5 expression. Hence, KDACs and KATs can regulate lipid metabolism. PEPCK-C is acetylated by p300 to induce its degradation and attenuate gluconeogenesis. Conversely, PEPCK-C is deacetylated and stabilized by Sirt2 through Sirt2 deacetylase. HDAC6 also plays an important role in gluconeogenesis regulation. PPARγ: Peroxisome proliferation activator receptor gamma; HDAC: Histone deacetylase; PRDM16: PR domain containing zinc finger protein 16.