

Review Article

Concise Review: The Regulatory Mechanism of Lysine Acetylation in Mesenchymal Stem Cell Differentiation

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Nowadays, the use of MSCs has attracted considerable attention in the global science and technology field, with the self-renewal and multidirectional differentiation potential for diabetes, obesity treatment, bone repair, nerve repair, myocardial repair, and so on. Epigenetics plays an important role in the regulation of mesenchymal stem cell differentiation, which has become a research hotspot in the medical field. This review focuses on the role of lysine acetylation modification on the determination of MSC differentiation direction. During this progress, the recruitment of lysine acetyltransferases (KATs) and lysine deacetylases (KDACs) is the crux of transcriptional mechanisms in the dynamic regulation of key genes controlling MSC multidirectional differentiation.

1. Introduction

Mesenchymal stem cells (MSCs), a kind of adult stem cell with multidirectional differentiation potential, can differentiate into many mesodermal lineages, including adipocytes, osteoblasts, chondrocytes, muscle cells, and nerve cells [1–4]. Based on its pluripotency, MSCs represent a wide range of cell sources for the treatment of diseases such as diabetes, obesity, and autoimmune diseases and have become the focus of global scientific and technological attention [5–7]. The differentiation of MSCs is regulated by many factors [8, 9]. Epigenetics, one of the main regulatory mechanisms of MSC differentiation, plays an important role in determining cell fate [10–14]. Among them, lysine acetylation, a kind of posttranslational modification (PTM) of proteins, has been extensively studied on the regulation of transcription [15–17].

Precise control of proteins is essential to organism function. Lysine acetylation is one of the major protein modifications after translation, which has multiple effects on protein and metabolic components [18]. It can regulate the expression of genes related to multidirectional differentiation and represent the pluripotency of MSCs to a certain extent. At the same time, the degree of lysine acetylation can also affect the differentiation direction and biological function of MSCs.

In this paper, the recent progress in the research of lysine acetylation modification in terms of MSC differentiation is reviewed from the above aspects.

2. Brief Summary of Lysine Acetylation

Lysine acetylation is a reversible process of transferring acetyl group from acetyl coenzyme A to the E-amino side chain of lysine [19]. Lysine acetylation modification is an evolutionarily conserved PTM, which exists in both prokaryotes and eukaryotes. Lysine acetylation is involved in a variety of major key cellular processes related to physiology and disease, such as gene transcription and expression, DNA damage repair, cell signal transduction, protein folding, and autophagy [20–22]. At the same time, it affects protein function through a variety of mechanisms, including protein stability, enzyme activity, subcellular localization, and other posttranslational modifications, as well as protein-protein and protein-DNA interaction, ultimately affecting cell cycle and cell differentiation [23, 24].

Acetylation of histones or nonhistones is mainly reversibly regulated by lysine acetyltransferase (KAT) and lysine deacetylase (KDAC), which are sometimes referred as histone acetyltransferase (HAT) and deacetylase (HDAC).

KAT can relax the structure of nucleosomes, promoting the expression of transcription factors and synergy. Transcription factors can contact with DNA molecules to activate transcription of specific genes. Deacetylation is the process in which KDACs make the promoter barely access to transcriptional regulatory elements to inhibit transcription followed by gene inactivation (Figure 1).

2.1. Lysine Acetyltransferases (KATs) and Lysine Deacetylases (KDACs). At present, it shows that 13 KATs have been identified in the human proteome (canonical), and most of them can be classified into three families: GCN5, p300, and MYST19 [18]. In addition, there are α -tubulin N-acetyltransferase 1 (TAT1/ATAT1), establishment of cohesion 1 homologue 1 (ESCO1) and ESCO2, and histone acetyltransferase 1 (HAT1/KAT1), and there is no homology. Besides TAT1, all classical KATs are mainly localized in the nucleus acetylating histones and nonhistones.

The deacetylation of proteins is catalyzed by deacetylase. At present, 18 kinds of KDACs have been found in human proteome. According to the homology of KDAC domain, it 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 (SirT 1-7), and class IV (including only one member, HDAC11) [25, 26]. Class I and IV KDACs are mainly distributed in the nucleus of cells, and class II KDACs are distributed in both the cytoplasm and nucleus, with exported to cytoplasm after signal activation [27]. Similarly, class III KDACs, also known as sirtuin deacetylases, are located in different cell compartments: Sirtuin 1 (SIRT1) and SIRT6 are in the nucleus, SIRT7 is in the nucleolus, SIRT2 is in the cytoplasm, and SIRT3, SIRT4, and SIRT5 are in mitochondria [28]. Moreover, class I, class II, and class IV KDACs are zinc-dependent enzymes, while class III HDACs require NAD⁺ as a cofactor of catalytic activity. Therefore, KDACs can also be classified into two categories: zinc-dependent HDACs and NAD⁺-dependent sirtuin deacetylases [29]. Zinc-dependent HDACs possess a highly conserved deacetylase domain, commonly referred as classical HDACs or classical KDACs (Table 1).

2.2. Functional Lysine Acetylation Networks. About 70% of the known acetylation sites of KATs are the targets of CBP and/or p300. Acetylation in most of the acetylated proteins is catalyzed by five KATs (CBP, p300, GCN5, PCAF, and TIP60) [27]. Similarly, for the networks regulated by KDACs, more than two-fifths of the acetylation sites are SIRT1 targets, and more than 60% are sirtuin deacetylase targets. Consistent with the location of sirtuins in cells, there are many nuclear proteins consisting of SIRT1 targets, such as transcription regulators, while SIRT3 targets are located in mitochondria, with most SIRT3 targets being involved in the regulation of mitochondrial metabolism. In contrast, KAT-regulated networks contain more transcriptional regulators, with fewer proteins being involved in metabolism.

Acetyl coenzyme A (Acetyl-CoA, ACA) is a key metabolite of cell function, including energy production in mitochondria and lipid biosynthesis in the cytoplasm. Acetylation is

directly related to the level of ACA. The specific production of ACA in cells can locally affect the acetylation of proteins. For example, nuclear ACLY, ACSS2, and PDC regulate histone acetylation by locally producing ACA and thereby affecting gene transcription [30]. In yeast, the consumption of mitochondrial ACA only eliminates the acetylation of mitochondrial proteins besides nucleoproteins [31]. In mice, the loss of ACA carboxylase 1 (ACC1) and ACC2 converts ACA into malonyl coenzyme A, resulting in increased protein acetylation, which may be achieved by increasing the level of ACA [32]. By genetic and restrictive dietary methods, the researchers confirmed the correlation between the fluctuation of ACA level and the change of acetylation level, which further indicated that ACA was the limiting factor for many acetylation events [33].

2.3. Cellular Roles of Lysine Acetylation. Protein acetylation is associated with many cellular processes and human diseases. Line mutations in several KATs and KDACs, such as KAT6A, SMC3 (coding chromosome protein 3), and HDAC8 (coding histone deacetylase 8, SMC3 deacetylase), are related to developmental retardation, abnormalities, and mental disabilities [34, 35]. Studies have found that acetylation is also closely related to cancer, inflammation, immune, and neurometabolic diseases such as diabetes [36–38]. The fact that KATs and KDACs are deregulated in various cancers gives us a clear hint that anomalous acetylation takes place and it might be corrected by therapeutic KDAC inhibitor treatment [39, 40]. At present, many small molecule inhibitors of KDACs and KATs have been attractive therapeutic candidates [38].

Prior to the discovery of KDAC, histone deacetylase inhibitors (HDACi) also advanced protein acetylation [41, 42]. Sodium butyrate, the first compound identified to induce histone acetylation, Trichostatin A (TSA, a fungal antibiotic), valproic acid (VPA), and several other compounds were identified initially as HDACi [43–45]. Since epigenetic changes critically contributed to cancer onset and progression, HDACi were quickly recognized as promising anticancer drugs [39, 46, 47].

HDACi equally promote the acetylation of nonhistone proteins, which can determine the interactions, localization, and stability of these proteins [42]. At the cellular level, HDACi induce cell differentiation, cell cycle arrest, senescence, apoptosis, reactive oxygen species (ROS) production, and mitotic cell death. *In vivo*, HDACi can reduce the invasiveness, angiogenesis, and metastasis of tumors, thus inhibiting the development of tumors. In contrast, CBP, KAT inhibitors discovered recently, and A485, p300 inhibitor, showed antiproliferative effects on lineage-specific tumor cell lines [48]; however, KAT6A and KAT6B inhibitors induced cell senescence and inhibited mouse lymphoma growth [49].

3. Acetylation Modification in Differentiation of MSCs

Mesenchymal stem cells (MSCs) are pluripotent progenitor cells that have the potential to differentiate into multiple

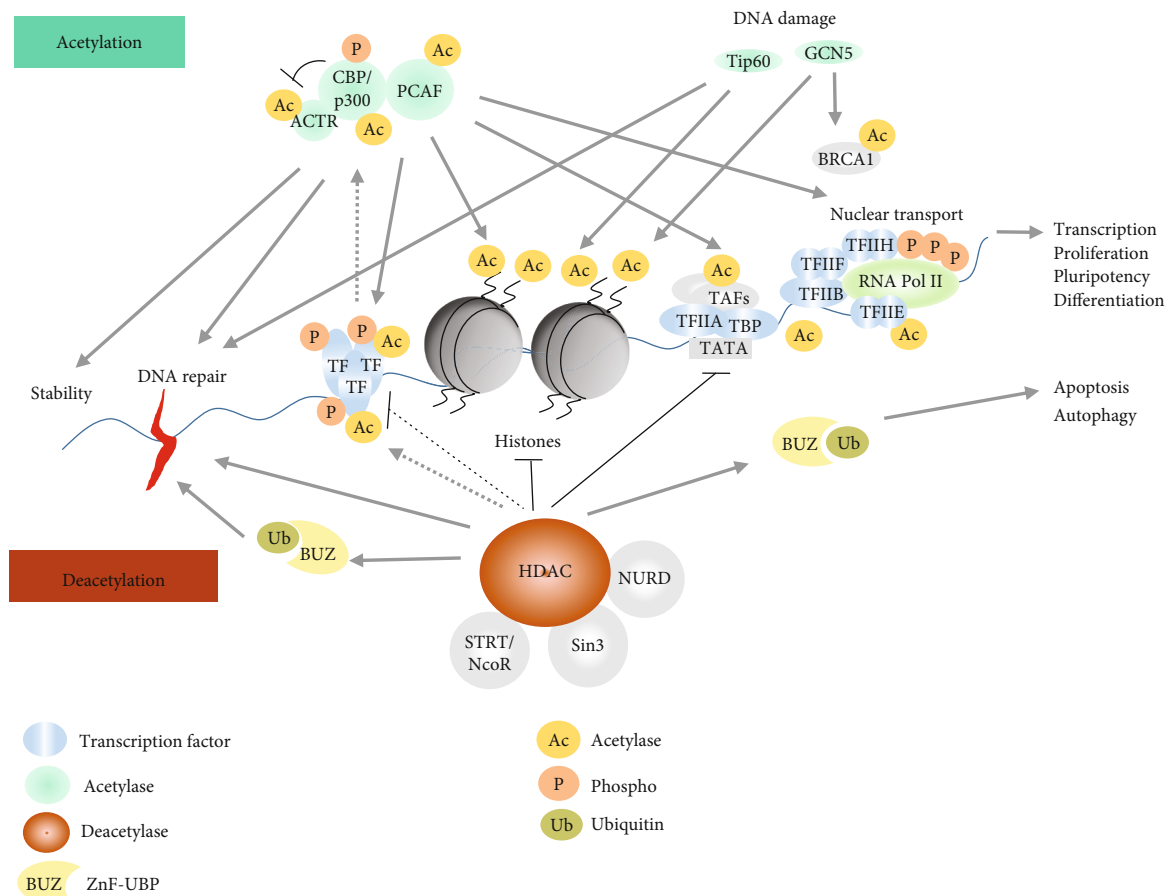


FIGURE 1: Pathway description of protein acetylation. Protein acetylation is involved in the regulation of chromatin structure and transcriptional activity. Acetylation complexes (such as CBP/p300 and PCAF) or deacetyl complexes (such as Sin3, NuRD, NcoR, and SMRT) are recruited to DNA-binding transcription factors (TFs) in response to signaling pathways. HATs induced histone hyperacetylation, which was associated with transcriptional activation, whereas HDACs induced histone deacetylation, which was associated with transcriptional repression. Many transcriptional coactivators have intrinsic acetylase activity, and transcriptional copressurization factors are associated with deacetylase activity. Histone acetylation stimulates transcription by remodeling advanced chromatin structures, attenuating histone-DNA interactions and providing binding sites for transcriptional activation complexes with proteins with containing brominated domains.

mesodermal lineages, including adipocytes, osteoblasts, and chondrocytes. During aging and osteoporosis, adipogenesis is superior to osteogenesis, which means that under these conditions, the balance of MSC differentiation is dysregulated. Numerous transcription factors are involved in the lineage selection and terminal differentiation of MSCs.

Lysine acetylation regulation is involved in many cell differentiation processes. It is also one of the major regulatory mechanisms of epigenetic regulation of MSCs to adipose differentiation and osteogenic differentiation [50]. Histone acetyltransferases are involved in initiating transcription primarily by the addition of acetyl groups, which leads to DNA denaturation. HDAC can reverse the acetylation process in cells (Figure 2).

3.1. The Role of Acetylation Modification in Adipogenic Differentiation of MSCs (Table 2). Lysine acetylation modification and its modified enzyme are basically involved in the epigenetic regulation of lipogenesis [51–53]. Lysine acetylation is gene-specific at adipogenic regulator genes, which play

different roles in regulating transcriptional networks during adipogenesis.

Pretreatment with HDAC inhibitors VPA and sodium butyrate (NaBu) inhibited the adipogenic differentiation of human umbilical cord blood and adipose-derived mesenchymal stem cells [54]; HDAC inhibitors TSA and suberoylanilide hydroxamic acid (SAHA) could inhibit the adipogenic differentiation of human preadipocytes [55]; the differentiation of fat cells could be promoted with *hdac3* knockout or the expression of *hdac1* interfered with siRNA [56–58]. In addition, high expression level of HDAC5 and HDAC6 is required for adequate adipocyte function [59]. HDAC9 has been demonstrated to repress adipogenesis. In the case of a chronic high-fat diet, proper adipogenic differentiation is impaired, and the expression of a negative regulator of adipogenic HDAC9 is increased. Ablation of HDAC9 in mice can prevent adverse health effects of chronic high-fat diets, including weight gain, impaired glucose tolerance, and insulin insensitivity [12, 60, 61]. Therefore, HDAC inhibitions hold great promise for clinical targeting of obesity-related diseases.

TABLE 1: The classification of KATs/KDACs.

Enzyme	Family	Abbreviations	Subcellular localization	
KATs	GCN5	GCN5 (KAT2A), PCAF (KAT2B)	Nucleus	
	P300	CBP (KAT3A), P300 (KAT3B)	Nucleus	
	MYST	Tip60 (KAT5), MOZ (KAT6A) MORF (KAT6B), HBO1 (KAT7), MOF (KAT8)	Nucleus	
	Other	ESCO1, ESCO2, HAT1 TAT1	Nucleus Cytoplasm	
KDACs	Class I	HDAC1, HDAC2, HDAC3, HDAC8	Nucleus	
	Class II	Class IIa	HDAC4, HDAC5, HDAC7, HDAC9	Nucleus
		Class IIb	HDAC6, HDAC10	Cytoplasm
	Class IV		HDAC11	Nucleus
			SIRT1, SIRT6	Nucleus
	Class III (SIRT)	SIRT2	Cytoplasm	
	SIRT3, SIRT4, SIRT5	Mitochondria		
		SIRT7	Nucleolus	

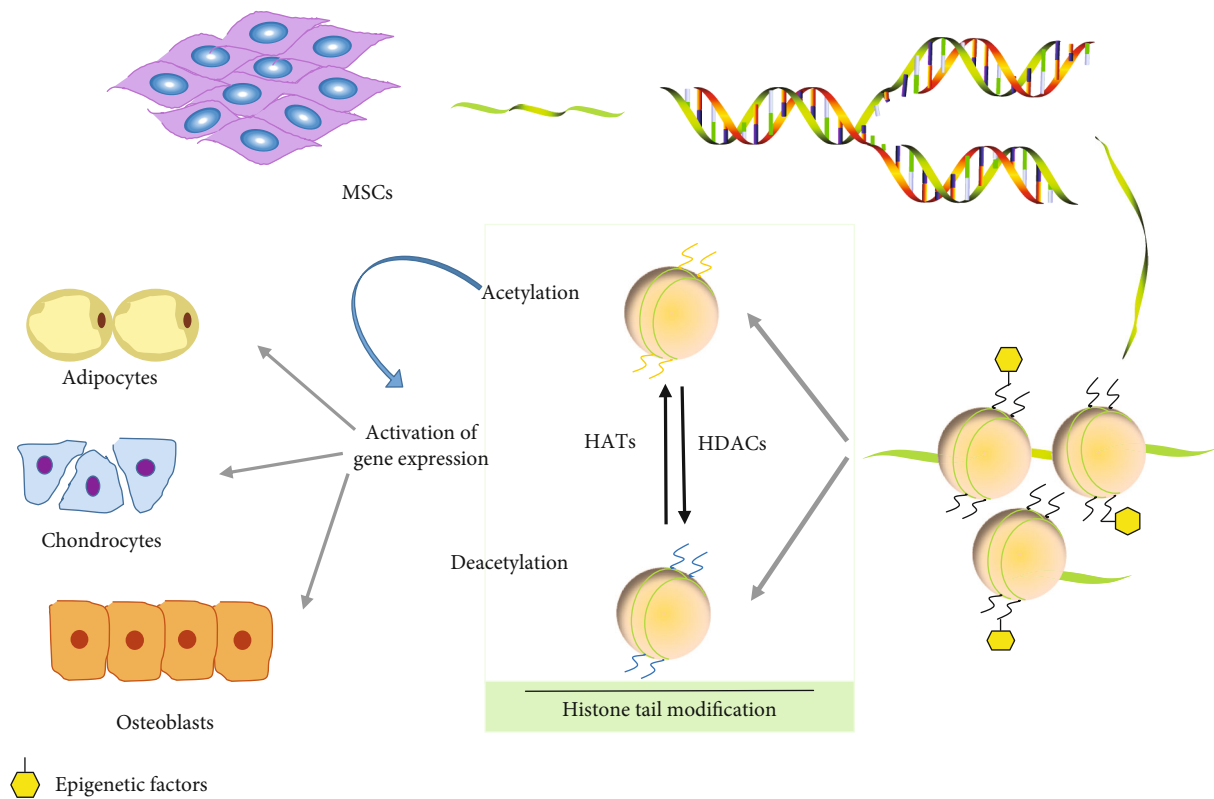


FIGURE 2: Acetylation regulation of mesenchymal stem cell differentiation along the adipocytic, osteogenic, and cartilage lineages.

Multiple transcription factors are involved in the lineage selection and terminal differentiation of MSCs. Peroxisome proliferator-activated receptor γ (PPAR γ), CCAAT/enhancer-binding proteins (C/EBPs), adipocyte assay and differentiation-dependent factor 1/sterol response element-binding protein 1c (ADD1/SREBP1c) are key regulators of mammalian adipocyte differentiation and also participate in the spectrum selection and terminal differentiation of MSCs [62, 63]. Studies have shown that the activation of PPAR γ and C/EBP α is accomplished by the interaction of transcrip-

tion factors, coactivators, and coinhibitors. Among them, lysine acetylation and deacetylation play an important role by genetic regulation. Zhang et al. (2012) detected the distribution patterns of acetylation modification of five key adipose formation regulatory genes, Pref-1, C/EBP β , C/EBP α , PPAR γ 2, and aP2, during the adipogenesis of C3H 10T1/2 mouse mesenchymal stem cells (MSCs) and 3T3-L1 preadipocytes, in order to determine the role of acetylation modification of lysine and its “division of labor” in adipocyte differentiation. The results showed that the detected lysine

TABLE 2: Acetylation regulation of mesenchymal stem cell differentiation into adipose cells.

Epigenetic mark/enzymatic function	Specific chromatin modifier	Targeted stem cell population	Adipogenesis	
			Differentiation	Refs.
Deacetylation				
	HDAC3	3 T3-L1	Attenuates adipogenesis by binding to the master regulator PPAR γ and attenuating PPAR γ 's capacity to drive gene expression	[56, 58]
			Attenuates adipogenesis by binding to the master regulator PPAR γ and repressing its target genes	[62, 63, 65–72]
	Sirt1	Mice bone marrow	Sirt1 ↓ { <ul style="list-style-type: none"> Increase of acetylated PPARγ → increased C/EBPα expression → promoted adipogenesis increase of acetylated Sox9 → reduction of collagen 2α1 → impaired chondrogenic differentiation 	[66]

TABLE 3: Acetylation regulation of mesenchymal stem cell differentiation into osteoblasts.

Epigenetic mark/enzymatic function	Specific chromatin modifier	Osteoblastogenesis		Ref.
		Nonhistone substrates or interacting proteins	Differentiation function	
Deacetylation				
	HDAC1, HDAC2		↓	[85–87, 89]
	HDAC3, HDAC7	Interaction with RUNX2	↓	[84, 88]
	HDAC4, HDAC5	RUNX2 deacetylation; interaction with SMAD3	↓	[68, 69]
	HDAC8	H3K9ac	↓	[83]
	SIRT1	Beta-catenin deacetylation	↑	[90–92]
	SIRT3	SOD deacetylation	↑	[89]

↑: promotion of differentiation; ↓: suppression of differentiation.

acetylation modification was globally stable throughout the adipogenesis process but showed a unique and highly dynamic distribution pattern for specific genes. For example, PPAR α 2 and aP2 genes in MSCs showed increased histone acetylation in the tails of H3 and H4 during adipogenesis, and increased histone acetylation levels activate the transcription of PPAR α 2 and aP2 genes [64]. In addition to directly binding to the lipid modifier genes, histone-modifying enzymes also modulate adipogenesis by interacting with adipogenic regulators. For example, SIRT1, a representative member of the mammalian sirtuin family, attenuates adipogenesis by binding to the major regulatory factor PPAR γ and inhibiting its target genes when food is restricted [65–71]. Conversely, decreased Sirt1 leads to an increase in PPAR γ acetylation, thereby increasing C/EBP α expression and promoting the development of lipogenesis [72]. In addition, KATs p300/CBP and Tip60 promote the activation of adipogenic-related genes by directly interacting with PPAR γ , thereby enhancing its transcriptional processes [73, 74]. Moreover, HDAC1 has been shown to be associated with regulatory elements of BAT-specific genes, leading to a decrease in the degree of histone H3K27 acetylation and thus to transcriptional inhibition [75]. And HDAC3 is also involved in the regulation of BAT gene expression and thermogenesis in mice [76–78]. Interestingly, unlike HDAC1- and HDAC3-

mediated regulation, HDAC9-mediated regulation of adipogenic gene expression is not dependent on its deacetylase domain, but instead on the control of amino-terminal cofactor interaction module of the protein [76].

3.2. The Role of Acetylation Modification in Osteogenic Differentiation of MSCs (Table 3). The degree of histone acetylation of related regulatory genes may reflect the maintenance and differentiation status of MSCs [79]. The acetylation of H3K9 and H3K14 (H3K9ac, H3K14ac) is a marker of gene activation [80]. During the osteogenic differentiation of bone marrow mesenchymal stem cells (BMMSCs), the expression of osteogenesis-related genes RUNX2 and alkaline phosphatase (ALP) gradually increased, while the expression of stem factors Oct4 and Sox2 related to stem cell self-renewal decreased significantly, and the variation was closely related to H3K9ac and H3K14ac [81].

In the current study, class I HDAC1, HDAC2, HDAC3, and HDAC8 and class III SIRT1 and SIRT3 played an important role in the differentiation direction of BMMSCs [82–89]. In the myocardial microenvironment, BMMSCs can differentiate into cardiomyocytes. During this process, the expression of HDAC1 is significantly decreased. At the same time, knockdown of HDAC1 can promote the direct differentiation of BMMSCs into cardiomyocytes [90]. HDAC8 reduces

the osteogenic differentiation of rat BMMSCs by inhibiting the acetylation of H3K9 and the activity of RUNX2 [83].

SIRT1 can directly regulate the factor Sox2 to maintain the self-renewal and pluripotency of BMMSCs. The decrease of its activity reduces the expression of Sox2, which leads to the degradation of self-renewal and differentiation ability of BMMSCs. The activated SIRT1 can dose-dependently promote the ability of BMMSCs to clone and differentiate into osteogenic adipogenic differentiation [91]. Similarly, SIRT1 can regulate the transcription of genes involved in BMMSC differentiation by deacetylating β -catenin to accumulate in the nucleus [92]. In addition, SIRT1 promotes the cartilage differentiation process of BMMSCs by activating the deacetylation of Sox9 and NF- κ B [93].

Histone deacetylase inhibitors have a strong influence on the differentiation of BMMSCs. Treatment of BMMSCs with histone deacetylase inhibitors VPA and NaBu increased histone H3 and H4 acetylation levels and significantly promoted liver-specific gene expression, suggesting that the agent promotes the differentiation of BMMSCs into the liver by inhibiting deacetylase [94]. At the same time, NaBu inhibited the expression of HDAC2 in rat BMMSCs and its recruitment on smooth muscle-specific genes could further induce high levels of H3K9ac and H4ac, which promoted the expression of smooth muscle-specific genes and induced BMMSCs to differentiate into smooth muscle [95]. Another histone deacetylase inhibitor, TSA, significantly inhibits the decreasing of Oct4, Sox2, and Nanog to stabilize the expression of pluripotency genes in BMMSCs [96]. Other studies have found that TSA treatment increases the level of acetylation of histone H3 and inhibits adipogenic differentiation of BMMSCs [97].

In addition, Tan et al. studied the H3K9ac modification of the gene promoter region of hBMMSCs at the genome-wide level. The results showed that the modification of H3K9 in the promoter region of hBMMSCs correlated well with mRNA expression. Functional analysis showed self-renewal in hBMMSCs. Multiple key intracellular signal transduction pathways can be regulated by H3K9 modification [98]. In the process of osteogenic differentiation of hBMMSCs, the overall enrichment of H3K9ac in the promoter region of the gene is gradually reduced [99]. *In vitro*, chondrogenic differentiation of hBMMSCs significantly increased the level of chromatin marker H3K9ac at the promoter and 5' end regions of the gene [100]. These results suggested that gene activation and silencing affected by H3K9ac may be critical for self-renewal, pluripotency maintenance, and osteogenic differentiation of MSCs.

3.3. The Role of Acetylation Modification in the Differentiation of MSCs into Chondrocytes. In cartilage tissue regeneration medicine, lysine acetylation is involved in the regulation of chondrocyte differentiation and terminal differentiation of mesenchymal stem cells. Cartilage tissue is a vascularless tissue composed of chondrocyte and extracellular matrix. Hence, cartilage tissue has limited repair ability [101]. Mesenchymal stem cells (MSCs) are promising alternative sources of chondrocytes because of their long-term self-renewal and multidirectional differentiation potential.

The differentiation of mesenchymal stem cells into chondrocytes is essentially a process of chondrocyte-specific phenotype gene expression in the mesenchymal stem cell genome. Various signaling pathways including transforming growth factor- β 1 (TGF- β 1)/SMAD pathway and Wnt/ β -catenin pathway have been proved to be related to this process [102–107]. Lysine acetylation plays an important role in regulating the expression of cartilage-specific genes [108–111]. Histone modification controls expression of key genes in cartilage formation by altering the spatial structure of chromatin, ultimately regulating the process of chondrogenesis of stem cells.

Histone modification plays an important role in regulating the early chondrogenic differentiation of mesenchymal stem cells [112]. Coactivator P300 has histone acetylase activity, which can directly mediate histone acetylation of Sox9 and activate Sox9 for cartilage formation. P300 can also interact with cyclic adenosine phosphate effector binding protein (CREB) to form coactivator and Sox9 to enhance the expression of chondrocyte-specific phenotype gene Col2a1. Thus, the histone acetylation modification associated with P300 can regulate the expression of chondrocyte-specific genes Sox9 and Col2a1 [113–115]. HDAC1 can not only directly bind to the promoter region of β -catenin to inhibit the expression of β -catenin gene but also degrade β -catenin through the interaction between the domain of deacetylase and the deacetylated β -catenin protein, which leads to the downregulation of the classical Wnt/ β -catenin signaling pathway and promotion of the cartilage differentiation process of mouse mesenchymal stem cells induced by TGF- β 1 [116]. HDAC4 can also promote the chondrogenesis of porcine synovial-derived mesenchymal stem cells (SDSCs) induced by TGF- β 1; meanwhile, HDAC4 can inhibit the expression of X hypertrophic phenotype X (SDSCs) [117].

Cartilage damage is usually accompanied by the occurrence of bone lesions. Multiple local factors are involved in regulating the physiological remodeling of cartilage, and the loss of balance of these factors may result in higher cartilage catabolism. Molecules of the Wnt pathway have become key regulators of bone and cartilage. Activation of Wnt/ β -catenin induces an imbalance in cartilage homeostasis [102, 118]. *In vitro* chondrogenesis experiments using C3H10T1/2 cells showed that mRNA and protein levels of β -catenin were inhibited during chondrogenesis, while expression levels of HDAC1 was elevated. The opposite expression pattern between β -catenin and HDAC1 suggests that there may be novel regulatory mechanisms involved in cartilage formation between these two factors [116].

4. Conclusion and Outlook

Lysine acetylation regulation is involved in many cell development and differentiation processes.

In the complex and delicate internal environment of organisms, epigenetic regulation often does not work in a single way. Different histone modifications can interact with each other and play a synergistic role. Histone modification can also be coupled with DNA methylation to produce complex epigenetic effects. The network regulation pattern of

epigenetic modification is also involved in the fine regulation of adipogenic differentiation of MSCs. The balance between osteogenic and adipogenic differentiation of MSCs is regulated by DNA methylation and histone acetylation in the promoter region of *C/EBP α* [119, 120]. At the end of osteogenic differentiation, the hypermethylation of *C/EBP α* promoter region prevents binding of PPAR γ with HDAC1 binding to this region further, reducing histone acetylation levels, and PPAR γ establishes DNA methylation in the promoter region of *C/EBP α* and the bridge of histone acetylation [120]. Histone modification factor YY1 and transcriptional coactivator p300 can alter the expression of chondrocyte-specific gene ChM-I in BMMSCs by regulating the level of histone acetylation, inhibiting YY1 and increasing p300 and hypomethylation of the promoter region. The expression of basic transcription factor Specificity 3 (Sp3) maintains the expression of ChM-I but does not function in the same way in hypermethylated cells, suggesting that there is synergistic negative regulation of ChM-I by histidine deacetylation and methylation during BMMSC cartilage differentiation [121].

RUNX2 was upregulated in BMMSCs during osteogenic differentiation, and both transcriptional activation-related H3K9ac and H3K4me3 modification levels and recruitment in the RUNX2 promoter region were elevated, while H3K9me3 modification levels associated with transcriptional repression were observed in the RUNX2 promoter region. The recruitment was reduced, and the degree of DNA methylation in the RUNX2 promoter region was reduced [97]. These findings suggested that different epigenetic modifications can synergistically regulate the differentiation process of BMMSCs.

Epigenetic modulators affect the function of adult tissue stem cells primarily by modulating the function of tissue-specific master regulators. However, for us, it is still far away to understand the specific role of individual epigenetic factors; more importantly, their combined activity in adult stem cells and their communication is unclear. We face many technical challenges, such as *in vivo* generation of models to specifically study stem cells and their molecular regulatory mechanisms of adult origin, as well as the lack of stem cell-specific inducible targeting strains and conventional methods for epigenetic analysis from very small amounts and powerful calculation methods to understand the large amount of data generated.

In recent years, a variety of epigenetic modifications have been found to participate in the differentiation of MSCs. Based on these modifications, drugs have been developed to effectively regulate these modifications, providing precise differentiation conditions for MSCs and enabling MSCs to differentiate in a controllable and predictable direction. At present, some small-molecule drugs that can regulate stem cell differentiation and proliferation are in the stage of detection and development, which can participate in various aspects of regulatory programming and development signaling pathways [122]. These results also have promising value for the study of differentiation mechanism and clinical application of mesenchymal stem cells derived from the bone marrow, fat, and umbilical cord blood.

In conclusion, the regulation of lysine acetylation plays an important role in the process of MSC adipogenesis and differentiation, but the specific mechanism is not yet fully understood, and a new regulatory modification network needs to be found. Further research in this field will provide clues for the fate of MSC differentiation and will have broad application prospects in clinical tissue engineering and cell therapy.

Conflicts of Interest

The authors confirm that there are no conflicts of interest.

Acknowledgments

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