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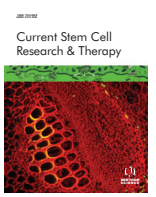
REVIEW ARTICLE

MicroRNA Regulation in Osteogenic and Adipogenic Differentiation of Bone Mesenchymal Stem Cells and its Application in Bone Regeneration



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Abstract: ***Background:*** Bone mesenchymal stem cells (BMSCs) are multipotent stromal cells providing a useful cell source for treating bone diseases and metabolic disorders. BMSCs fate determination and lineage progression are controlled by multiple cytokines, transcriptional factors, signaling pathways, and microRNAs (miRNAs). MiRNAs are small non-coding RNAs that inhibit the post-transcriptional gene expression or degrade their targets. They are closely involved in controlling the key steps of osteogenesis and adipogenesis of BMSCs.

Objective: We aim to summarize the roles of miRNAs and their pathways in regulating osteogenic and adipogenic differentiation of BMSCs, and sketch its preliminary applications in bone regeneration.

Method: We reviewed the published literature about the microRNA regulation in osteogenic and adipogenic differentiation of BMSCs.

Results: Most of miRNAs are expressed in BMSCs, perform as negative regulators of osteogenesis and have bidirectional effects on adipogenesis. Runx2 and PPARγ are two key transcriptional factors in osteogenesis and adipogenesis, respectively.

Conclusion: Anti-miRNAs or miRNA mimics is potential therapeutic strategy to repress pathological miRNAs for cellular therapies to bone diseases. The preliminary applications of miRNAs in BMSCs strongly suggested their bright future in bone regeneration.

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1. INTRODUCTION

Bone mesenchymal stem cells (BMSCs) are multipotent stromal cells that can differentiate into a variety of cell types, such as osteoblasts, adipocytes, chondrocyte, and *etc*. [1, 2]. Accumulating evidences support this theory that heightened osteoblastic and diminished adipocytic differentiation occurs through a "switch-like" diversion of BMSCs to osteoblastogenesis [3]. Many factors strictly govern the BMSCs' fates, and therefore they comprise a complex network to regulate the differentiation of BMSCs to a precisely-tuned extent. For example, multiple cytokines, such as TGFβ and BMP, induce osteogenic and adipogenic differentiation [4-6]. A variety of transcription factors including Runx2, Osterix, and SATB2 is related with osteogenesis [7, 8]. In addition, the pathways of Smad, MAPK, Wnt, Notch, and Hedgehog are involved in the above [9, 10]. What is worthy taking special consideration is that microRNAs (miRNAs) have been newly identified to play a crucial role in BMSCs' differentiation.

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MiRNAs are small non-coding RNAs serving as repressors of the post-transcriptional gene expression or degraders of their targets [11, 12]. Mature miRNAs are composed of approximately 22 nucleotides by binding with the 3' untranslated region (3'UTR) imperfectly complementarily [13]. They widely existed in eukaryotic organisms and had a tight relationship with cell differentiation, proliferation, and survival [14]. Moreover, miRNAs are stably expressed in body fluids, such as serum, plasma, urine, and saliva. So they are regarded as reliable markers of curative effect and prognosis [12]. At the same time, miRNAs are important regulators of bone remodeling in multiple aspects and signaling pathways. Therefore, it is needed to study the regulation roles of miRNAs on bone metabolism.

Of note, there is a complex circuit between miRNAs and bone homeostasis. Based on the published literature, different miRNAs interact with different genes and differentiation direction. In this review, we will summarize the recent development about the miRNAs that control BMSCs' fate and the related circuitry. It will shed light on the potential application of miRNAs to bone defect and provide a new clue for bone regeneration.

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2. MIRNAS CONTROL THE PHENOTYPES OF BMSCS

Although lots of miRNAs have been detected to either increase or decrease during BMSCs formation, differentiation and maturation, only a few of them are significantly changed and have meaningful targets related with bone metabolism, and then are valuable for us to further study. Long-term culture of BMSCs tended to increase miR-29C, miR-369-5P, miR-371, miR-499, and miR-LET-7F [15]. Data showed that miR-335 orchestrated cell proliferation, migration and differentiation of BMSCs. It controlled the switch between the resting and reparative phenotypes of BMSCs [16]. Differential miRNA expression analyses revealed that miR-335 was significantly downregulated during BMSCs differentiation. MiR-335 overexpression inhibited BMSCs' proliferation and migration, and differentiation. Wnt upregulated miR-335 expression, while interferon-c downregulated it. RUNX2 was witnessed as a direct target of miR-335 in BMSCs. However, Yang *et al*. found that increased miR-21 expression was associated with an elevated differentiation potential of adipogenesis and osteogenesis [17]. MiR-21 overexpression elevated PPARγ and runx2 expression during BMSC differentiation, whereas miR-21 knockdown reduced the two genes. The ERK–MAPK signaling pathway activity varied accordingly followed the up and down of miR-21 during the first 4 days of adipogenesis and osteogenesis.

3. THE REGULATION ROLES OF MIRNAS ON OSTEOGENESIS

Lots of studies have delineated various miRNAs expression in bone tissue or bone diseases. More and more findings indicated that miRNAs are key regulators of bone formation and have a close correlation with signal pathways and transcription factors that control osteoblast formation and differentiation. MiR-138 was expressed in mouse calvaria, but was very low in long bones and bone marrow [18]. A set of miR-22-3p, miR-328-3p, and let-7g-5p was obviously changed in 175 serum miRNAs obtained from osteoporosis patients and confirmed their effects on osteogenic differentiation BMSCs *in vitro* [19]. These reports implied a key role of miRNAs in osteoblastogenesis. Generally, the related miRNAs acted with osteo-related genes through the following three principal pathways (Fig. **1**). It is deduced that they have cross-talks between each other, but few literature about it.

3.1. MiRNAs Regulation on BMP-Smad1/5/8 Signaling Pathways of Osteogenic Differentiation

BMPs are members of the transforming growth factor beta (TGFβ) superfamily, and are the most effective osteogenic activators until now [4-6]. BMP receptors activate intracellular downstream Smads and then trigger a series of cascade reactions. MiR-214 was down-regulated during osteogenesis and up-regulated in BMSCs of osteoporotic mice [20]. MiR-214 overexpression suppressed BMSCs' osteoblast differentiation of *in vitro*, whereas inhibition of miR-214 function accelerated this process. FGFR1 was suggested as a direct target of miR-214. Similarly, miR-31 expression decreased progressively during BMSCs differentiation. Inhibition of miR-31 obviously enhanced ALP activity and mineralization in BMSCs cultures [21]. Overexpression

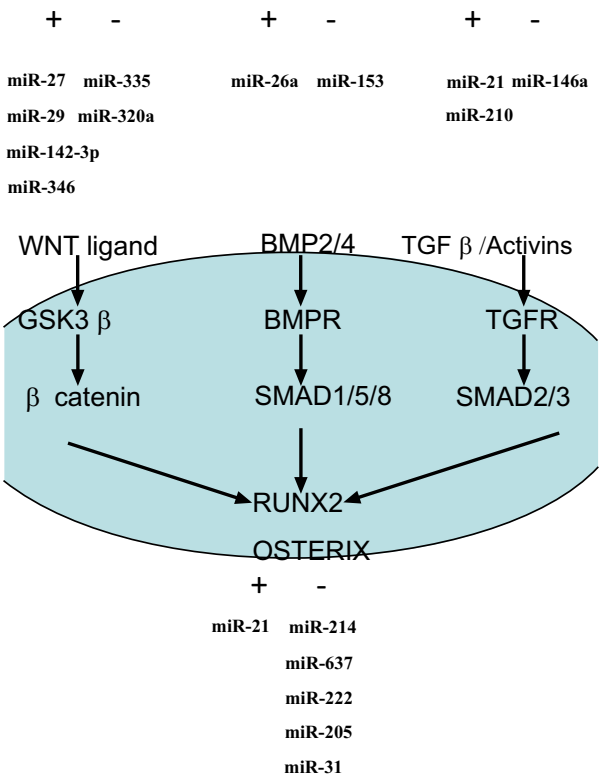

Fig. (1). Osteo-specific miRNAs through three main signaling pathways and their target genes. +: miRNAs acted as activators; -: miRNAs acted as suppressors.

of miR-31 obviously reduced expression of osteopontin (OPN), bone sialoprotein (BSP), osterix, and osteocalcin, but not Runx2 [22]. Based on in silico analysis, six BMPs gene homeobox a10 (HOXA10)-targeting miRNAs were identified among which miR-320a was downregulated during osteogenic induction [23]. MiR-320a overexpression downregulated HOXA10 and significantly inhibited bone formation in BMSCs. Ectopic expression of HOXA10 rescued the effects of miR-320a on osteogenesis. MiR-153 was a mechano-sensitive miRNA that regulated osteoblast differentiation by directly targeting BMPR2. Its overexpression inhibited the osteogenic differentiation of BMSCs [24]. Knockdown of BMPR2 by RNA interference suppressed the osteogenesis, with a similar effect to the upregulation of miR-153. Adversely, another group found that miR-26a treatment could effectively increase the osteogenesis [25]. It was found that miR-26a conducted its effect through BMP/Smad signaling pathway and then repressing Tob1 protein expression.

3.2. MiRNAs Regulation on WNT-βCATENIN Signaling

Wnt pathway, as a positive regulator of BMSCs self-renewal, was involved in diverse process, such as embryonic development, tissue homeostasis, and cancer pathogenesis. Zhang *et al*. reported that regulation of Wnt pathway by miR-27 and miR-29 stimulated human oseogenesis [26, 27]. Other reports also suggested that miR-27 and miR-142-3P upregulated and miR-335-50 downregulated during osteoblast differentiation [28]. In addition, miR‑346 promoted the osteogenic

differentiation of BMSCs by targeting glycogen synthase kinase-3β (GSK-3β) [29] through the Wnt/β-catenin pathway [30, 31].

3.3. miRNAs Regulation Through TGFβ/Activins-Smad2/3 Pathway

Activin is another member of TGFβ superfamily. Data showed that miR-21 played an important role in the stretch-induced osteogenesis of human periodontal ligament stem cells by directly targeting the activin receptor type IIB (ACVR2B), a transmembrane serine/threonine receptor kinase [32]. Similarly, miR-210 was suggested as a positive regulator of osteoblastic differentiation by suppressing the TGFβ/activin pathway through inhibition of AcvR1B [33]. In addition, enhanced miR-146a expression was found in osteogenesis targeting SMAD2 and SMAD3 of the TGFβ pathway [34].

3.4. Target Genes Involved in the Above Pathways

Runx2 is a bone transcription factor essential for bone formation and bone mineralization [35]. Osterix, another important transcription factor, also regulates osteogenesis in multi-dimension [36]. MiR-23a, miR-30c, miR-34c, miR-133a, miR-135a, miR-205, and miR-217 were reported as seven Runx2-targeting miRNAs [37]. At the same time, miR-214, miR-31, and miR-637 directly inhibited osterix expression [38-40]. Data demonstrated that miR-222 expression significantly decreased during the osteogenesis [41]. Inhibition of miR-222 stimulated osteogenic differentiation. Over expression of miR-222 acted opposite. Inhibition of miR-222-3p function by lentiviruses in BMSCs promoted expression of ALP activity and matrix mineralization through Smad5- RUNX2 signaling axis. Microarray assay from 3 donors demonstrated that miR-31 and Osterix inversely correlated during osteogenic differentiation [22]. Also, miR-205 expression was down-regulated in a time-dependent manner during osteogenesis [42]. Decreased miR-205 enhanced bone formation by up-regulating BSP and OPN protein levels and increasing ALP activity and osteocalcin secretion. Furthermore, miR-205 regulated protein expression of SATB2 and Runx2. Overexpression of SATB2 activated Runx2 and could rescued the negative effects of miR-205 on osteogenesis.

4. EFFECTS OF miRNAs ON ADIPOGENESIS

The essential transcriptical event involved in adipocytic phenotype is PPARγ and the expression of CCAAT/enhancer-binding protein α (C/EBPα) [43]. MiR-369-5p and miR-371 were antagonistic up-stream regulators of adipogenesis [15]. MiR-20a and miR-548d-5p repressed adipogenic differentiation of BMSCs through PPARγ signaling [44, 45]. Data suggested that miR-320 family including miR-320a, 320b, 320c, 320d and 320e, were upregulated during adipogenic differentiation of BMSCs [46]. MiR-320c overexpression in BMSCs promoted adipocytic differentiation and prompted adipocytes formation. Stable expression of miR-320c at physiological levels (~1.5-fold) accelerated adipogenesis and suppressed osteogenesis of BMSCs. In addition, it was demonstrated that miR-31, miR-27, and miR-130 all inhibited adipogenesis of BMSCs. A study about miR-155, miR-221, and miR 222 had validated that they may act as the negative regulators of PPARγ and C/EBPα, and as a result inhibited the adipogenic differentiation of BMSCs [47].

Moreover, some other miRNAs affected directly or indirectly interacted with PPARγ, and performed enhancing or inhibition effects on adipogenesis as a result. MiR21 increased adipogenesis of hBMSCs by blocking TGFβ [48]. However, Kang *et al*. believed that miR-21 promoted preadipocytes commitment by directly targeting AP1 gene [49]. In addition, miR-140 stimulated adipogenesis through down-regulating osteopetrosis-associated transmembrane protein (Ostm1), which may lead to the decrease of Wnt/β-catenin pathway [50]. Some studies explored the role of miR-210, miR-148a, miR-194, and miR-322 in regulating adipogenesis by WNT pathway [51] which were suggested to inhibit adipocyte formation by blocking the expression of PPARγ and C/EBPα.

5. miRNAs AND THE BALANCE BETWEEN OSTEOGENESIS AND ADIPOGENESIS

As osteoblasts and adipocytes share BMSCs as a precursor, a reciprocal association between bone formation and bone marrow adiposity has been noted frequently. Overexpression of miR-204, miR-17-5p, miR-106a suppressed osteoblast differentiation and promoted adipocyte differentiation [52]. However, miR-637 had an opposite effect. As the description above, miRNAs are suggested to regulate lots of genes involving bone status. Their effects on the multi-differentiation are likely to be complex. In coculture system, adipocytes transferred anti-osteoblastic miR-138, miR-30c, miR-125a, miR-31 to osteoblasts through extracellular vesicles (EVs) [53]. However, it's not clear whether EV was the key element in the balance of osteogenesis and adipogenesis.

MiR-30e was induced in primarily cultured mouse BMSCs [54], which is crucial for maintaining the balance of adipocytes and osteoblasts by targeting the Wnt/beta-catenin signaling. In addition, adipogenic stimuli reduced miR-194 expression with increases in COUP-TFII expression [55]. Osteogenic stimuli increased miR-194 expression with decreases in COUP-TFII expression. Therefore, miR-194 acted as a critical regulator of COUP-TFII, and could determinate the fate of MSCs to differentiate into osteoblasts and adipocytes. MiR-223 was involved in the reciprocal regulation of adipocyte and osteoblast differentiation through a novel C/EBPs/miR-223/FGFR2 regulatory feedback loop [56]. Let-7 was proved to obviously promote osteogenesis and inhibit adipogenesis of BMSCs *in vitro* through the repression of high mobility group AT-hook2 (HMGA2) expression [57].

Based on the published literature, it was drawn that miRNA- targeted osteoblast-related gene is RUNX2, while miRNA-targeted adipocyte-related gene is PPARγ and C/EBPα [58]. MiR-17-5P and miR-106a could promote adipogenesis and suppress osteogenesis by inhibiting runx2 expression, and increasing PPARγ and C/EBPα expression.

Most of the miRNAs were founded to inhibit or facilitate osteoblast differentiation and adipocyte differentiation. Even, some of their expression pattern overlapped more. However, there is still a few of miRNAs silencing both osteoblasts and adipocyte genes. We inferred that the ultimate biological effects of miRNA depended on the comprehensive aspects of their routine, targets, expression levels *etc*.

6. APPLICATIONS OF miRNAs IN BONE REGENERATION

It is well known that BMSCs are excellent precursors suitable for regeneration. An increasing number of miRNAs have been identified to participate in the differentiation of BMSCs. MiRNAs are detected in every progress as either a blocker or an activator in osteogenesis. They showed great promise to serve as biomarkers and potential therapeutic targets for bone. The putative targets of miRNAs should been identified by bioinformatics database and luciferase assay. Knowledge of these miRNAs roles, pathways and targets in BMSCs differentiation provides an important foundation for their application in bone regeneration. In experiments, overexpression of a specific miRNA was conducted by viral-based methods with viral vectors [59], antisense miRNA oligonucleotides (antagomirs), and microRNA sponges [60]. Additionally, the delivery system included silver nanoparticle complex [61], cell-penetrating peptide rich in arginine [62], and some materials with lower toxicity and less off-target effects.

The findings suggested that the potential utilization of miRNA mimics/ inhibitors or sponge to treat bone metabolic disorders is just a beginning. There is a long way between bench to clinic. It was reported that treating osteoblasts with miR-30c led to coordinate effects on the expression of a cluster of genes encoding components [63]. Another study conducted by Chen *et al*. reported that newly formed bone was detected with anti-miR-34a transfected cells [64]. Additionally, silencing of miR-542-3p resulted in bone formation by microCT scanning in overiectomized rats [65]. Wei *et al*. put miR-34 family into miR-34s-deficient mice and miR-34c transgenic mice, and found that they finally inhibited the proliferation and terminal differentiation of osteoblasts [66]. In a hind limb uploading mouse model, microCT suggested bone loss was partly reversed by anti-miR-103a treatment [67].

CONCLUSION

Hundreds of miRNAs are expressed in BMSCs and aid in modulating gene expression during BMSCs formation, development and differentiation. Most of them performed as negative regulators of osteogenesis through BMP/Smad, WNT/β-catenin, and Activin/Smad signaling pathways. Meantime, they have bidirectional effects on adipogenesis. Runx2 and PPARγ are two crucial transcriptional factors in osteogenesis and adipogenesis, respectively. Further, anti-miRNAs or miRNA mimics is potential therapeutic strategy to repress pathological miRNAs for cellular therapies. The preliminary applications of miRNAs strongly suggested their bright future in bone regeneration.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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