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The role of microRNAs in cell fate determination of mesenchymal stem cells : balancing adipogenesis and osteogenesis

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Mesenchymal stem cells (MSCs) are multipotent stem cells capable of differentiating into adipocytes, osteoblasts, or chondrocytes. A mutually inhibitory relationship exists between osteogenic and adipogenic lineage commitment and differentiation. Such cell fate decision is regulated by several signaling pathways, including Wnt and bone morphogenetic protein (BMP). Accumulating evidence indicates that microRNAs (miRNAs) act as switches for MSCs to differentiate into either osteogenic or adipogenic lineage. Different miRNAs have been reported to regulate a master transcription factor for osteogenesis, such as Runx2, as well as molecules in the Wnt or BMP signaling pathway, and control the balance between osteoblast and adipocyte differentiation. Here, we discuss recent advancement of the cell fate decision of MSCs by miRNAs and their targets. [BMB Reports 2015; 48(6): 319-323]

INTRODUCTION

MSCs are capable of differentiate into several distinct cell types, including osteoblasts and adipocytes. The osteogenesis and adipogenesis of MSCs maintain a homeostasis under physiological conditions. It is often found that the signal, which promotes one cell fate, actively represses the alternative fate (1). The balance between osteogenic and adipogenic differentiation is tightly regulated by multiple signaling pathways. Dysregulation of this balance is known to lead to various human diseases, such as osteoporosis which is often associated with a significant increase in adipocytes accumulation at an expense of bone loss (2). On the contrary, patients with a high bone mass phenotype often exhibit reduced fat tissue volume (3). Therefore, clear understanding of the control mechanism of maintenance of this balance between osteogenic and adipo-

genic differentiation of MSCs is of great importance to elucidate the pathogenesis and a development of novel and effective therapies for bone diseases.

The adipogenic and osteogenic differentiation from MSCs is regulated by multiple regulatory factors and signaling pathways, such as the Wnt/ β -catenin, TGF β /BMPs/Smads, Notch, JAK/STAT, MAPK, phosphatidylinositol-3 kinase (PI3K)/Akt and Hedgehog pathways (4-6). Osteoblast development is governed by the activation of Wnt/ β -catenin signaling. Wnt signaling through Frizzled and its co-receptors, low-density lipoprotein receptor-related protein (LRP) 5/6, inhibits the Axin/GSK3/APC complex, and β -catenin accumulates in the nucleus, which then directly regulates osteoblast activity (7). Transcription factors, such as Runt-related transcription factor 2 (Runx2) and Osterix (Osx), lead to the terminal osteoblast differentiation, which is characterized by the calcification of the extracellular matrix (5). Alkaline phosphatase (ALP), osteopontin (Opn) and osteocalcin (Ocn) are involved in the mineralization process. BMP signaling is also a central signaling pathway involved in the induction of osteogenic differentiation and regulation of bone formation. Specifically, BMP-2 is the most frequently studied ligand of BMPs that promotes osteogenic commitment and terminal osteogenic differentiation in MSCs. Gene regulation mediated by several transcription factors play a critical role to form mature adipocytes from MSCs (8, 9). CCAAT/enhancer binding protein (C/EBP) β and δ activate C/EBP α and peroxisome proliferator-activated receptor γ (PPAR γ) to coordinate the expression of adipogenic genes characteristic of terminally differentiated adipocytes. PPAR γ is regarded as a master transcriptional regulator of both adipocyte differentiation and lipid storage in mature adipocytes.

miRNAs are evolutionarily conserved short (19-25 nt) non-coding RNAs that mainly regulate gene expression in a post-transcriptional manner. miRNAs function via partially complementary base pairing with the 3'-untranslated region (UTR) of target mRNAs. miRNA and target mRNA pairing typically results in gene silencing via translational repression and/or destabilization of mRNA (10). Many studies suggest that miRNAs critically regulate fate decisions of stem cells, including self-renewal and differentiation. Conversely, miRNAs also critical during the reprogramming of differentiated somatic cells to generate induced pluripotent stem (iPS) cells (11). During a

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pluripotent state, transcription factors which are required to promote cellular differentiation are downregulated by miRNAs. Once decision to exit from a pluripotent state is made, lineage-specific miRNAs are induced, which inhibit transcription factors specific for the pluripotent state, such as Sox2, Oct4 and Nanog.

Emerging evidence suggests that miRNAs are involved in regulating the differentiation and cell fate decisions of MSCs (12). In human bone marrow-derived MSCs, silencing of Dicer or Drosha, two key enzymes in the miRNA biogenesis pathway, inhibits both osteogenic and adipogenic differentiation (13). Recently, miR-196a, -29b, -2861, -3960 and -335-5p are reported to enhance osteogenic differentiation (14-17), while miR-26a, -133, -135, -141 and -200a could impede osteogenic differentiation (18-20), and miR-143, -24, -31, -30c and -642a-3p are involved in regulating adipogenesis (21-24). Although many miRNAs have been identified to regulate either adipogenesis or osteogenesis, only a few were implicated in both processes and play a role in balancing these two cell fates.

This review focuses on miRNAs that function as mediators of the balance between the adipogenesis and osteogenesis of MSCs. These miRNAs determine the adipogenic versus osteogenic fates of MSCs by modulating Wnt or BMP signaling via the repression of components of the signaling pathway or regulating key transcription factors in the differentiation of MSCs, such as Runx2 (Table 1).

miRNAs THAT DETERMINE ADIPOGENIC DIFFERENTIATION

Each member of the miR-30 family (miR-30a-e) is differentially regulated during adipocyte and osteoblast differentiation (25). miR-30e is the most prominently regulated during adipogenesis and osteogenesis (26). miR-30e is induced in the mesenchymal cell line C3H10T1/2 and the pre-adipocyte 3T3-L1 in response to treatment of adipocyte-inducing medium. Conversely, the expression of miR-30e is reduced in the mouse stromal line ST2 and pre-osteoblast MC3T3-E1 after treatment of osteocyte-inducing medium. The overexpression of miR-30e promotes pre-adipocytes to differentiate into ma-

Table 1. miRNAs that reciprocally regulate the differentiation of adipocytes and osteoblasts

	miRNA	Target mRNA
Osteogenic switch (Increased expression during osteogenesis)	miR-21	Sox2, Spry2
	miR-22	HDAC6
Adipogenic switch (Increased expression during adipogenesis)	miR-204	Runx2
	miR-211	Rnux2
	miR-17-5p	BMP2
	miR-106a	BMP2
	miR-30e	LPR6
	miR-637	Osx

ture adipocytes, along with increased expression of adipocyte-specific transcription factors, such as PPARc, C/EBP α and C/EBP β (26). The overexpression of miR-30e inhibits osteoblast differentiation, characterized by reduced expression of pro-osteogenic transcription factors, such as Runx2, Osx, Ocn, ALP and bone sialoprotein (BSP). The inhibition of the endogenous miR-30e represses the differentiation of pre-adipocytes and potentiates the osteoblast differentiation (26). LRP6 is shown to be a direct target of miR-30e (26). The knockdown of LRP6 in 3T3-L1 cells downregulates β -catenin/T-cell factor (TCF)-mediated gene expression and potentiates the differentiation into mature adipocytes. These results demonstrate that miR-30e controls the balance of adipocyte differentiation and osteoblast differentiation by modulating the canonical Wnt signaling (Fig. 1). The levels of miR-30c and miR-30d are also increased during adipocyte differentiation, but decreased during osteoblast differentiation similar to miR-30e (25). miR-30c and miR-30d are found to target Smad1, a signal transducer of BMP signaling pathway, and inhibit BMP-mediated osteoblast differentiation. Therefore, miR-30c and miR-30d are also mediators to balance the osteogenesis and adipogenesis via regulating BMP signaling (Fig. 1).

miRNA expression profiling in human adipose-derived mesenchymal stem cells (hADSCs) find that the miR-17 cluster of family of miRNAs, miR-17-5p, miR-106a and miR-20a, are downregulated when the cell undergoes osteogenic differentiation while upregulated during adipocyte differentiation (27). The overexpression of miR-17-5p and miR-106a inhibits the ALP activity, mineralization and expression of the osteogenic transcription factors, such as Runx2, Osx, Ocn and Ocn. The downregulation of the endogenous miR-17-5p and miR-106a

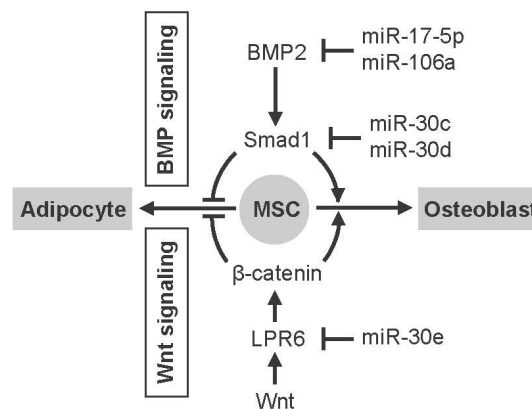


Fig. 1. miRNAs that control signaling governing osteogenesis and adipogenesis. BMP and Wnt signaling pathways have been demonstrated to preferentially induce the osteogenesis of MSCs at the expense of adipogenesis. miR-17-5p/miR-106a and miR-30c/miR-30d inhibit BMP signaling by targeting key components of the pathway, such as BMP2 and Smad1, respectively. miR-30e inhibits Wnt signaling via the repression of LPR6, a key coreceptor of Wnts.

promotes osteogenic differentiation and suppresses the adipogenic differentiation in hADSCs (27). BMP2 is identified as a direct target of miR-17-5p and miR-106a (27). Therefore, miR-17-5p and miR-106a balance the osteogenic and adipogenic lineage commitment in hADSCs by modulating BMP signaling (Fig. 1).

Runx2 is identified as a key transcription factor that regulates osteogenesis and chondrogenesis (28, 29). Regulation of Runx2 also affects the adipogenic potential of MSCs. miRNAs that regulate MSC differentiation via the modulation of Runx2 were investigated. miR-204 and miR-211 are induced during adipocyte differentiation, which downregulate Runx2 expression (30) (Fig. 2). miR-204 and miR-211 act as endogenous repressors of Runx2 in MSCs (30). The perturbation of miR-204 results in upregulation of osteogenesis and downregulation of adipogenesis, characterized by suppression of adipocyte marker genes, such as adipocyte protein 2 (aP2), adiponin and PPAR γ (30). Conversely, when miR-204 was overexpressed, the expression levels of aP2, adiponin and PPAR γ are increased, which adipocyte differentiation is promoted and osteoblast differentiation is inhibited (30). However, miR-204 inhibitor did not reverse the decrease of Runx2 levels during adipocyte differentiation, although miR-204 perturbation did significantly affect the Runx2 levels. This finding suggests that Runx2 expression is not exclusively regulated by miRNAs in MSC differentiation.

Osx, as a downstream of Runx2, is induced by BMP2 in MSCs and required for the differentiation of pre-osteoblasts into mature osteoblasts (31, 32). The cartilage is formed normally in Osx-null embryos, but they completely lack bone for-

mation (33). miR-637 is shown to target Osx (34). The expression of miR-637 is increased during adipocyte differentiation, and decreased during osteoblast differentiation. The expression of adipogenic markers, such as PPAR γ , C/EBP α and sterol regulatory element-binding protein 1c (SREBP-1c), are significantly increased in miR-637-overexpressing MSCs, but are decreased in response to a miR-637 inhibitor. Moreover, the levels of both BMP2 and Runx2 are downregulated by miR-637 and upregulated by inhibition of miR-637. These results indicate that miR-637 promotes the adipogenesis and suppresses the osteogenesis of MSCs, and maintains the balance of these two cell fates.

miRNAs THAT PROMOTE OSTEOGENIC DIFFERENTIATION

miR-22 is also found to regulate the adipogenic and osteogenic differentiation in hADSCs (35) (Fig. 2). The expression of miR-22 is decreased during adipogenic differentiation but increased during osteogenic differentiation. Consistently, the overexpression of miR-22 in hADSCs inhibits the accumulation of lipid droplets and represses the expression of adipogenic transcription factors and adipogenic-specific genes. Conversely, the enhanced ALP activity and matrix mineralization, as well as the increased expression of osteo-specific genes, indicate a positive role of miR-22 in regulating osteogenic differentiation. Histone deacetylase 6 (HDAC6), a co-repressor of Runx2 (36), is identified as a target of miR-22. Silencing endogenous HDAC6 expression in hADSCs enhances osteogenesis but represses adipogenesis, suggesting a role of the miR-22-HDAC6 axis which in turn activates Runx2 activity and osteogenic differentiation.

The ERK-MAPK signaling pathway plays a pivotal role in initiating and maintaining cell differentiation (37). The elimination of ERK activity is sufficient to maintain the self-renewal ability of embryonic stem cells, and the inhibition of MAPK signaling can convert terminally differentiated cells to a pluripotent state (37, 38). The ERK-MAPK signaling pathway has also been shown to be a major regulator of adipogenesis and osteogenesis in MSCs (39). Sprouty 1 and 2 (Spry1 and Spry2) are negative regulators of the ERK signaling pathway, and Spry2 is identified as a target of miR-21. miR-21 expression is elevated during adipogenesis and osteogenesis (40). These results suggest that miR-21 plays a critical role in maintaining the duration of the ERK-MAPK signaling pathway by repressing Spry2 expression to increase the differentiation potential of MSCs.

Furthermore, miR-21 targets Sox2 (41). Sox2 is one of four genes used to promote iPS cells and repress cell differentiation in concert with Oct4 and Nanog (42). The expression of osteogenic markers, such as Ocn and Runx2, is increased in MSCs when miR-21 is overexpressed. These results demonstrate that miR-21 not only suppresses the pluripotency but also accelerates osteogenic differentiation (Fig. 2).

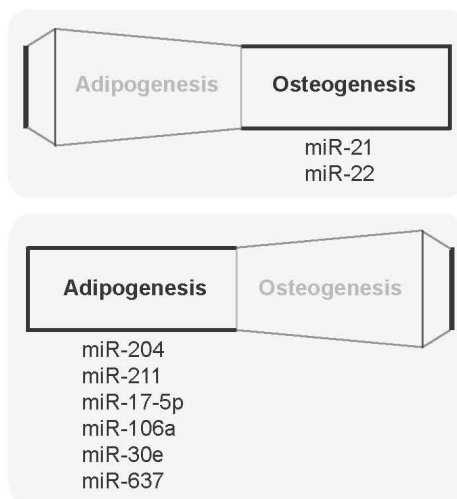


Fig. 2. miRNA switch of mesenchymal stem cell fate. The differentiation of an MSC into either an adipocyte or osteoblast can be controlled by miRNA switches. miR-21 and miR-22 switch on osteogenesis, while miR-204, miR-17-5p, miR-106a, miR-30e and miR637 switch on adipogenesis.

CONCLUSIONS AND PERSPECTIVES

The differentiation of mesenchymal stem cells into a particular lineage is tightly regulated, and a malfunction in this regulation could lead to pathological consequences. Specifically, an inverse relationship exists between the osteogenic and adipogenic lineage commitment and differentiation, suggesting a switch between these two processes. Recent miRNA expression profiling studies during both the adipogenic and osteogenic differentiation of MSCs have found several miRNAs with an inverse expression pattern between adipogenesis and osteogenesis. These miRNAs act as switches during the fate determination of MSCs by regulating molecular signaling pathways, such as Wnt/ β -catenin and BMP signaling, and multiple transcription factors. Therefore, modulation of levels of these miRNAs could serve as novel therapies for osteogenesis- or adipogenesis-related disorders. Further understanding of the miRNAs that modulate signaling pathways other than Wnt or BMP, including the TGF β , Notch, JAK/STAT, PI3K/Akt and Hedgehog signaling pathways during MSC differentiation will provide more complete picture of the mechanisms of the cell fate decision in MSCs.

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