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REVIEW ARTICLE

Epigenetic regulation of mesenchymal stem cell aging through histone modifications

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Abstract Stem cell senescence and exhaustion, a hallmark of aging, lead to declines in tissue repair and regeneration in aged individuals. Emerging evidence has revealed that epigenetic regulation plays critical roles in the self-renew, lineage-commitment, survival, and function of stem cells. Moreover, epigenetic alterations are considered important drivers of stem cell dysfunction during aging. In this review, we focused on current knowledge of the histone modifications in the aging of mesenchymal stem cells (MSCs). The aberrant epigenetic modifications on histones, including methylation and acetylation, have been found in aging MSCs. By disturbing the expression of specific genes, these epigenetic modifications affect the self-renew, survival, and differentiation of MSCs. A set of epigenetic enzymes that write or erase these modifications are critical in regulating the aging of MSCs. Furthermore, we discussed the rejuvenation strategies based on epigenetics to prevent stem cell aging and/or rejuvenate senescent MSCs.

Introduction

Aging is a universal phenomenon in animals, leading to the decline of tissue and organ regenerative capacities.[1–3] Degenerative diseases of nearly all organs emerge during aging, leading to suffering and death in the aged population.[4] Along with the progress of global aging, how to prolong the lifespan and maintain healthy aging is becoming a critical issue in medicine.[5] Age-related diseases, such as osteoporosis are attributed to the imbalance between tissue resorption and formation.[6] MSCs as the source of progenitor cells are essential in tissue formation.[7] However, aging usually led to the dysfunction and exhaustion of stem cells.[8] The decline of proliferation, migration, differentiation, and other biological characteristics of MSCs results in reduced tissue regeneration.[9,10] Rejuvenating senescent MSCs in the aged population is a promising strategy for age-

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related diseases. However, no efficient anti-aging treatments are available in the clinic, due majorly to the elusiveness of the mechanism of aging.

Although several theories have been developed, it remains difficult to understand the mechanism and process of aging. A critical question in aging is why young and aging cells with the same genome possess distinct characteristics and capacities. In the last decade, emerging evidence uncovered a close relationship between epigenetic regulation and stem cell aging.[11] Epigenetics are the mechanisms that control gene expression and protein expression without changing the sequence of DNA.[12] Epigenetic modifications of DNA and histones are considered to be a key mechanism that determines the lineage differentiation, function, and fate of stem cells. Emerging evidence demonstrated that aberrant epigenetic modifications on chromatin, DNA, or RNA are hallmarks of aging, indicating that epigenetic regulation is closely related to stem cell aging.[13]

Epigenetic modification on histones, one of the most important epigenetic regulations on cells, plays an important role in the functionality and fate of MSCs. Several specific epigenetic modifications on histones are changed during aging, leading to aberrant expression of key transcription factors or signaling molecules. Importantly, these aberrant epigenetic modifications are reversible by targeting key regulators of epigenetic modification, providing a possibility to prevent or even rescue the process of cellular aging.[14]

In this review, we summarized recent findings of histone modifications on MSC aging and discussed the rejuvenation strategies based on epigenetics to prevent stem cell aging and/or rejuvenate senescent MSCs.

Mesenchymal stem cells

MSCs are multipotent stem cells isolated from bone marrow, adipose, and other tissues with potent proliferative capacity and multi-lineage differentiation potential. The International Society of Cellular Therapy (ISCT) defined criteria to standardize and identify MSCs as followed: (i) Upon proper stimulation, MSCs are able to differentiate into classic mesodermal lineages of bone, fat, and cartilage *in vitro*.[15] (ii) MSCs express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79 alpha or CD19 and HLA-DR surface molecules.[16] In general, MSCs derived from different origins are heterogeneous populations exhibiting several distinct characteristics and properties.[17]

MSCs perform a broad range of biological functions in tissue homeostasis maintenance and regeneration. MSCs are able to differentiate into cells derived from all three embryonic germ layers under strictly controlled culture conditions *in vitro*.[18] Besides, MSCs secrete a wide range of paracrine factors supporting regenerative processes in damaged tissues. These paracrine factors comprise the components of the extracellular matrix, proteins involved in the adhesion process, enzymes as well as their activators and inhibitors, growth factors and binding proteins, cytokines, and chemokines, *etc*.[19] In addition, MSCs grown *in vitro* have the ability to interact and regulate the function of the majority of effector cells involved in the processes of the primary and acquired immune response.[20] Depending on these capacities of self-renewal and proliferation ability, multilineage differentiation potentials, paracrine regulation, and immunomodulatory effects, MSCs play an important role in tissue homeostasis, repair, and regeneration.

Aging

Aging is broadly defined as the time-dependent functional decline that affects most living organisms. Aging is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death.[1] This deterioration is the primary risk factor for major human age-related pathologies including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases.[1,21,22] Nine tentative hallmarks represent common denominators of aging, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication.[1] Among these hallmarks, epigenetic alterations, telomere attrition, genomic instability, and loss of proteostasis are primary hallmarks that are considered to be the primary causes of cellular damage, which resulted in stem cell exhaustion and altered intercellular communication.

Mesenchymal stem cell aging

The decline in the regenerative potential of tissues is one of the most obvious characteristics of aging. MSC senescence and exhaustion unfold as the integrative consequence of multiple types of aging-associated damages and likely constitute the ultimate culprits of tissue and organismal aging. [1]In aged individuals, MSCs suffer from cellular senescence, which is in accordance with the decline of tissue formation.[9,23,24] MSCs derived from aged tissues usually expressed increased markers of cellular senescence such as SA β-Gal, $P16^{INK4a}$, P53, *etc*.[25] Furthermore, an overall age-related molecular change was observed in MSCs during aging. Decreased expression of genes involved in differentiation, proliferation, and migration is observed in aging MSCs when compared with normal MSCs.[26] Along with the senescence phenotype, the function of MSCs declines dramatically during aging.[9] Mounts of evidence demonstrated that aging lead to dramatic changes in the properties of self-renew, multipotent differentiation, migration, survival, and inter-cellular communications.[9] A striking phenomenon is the decline of the proliferation of aging MSCs. The cell cycle of aging MSCs was arrested in G0/G1 phase, and the proportion of cells in the S phase was reduced during aging.[27] Another dominant characteristic of aged MSCs is the decline of differentiation into mature functional cells.[24] For example, a study showed that the lineage commitment of bone marrow MSCs shifted from osteoblast to adipocyte during aging, which was in accordance with the increase of bone marrow fat in the aged population.[28] Besides, other functionalities of MSCs such as immunoregulation, paracrine effect on regulating hematopoiesis, and therapeutic effects in cytotherapy[10] are all dramatically decline during aging, suggesting an overall age-related decline in the "fitness" of MSCs during aging.[23]

In theory, the aging of MSCs would ultimately lead to two main outcomes: (i) exhaustion of stem cells due to the decline of the self-renew ability of MSCs; (ii) decline of precursor cells due to the decline of the differentiation capacity of MSCs. The exhaustion of MSCs and precursor cells ultimately leads to the loss of homeostasis in tissues. At present, no effective drugs are available to prevent stem cell aging in aged populations. Therefore, understanding the mechanism of MSC aging is crucial to finding novel therapy for age-related diseases.

Epigenetic regulation is closely related to MSC aging

The term "epigenetic regulation" refers to the direct impact upon phenotypes without changing the underlying sequence of DNA, including modification of acetylation and methylation on histone, DNA methylation, and post-transcriptional regulation of non-coding microRNA. Different modifications on the chromosomes and DNA determine the expression of specific genes,[12] which eventually modulate the function of stem cells, including self-renew, lineage commitment, and regulatory function. Moreover, diverse epigenetic regulatory modifications form a complicated dynamic network, which ensures the suitable response of MSCs to the microenvironment.[29]

But during aging, dramatic changes in the external cues and internal cues lead to aberrant expression of key epigenetic modifications. Emerging evidence revealed the landscape of epigenetic modifications on DNA and chromatin is varied between young and aging stem cells, which is considered to be a hallmark of aging.[29] Using the technique to detect epigenetic signatures on single-cell levels, the heterogeneity of histone modifications was detected in young and aging cells derived from the same tissue.[30] In accordance, these epigenetic defects or epimutations accumulated throughout life may specifically affect the behavior and functionality of stem cells.[1]

Several remarkable pieces of evidence come from gene-modification animal models overexpressing specific enzymes in epigenetic modification.[31] Another important line of evidence that supports the epigenetic modification of aging comes from the studies of metabolism, which is the most critical factor that controls the life span. DNA methylation increases at genes related to DNA replication, cell cycle, and adipogenic differentiation due to long-term culture, which may in part affect bone marrow MSC senescence.[32] Dysregulated expression of BRG1, a chromatin remodeling factor, induces the senescence of bone marrow MSCs, which is associated with the silencing of *NANOG* and changes in the levels of chromatin proteins.[33] Consistently, functional data indicate that alteration of several chromatin modifiers extends lifespan, suggesting that specific epigenetic regulation enzymes can be key to longevity.[34] Treatment of miR-26a, which represses *Tob1* expression, could effectively improve the osteogenic differentiation capability of bone marrow MSCs isolated from osteoporotic mice both *in vitro* and *in vivo*.[35] DNA methylation inhibitor 5-aza-2′-deoxycytidine ameliorates disuse-induced osteopenic bone development.[36] Taken together, these findings reveal that the changes in the landscape of epigenetic modification led to the loss of homeostasis in MSCs during aging.

Histone modification regulating MSC aging

DNA is packaged inside a nucleus with the help of proteins called histones, forming a DNA-protein complex called chromatin. The fundamental unit of chromatin is called the nucleosome, which consists of DNA and the core histones (H2A, H2B, H3, and H4). Histone H1 links the nucleosomes together to form a chromosome.[37] There are many different covalent modification types of the N-terminal amino acids of basic residues, including methylation, acetylation, phosphorylation, ubiquitylation, and sumoylation.[38] The modifications on histone are determined by a set of enzymes. For example, histone methylation levels depend on the balance between histone methyltransferases (HMTs) and histone demethylases (HDMTs), while the acetylation levels rely on the balance between histone deacetylases (HDACs) and histone acetyltransferases (HATs) (Fig. 1).[31]

Histone modifications play fundamental roles in most biological processes that are involved in the manipulation and expression of DNA.[39] Changes in histone modifications, especially the role of histone methylation and histone acetylation, have been extensively studied during aging.[14] Compared with young MSCs, the types and levels of histone modifications in aging MSCs have critical differences. Recent studies have highlighted histone modification state as an important modulator in MSC aging. Modifications at different sites of histones can change the manner that DNA wrap around them, which leads to changes in the folding or exposure conditions of gene promoter regions, thereby inhibiting or promoting gene expression.

Histone modification regulates cellular senescence by affecting the transcription activity of surrounding DNA related to the cell cycle. Since the relevance of most of these changed histone modifications with the aging of MSCs remains unknown, we only focused on the epigenetic mechanisms that have been verified to be closely related to the functional decline of aged MSCs. The related histone modifications associated with the aging of MSCs should satisfy the following three criteria: (i) its expression levels changed during aging; (ii) its change leads to the decline of the function of aged MSCs; (iii) recovery of its expression should, at least partly, rescue the functional decline of aged MSCs. Major changes in histone modifications are summarized in Table 1.

Impact of histone methylation on MSC aging

Histone methylation can occur on arginine, lysine, and histidine by adding methyl groups. Histone methylation usually occurs at the lysine (K) residues of histone H3 and H4. Histone methylation can be divided into mono- (me1), di- (me2), or tri-methylated (me3) states depending on the number of methylated lysine residues.[38] Histone methylations at different sites are linked to gene activation or repression.[40] Senescence-associated changes in histone methylation on H3K27, H3K9, H3K4, and other sites of histone are correlated with gene expression changes during

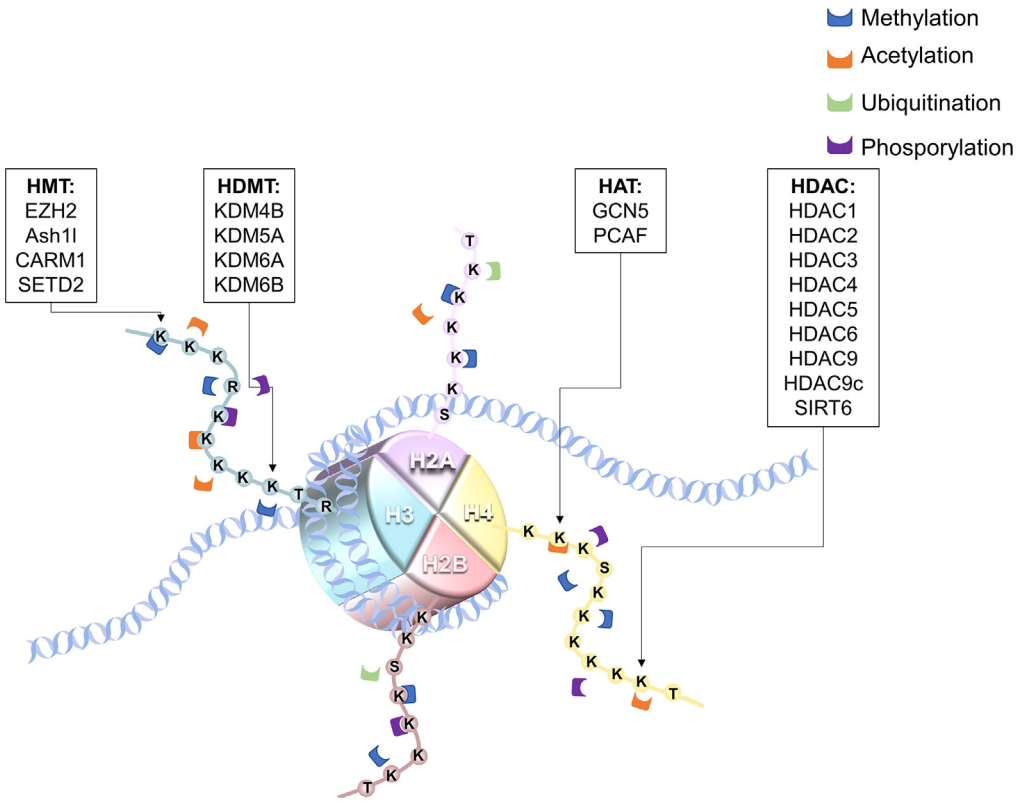

Figure 1 Epigenetic histone modifications. The methylation levels of N-terminal amino acid residues can be regulated by HMT and HDMT. The acetylation levels of N-terminal amino acid residues can be regulated by HAT and HDAC. HMT: histone methyltransferase, a class of enzymes that catalyze the methyl groups of histone proteins. HDMT: histone demethylase, a class of enzymes that catalyze the removal of methyl groups of histone proteins. HAT: histone acetyltransferase, a class of enzymes that catalyze the acetyl groups of histone proteins. HDAC: histone deacetylase, a class of enzymes that catalyze the removal of acetyl groups of histone proteins.

aging. To further clarify the exact roles of histone methylation, we will discuss how specific histone modifications impact the senescence of MSCs.

H3K27 methylation related to MSC aging. The H3K27me3 levels in MSCs are related to age. The H3K27me3 levels in bone marrow MSCs of aging mice and ovariectomized osteoporotic mice were significantly increased compared to the normal mice.[41–43] In another research, loss of H3K27me3 was reported to be associated with bone marrow MSC aging.[44]

H3K27me3 levels are majorly regulated by EZH2, KDM6A (also known as UTX), and KDM6B (also known as JMJD3). EZH2 is a histone methyltransferase within the PcG protein complex, which methylates histone H3 on lysine 27.[45] Redundant EZH2 increased H3K27me3 levels, leading to the shift of bone marrow MSC cell lineage commitment to adipocytes during osteoporosis.[42] With further research, EZH2 and H3K27me3 were highly enriched in promoters of *Wnt1*, *Wnt6*, and *10a* in bone marrow MSCs of ovariectomized mice. EZH2 decreased the enrichment of H3K27me3 on *Wnt1*, *Wnt6*, and *Wnt10a* promoters in bone marrow MSCs to repress the expression of these *Wnt* genes.[42] Overexpression of EZH2 resulted in increased H3K27me3 at the transcription start sites (TSS) of *Runx2* and *Bglap*, both are the matter transcription fate triggering osteogenesis. Enforced expression of EZH2 in bone marrow MSCs inhibits osteogenesis but promotes adipogenesis *in vitro*.[46] Contrary to the consequences above, another study found that deletion of *EZH2* in early pubertal mice results in premature cellular senescence, depleted MSC pool, and impaired osteogenesis as well as osteoporosis in later life.[44] Mechanistically, EZH2 increases H3K27me3 in the promoter regions of cell cycle inhibitor genes including $P15^{INK4b}$, $P16^{INK4a}$, $P21^{CIP1}$, and $P27^{KIP1}$.[44]

KDM6A and KDM6B lead to reduced di- and tri-methylation on H3K27 in cells.[47] KDM6B was significantly reduced in bone marrow MSCs from old mice compared to those from young mice. Knock-down of *KDM6B* reduced bone marrow MSC-mediated bone formation *in vivo* via increasing H3K27me3 levels at the promoters of *BMP2*, *BMP4*, and *HOXC6-1*.[41] Knockdown of *Kdm6a* gene expression in bone marrow MSCs leads to increased adipogenesis and decreased osteogenesis *in vitro*. Enforced expression of KDM6A in bone marrow MSCs decreased H3K27me3 marks at the TSS of *Runx2* and *Bglap*.[46]

Increasing H3K9 methylation related to MSC aging. The methylation status of H3K9 may be a critical epigenetic factor that determines the fate of MSCs. Increasing

Table 1 Histone modification on MSC aging.

Histone modification	Modification site	Regulatory factors	Mechanism	Influence	Reference
Histone methylation	H3K27 me3↑	EZH2↑	Increase H3K27me3 at the *Runx2* and *Bglap* TSS	Promote adipogenesis	[46]
	H3K27 me3↑	EZH2↑	Increase H3K27me3 on the *Wnt* promoters	Promote adipogenic differentiation	[42]
	H3K27 me3↓	EZH2↓	Decrease H3K27me3 on senescence inducer genes and cell cycle inhibitor genes promoters	Cellular senescence, osteoporosis	[44]
	H3K27 me3↑	KDM6A↓	Increase H3K27me3 at the *Runx2* and *Bglap* TSS	Promote adipogenic, decrease osteogenesis	[46]
	H3K27 me3↑	KDM6B↓	Increase H3K27me3 at the promoters of *BMP2*, *BMP4*, and *HOXC6-1*, inhibit the expression of *RUNX2* and *BGLAP*	Reduce bone formation	[41]
	H3K9 me3↑	KDM4B↓	Increase H3K9me3 levels on the promoters of *Wnt* target genes, *Runx2* and *Ccnd1*	Exacerbate bone-fat imbalance in skeletal aging and osteoporosis	[48]
	H3K4 me3↓	KDM5A↑	Demethylate H3K4me3 on *Runx2* promoter	Osteoporosis	[51]
	H3K4 me3↓	ASH1L↓	Reduced H3K4me3 at the *Hoxa10*, *Osx*, *Runx2*, and *Sox9* promoters	Osteoporosis	[54]
	H3R17 me↓	CARM1↓	Decrease H3R17 methylation on the *DDR2* promoter region	Exhibit phenotypical changes associated with cellular senescence	[58]
	H3K36 me3↓	SETD2↓	Alter the H3K36me3 level on the genomic regions	Increase bone marrow adipocytes and decrease bone mass	[56]
Histone acetylation	H3K9 ac↓	GCN5↓	Decrease H3K9ac on the promoters of *Wnt* genes.	Osteoporosis	[65]
	H3K9 ac↓	PCAF↓	Decrease H3K9 ac at *BMP2*, *BMP4*, *BMPR1B*, and *RUNX2* promoters	Osteoporosis	[67]
	H3K9 ac↓	HDAC9↑	Decreased level of H3K9 acetylation at the promoters of autophagy-related genes	Bone mass loss	[62]
	H3K56 ac↑	SIRT6↓	Increase H3K56ac at the *HO-1* promoter	Impairment of differentiation potential of MSCs into bone and cartilage, upregulation of P16 and P21 proteins	[70]
	unknown	HDAC1, HDAC2, HDAC3↑	Decreases histone acetylation	Osteoarthritis	[71]
	H3/H4 ac↓	HDAC4, HDAC5, HDAC6↑	Histone acetylation in *OCT4*, *SOX2*, and *TERT* promoters was reduced	Mean telomere length reduces	[59]
	H3 ac↑	HDAC1, HDAC2↓	Promote H3 acetylation on the *KDM6B* promoter	Upregulation of $P16^{INK4A}$	[60]
	unknown	HDAC9c↓	EZH2 attenuates the expression of HDAC9c	Attenuate osteogenic, enhance adipogenic	[72]
	H3/H4 ac↑	HDAC inhibitor↑	Increase the acetylation of histone H3 and H4	Inhibit proliferation and enlarge the cell shape	[61]

Notes: ↑: increase; ↓: decrease.

H3K9me3 has been proposed to be associated with bone marrow MSC aging.[48] KDM4B (also known as JMJD2B) is a histone demethylase with catalytic activity against the histone residues H3K9me3, H3K9me2, H3K36me3, H3K36me2, H4K20me2, and H1.4K26me3.[49] The status of H3K9me2 is important to the maintenance of stem cell self-renewal and proliferation.[50] Down-regulate of KDM4B impairs MSC self-renewal and promotes MSC exhaustion. Loss of *KDM4B* exacerbates bone-fat imbalance in skeletal aging and osteoporosis.[48] A study of genome-wide histone H3K9me2 profiles at gene promoters in human bone marrow MSCs found that genes involved in canonical signaling pathways, cell cycle pathways, and cytokine-related pathways might be regulated by H3K9 modifications.[50] Further, KDM4B was present on the promoters of *Wnt* target genes, *Runx2* and *Ccnd1* in mouse MSCs. Deletion of KDM4B significantly increased H3K9me3 levels on their promoters, respectively.[48] However, the detailed mechanisms of the cellular pathways affected by H3K9 modifications in human MSC self-renewal and differentiation need to be investigated by further investigation.[50]

Decreasing H3K4 methylation related to MSC aging. The expression level of H3K4me3 was markedly decreased in an ovariectomized mouse model.[51] A study showed MSCs derived from obese swine abdominal adipose tissue displayed decreased H3K4me3 compared to lean pig MSCs, suggesting H3K4me3 is related to adipogenesis of MSCs.[52] H3K4me3 levels are regulated by KDM5A and ASH1L. KDM5A was shown to act specifically on H3K4 methylations (me1/2 and 3), and H3K4me3 is the preferred substrate.[53] KDM5A was significantly increased in bone marrow MSCs isolated from ovariectomized mice. In KDM5A overexpressed bone marrow MSCs, the levels of H3K4me3 modification at the *Runx2* promoter were lower than that in bone marrow MSCs from normal mice.[51] ASH1L is a member of the Trx family, which has been reported to activate gene expression through the H3K4 methyltransferase activity of its SET domain.[54] ASH1L expression was decreased in the ovariectomized mouse model, and the amount of H3K4me3 was also decreased. Knockdown of *Ash1l* resulted in reduced H3K4me3 at the *Hoxa10*, *Osx*, *Runx2*, and *Sox9* promoters. But the silence of *Ash1l* had no detectable impact on the levels of H3K4me3 in the PPARγ promoter region, suggesting that ASH1L does not directly bind to its promoter region.[54]

Other types of histone methylation related to MSC aging. Besides H3K27, H3K9, and H3K4, other types of histone methylation are involved in the regulation of MSC differentiation and aging. The H3K36me3 level is decreased during adipocyte differentiation of MSCs *in vitro*. SET-domain-containing 2 (SETD2) is a methyltransferase responsible for H3 lysine 36 tri-methylation.[55] Loss of *SETD2* in bone marrow MSCs *in vitro* facilitated differentiation propensity to adipocytes rather than to osteoblasts. Deletion of *Setd2* in mouse bone marrow MSCs resulted in bone loss and marrow adiposity. Integrating the ChIP-seq data with the RNA-seq data found that 983 genes showed both the change of expression level and the loss of H3K36me3 peaks after SETD2 deficiency, indicating that the altered H3K36me3 level on the genomic regions might regulate the expression levels of these genes.[56] CARM1 methylates arginine residues 2, 17, and 26, on histone H3. H3R17 is the major CARM1 target site.[57] The late-passage human bone marrow MSCs exhibited phenotypical changes associated with cellular senescence compared to their early-passage counterpart during *in vitro* culture. Inhibition of CARM1 expression in human bone marrow MSCs induced cellular senescence and DDR2 downregulation. Further, bone marrow MSCs treated with CARM1 inhibition demonstrated a significant decrease in CARM1 accumulation and H3R17 methylation in the promoter region of *DDR2*.[58]

Impact of histone acetylation on MSC aging

MSCaging appears to be associated with changes in histone acetylation levels in the gene promoter. Changes in global histone acetylation levels are detected in aging MSCs. Recent studies were unclosed that some sites of histone acetylation, such as H3K9, H3K14, and H3K56, are closely related to the aging of MSCs.

Pan-acetylation related to MSC aging. Human fetal placental MSCs gradually age during *in vitro* culture. After long-term culturing, the expression of HDAC4, HDAC5 and HDAC6 was significantly up-regulated, and acetylation of both histone H3 and histone H4 was significantly reduced. Histone acetylation in *OCT4*, *SOX2*, and *TERT* (telomerase reverse transcriptase) promoters was reduced in fetal placental MSCs at passage 8 of *in vitro* culture relative to cells at passage 3.[59] The expression levels of HDAC1, HDAC2, c-MYC, and PcGs were downregulated upon replicative senescence of human adipose tissue-derived MSCs and human umbilical cord blood-derived MSCs *in vitro*. Inhibition of HDAC1 and HDAC2 promotes H3 acetylation on the *KDM6B* promoter, to upregulate KDM6B expression, resulting in decreased expression of H3K27me3 on the $P16^{INK4A}$ promoter region and an increase in $P16^{INK4A}$ expression.[60]

HDAC inhibitors inhibit the proliferation and relatively enlarge the cell shape of MSCs that originated from adipose tissue or umbilical cord blood MSCs. These changes are essential characteristics of cell senescence, although the HDAC inhibitors did not increase the senescence biomarker, SA-β-gal, in MSCs. HDAC inhibitor activated the transcription of $P21^{CIP1/WAF1}$ by increasing the acetylation of histone H3 and H4 and eventually blocked the cell cycle at the G2/M phase.[61]

Decreasing H3K9 acetylation related to MSC aging. Decreased acetylation level of H3K9 was observed in aged bone marrow MSCs.[62] Histone H3K9 acetylation level can be regulated by general control non-derepressible 5 (GCN5, also known as KAT2A), P300/CBP-associated factor (PCAF, also known as KAT2B), and HDAC9. GCN5 is likely to acetylate the majority of lysine sites in H3 and H2B except for H3K14. Histone H3K9 is a primary target of GCN5 HAT activity *in vivo*.[63] GCN5 was decreased significantly in bone marrow MSCs of aged mice. Compared with sham-operated mice, MSCs from ovariectomized mice also displayed significantly reduced *Gcn5* expression at the early stage of osteoporosis,[64] suggesting that GCN5 is closely

related to the dysfunction of osteoporotic bone marrow MSCs. Knockdown of *Gcn5* significantly decreased H3K9ac levels in bone marrow MSCs and inhibited osteogenic differentiation of bone marrow MSCs *in vivo*. Mechanistically, GCN5 regulates osteogenesis partly by influencing acetylation on H3K9 on the promoters of *Wnt* genes.[65]

PCAF is competent in both histone H1 and histone H3 acetylation.[66] Knockdown of *PCAF* reduced PCAF binding to the *BMP2*, *BMP4*, *BMPR1B*, and *RUNX2* promoters, which was associated with decreased levels of H3K9 acetylation.[67] PCAF is enquired for MSC-mediated bone formation *in vitro* and *in vivo* through bone morphogenetic protein signaling and might be involved in osteoporosis.

Gene activation affected by H3K9 acetylation may be essential to the maintenance of MSC self-renewal and multi-potency.[50] Increased expression of HDAC9 and decreased level of H3K9 acetylation were observed during age-related bone mass loss. HDAC9-induced imbalance in the differentiation of aged bone marrow MSCs was partially attributed to impaired autophagy. Aged bone marrow MSCs possessed fewer autophagosomes than young cells and the expression levels of the autophagy-related proteins were reduced in aged bone marrow MSCs, which suggests impaired autophagic activity in aged cells. The levels of HDAC9 binding to the promoters of autophagy-related genes, such as *ATG7*, *BECN1*, *LC3a*, and *LC3b*, significantly increased in aged bone marrow MSCs. In contrast, the level of H3K9ac binding to these promoters was significantly reduced in aged bone marrow MSCs.[62] Therefore, H3K9 acetylation levels are related to age-related bone loss.

Decreasing H3K14 acetylation related to MSC aging. H3K14 acetylation levels are changing on different gene promoters during aging, which is in accordance with the fact that the expression of genes associated with stem cell pluripotency and proliferation decreased markedly during culture expansion. When comparing MSCs at passage 1 with MSCs at passage 6 during *in vitro* culture, dramatic alterations in histone H3 acetylation in K 9 and K14 were observed in *TERT*, *SOX2*, *OCT4*, *RUNX2*, and *ALP* genes. Down-regulation in the expression of *TERT*, *SOX2*, and *OCT4* genes was closely associated with decreases in H3K9 and H3K14 acetylation levels in the promoter regions of the corresponding genes. Moreover, up-regulated expression of *RUNX2* and *ALP* genes was accompanied by increases in H3K9 and acetylation levels in the promoter of the corresponding genes. However, the role of the acetylation levels of H3K14 alone in MSC aging is not discussed in this study.[68]

Increasing H3K56 acetylation related to MSC aging. H3K56 acetylation levels increased during aging. SIRT6 has been well-defined as an NAD^+-dependent histone deacetylase targeting specific sites on histone H3. H3K56 has been determined to be a substrate of SIRT6.[69] SIRT6 deficiency leads to an increase in ROS levels and vulnerability to oxidative insults, which results in impairment of the differentiation potential of MSCs into bone and cartilage. *SIRT6* depletion in human MSCs upregulates P16 and P21 proteins and accelerates cellular senescence during *in vitro* culture. SIRT6 binds with both nuclear factor erythroid 2-related factor 2 (NRF2) and RNA polymerase II to form a protein complex, which was required for the transactivation of NRF2-regulated antioxidant genes, including heme oxygenase 1 (*HO-1)*. SIRT6 is responsible for the deacetylation of H3K56ac at the *HO-1* promoter in MSCs, which is important for the recruitment of RNA polymerase II transcriptional machinery.[70]

Other types of histone acetylation related to MSC aging. A few studies found that histone acetylation levels are decreased during aging. Histone acetylation levels are significantly lower in cells in the bone degenerative disease tissue compared to healthy bone tissue.[71] Osteoarthritic bone contains more senescent cells compared to healthy bone tissue. In osteoarthritis, HDAC1, HDAC2, and HDAC3 were highly up-regulated. Increased HDAC activity, in turn, decreases histone acetylation.[71] The increasing and decreasing trend in EZH2 and HDAC9c expression was observed with age, respectively. Increased EZH2 was found to attenuate the expression of HDAC9c in aged MSCs, leading to weaker osteogenic potential and stronger adipogenic potential.[72]

Epigenetics-based strategies for MSC aging and age-related diseases

Comprehending detailed epigenetic mechanisms could provide us with a novel horizon for dissecting MSC-related pathogenesis and further optimizing MSC-mediated regenerative therapies. These alterations of histone modification during aging and age-related diseases are theoretically reversible by using the epigenetic modulator, suggesting epigenetic modifications as therapeutic targets for aging. By regulating histone methylation levels and histone acetylation levels, novel therapies for MSC aging and age-related diseases, especially osteoporosis, are provided (Table 2).

Epigenetic therapy targeting histone methylation

Restoration of the decreased levels of histone methylation during aging. According to recent studies, up-regulation of the decreased H3K27, H3K4, H3K36, H3R17, and H3K9 methylation levels provided a novel therapeutic strategy for improving bone formation. Histone methylation levels can be up-regulated by increasing histone methyltransferases (HMTs) and inhibiting histone demethylases (HDMTs).

HMTs, including EZH2, ASH1L, SETD2, and CARM1, have been tested as therapeutic targets. Basic helix-loop-helix transcription factor Twist-1, a regulator of Ezh2 recruitment, can greatly enhance the life span of bone marrow MSCs.[73] Twist-1 overexpression dramatically reduced the levels of *P14*, *P16*, and *P21* transcripts in bone marrow MSCs *in vitro*. In a later passage, Twist-1-overexpressing human bone marrow MSCs exhibited a significantly reduced ratio of senescent cells. Twist-1 induces EZH2 recruitment to increase H3K27me3 along the *Ink4A/Arf* locus, repressing the senescence of human bone marrow MSCs.[43] $1,25(OH)_2D_3$ inhibited the senescence of bone marrow MSCs and osteocytes. Comparison between $1,25(OH)_2D_3$-treated old mice and those control mice demonstrated that supplementation

Table 2 Epigenetics-based strategy for MSC aging and age-related diseases.

Histone modification	Drugs or regulator	Histone modifier	Mechanism	Therapeutic effect	Reference
Up-regulation of the decreased levels of histone methylation	Twist-1	EZH2	Increase H3K27me3 along the *Ink4A/Arf* locus, repress transcription of both P16/P14	Reduce the ratio of senescent cells and continued to proliferate	[43]
	1,25(OH)2D3	EZH2	Up-regulate Ezh2 via the vitamin D receptor (VDR)-mediated transcription to increase H3K27me3.	Prevent bone loss	[74]
	1,25(OH)2D3	unknown	Up-regulate H3K9me2 and down-regulate H3ac at the *BMP2* promoter region and activated the Wnt/β-catenin signaling.	Prevent bone loss	[75]
	Lentivirus transduction	ASH1L	Regulate the enrichment of H3K4me3 at the *Hoxa10*, *Osx*, *Runx2*, and *Sox9* promoters	Facilitate osteogenesis and chondrogenesis, suppress adipogenesis	[54]
	LBP	SETD2	This leads to a decrease of H3K36me3 in the *Lbp* gene body	Rescue the lack of osteogenesis and enhanced adipogenesis	[56]
	siRNA Transfection	CARM1	Bind to the promoter region of *DDR2* and methylated H3R17	Senescence cells were decreased	[58]
	BMP2	KDM5A	Increase the H3K4me3 level at the *Runx2* promoter	Rescue bone loss and the decrease in bone formation	[51]
	Pargyline	LSD1	Enhance the dimethylation level of H3K4 at the promoter regions of *Runx2* and *Bglap* genes	Rescue or prevent the osteoporotic	[77]
Down-regulation of the increased levels of histone methylation	Retroviral transduction	KDM6A	Remove H3K27me3 at the *Runx2* and osteocalcin TSS.	Promote bone formation	[46]
	BMP-4/7	KDM4B KDM6B	Promote *DLX* expression by demethylating H3K9me3. Control *HOX* gene expression by demethylating H3K27me3.	Promote bone regeneration and repair	[41]
	DZNep	EZH2	Reduce EZH2 expression, decreased H3K27me3 levels on promoters of *Wnt1*, *Wnt6*, and *Wnt10a*	Increase trabecular bone, enhance osteogenic differentiation, and inhibit adipogenesis	[42]
	DZNep	EZH2	Reduce EZH2 expression, decrease H3K27me3 at the *Runx2* and *Bglap* TSS	Increase mineral deposit	[46]
	Chaetocin	SUV39 family	Suppress H3K9 methylation via Wnt/β-catenin signaling pathways	Increase in bone tissue	[78]
Up-regulation of the decreased levels of histone acetylation	Lentivirus transduction	GCN5	Increasing H3K9ac on the promoters of *Wnt* genes	Attenuate bone loss during osteoporosis	[65]
	Retroviral infection	PCAF	Control BMP signaling gene expression by increasing H3K9ac.	Inhibit adipocyte	[67]
	AA	PcG KMD6B	Repress KDM6B expression and sustain PcG expression	Downregulate $P16^{INK4A}$ expression levels	[60]
	VPA	HDAC8	Promote the level of H3K9ac and enhance the expression of osteogenesis-related genes	Accelerate the osteogenic mineralization	[79]

Table 2 (*continued*)

Histone modification	Drugs or regulator	Histone modifier	Mechanism	Therapeutic effect	Reference
	HDAC9 inhibitors	HDAC9	Maintains the levels of acetylation modifications on H3K9 of autophagy-related genes	Promote osteogenic differentiation and repress adipogenic	62
	bFGF	unknown	Regulate H3K9ac and H3K14ac at *TERT*, *OCT4*, *SOX2*, *RUNX2*, and *ALP* promoter regions	Promote MSC proliferation	68
Down-regulation of the increased levels of histone acetylation	Lentiviral transduction	SIRT6	Deacetylate H3K56 at the *HO-1* promoter.	Delayed cellular senescence	70
	GSK343	HDAC9c	Regulate the EZH2-HDAC9c regulatory axis, sequester PPARγ-2 and allow *RUNX2* to turn on *OPN* transcription.	Enhance osteogenesis and suppress adipogenesis	72

of exogenous $1,25(OH)_2D_3$ could prevent bone loss induced by natural aging by stimulating osteogenic differentiation and bone formation. Mechanistically, $1,25(OH)_2D_3$ can influence histone methylation in several ways. $1,25(OH)_2D_3$ prevents age-related osteoporosis partly by up-regulating EZH2 via the vitamin D receptor (VDR)-mediated transcription, resulting in increased H3K27me3 and repressed *P16*/*P19* transcription.[74] Bone marrow MSCs treated with $1,25(OH)_2D_3$ exhibited a significant increase in H3K9me2 level at the *BMP2* promoter region and activated the Wnt/β-catenin signaling, providing a new way for osteoporosis treatment. This study also demonstrated that $1,25(OH)_2D_3$ decreases the acetylation of histone H3 at the same *BMP2* promoter region.[75] Treatment of lentivirus vector over-expressing *Ash1l* facilitated osteogenesis and chondrogenesis but suppressed adipogenesis in mouse embryonic fibroblast cell line with multipotency *in vitro*, which was associated with an increase of H3K4me3 at the TSS of *Hoxa10, Osx, Runx2*, and *Sox9*.[54] Bone marrow MSCs infected with lentivirus overexpressing lipopolysaccharide-binding protein (LBP) could partially rescue adipogenesis and promote osteogenesis *in vitro*. LBP might be an attractive reagent for rejuvenating bone because it is a downstream target of *SETD2*.[56] The expression of discoidin domain receptor 2 (DDR2) in adult donor-derived human bone marrow MSCs was lower than it was in the young donor-derived human bone marrow MSCs. siRNA inhibition of DDR2 expression recapitulated features of senescence in early-passage bone marrow MSCs. Direct binding of CARM1 to the DDR2 promoter region with a high level of H3R17 methylation in early-passage bone marrow MSCs, and inhibition of CARM1-mediated histone arginine methylation decreased DDR2 expression and led to cellular senescence.[58] Vitamin-C treatment substantially alleviated SASP in premature senescence of subchondral bone marrow MSCs.[76] Co-incubation of obese MSCs with vitamin-C enhanced 5-hydroxyemthycytosine (5hmC) marks and reduced their global levels of H3K9me3 and H3K27me3. Contrarily, Vitamin-C did not affect 5hmC and decreased H3K4me3 in lean MSCs.[52]

Studies about inhibitors of HDMTs are limited. BMP2 was reported to inhibit KDM5A to remove methyl groups of histone proteins. Treatment of BMP2 partly rescued bone loss in ovariectomized mice by enhancing bone formation, which is possibly attributed to an increased H3K4me3 level at the *Runx2* promoter.[51] Pargyline, an inhibitor of lysine-specific demethylase 1 (LSD1), partially rescued or prevented osteoporotic conditions in aged or ovariectomized mouse models by increasing bone density. Pargyline functioned partly by enhancing the dimethylation level of H3K4 at the promoter regions of *Runx2* and *Bglap* genes.[77]

Down-regulation of the increased levels of histone methylation during aging. Histone demethylation can be promoted by histone demethylases (HDMTs) and inhibitors of histone methyltransferases (HMTs). Several studies indicate decreasing H3K27 and H3K9 methylation levels can prevent MSC aging.

HDMTs, such as KDM4B, KDMA6A, and KDMA6B, are required for the osteogenic differentiation of MSCs.[41,46] Bone marrow MSCs over-expressing KDM6A produced significantly higher levels of bone formation compared to vector control bone marrow MSCs in mice. KDM6A regulated osteogenic differentiation by removing H3K27me3 at the *Runx2* and *Bglap* TSS.[46] BMP-4/7, potent inducers of osteogenic differentiation of MSCs, strongly induced KDM4B and KDM6B expression in human bone marrow MSCs cultured *in vitro*. KDM4B promoted *DLX* expression by demethylating H3K9me3, while KDM6B controlled *HOX* gene expression by directly demethylating H3K27me3.[41] These studies provide us with a new therapeutic target for regulating MSC senescence.

Inhibitors of histone methyltransferases, DZNep and chaetocin, might provide some inspiration for osteoporosis treatment. Bone marrow MSCs treated with 20 μM DZNep showed a significant decrease in oil red O-positive lipid formation and a significant increase in mineral deposits,[46] suggesting that DZNep enhanced osteogenic differentiation and inhibited adipogenesis of bone marrow MSCs of ovariectomized mice. DZNep treatment reduced EZH2 mRNA and

protein levels in bone marrow MSCs and increased trabecular bone of ovariectomized mice.[42] Chaetocin is an inhibitor of the histone methyltransferase SUV39 family. Chaetocin mediates the status of H3K9 methylation via Wnt/β-catenin signaling pathways and its downstream targets. Treatment of chaetocin promotes osteogenic differentiation and inhibits adipogenic differentiation, leading to the increase of bone tissue *in vivo*.[78] All of the studies suggest that epigenetic rejuvenation might be a new strategy for preventing aging and age-related diseases by inhibiting HMTs in MSCs.

Epigenetic therapy targeting histone acetylation

Restoration of decreased histone acetylation during aging. HATs, such as GCN5, and PCAF, upregulate the decreased levels of H3K9ac. Injection of Gcn5-lentiviral expression vector into ovariectomized mice enhanced biomarkers of bone formation and attenuated the decline of bone formation in trabecular bone. H3K9ac levels in promoter regions of *Wnt* genes were also increased after overexpression of GCN5.[65] GCN5 also can inhibit NF-κB signaling by mediating the proteasomal degradation of P65.[64] PCAF may have the potential as a therapeutic target for the treatment of osteoporosis. PCAF controls BMP signaling gene expression by increasing H3K9 acetylation. The levels of PCAF were upregulated in bone marrow MSCs after osteogenic induction. PCAF is required for osteogenic differentiation and inhibits adipocyte lineage.[67]

Some inhibitors of HDACs have been studied and applied to delay aging. Anacardic acid (AA) is an inhibitor of HDAC1 and HDAC2. The $P16^{INK4A}$ expression levels were downregulated by AA treatment in human umbilical cord blood-derived MSCs *in vitro*. AA plays a role in sustaining PcG and repressing KDM6B expression levels by regulating histone acetylation status at its promoter regions.[60] Valproic acid (VPA) treatment accelerated the osteogenic mineralization of bone marrow MSCs *in vitro*. The mRNA levels of multiple osteogenic markers were increased in VPA-treated bone marrow MSCs compared to the control. VPA inhibited HDAC8 expression, which decreased the level of H3K9ac and significantly inhibited the expression of osteogenesis-related genes *RUNX2*, Osterix, *OCN*, *OPN*, and *ALP*. The HDAC8-overexpressed bone marrow MSCs treated with VPA exhibited higher osteogenic mineralization compared to the group without VPA addition, indicating that the inhibitory effect of HDAC8 on the osteogenic differentiation of bone marrow MSCs could be rescued by VPA.[79] Trichostatin A (TSA), sodium butyrate (NaB), and HDAC9 siRNA (siHDAC9) are inhibitors of HDAC9. The low expression level of HDAC9 maintains the high levels of acetylation modifications on H3K9 of autophagy-related genes which promotes intracellular autophagosome formation and subsequently facilitates osteogenic differentiation of bone marrow MSCs.[62] HDAC9 inhibition promoted osteogenic differentiation and repressed adipogenic differentiation and reduced the expression of senescence-related proteins P53 and p-P53 in aged bone marrow MSCs. Treatment of HDAC9 inhibition reduced the number of senescence cells and adipocytes and increased the number of OCN-positive cells in the bone marrow, resulting in increased bone mass in aged mice. Largazole or TSA is a novel histone deacetylase inhibitor. The expression levels of H3K9/K14 acetylation and H3K4 dimethylation in the *OCT4*, *NANOG*, *TERT*, and *CXCR4* gene promoters were higher in the TSA and largazole groups than in the control group. Treatment of TSA and largazole promoted the proliferative abilities of human umbilical cord MSCs.[80] Besides, basic fibroblast growth factor (bFGF), a potent cytokine being used as a supplement to MSCs culture, was found to suppress alterations in H3K9ac and H3K14ac in *TERT*, *OCT4*, *SOX2*, *RUNX2*, and *ALP* genes promoter. MSCs grown in a growth medium supplemented with bFGF had a 10-fold increase in cumulative cell number at passage 4 compared to MSCs grown in a growth medium alone.[68]

Down-regulation of redundant histone acetylation during aging. In recent studies, different HDACs have been studied to down-regulate histone acetylation levels. SIRT6 diminished cellular ROS levels and delayed cellular senescence by regulating the transactivation of NRF2-regulated antioxidant genes, including heme oxygenase 1 (HO-1). SIRT6-deleted human MSCs could be alleviated by lentiviral vector-mediated reconstitution of exogenous SIRT6.[70] EZH2 binds to the HDAC9c promoter to suppress its gene expression. Lower expression of EZH2 promotes expression of HDAC9c, which sequesters PPARg-2 and allows *RUNX2* to turn on *OPN* transcription, leading to increased osteogenesis. Treatment of GSK343, an EZH2 inhibitor, enhanced the osteogenic potential of and inhibited the adipogenic potential of MSCs in aged mice.[72]

Conclusions and future directions

In summary, the aging of MSCs is closely associated with aberrant epigenetic modifications. The landscape of epigenetic modifications could be used to predict the aging of cells, tissues, or individuals. Several key regulators of histone modification have been demonstrated to play a role in regulating MSC function and aging. The dysregulation of specific epigenetic modifications would lead to the decline of MSC function and aging. Due to the reversibility of epigenetic regulation, it would be a potential target for antiaging treatment (Fig. 2). Histone modification is involved in diverse physiological functions and the aging process of MSCs. In this context, we reviewed the histone modification in the process of aging and the regenerative strategies based on epigenetic regulation of MSC aging. However, further studies need to be done to fully understand the epigenetic changes and provide clues for age-related disease therapy.

Other types of histone modification on MSC aging

Histone modifications are diverse and complicated, including methylation, acetylation, phosphorylation, acetylation, sumoylation, et al. Several types of histone modifications that participated in the aging process of MSCs have been concluded in this review. However, the understanding of other types of modification to stem cell aging is limited. Emerging evidence showed that histone modifications other than methylation and acetylation, also play a profound effect on stem cells. For example, the phosphorylation of serine 10 on histone H3 (H3S10ph) is important for chromosome condensation and cytokinesis during mitosis in mammals. Aurora kinase B (*AURKB*) regulates H3S10ph.

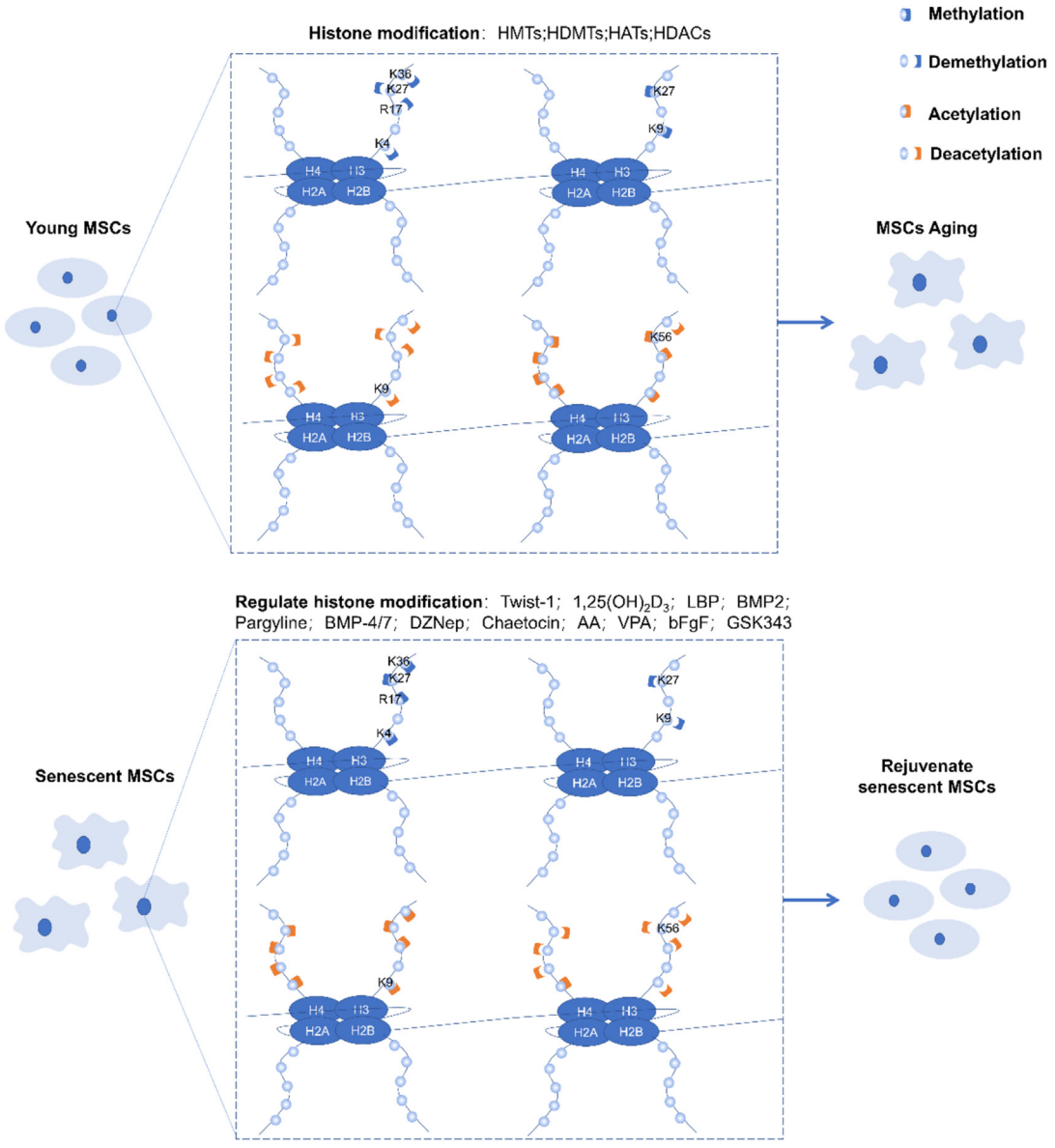

Figure 2 Histone modification regulating MSC aging and epigenetic therapy targeting histone modification. There are changes in histone modification during aging. It is characterized by the senescence and exhaustion of MSCs. Through drugs or other regulators, it can reverse the histone modification in the process of aging and rejuvenate senescent MSCs, to achieve the purpose of anti-aging.

Silencing *AURKB* expression inhibits gastric cell proliferation and arrests the cell cycle in the G2/M phase.[81] The histone H3 threonine 11 phosphorylation (H3pT11) functions as a marker for nutritional stress and aging. *H3pT11* defective mutants prolonged their chronological lifespan by altering nutritional stress responses. Sch9 and CK2 kinases cooperatively regulate H3pT11 under stress conditions.[82] Therefore, the investment of other types in histone modification is necessary to understand the complicated epigenetic code on stem cell function and destiny.

Epigenetic networks in regulating MSC aging

We can conclude from the above that histone modification often does not work individually. Furthermore, histone modification can also be coupled with other epigenetic modifications to produce complex epigenetic effects. DNA methylation sites have been discovered to be associated with histone modification. MSCs showed highly consistent senescence-associated modifications at specific CpG sites. These DNA-methylation changes correlated with histone marks, such as H3K9me3, H3K27me3, and other EZH2 targets.[83] The INO80 chromatin remodeling complex interacts with WD repeat-containing protein 5(Wdr5) protein that catalyzes H3K4me3 formation to positively regulate the canonical Wnt pathway.[84] The crosstalk and interplay between different epigenetic modifications make it much more difficult to comprehensively understand the mechanism of aging.

The discrepancy between histone modifications on MSC aging

It has been found that the effect of specific histone modifications on stem cell aging is complex. On the one hand, different modification sites on the same genes and the same modification sites on different genes can have different influences. On the other hand, different modification sites on different genes can even produce the same effect. This evidence suggests that epigenetic modification co-operated with other regulatory mechanisms to orchestrate the destiny and function of MSCs. The evidence also suggests the choice of targets for epigenetic therapy should be prudent and consider the specific situations of aging.

Specific regulation of tri-methylated (me3) histone

Tri-methylation has been studied extensively because it is generally associated with gene repression. The H3K27me3 signals were modestly elevated at the silent promoter and reduced at active promoters and genic regions, whereas not much change was observed in intergenic regions. Recent studies showed that, besides H3K27me3, H3K27me1 and H3K27me2 also take part in the regulation of MSC function. H3K27me2 signals were higher at active promoters than silent promoters, particularly downstream of the TSS, H3K27me2 had a similar distribution with H3K27me3, though less biased toward silent genes.[40] However, the present strategies would affect the whole process of methylation and simultaneously regulate the mono-methylation, di-methylation, and tri-methylation of histone. Further studies are needed to elucidate the effect of these different types of histone methylation on MSC aging.

Clinical trials of epigenetic therapy on age-related diseases

The US FDA has accepted HDAC inhibitors, EZH2 methyltransferase inhibitors, HDAC6 inhibitors, and other epigenetic agents as therapies for different cancers. Tazemetostat (Tazverik™), a first-in-class, small-molecule EZH2 inhibitor, received accelerated approval for the treatment of adults and adolescents with locally advanced or metastatic epithelioid sarcoma not eligible for complete resection.[85] Belinostat is an intravenously administered histone deacetylase inhibitor and antineoplastic agent that is approved for use in refractory or relapsed peripheral T-cell lymphoma.[86] HDAC inhibitor valproic acid (VPA) has been shown to alter the proliferation, survival, cell migration, and hormone receptor expression of breast cancer cells in both the pre-clinical and clinical settings.[87] But more extensive studies are necessary to investigate the potential of epigenetic therapy for other age-related diseases.

Author contributions

Y. Sun: collected and collated documents, and wrote the manuscript; H. Zhang: collected and collated documents, and wrote the manuscript; L. Liao: revised and modified the manuscript; X. Su: collected and collated documents, and wrote and proofed this review. All authors read and approved the final version of the manuscript.

Conflict of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interests.

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