Chapter

Epigenetic Control of Mesenchymal Stromal Cell Fate Decision

Haoli Ying, Ruolang Pan and Ye Chen

Abstract

Mesenchymal stem cells (MSCs) are progenitors of connective tissues, which have emerged as important tools for tissue engineering owing to their differentiation potential in various cell types. The therapeutic utility of MSCs hinges upon our understanding of the molecular mechanisms involved in cellular fate decisions. Thus, the elucidation of the regulation of MSC differentiation has attracted increasing attention in recent years. A variety of external cues contribute to the process of MSC differentiation, including chemical, physical, and biological factors. Among the multiple factors that are known to affect cell fate decisions, the epigenetic regulation of MSC differentiation has become a research hotspot. In this chapter, we summarize recent progress in the determination of the effects of epigenetic modification on the multilineage differentiation of MSCs.

Keywords: mesenchymal stromal cells, post-translational modifications, differentiation, cellular fate decision, epigenetic regulation

1. Introduction

Mesenchymal stromal cells (MSCs) are progenitors of connective tissues, initially characterized as plastic adherent, fibroblastic cells, with the potential to differentiate into many types of cells, including predominantly osteoblasts (cells that secrete the matrix of the bones), chondrocytes (cells embedded in the lacunae of the cartilage matrix), and adipocytes (fat-storing cells), under appropriate conditions. MSC studies have progressed rapidly since the initial report of human MSC isolation from bone marrow. MSCs have been shown to reside within the connective tissues of most organs. Owing to their ease of isolation and unique characteristics, MSCs have been widely regarded as potential candidates for tissue engineering and repair. Further, the fate decision of MSCs has also piqued the interest of scientists. During the last two decades, various signaling molecules important to MSC differentiation have been identified, and the epigenetic regulation of MSC differentiation has recently become a research hotspot.

The transformation process of MSCs from a self-renewing state to a specific lineage is always accompanied by changes in cell morphology and function, which are largely determined by the differential expression of genes. Specifically, genes related to self-renewal are turned off, and transcription of cell type-specific genes is activated. Epigenetic regulation refers to the phenotypic change through gene

differential expression without DNA sequence alteration, including four main categories: [1] DNA methylation, [2] histone modifications, and [3] chromatin remodeling (nucleosome positioning); and [4] non-coding RNAs. It has been widely reported that epigenetic and post-translational modifications have a broad and far-reaching influence on MSC differentiation at multiple levels. Here, we provide an overview of the recent findings regarding the roles of epigenetic modification in the fate decision of MSCs.

2. DNA methylation

DNA methylation is an important epigenetic modification referring to the addition of a methyl (-CH3) group to the fifth carbon atom of a cytosine ring to form 5-methylcytosine (5-mC). The process is catalyzed by enzymes known as DNA methyltransferases (DNMTs). DNA methylation was the first epigenetic mark to be discovered, and it plays an important role in normal human growth, development, aging, tumorigenesis, and other genetic and epigenetic diseases. This epigenetic mark has the ability to turn genes on or off and can be inherited through cell division. Recent studies have suggested that methylation and demethylation of specific genes, such as *Runx2*, osteopontin (*Opn*), distal-Less homeobox 5 (*Dlx5*), osterix, collagen type 2 (*Col2*), and *Col10*, play key roles in the multi-lineage differentiation of MSCs.

2.1 Osteogenic differentiation of MSCs and DNA methylation

According to numerous studies, DNA methylation is dynamically involved in the osteogenesis of MSCs. Generally, it may be considered that DNA methylation has a repressor role in the promoter regions with CpG islands, blocking gene expression. During osteogenic differentiation, demethylation was observed at specific CpG regions in the promoters of osteogenic lineage-specific genes, such as *RUNX2, DLX5, SP7, SPP1 OPN, COX2,* alkaline phosphatase *(ALP)*, and osteocalcin *(OCN)*, and the expression of these genes was sequentially increased, whereas the expression of pluripotent genes and hypermethylated promoters was downregulated.

Villagra *et al.* observed a significant hypermethylation at the osteocalcin gene locus in undifferentiated MSCs, and the CpG methylation of the osteocalcin promoter significantly decreased upon osteogenesis induction [1]. Dansranjavin *et al.* reported that the differentiation of MSCs was accompanied by a reduced expression of stemness genes such as LIN28, via the hypermethylation of their promoter regions [2]. Arnsdorf *et al.* reported a protocol to promote MSC osteogenic differentiation by applying a mechanical stimulus [3]. According to their results, mechanical stimulation causes the release of Dnmt3b from bone-specific genes, thus leading to promoter demethylation and upregulated gene expression. Yang *et al.* found that the depletion of demethylase Tet1 and Tet2 may hinder the demethylation of the P2rX7 promoter, resulting in a decrease in the osteogenic differentiation capacity of MSCs [4]. Furthermore, the involvement of DNA methylation in the osteogenic differentiation of MSCs has been supported by differentiation studies using demethylating agents. For example, Zhou *et al.* reported that pretreatment with 5-aza-2′-deoxycytidine (5-ADC) drives the osteogenic differentiation of MSCs by enhancing the expression of osteogenic genes (such as *Dlx5*) associated with the demethylation of the CpG shore [5]. Abnormal changes in the methylation modification mechanism in osteogenic differentiation are associated with the occurrence and development of many common skeletal diseases. García-Ibarbia *et al.* analyzed hip fracture samples from patients with osteoporosis and found that the activity of the Wnt signaling pathway

in osteoblasts was reduced. The methylation statuses of *Fzd10, Tbl1x, Csnk1e, Wnt8A, Csnk1a1l*, and *Sfrp4* were also observed to be significantly different from those found in normal bone tissues [6]. Another study explored the differences in gene-wide DNA methylation patterns in osteoporosis and osteoarthritis. The results showed that there was a total of 241 Cpg sites with significant differences in the methylation status. Bioinformatic analysis showed that the sites of difference were mostly related to cell osteogenic differentiation and skeletal embryonic development, especially the homeobox family genes [7]. Sun *et al.* reported that abnormal CpG island hypermethylation of the ABCB1 gene promoter was correlated with glucocorticoid (GC)-associated osteonecrosis of the femoral head (ONFH) in patients [8].

2.2 Adipogenic differentiation of MSCs and DNA methylation

Adipogenesis is highly regulated by a sequential cascade of transcriptional events. Key transcriptional factors controlling adipogenesis include several CCAAT/enhancer-binding protein (C/EBP) family members, including C/EBPα, β, and δ, and the nuclear receptor peroxisome proliferator γ (PPARγ). On the other hand, a number of negative transcriptional factors have also been identified, including GATA2/3, chicken ovalbumin upstream promoter transcription factor (COUP-TF), interferon regulatory factors (IRFs), and Wnt family proteins.

Barrand *et al.* showed that the promoters of stemness genes (*OCT4, NANOG*, and *SOX2*) were hypermethylated in adipose-derived MSCs [9]. In addition, the promoters of adipogenic genes, including leptin (LEP), PPARγ2, fatty acid-binding protein 4 (FABP4), and lipoprotein lipase (LPL), are hypomethylated in these undifferentiated MSCs, as revealed by Noer *et al* [10]. It was reported by Fujiki *et al.* and Melzner *et al.* that the promoters of PPARγ2 and LEP were progressively demethylated along with the terminal differentiation of adipocytes [11, 12]. Studies also revealed that the levels of the DNA methyltransferase 1 (*DNMT1*) gene transcript increased at the beginning of adipogenesis and then decreased [13], while the levels of *DNMT3a* and *DNMT3b* transcripts increased during differentiation [14]. Wnt signaling is a key determinant of the fate between adipogenic and osteogenic differentiation. Chen *et al.* proved that the methylation level of the Wnt10a 5-region was markedly reduced in MSCs after 5-Aza-dC treatment, which likely significantly inhibited adipogenesis and promoted osteogenesis [15].

2.3 Chondrogenic differentiation of MSCs and DNA methylation

DNA methylation and demethylation status also influence MSC chondrogenic differentiation. DNA methylation at specific CpGs has been shown to influence genes such as *MMP13, IL1, iNOS*, chondromodulin, collagen 9, and *GDF5* in chondrocytes. Similarly, induction of *COL10A1* expression during chondrogenesis of MSCs is correlated with the demethylation of two CpG sites in the *COL10A1* promoter. In addition, Kim *et al.* also showed an elevated extent of DNA methylation in the *SOX9* promoter in damaged chondrocytes of osteoarthritis (OA) patients compared to the observation in normal chondrocytes [16]. More recently, Barter *et al.* characterized the DNA methylation changes during the chondrogenesis of MSCs using an Infinium 450 K methylation array. A chondrocyte-specific methylation profile was established by comparison with cartilage and non-cartilage tissue methylation profiles, and they also identified significant changes in DNA hypomethylation at many key cartilage gene loci during chondrogenic differentiation, including *COL11A2, SOX9*, and *ACAN* [17]. Further studies analyzing these epigenetic changes during chondrogenesis are needed.

3. Histone epigenetic modification

Histone modification, a common form of epigenetic regulation, refers to post-translational modifications that are added to the N-terminal tail of histones. Histone modification has been shown to play important roles in regulating cell-specific gene expression. So far, more than sixty different residues on core histones (H2A, H2B, H3, H4) with potential to be modified have been reported. These modifications made to histones, including acylation, methylation, phosphorylation, ubiquitination, and sumoylation, can impact gene expression by altering the chromatin structure or recruiting histone modifiers. Histone proteins function to package DNA, which wraps around the eight histones, into chromosomes. In general, it has been well established that histones on the promoter regions of master transcription factors associated with MSC cell fate commitment, such as *RUNX2* and *OSX* in osteogenic differentiation, *PPARG* and *CEBPA* in adipogenic differentiation and *SOX9* in chondrogenic differentiation, are dynamically modified (**Table 1**). In response to appropriate developmental and/or differentiation signals, histone modifications act in diverse biological processes such as transcriptional activation/inactivation, chromosome packaging, and DNA damage/repair. Furthermore, different types of modifications may have synergistic or antagonistic effects to regulate specific gene expression.

3.1 Histone acetylation modification

Histone acetylation is an epigenetic modification characterized by the addition of an acetyl group ($COCH_3$) to histone proteins, specifically to lysine residues within the N-terminal tail. Histone acetylation is one of the most common epigenetic modifications, which leads to the neutralization of the positive charge on the histone proteins, weakening their interaction with DNA, and finally promoting the opening of chromatin structure and activating gene transcription. On the other hand, histone deacetylation is related to chromatin transcription inhibition. The level of histone acetylation is mainly regulated by histone acetylase (HAT) and histone deacetylase (HDAC).

The degree of histone acetylation of related regulatory genes can reflect the maintenance of stemness and the differentiation status of MSCs. During the process of osteogenic differentiation, the expression of osteogenic genes (such as *RUNX2, OSX*, and *ALP*) gradually increases in MSCs, while the expression of stemness-related genes (such as *OCT4* and *SOX2*) is significantly decreased. These changes in gene expression were found to be closely related to H3K9Ac and H3K14Ac, which can be used as marks of gene activation. The regulatory roles of histone acetylases in osteoblast differentiation have been increasingly recognized. According to previous and existing studies, *HDAC1, HDAC6, HDAC8*, and *SIRT1* play important roles in the differentiation of MSCs. For example, Wang *et al.* found that inhibiting the expression of histone deacetylase 1 (HDAC1) can effectively enhance the osteogenic differentiation, gene expression, and the bone formation activity of bone marrow MSCs under mechanical stimulation [25]. Lu *et al.* showed that HDAC1 has a negative correlation with cardiac cell differentiation of MSCs under a myocardial microenvironment. During this process, the expression of HDAC1 in MSCs was significantly decreased in a time-dependent manner. In addition, their data proved that the knockdown of HDAC1 promoted the directed differentiation of MSCs into cardiac cells [26]. Several studies have shown that the expression of HDAC6 is reduced during osteogenic differentiation, and HDAC6 negatively regulates the expression of *OC,* osteopontin *(OPN), BSP2, OSX*, and *ALP* partly by binding to the *RUNX2* C-terminus and adjusting *RUNX2* activity [27–29]. Fu *et al.* reported

Involved epigenetic histone modification	Factor	Mechanism	Result	Reference
Histone deacetylation	HDAC inhibitor	Stimulate the transcription of $p21_{CIP1/WAF1}$ through enhancing the H3 and H4 acetylation	Arrest the cell cycle at the G2/M check point, inhibit adipogenic, chondrogenic, and neurogenic differentiation; promote osteogenesis	[18]
Histone acetylation	Knockdown of PCAF (histone H3K9 acetyltransferase)	Insufficient to increase H3K9 acetylation at promoters of BMP2, BMP4, BMPR2B, and Runx2	INHIBIT adipogenic differentiation and promote osteogenic differentiation in MSCs; reduce the bone formation both in vitro and in vivo	[19]
Histone acetylation	GCN5 knockdown	Insufficient to inhibit NF-κB signaling by mediating the proteasomal degradation of p65 (acetyl K310)	Inhibits osteogenic differentiation of MSCs	[20]
Histone deacetylation	SIRT1 knockout	Insufficient to deacetylate β-catenin to promote its accumulation in the nucleus	Reduce differentiation towards osteoblasts, and chondrocytes	[21]
Histone demethylation	Overexpression of KDM5A	Decrease H3K4me3 levels on promoters of Runx2 by demethylating H3K4me3	Inhibit osteogeninsis; lead to osteoporosis	[22]
Histone methylation	G9a inhibitor	Unclear (correlate with PPARγ and C/EBPα expression)	Impair the proliferation but the anti-proliferative effect is not sustained; increase adipogenic potential and decrease osteogenic potential of MSCs	[23]
Histone methylation	Downregulation of BMI1	Insufficient to recruit and stabilize PRC2 which trimethylate H3K27	Cellular senescence	[24]
Histone methylation	Downregulation of EZH2	Insufficient to trimethylate H3K27 as catalytic subunit of PRC2 and keep a high extent of H3K27me3 to suppress $p16^{INK4A}$-induced senescence	Cellular senescence	[24]

Table 1.
Histone modification in MSC differentiation and aging.

that the pharmacological inhibition of HDAC8 by the HDAC inhibitor, valproic acid (VPA), increased the levels of H3K9Ac and significantly enhanced the expression of the osteogenesis-related genes *RUNX2*, osterix, *OCN*, OPN, and *ALP*. Similarly, knockdown of HDAC8 enhanced the osteogenic differentiation of MSCs [30]. Furthermore, SIRT1, an NAD + -dependent deacetylase, also acts as a key regulator of MSC differentiation. The decrease in its activity reduces the expression of the stemness factor Sox2, which leads to the degradation of the self-renewal and differentiation ability of MSCs [31]. It was reported that SIRT1 knock-out MSCs showed reduced differentiation toward osteoblasts and chondrocytes in vitro, but showed no difference in proliferation or apoptosis. Petra *et al.* showed that SIRT1 deacetylates β-catenin to promote its accumulation in the nucleus, leading to the transcription of genes for MSC differentiation [21]. Additionally, epigenetic research has shed light on the effects of histone acetylation on adipogenesis and chondrogenesis. Chemically distinct HDAC inhibitors have been shown to prevent adipocyte differentiation [32]. It has been reported that SIRT2 acts as an important regulator of adipocyte differentiation through the deacetylation of forkhead box protein O1 (FOXO1), peroxisome proliferator-activated receptor gamma (PPARγ), and CCAAT/enhancer binding protein β (C/EBPβ) [33, 34]. Nakade *et al.* showed that Jun dimerization protein 2 (JDP2) plays a key role as a repressor of adipocyte differentiation by regulating the expression of C/EBPδ via the inhibition of histone acetylation [35]. In addition, SIRT1 can also promote the cartilage differentiation process of MSCs by activating the deacetylation of Sox9 and NF-κB [36].

3.2 Histone methylation

Histone methylation is another common post-translational modification by which methyl groups are transferred to the amino acids of histone proteins that make up the nucleosomes. Histone methylation can occur at various sites in histone proteins, primarily on lysine and arginine residues, and it can be governed by multiple positive and negative regulators, even at a single site, to either activate or repress transcription. Histone methylation is regulated by histone methyltransferase (HMT) and histone demethylase (HDM), which can be monomethylated, dimethylated, or trimethylated.

The increase in methylation usually promotes the affinity of histones to DNA and increases the degree of transcriptional inhibition, such as H3K9 methylation and H3K27 methylation. H3K9 dimethylation and trimethylation are typical repressive histone modifications that mediate the formation of heterochromatic regions. It was reported that the knockdown of ESET, a H3K9 methyltransferase, causes an aberrant expression of Runx2 and finally leads to the impairment of osteogenic differentiation and bone defects in mice. On the other hand, the knockdown of EHMT1, a H3K9 specific methyltransferase, resulted in decreased H3K9me2 levels on the promoters of Runx2, thereby upregulating transcription in mouse tissues. With respect to the adipogenic differentiation of MSCs, it was found that the enrichment of H3K9me1 and H3K9me2 on the promoters of C/EBP and PPARγ was negatively associated with adipogenic differentiation. Lowering the H3K9 methylation levels in these regions by either H3K9 demethylase or HMT inhibitors ultimately promoted adipogenic differentiation. In addition, H3K9me3 levels in the promoter region of Sox9, as well as its target genes *Col2a1* and *aggrecan*, were found to be negatively correlated with the chondrogenic differentiation of MSCs. H3K27 methylation is another heterochromatic histone modification associated with transcriptional repression. H3K27me3 on the promoters of Wnt family genes, including *Wnt1, Wnt6, Wnt10a,* and *Wnt10b*, was increased during osteogenesis. Knockdown of the H3K27me3 demethylases such as KDM4B

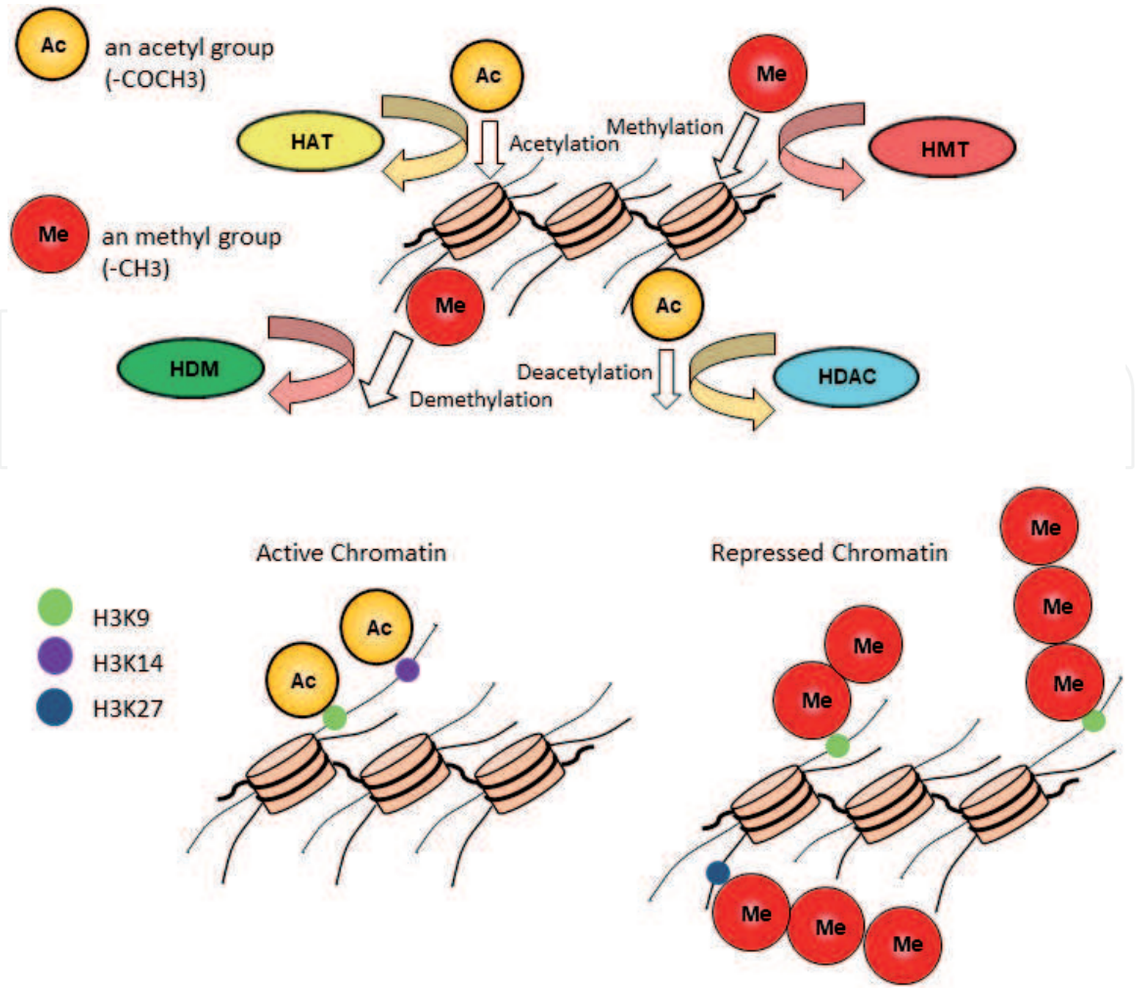

Figure 1.
The mechanisms of histones modification.

and KDM6B attenuated osteogenesis. Moreover, H3K27me3 demethylases have also been reported to facilitate the adipogenic and chondrogenic differentiation of MSCs. Hemming *et al.* found that high expression of the enhancer of zeste homolog 2 (EZH2), a histone methyltransferase catalyzes the tri-methylation of chromatin H3K27, promotes the adipogenic differentiation of MSCs, and inhibits the osteogenic differentiation, while demethylase KDM6A has the opposite effect. In contrast to H3K9 and H3K27 methylation, methylation of H3K4 is associated with an active transcription state. It has been reported that H3K4me3 levels are negatively associated with osteogenesis. HDMs, such as KDM5A and KDM5B, which specifically demethylate H3K4, were found to influence MSC osteogenic differentiation. A recent study also reported that silencing of Ash1l, an H3K4 methyltransferase, promotes adipogenesis while suppressing osteogenesis and chondrogenesis (**Figure 1**).

4. Chromatin remodeling

Chromatin remodeling is the dynamic modification of chromatin architecture, which is an important mechanism for regulating gene expression. In eukaryotes, DNA is tightly wound into a complex called chromatin. Chromatin remodeling allows the access of tightly condensed DNA to various regulatory factors, such as transcription factors and components of DNA replication, so that specific genes can be expressed. The basic mechanism of chromatin remodeling depends on the three

dynamic properties of nucleosomes: reconstruction, enzyme-induced covalent modification, and repositioning. In addition, the aforementioned histone modification is another important aspect of chromatin remodeling. Aberrations in chromatin remodeling proteins are associated with various human disorders and diseases. The major activities involved in nucleosome structure alterations use the energy supplied by ATP hydrolysis to affect nucleosomes. These enzymes are called ATP-dependent chromatin (or nucleosome) remodeling factors. The system involves four subfamilies of ATP-dependent chromatin remodeling complexes, namely switch/sucrose non-fermentable (SWI/SNF), nucleosome remodeler deacetylase (NuRD), INO80, and imitation switch (ISWI).

Several studies have demonstrated that functional SWI/SNF machinery plays an important role in regulating MSC tri-lineage differentiation by interacting with tissue-specific transcription factors and crosstalk with cell signaling pathways. Brahma-associated factor (BAF) complex subunits have been implicated in MSC osteo-lineage commitment. For example, depletion of BRG1 leads to constitutive osteo-lineage gene expression [37]. BRM negatively regulates osteocalcin expression [38]. Loss of the classical BAF restricted subunit Pbrm1/Arid2/Brd7 leads to reduced osteogenesis without compromising adipogenesis [39]. It has also been reported that SWI/SNF-dependent chromatin remodeling is involved in MSC adipogenic differentiation. BRG1 overexpression was associated with promoted adipogenic differentiation, which was associated with a marked increase in the differentiation markers PPARγ and LPL [40]. BAF45A was identified as an important regulator of adipogenic differentiation in human MSCs [41]. In addition, other ATP-dependent chromatin remodelers, such as chromodomain helicase DNA binding (CHD) proteins, are also involved in MSC lineage commitment. CHD4 was reported to be implicated in chondrogenesis. Simon *et al.* reported that CHD1 is required for the induction of osteoblast-specific gene expression, extracellular-matrix mineralization, and ectopic bone formation in vivo [42]. CReMM plays a role in mediating the transcriptional response to hormones that coordinate osteoblast function [42, 43]. It was proved by Kumar *et al.* that metastasis-associated gene 1(MTA1) negatively regulates osteo-lineage gene expression [44]. Together, chromatin remodeling plays an important role in MSC lineage commitment.

5. Non-coding RNAs

The RNA world is divided into two classes: 1) RNAs that have coding potential (mRNAs) and 2) RNAs without coding potential, referred to as non-coding RNAs (ncRNAs). Although mRNAs have been extensively studied, ncRNAs span more than 98% of DNA transcripts. In the past, these molecules were considered as "evolutionary junk" but increasing evidence suggests that these molecules spatiotemporally regulate protein-coding gene expression in several molecular mechanisms. With improved RNA-sequencing techniques, in recent years, there have been great advances in identifying and understanding ncRNAs. Epigenetic ncRNAs, including microRNAs (miRNAs), small interfering RNAs (siRNAs), piwi-interacting RNA (piRNA), and long noncoding RNAs (lncRNA), have been reported to play key roles in the regulation of various diseases and biological processes, including cellular differentiation, proliferation, apoptosis, gene regulation, and cancer development.

5.1 Long non-coding RNA

lncRNA is a novel class of noncoding RNAs longer than 200 nt, which can regulate gene expression at the transcriptional and post-transcriptional levels. LncRNAs

are mainly located in the cell nucleus or cytoplasm, affecting the status and fate of cells through different post-transcriptional mechanisms. Nuclear lncRNAs guide chromatin modifiers, such as DNA methyltransferase, histone methyltransferase, and heteronuclear ribosome protein, to a specific genetic locus and induce chromatin structure remodeling, which in turn regulates gene expression either positively or negatively. Cytoplasmic lncRNAs can either block the functional site or alter the structure and modification of specific proteins, thereby regulating the function and stabilization of these proteins, and ultimately alter the fate and function of cells. During the last decade, multiple studies have demonstrated that lncRNAs are widely involved in growth and development by controlling the fate of cells, including MSCs.

Studies have demonstrated the importance of lncRNAs in bone regeneration and bone formation. Many lncRNAs regulating the osteogenic differentiation of MSCs have been identified, including ANCR, AK141205, AK028326, DANCR, MALAT1, MEG3, MORD, and POIR; these either promote or inhibit osteogenic differentiation through diverse pathways. For example, MALAT1 promotes *OSX* expression and the osteogenesis of MSCs by sponging miR-143, and MALAT1 can be used as a biomarker for the detection of osteoporosis [45]. MEG3 inhibited bone morphogenetic protein 2 (BMP2) through interaction with hnRNPI, which plays an active role in mRNA splicing, and finally suppresses osteogenic differentiation [46]. Exosome-transferred lncRNA, RUNX2 antisense RNA 1 (RUNX2-AS1), decreases the expression of RUNX2 in MSCs by forming an RNA duplex with RUNX2, consequently suppressing osteogenesis [47]. MEG3 has also been shown to promote osteogenic differentiation in MSCs via the BMP4 signaling pathway [48]. Moreover, lncRNAs are associated with osteogenesis through the regulation of classical signaling pathways, including the Wnt/β-catenin pathway, p38 mitogen-activated protein kinase (MAPK) pathway, Notch signaling pathway, and nuclear factor-κB (NFκB) signaling pathway. Currently, few studies have focused on lncRNA expression and their functions in the chondrogenic and adipogenic differentiation of MSCs. Xiao *et al.* reported that adipogenic differentiation-induced noncoding RNA (ADINR) was significantly upregulated in MSCs after adipogenic induction. Knocking out ADINR significantly inhibited the ability of MSCs to differentiate into adipocytes. A mechanistic study revealed that ADINR positively regulates the expression of the transcription factor C/EBPα [49]. Wang *et al.* reported that the expression of the lncRNAs ZBED3-AS1 and CTA-941F9.9 was significantly upregulated during the differentiation process of MSCs toward cartilage [50].

5.2 MicroRNA

MicroRNAs are the most abundant class of small ncRNAs with a length of 21–25 nt, and have been studied extensively. miRNAs are also involved in the epigenetic regulation of genes in both the cytoplasm and nucleus through different mechanisms. Their main action is the negative regulation of gene expression by specifically binding to a target mRNA through base complementary pairing and inducing its degradation or the inhibition of its translation.

Accumulating evidence indicates that miRNAs play an important role in the maintenance of stemness and differentiation of MSCs (**Table 2**). As mentioned above, lineage differentiation of MSCs is a complex biological process. For example, MSCs differentiate into osteogenic progenitor cells and subsequently osteoblasts, and then gradually become mature bone cells along with a variety of extracellular matrix mineralization. This process involves a large number of secretory and transcription factors. In addition, the differentiation and maturation of MSCs also involves signaling pathways such as WNT, BMP, and PI3K/Akt. The key effector

Involved miRNA	Mechanism	Result	Reference
miR-23a	targets LRP5 and subsequently suppress the Wnt/β-catenin signaling pathway	Inhibit osteogenesis of MSCs	[51]
miR-26a	in BMSCs: targets GSK3*β* mainly and activates Wnt/β-catenin signaling pathway; in ADSCs: targets Smad1 mainly and inhibits BMP signaling pathway	Inhibit osteogenesis of ADSCs and promote osteogenesis of BMSCs	[52]
miR-30c	reduces Runx2 protein	Inhibit osteogenesis of MSCs	[53]
miR-34c			
miR-133a			
miR-135a			
miR-137			
miR-204			
miR-205			
miR-217			
miR-338			
miR-20b	Activate the BMPs/Runx2 signaling pathway at four levels, which consists of repressing $PPAR_{\gamma}$, Bambi and Crim1	Promote ostegenesis	[54, 55]
miR-29b	activates the AKT/β-catenin signaling pathway by inhibiting PTEN expression	Promote osteogenesis of hADSCs	[56]
miR-196a	targets HOXC8 (a negative regulator of SMAD1)	Inhibit proliferation and promote osteogenesis of hDASCs	[57]
miR-17-5p	Represses the Wnt signaling pathway effector Tcf7l2	Promote adipogenesis of BM-MSCs	[58, 59]
miR-21	Alters SMAD3 phosphorylation without affecting total levels of SMAD3 protein and modulate TGF-β signaling pathway		[59, 60]
miR-143	Directly represses MAP2K5 (a key member of the MAPKK family in the MAPK signaling pathway)		[59, 61]
miR-30a	Targets Runx2	Promote adipogenesis	[62]
miR-30d			
miR-642a-3p	unknown	In a high level in adipogenesis	[62]

Table 2.
miRNA and MSCs differentiation.

molecules in these pathways can be regulated by miRNAs, which in turn affects MSC fate decisions. Recently, various miRNAs, including miR-20b, -29b, -30a-5p, -142-3p, -196a, -210, -746-5p, -2861, -3960, -335-5p, etc., have been reported to enhance osteogenic differentiation, whereas miR -23a, -26a, -30c, -34b, -34c, -125, -133a, -135a, -137, -141, -148, -200a, -204, -205, -206, -217, and -338 could impede osteogenic differentiation, and miR-143, -24, -31, -30c, and -642a-3p are involved in regulating adipogenesis. Oskowitz *et al.* reported that silencing of Dicer or Drosha, two key enzymes in the miRNA biogenesis pathway, inhibits both the osteogenic and adipogenic differentiation of MSCs [63]. Some miRNAs have been

reported to act as switches for MSCs to differentiate into different lineages. For example, the miR-17 cluster of the miRNA family, miR-17-5p, miR-106a, and miR-20a, are downregulated when the cell undergoes osteogenic differentiation and is upregulated during adipocyte differentiation [64]. Miyaki et al. also demonstrated that the expression of miR-140 increased during chondrocytic differentiation along with the expression of Sox9, Aggercan, and Col2A1 [65]. In addition, recent research has found that miRNAs can form a competitive endogenous RNA regulation network with lncRNAs and circRNAs. Some research groups have started paying more attention to this regulatory network, which will further improve our understanding of the role of ncRNAs in MSC maintenance and differentiation.

6. Conclusions

"Epigenetics" was first used to define the complex interactions between the genome and the environment that are involved in the development and differentiation of organisms. Nowadays, the term refers to heritable alterations in gene expression that are not mediated at the DNA sequence level. Accumulating evidence has suggested that the processes of epigenetic modifications are crucial and largely responsible for the variable activation and repression of specific genes at specific time points during the lifespan of stem cells, allowing for the terminally differentiated phenotype. With the advances in biological and experimental technologies, a variety of epigenetic modifications involved in the cell fate determination of MSCs have been discovered in recent years. In addition to the types of epigenetic modifications introduced in the article, some researchers have suggested the role of histone phosphorylation, ubiquitination, and other modifications in the differentiation of MSCs. On this basis this information, drugs that effectively regulate these modifications have been developed to provide precise differentiation conditions for MSCs and make them more effective in clinical treatment. The disadvantage of epigenetic therapy using small molecule drugs is the lack of specificity, which needs to be further studied. In summary, epigenetic modifications play an important regulatory role in the cell fate determination of MSCs, but the precise function of these modifications in different MSC types, as well as the associated underlying mechanisms, remain to be thoroughly investigated. In-depth research in this field would provide important reference data for the differentiation mechanism research and clinical application of MSCs.

Acknowledgements

The authors were supported by the National Key Research and Development Program of China (2020YFA0113003, 2018YFC1004803) and the Fundamental Research Funds for the Central Universities.

Conflict of interest

The authors declare no competing financial interests.

Author details

Haoli Ying[1,2], Ruolang Pan[3] and Ye Chen[1,2*]

1 Institute of Genetics and Division of Medical Genetics and Genomics, The Children's Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China

2 Zhejiang Provincial Key Laboratory of Genetic and Developmental Disorders, Hangzhou, Zhejiang, China

3 Key Laboratory of Cell-Based Drug and Applied Technology Development in Zhejiang Province, Hangzhou, Zhejiang, China

*Address all correspondence to: yechency@zju.edu.cn

References

[1] Villagra A, Gutirrez J, Paredes R, Sierra J, Puchi M, Imschenetzky M, et al. Reduced CpG methylation is associated with transcriptional activation of the bone-specific rat osteocalcin gene in osteoblasts. J Cell Biochem. 2002;

[2] Dansranjavin T, Krehl S, Mueller T, Mueller LP, Schmoll HJ, Dammann RH. The role of promoter CpG methylation in the epigenetic control of stem cell related genes during differentiation. Cell Cycle. 2009;

[3] Arnsdorf EJ, Tummala P, Kwon RY, Jacobs CR. Mechanically induced osteogenic differentiation - The role of RhoA, ROCKII and cytoskeletal dynamics. J Cell Sci. 2009;

[4] Yang R, Yu T, Kou X, Gao X, Chen C, Liu D, et al. Tet1 and Tet2 maintain mesenchymal stem cell homeostasis via demethylation of the P2rX7 promoter. Nat Commun [Internet]. 2018;9(1). Available from: http://dx.doi.org/10.1038/s41467-018-04464-6

[5] Zhou GS, Zhang XL, Wu JP, Zhang RP, Xiang LX, Dai LC, et al. 5-Azacytidine facilitates osteogenic gene expression and differentiation of mesenchymal stem cells by alteration in DNA methylation. Cytotechnology. 2009;

[6] García-Ibarbia C, Delgado-Calle J, Casafont I, Velasco J, Arozamena J, Pérez-Núñez MI, et al. Contribution of genetic and epigenetic mechanisms to Wnt pathway activity in prevalent skeletal disorders. Gene. 2013;

[7] Delgado-Calle J, Fernández AF, Sainz J, Zarrabeitia MT, Sañudo C, García-Renedo R, et al. Genome-wide profiling of bone reveals differentially methylated regions in osteoporosis and osteoarthritis. Arthritis Rheum. 2013;

[8] Sun Z, Yang S, Ye S, Zhang Y, Xu W, Zhang B, et al. Aberrant CpG Islands' hypermethylation of ABCB1 in mesenchymal stem cells of patients with steroid-associated osteonecrosis. J Rheumatol. 2013;40(11):1913-20.

[9] Barrand S, Collas P. Chromatin states of core pluripotency-associated genes in pluripotent, multipotent and differentiated cells. Biochem Biophys Res Commun. 2010;

[10] Noer A, Serensen AL, Boquest AC, Collas P. Stable CpG hypomethylation of adipogenic promoters in freshly isolated, cultured, and differentiated mesenchymal stem cells from adipose tissue. Mol Biol Cell. 2006;

[11] Fujiki K, Kano F, Shiota K, Murata M. Expression of the peroxisome proliferator activated receptor γ gene is repressed by DNA methylation in visceral adipose tissue of mouse models of diabetes. BMC Biol. 2009;

[12] Melzner I, Scott V, Dorsch K, Fischer P, Wabitsch M, Brüderlein S, et al. Leptin gene expression in human preadipocytes is switched on by maturation-induced demethylation of distinct CpGs in its proximal promoter. J Biol Chem. 2002;

[13] Stachecka J, Lemanska W, Noak M, Szczerbal I. Expression of key genes involved in DNA methylation during in vitro differentiation of porcine mesenchymal stem cells (MSCs) into adipocytes. Biochem Biophys Res Commun. 2020;

[14] Nomura Y, Hara ES, Yoshioka Y, Nguyen HT, Nosho S, Komori T, et al. DNA Methylation-Based Regulation of Human Bone Marrow-Derived Mesenchymal Stem/Progenitor Cell Chondrogenic Differentiation. Cells Tissues Organs. 2020;207(3-4):115-26.

[15] Chen YS, Wu R, Yang X, Kou S, MacDougald OA, Yu L, et al. Inhibiting

DNA methylation switches adipogenesis to osteoblastogenesis by activating Wnt10a. Sci Rep. 2016;

[16] Kim K Il, Park YS, Im G Il. Changes in the epigenetic status of the SOX-9 promoter in human osteoarthritic cartilage. J Bone Miner Res. 2013;

[17] Barter MJ, Bui C, Cheung K, Falk J, Gómez R, Skelton AJ, et al. DNA hypomethylation during MSC chondrogenesis occurs predominantly at enhancer regions. Sci Rep. 2020;

[18] Lee S, Park JR, Seo MS, Roh KH, Park SB, Hwang JW, et al. Histone deacetylase inhibitors decrease proliferation potential and multilineage differentiation capability of human mesenchymal stem cells. Cell Prolif. 2009;

[19] Zhang P, Liu Y, Jin C, Zhang M, Lv L, Zhang X, et al. Histone H3K9 Acetyltransferase PCAF Is Essential for Osteogenic Differentiation Through Bone Morphogenetic Protein Signaling and May Be Involved in Osteoporosis. Stem Cells. 2016;

[20] Zhang P, Liu Y, Jin C, Zhang M, Tang F, Zhou Y. Histone Acetyltransferase GCN5 Regulates Osteogenic Differentiation of Mesenchymal Stem Cells by Inhibiting NF-κB. J Bone Miner Res. 2016;

[21] Simic P, Zainabadi K, Bell E, Sykes DB, Saez B, Lotinun S, et al. SIRT1 regulates differentiation of mesenchymal stem cells by deacetylating β-catenin. EMBO Mol Med. 2013;

[22] Wang C, Wang J, Li J, Hu G, Shan S, Li Q, et al. KDM5A controls bone morphogenic protein 2-induced osteogenic differentiation of bone mesenchymal stem cells during osteoporosis. Cell Death Dis. 2016;

[23] Khanban H, Fattahi E, Talkhabi M. In vivo administration of G9A inhibitor A366 decreases osteogenic potential of bone marrow-derived mesenchymal stem cells. EXCLI J. 2019;

[24] Jung JW, Lee S, Seo MS, Park SB, Kurtz A, Kang SK, et al. Histone deacetylase controls adult stem cell aging by balancing the expression of polycomb genes and jumonji domain containing 3. Cell Mol Life Sci. 2010;

[25] Wang J, Wang CD, Zhang N, Tong WX, Zhang YF, Shan SZ, et al. Mechanical stimulation orchestrates the osteogenic differentiation of human bone marrow stromal cells by regulating HDAC1. Cell Death Dis. 2016;

[26] Lu DF, Yao Y, Su ZZ, Zeng ZH, Xing XW, He ZY, et al. Downregulation of HDAC1 is involved in the cardiomyocyte differentiation from mesenchymal stem cells in a myocardial microenvironment. PLoS One. 2014;

[27] Yan GQ, Wang X, Yang F, Yang ML, Zhang GR, Wang GK, et al. MicroRNA-22 Promoted Osteogenic Differentiation of Human Periodontal Ligament Stem Cells by Targeting HDAC6. J Cell Biochem. 2017;

[28] Manzotti G, Torricelli F, Donati B, Sancisi V, Gugnoni M, Ciarrocchi A. HDACs control RUNX2 expression in cancer cells through redundant and cell context-dependent mechanisms. J Exp Clin Cancer Res. 2019;

[29] Ozaki T, Wu D, Sugimoto H, Nagase H, Nakagawara A. Runt-related transcription factor 2 (RUNX2) inhibits p53-dependent apoptosis through the collaboration with HDAC6 in response to DNA damage. Cell Death Dis. 2013;

[30] Fu Y, Zhang P, Ge J, Cheng J, Dong W, Yuan H, et al. Histone deacetylase 8 suppresses osteogenic differentiation of bone marrow stromal cells by inhibiting histone H3K9 acetylation and RUNX2 activity. Int J Biochem Cell Biol. 2014;

[31] Yoon DS, Choi Y, Jang Y, Lee M, Choi WJ, Kim SH, et al. SIRT1 directly regulates SOX2 to maintain self-renewal and multipotency in bone marrow-derived mesenchymal stem cells. Stem Cells. 2014;

[32] Catalioto RM, Maggi CA, Giuliani S. Chemically distinct HDAC inhibitors prevent adipose conversion of subcutaneous human white preadipocytes at an early stage of the differentiation program. Exp Cell Res. 2009;

[33] Wang F, Tong Q. SIRT2 suppresses adipocyte differentiation by deacetylating FOXO1 and enhancing FOXO1's repressive interaction with PPARγ. Mol Biol Cell. 2009;

[34] Jing E, Gesta S, Kahn CR. SIRT2 Regulates Adipocyte Differentiation through FoxO1 Acetylation/ Deacetylation. Cell Metab. 2007;

[35] Nakade K, Pan J, Yoshiki A, Ugai H, Kimura M, Liu B, et al. JDP2 suppresses adipocyte differentiation by regulating histone acetylation. Cell Death Differ. 2007;

[36] Choi SM, Lee KM, Ryu SB, Park YJ, Hwang YG, Baek D, et al. Enhanced articular cartilage regeneration with SIRT1-activated MSCs using gelatin-based hydrogel. Cell Death Dis. 2018;

[37] Nguyen KH, Xu F, Flowers S, Williams EAJ, Fritton JC, Moran E. SWI/ SNF-Mediated Lineage Determination in Mesenchymal Stem Cells Confers Resistance to Osteoporosis. Stem Cells. 2015;

[38] Flowers S, Nagl NG, Beck GR, Moran E. Antagonistic roles for BRM and BRG1 SWI/SNF complexes in differentiation. J Biol Chem. 2009;

[39] Sinha S, Biswas M, Chatterjee SS, Kumar S, Sengupta A. Pbrm1 Steers Mesenchymal Stromal Cell Osteolineage Differentiation by Integrating PBAF-Dependent Chromatin Remodeling and BMP/TGF-β Signaling. Cell Rep. 2020;

[40] Napolitano MA, Cipollaro M, Cascino A, Melone MAB, Giordano A, Galderisi U. Brg1 chromatin remodelling factor is involved in cell growth arrest, apoptosis and senescence of rat mesenchymal stem cells. J Cell Sci. 2007;

[41] van de Peppel J, Strini T, Tilburg J, Westerhoff H, van Wijnen AJ, van Leeuwen JP. Identification of Three Early Phases of Cell-Fate Determination during Osteogenic and Adipogenic Differentiation by Transcription Factor Dynamics. Stem Cell Reports. 2017;

[42] Baumgart SJ, Najafova Z, Hossan T, Xie W, Nagarajan S, Kari V, et al. CHD1 regulates cell fate determination by activation of differentiation-induced genes. Nucleic Acids Res. 2017;

[43] Marom R, Shur I, Hager GL, Benayahu D. Expression and regulation of CReMM, a chromodomain helicase-DNA-binding (CHD), in marrow stroma derived osteoprogenitors. J Cell Physiol. 2006;

[44] Kumar A, Salimath BP, Schieker M, Stark GB, Finkenzeller G. Inhibition of metastasis-associated gene 1 expression affects proliferation and osteogenic differentiation of immortalized human mesenchymal stem cells. Cell Prolif. 2011;

[45] Gao Y, Xiao F, Wang C, Wang C, Cui P, Zhang X, et al. Long noncoding RNA MALAT1 promotes osterix expression to regulate osteogenic differentiation by targeting miRNA-143 in human bone marrow-derived mesenchymal stem cells. J Cell Biochem. 2018;

[46] Liu Y, Zeng X, Miao J, Liu C, Wei F, Liu D, et al. Upregulation of long noncoding RNA MEG3 inhibits the osteogenic differentiation of

periodontal ligament cells. J Cell Physiol. 2019;

[47] Li B, Xu H, Han H, Song S, Zhang X, Ouyang L, et al. Exosome-mediated transfer of lncRUNX2-AS1 from multiple myeloma cells to MSCs contributes to osteogenesis. Oncogene. 2018;

[48] Chen S, Jia L, Zhang S, Zheng Y, Zhou Y. DEPTOR regulates osteogenic differentiation via inhibiting MEG3-mediated activation of BMP4 signaling and is involved in osteoporosis. Stem Cell Res Ther. 2018;

[49] Xiao T, Liu L, Li H, Sun Y, Luo H, Li T, et al. Long noncoding RNA ADINR regulates adipogenesis by transcriptionally activating C/EBPα. Stem Cell Reports. 2015;

[50] Wang L, Li Z, Li Z, Yu B, Wang Y. Long noncoding RNAs expression signatures in chondrogenic differentiation of human bone marrow mesenchymal stem cells. Biochem Biophys Res Commun. 2015;

[51] Li T, Li H, Wang Y, Li T, Fan J, Xiao K, et al. MicroRNA-23a inhibits osteogenic differentiation of human bone marrow-derived mesenchymal stem cells by targeting LRP5. Int J Biochem Cell Biol. 2016;

[52] Su X, Liao L, Shuai Y, Jing H, Liu S, Zhou H, et al. MiR-26a functions oppositely in osteogenic differentiation of BMSCs and ADSCs depending on distinct activation and roles of Wnt and BMP signaling pathway. Cell Death Dis. 2015;

[53] Zhang Y, Xie RL, Croce CM, Stein JL, Lian JB, Van Wijnen AJ, et al. A program of microRNAs controls osteogenic lineage progression by targeting transcription factor Runx2. Proc Natl Acad Sci U S A. 2011;

[54] Zhang ZJ, Zhang H, Kang Y, Sheng PY, Ma YC, Yang ZB, et al. MiRNA expression profile during osteogenic differentiation of human adipose-derived stem cells. J Cell Biochem. 2012;

[55] He J, Zhang J fang, Yi C, Lv Q, Xie W dong, Li J na, et al. miRNA-mediated functional changes through co-regulating function related genes. PLoS One. 2010;

[56] Xia T, Dong S, Tian J. miR-29b promotes the osteogenic differentiation of mesenchymal stem cells derived from human adipose tissue via the PTEN/AKT/β-catenin signaling pathway. Int J Mol Med. 2020;

[57] Kim YJ, Bae SW, Yu SS, Bae YC, Jung JS. miR-196a regulates proliferation and osteogenic differentiation in mesenchymal stem cells derived from human adipose tissue. J Bone Miner Res. 2009;

[58] Tian L, Song Z, Shao W, Du WW, Zhao LR, Zeng K, et al. Curcumin represses mouse 3T3-L1 cell adipogenic differentiation via inhibiting miR-17-5p and stimulating the Wnt signalling pathway effector Tcf7l2. Cell Death Dis. 2017;

[59] An X, Ma K, Zhang Z, Zhao T, Zhang X, Tang B, et al. MiR-17, miR-21, and miR-143 Enhance Adipogenic Differentiation from Porcine Bone Marrow-Derived Mesenchymal Stem Cells. DNA Cell Biol. 2016;

[60] Jeong Kim Y, Jin Hwang S, Chan Bae Y, Sup Jung J. MiR-21 regulates adipogenic differentiation through the modulation of TGF-β signaling in mesenchymal stem cells derived from human adipose tissue. Stem Cells. 2009;

[61] Chen L, Hou J, Ye L, Chen Y, Cui J, Tian W, et al. MicroRNA-143 regulates adipogenesis by modulating the map2k5-erk5 signaling. Sci Rep. 2014;

[62] Zaragosi LE, Wdziekonski B, Brigand KL, Villageois P,

Mari B, Waldmann R, et al. Small RNA sequencing reveals miR-642a-3p as a novel adipocyte-specific microRNA and miR-30 as a key regulator of human adipogenesis. Genome Biol. 2011;

[63] Oskowitz AZ, Lu J, Penfornis P, Ylostalo J, McBride J, Flemington EK, et al. Human multipotent stromal cells from bone marrow and microRNA: Regulation of differentiation and leukemia inhibitory factor expression. Proc Natl Acad Sci U S A. 2008;

[64] Li H, Li T, Wang S, Wei J, Fan J, Li J, et al. MiR-17-5p and miR-106a are involved in the balance between osteogenic and adipogenic differentiation of adipose-derived mesenchymal stem cells. Stem Cell Res. 2013;

[65] Miyaki S, Nakasa T, Otsuki S, Grogan SP, Higashiyama R, Inoue A, et al. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. Arthritis Rheum. 2009;