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MINIREVIEWS

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

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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

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.

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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

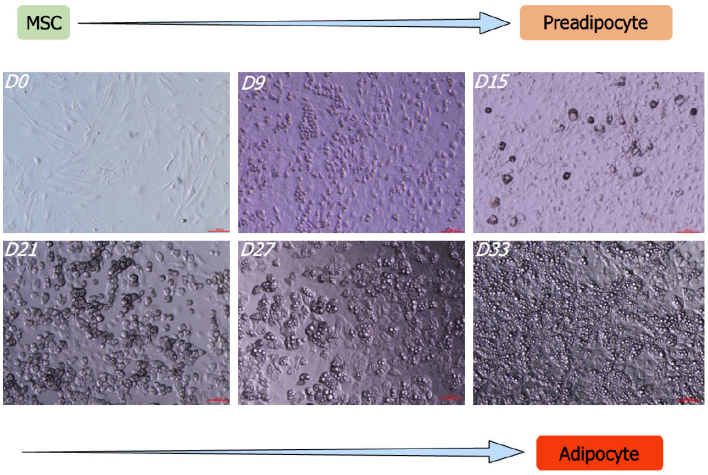

Figure 1 Morphological changes in differentiating mouse thymic mesenchymal stem cells. Schematic illustration of the adipogenic differentiation protocol and cellular morphological changes that occurred during the differentiation process 33 consecutive days after induction. Typically, mesenchymal stem 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.

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

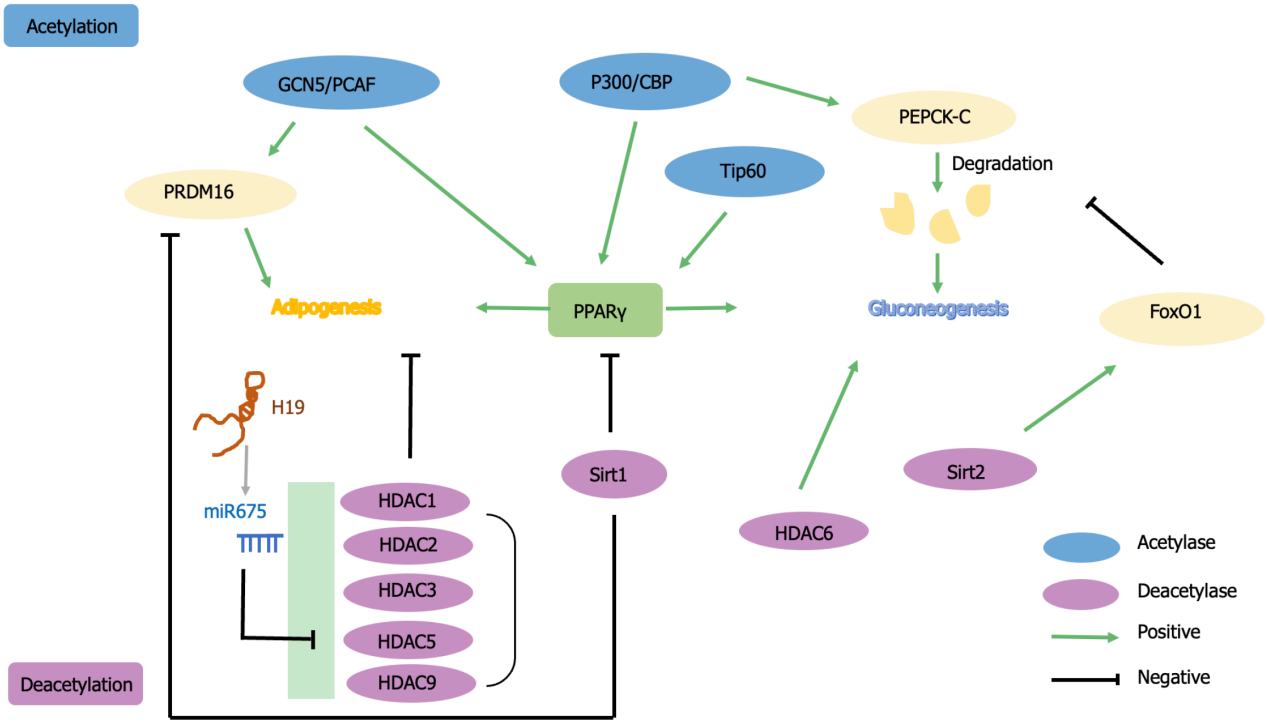

Figure 2 Regulation of adipogenesis and gluconeogenesis by lysine deacetylases, acetyltransferases and noncoding RNAs. Lysine deacetylases (KDACs) and acetyltransferases (KATs) are important regulators of adipocyte differentiation and gluconeogenesis. Peroxisome proliferation activator receptor gamma 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.

reduction in adipogenesis occurring in ASCs[90]. Although MSC transplantation has shown beneficial effects in treating autoimmune diseases, the ability of the BAMBI^{high}MFGE8^{high} 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.

FOOTNOTES

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