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Review Article

The emerging role of microRNAs in bone remodeling and its therapeutic implications for osteoporosis

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Osteoporosis, a common and multifactorial disease, is influenced by genetic factors and environments. However, the pathogenesis of osteoporosis has not been fully elucidated yet. Recently, emerging evidence suggests that epigenetic modifications may be the underlying mechanisms that link genetic and environmental factors with increased risks of osteoporosis and bone fracture. MicroRNA (miRNA), a major category of small noncoding RNA with 20–22 bases in length, is recognized as one important epigenetic modification. It can mediate post-transcriptional regulation of target genes with cell differentiation and apoptosis. In this review, we aimed to profile the role of miRNA in bone remodeling and its therapeutic implications for osteoporosis. A deeper insight into the role of miRNA in bone remodeling and osteoporosis can provide unique opportunities to develop a novel diagnostic and therapeutic approach of osteoporosis.

Introduction

Epidemiology of osteoporosis and bone fracture

Osteoporosis, a common and complex disease, is increasing dramatically with the aging of the population [1,2]. It is a multifactorial bone disorder with deterioration of microarchitecture and compromised bone strength, which predisposes bones to higher risks of bone fragility and bone fracture [3]. It is a chronic disease affecting both sexes and all races, exerting a strong influence on life quality, morbidity, and even mortality. It is estimated that the prevalence of osteoporosis is in more than 75 million people worldwide, and the number will increase to approximately 14 million by the year 2020 in the United States [1]. Osteoporosis-related fracture is over 1.5 million annually, and hip fracture is estimated to project to 6.3 million in 2050 [4]. Strikingly, the mortality rate is approximately 20% during the first year following a hip fracture [5]. Vertebral fractures are associated with increased risks of height loss, back pain, deformity, and mortality. It can increase the future risks of additional vertebral fractures by 5 to 10 times [6]. In the United States, direct healthcare costs of osteoporosis and its related bone fractures are estimated to be 19 billion USD per year [7]. However, as a global health concern, the condition remains severely underprevented, underdiagnosed, and undertreated.

Bone remodeling and pathogenesis of osteoporosis

The skeleton microstructure is composed of mineralized extracellular matrix and bone remodeling units, including osteocytes, osteoblasts, osteoclasts, and lining cells [1]. The function of osteoclasts and osteoblasts is critical in maintenance and remodeling of bones. Bone remodeling is a lifelong process with new bone tissues formed and mature bone tissues resorbed, also known as bone formation and bone resorption [8]. An imbalance of bone formation and bone resorption can result in metabolic bone diseases. If the process of bone resorption is faster than new bone formation, osteoporosis can finally occur

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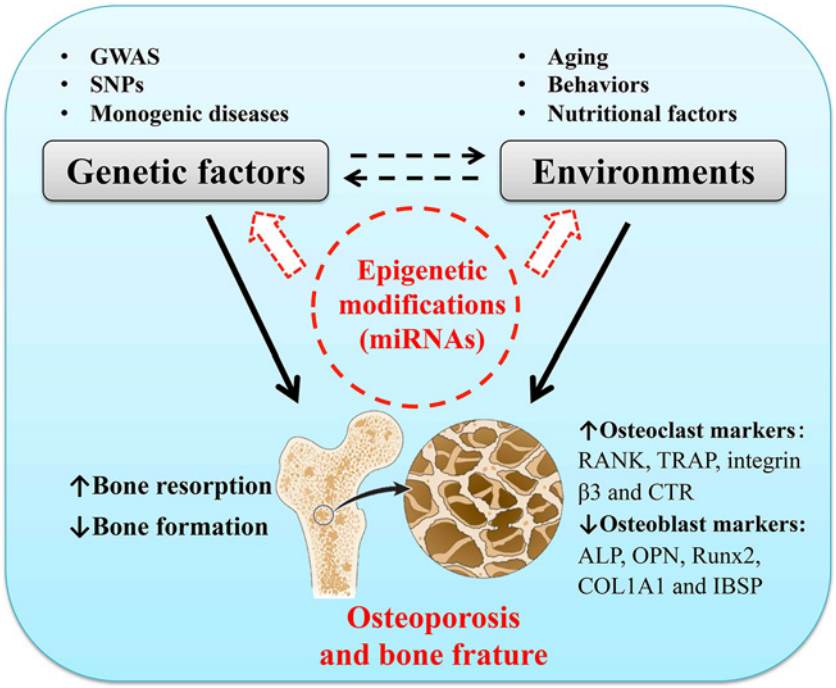

Figure 1. Epigenetic modifications underlying the risks of osteoporosis and bone fracture

Osteoporosis is a common and complex disease with multifactorial origin that is influenced by both genes and environments. Epigenetic modifications, especially miRNAs represent a promising area to link genetics and gene expressions with the risks of osteoporosis and bone fracture; ALP, alkaline phosphatase; COL1A1, collagen, type I, α 1; CTR, calcitonin receptor; IBSP, integrin binding sialoprotein; OPN, osteopontin; RANK, the receptor activator of nuclear factor-κ B; TRAP, tartrate-resistant acid phosphatase.

[9]. Osteoporosis is a multifactorial disease that can be regulated by both genetic factors and environments. Using genome-wide association studies, numerous studies about genetic risks for osteoporosis have been performed to assess bone mineral density (BMD) as a quantitative trait [10]. It reported that more than 60 genes were related with BMD and the development of osteoporosis [11]. Several studies have identified a number of single nucleotide polymorphisms associated with a low BMD or an increased risk of fracture [12], such as vitamin D receptor gene [13], insulin-like growth factor 1 gene [14], and estrogen receptor α gene [15]. It also has demonstrated that genetic causes of monogenic bone disorders with abnormal high or low bone mass and strength can induce osteoporosis [16]. Although genetic factors are important for the development of osteoporosis and other bone diseases, it is reported that the power of genetic variables in bone remodeling is less than 3% [17]. In addition to genetic factors, behaviors (such as low level of physical activity, cigarette smoking, and caffeine intake) together with nutrients (including dietary calcium intake and vitamin D deficiency) are critical determinants of osteoporosis and bone fracture [4]. Recently, emerging evidence suggests that epigenetic modifications may be the underlying mechanisms that link genetic and environmental factors with an altered risk of osteoporosis [18,19]. A hypothetical model was tentatively proposed to illustrate the interactions among genetic factors, environments and epigenetics, and the potential mechanism underlying the role of microRNAs (miRNAs) in bone remodeling and osteoporosis (Figure 1). A deeper insight into the epigenetic mechanisms underlying bone remodeling will provide opportunities to develop a novel therapeutic approach for osteoporosis and bone fracture.

Epigenetics and miRNAs

What is epigenetics?

Epigenetics is the study of heritable changes in gene function—a change in phenotype without a change in genotype, which was coined by Waddington in 1942 [20]. In recent years, epigenetics has been widely accepted as an important molecular process that can regulate the activity of genome and does not involve any changes to the underlying DNA sequence [21]. It can be inherited steadily by mitosis and meiosis through cell differentiation and division between

generations [22]. Epigenetics is characterized with three main features: without any alterations in the DNA sequence, heritability, and reversibility. Epigenetic regulations are involved in gene expressions that can produce permanent changes associated with cell differentiation and development [22]. There are three major categories of epigenetic modifications including DNA methylation, histone modification, and noncoding RNAs. For DNA methylation, the first genome-wide DNA methylation analysis of trabecular bone biopsies identified 241 CpG sites in 228 genes which were significantly differentially methylated between femur fracture patients and control osteoarthritis patients [24]. Zhang et al. [25] also examined histone acetylation and methylation in critical osteogenic genes during osteogenesis and found dynamic and distinct histone modifications of osteogenic genes during osteogenic differentiation. Noncoding RNAs are the latest discovered epigenetic modifications, such as miRNAs, long noncoding RNAs and circular RNA [26]. In our review, we mainly focused on the emerging role of miRNAs in bone remodeling, osteoporosis, and bone fracture.

A glimpse at miRNAs

MiRNAs are the most studied noncoding RNAs related with bone metabolism and bone diseases. MiRNAs are a major class of small RNA molecules (approximately 20–22 nucleotides), which can decrease the expressions of target genes at post-transcriptional level [27]. It can regulate post-transcriptional gene expressions by binding to the 3′-untranslated regions (3′-UTR) of target genes, resulting in mRNA degradation and transcription inhibition [28]. Each miRNA has a number of targets, and several miRNAs can target to the same mRNA. This phenomenon indicates that the process of miRNA regulation is complex [29]. To date, over 2000 miRNAs have been identified, and up to 60% of human genome can be regulated by miRNAs [30]. MiRNAs play a critical role in the regulation of most biological processes, including cell development [31], cell differentiation, cell proliferation [32], cell cycle regulation, and metabolism [26]. Numerous evidence demonstrated that miRNAs can regulate bone remodeling and the development of osteoporosis and bone fracture [33].

miRNA and its role in the process of bone remodeling

miRNAs and osteoblast differentiation

In the last decade, a large number of miRNAs have been clearly found and deeply involved in the regulation of bone remodeling, including bone resorption and formation [34-36]. The role of miRNAs in both osteoclasts and osteoblasts growth and differentiation has been largely investigated [37]. Osteoblast differentiation is an important process of bone homeostasis. Increasing studies indicated that miRNAs can regulate the biological process of osteoblast differentiation [33]. Several miRNAs can target the 3′-UTR of Runt-related transcription factor 2 (Runx2), the one important molecule that can regulate bone-related gene expressions [38]. Overexpression of miR-375 inhibited osteogenic differentiation by targeting Runx2, with decreased activity of several critical osteoblast markers, such as alkaline phosphatase (ALP), osteocalcin, and IBSP [39]. It also showed that miR-96 promoted osteogenic differentiation by suppressing heparin-binding epidermal growth factor-like growth factor (HB-EGF)–EGF receptor signaling in osteoblastic cells [40]. Li et al. [41] found that miR-194 regulated osteoblast differentiation through modulating signal transducer and activator of transcription 1 (STAT1)-mediated Runx2 nuclear translocation. Some miRNAs have been profiled to be able to promote and suppress distinct signaling pathway related with osteogenic differentiation. In mesenchymal stem cells (MSC), miR-124 could inhibit osteogenic differentiation and bone formation by targeting Dlx transcription factors, including Dlx5, Dlx3, and Dlx2 genes [42]. MiR-216a rescued osteogenesis, enhanced osteoblast differentiation and bone formation, by regulating c-Cbl-mediated phosphatidylinositol 3 kinase (PI3K)/protein kinase B/serine-threonine protein kinase (AKT) pathway [43]. In a human MSC, miRNA-153 could suppress osteogenic differentiation, with targeting bone morphogenetic protein receptor type II (BMPR2) [44]. MiR-542-3p suppressed osteoblast cell differentiation and proliferation, targeted bone morphogenetic protein-7 (BMP-7) signaling and then inhibited bone formation [45]. Dickkopf-1 (DKK1), as an important biomarker for osteoporosis, is an antagonist of WNT signaling pathway. One recent study showed that miRNA-433-3p promoted osteoblast differentiation with DKK1 as the target gene [46]. These data suggest that miRNAs play a significant role in the process of osteoblast differentiation targeting major genes and signaling pathways related with osteogenic differentiation. The relevant studies showing the role of miRNAs in osteoblast differentiation were summarized in Table 1 and Figure 2.

MiRNAs and osteoclast differentiation

Apart from an osteoblast differentiation, the expression pattern of miRNAs related with the osteoclast differentiation has also been explored. The ligand–receptor system of receptor activator of nuclear factor-kappa B (RANK)/RANK

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Table 1 Summary of the relevant studies showing the role of miRNAs in osteoblast differentiation

MiRNA ID	Cell types	Target genes and pathways	Effects on bone remodeling	Year	References
miR-375	C2C12 cell	Runx2	Inhibited osteogenic differentiation	2015	Du et al. [39]
miR-96	MC3T3-E1 cells and mouse MSCs	Heparin-binding EGF-like growth factor (HB-EGF)	Promoted osteogenic differentiation	2014	Yang et al. [40]
miR-194	Bone mesenchymal stem cells (BMSCs)	STAT1 and Runx2	Promoted osteoblast differentiation	2015	Li et al. [41]
miR-124	Human and mouse MSCs, MC3T3-E1 cells, and C2C12 cells	Dlx transcription factors: Dlx5, Dlx3, and Dlx2	Inhibited osteogenic differentiation	2015	Qadir et al [42].
miR-216a	Human adipose-derived MSCs (hAMSCs)	Osteoblast marker genes (ALP, OPN, Runx2, COL1A1 and IBSP), and c-Cbl-mediated PI3K/AKT pathway	Promoted osteoblast differentiation and enhanced bone formation	2015	Li et al. [43]
miR-153	Human mesenchymal stem cells (hMSCs)	BMPR2	Suppressed osteogenic differentiation	2015	Cao et al. [44]
miR-542-3p	Human osteoblast cells	BMP-7 and BMP-7/PI3K-survivin non-Smad pathway	Suppressed osteoblast cell proliferation and differentiation	2014	Kureel et al. [45]
MiR-433-3p	Rat bone marrow derived osteoblasts	DKK1	Promoted osteoblast differentiation	2017	Tang et al. [46]

Abbreviations: ALP, alkaline phosphatase; BMP-7, bone morphogenetic protein-7; BMPR2, bone morphogenetic protein receptor type II; COL1A1, collagen, type I, α 1; DKK1, Dickkopf-1; IBSP, integrin binding sialoprotein; MSC, mesenchymal stem cell; OPN, osteopontin; PI3K, phosphatidylinositol 3 kinase; Runx2, runt-related transcription factor 2; STAT1, signal transducer and activator of transcription 1.

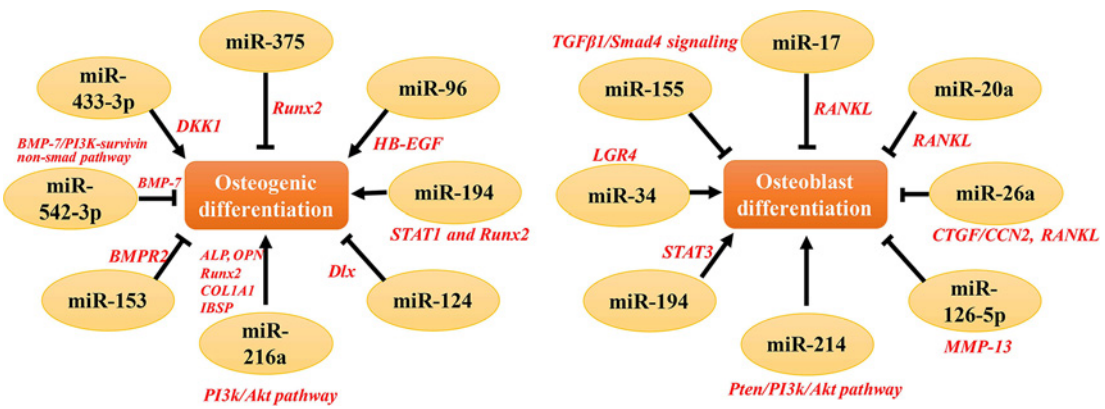

Figure 2. MiRNAs and their roles in osteoblast and osteoclast differentiation

A number of miRNAs have been clearly found and deeply involved in the regulation of osteoblast and osteoclast differentiation, by targeting to bone-related genes and different signaling pathways; ALP, alkaline phosphatase; BMP-7, bone morphogenetic protein-7; BMPR2, bone morphogenetic protein receptor type II; COL1A1, collagen, type I, α 1; CTGF/CCN2, connective tissue growth factor/CCN family 2; DKK1, Dickkopf-1; IBSP, integrin binding sialoprotein; LGR4, leucine-rich repeat-containing G-protein-coupled receptor 4; MMP-13, matrix metalloproteinase-13; OPN, osteopontin; PI3K, phosphatidylinositol 3 kinase; RANKL, the receptor activator of nuclear factor-κ B ligand; Runx2, runt-related transcription factor 2; STAT3, signal transducer and activator of transcription 3; TGFβ1, transforming growth factor β1.

ligand (RANKL)/osteoprotegerin (OPG) is a key component in bone tissue metabolism, which can regulate osteoclasts differentiation and osteolysis. MiRNA-17/20a could target RANKL and inhibit osteoclast differentiation induced by glucocorticoid in osteoblast cells [47]. MiRNA-26a mimic ectopic expression could attenuate osteoclast and actin-ring formation in osteoclast precursor cells, with decreased expression of connective tissue growth factor/CCN family 2 (CTGF/CCN2). Overexpression of miRNA-26a inhibitor promoted RANKL-induced osteoclast formation and function [48]. In addition to RANK/RANKL/OPG system, miRNAs also can regulate other signaling pathways related with osteoclast differentiation. Wu et al. [49] found that miR-126-5p overexpression could inhibit osteoclast

Table 2 Summary of the relevant studies showing the role of miRNAs in osteoclast differentiation

MiRNA ID	Cell types	Target genes and pathways	Effects on bone remodeling	Year	References
miR- 17/20a	Osteoblast cells	RANKL	Inhibited glucocorticoid-induced osteoclast differentiation and function	2014	Shi et al. [47]
miR-26a	Osteoclast precursor cells	CTGF/CCN2, RANKL	Attenuated osteoclast formation, actin-ring formation, and bone resorption	2015	Kim et al. [48]
miR- 126-5p	The stromal cells of giant cell tumor	MMP-13	Inhibited osteoclast differentiation	2014	Wu et al. [49]
miR-214	BMMs	Pten/PI3k/Akt pathway	Promoted osteoclastogenesis	2015	Zhao et al. [50]
miR-194	Primary mouse osteoclasts	STAT3	Promoted osteoclast differentiation	2014	Liu et al. [51]
miR-34	Osteoclast precursors	LGR4	Promoted osteoclast differentiation	2017	Cong et al. [52]
miR-155	Bone marrow-derived macrophages	TGFβ1/Smad4 signaling	Inhibited osteoclast differentiation	2017	Zhao et al. [53]

Abbreviations: CTGF/CCN2, connective tissue growth factor/CCN family 2; LGR4, leucine-rich repeat-containing G-protein-coupled receptor 4; MMP-13, matrix metalloproteinase-13; PI3K, phosphatidylinositol 3 kinase; RANKL, the receptor activator of nuclear factor-kappa B ligand; STAT3, signal transducer and activator of transcription 3; TGFβ1, transforming growth factor β1.

differentiation and decrease osteolysis formation in the stromal cells of a giant cell tumor. In bone marrow monocytes (BMMs), miRNA-214 overexpression promoted osteoclastogenesis by PI3K/Akt pathway, with phosphatase and tensin homolog (Pten) as the potential target [50]. Liu et al. [51] identified a novel miRNA (miR-9718) in primary mouse osteoclasts that could enhance osteoclast differentiation, with post-transcriptional repression of protein inhibitor of activated STAT3 (PIAS3). Recent studies found that miR-34c significantly promoted osteoclast differentiation targeting 3′-untranslated region of leucine-rich repeat-containing G-protein-coupled receptor 4 (LGR4) [52], while miR-155 mediated its suppressive effect on osteoclast differentiation by targeting transforming growth factor β1 (TGFβ1)/Smad4 signaling pathway [53]. Taken together, the aforementioned studies demonstrated that miRNAs can play a critical role in osteoclast differentiation. The relevant evidence was summarized in Table 2 and Figure 2.

Circulating miRNAs in osteoporosis and bone fracture

It reported that miRNAs can be secreted by different cells and have been discovered in the bloodstream and bodily fluids [54-58]. Circulating miRNAs have the potential to be utilized as novel biomarkers for the early diagnosis, treatment and prognosis of several diseases, such as cancer [59], cardiovascular diseases [60,61], obesity [62], and diabetes mellitus [63]. Recent studies demonstrated that serum miRNA levels were markedly up-regulated in patients with osteoporotic fractures and could impact osteogenic differentiation [56]. Nine miRNAs, including miR-93, miR-24, miR-23a, miR-124a, miR-122a, miR-21, miR-125b, miR-100, and miR-148a were significantly increased in the serum of 30 patients with osteoporosis, compared with 30 nonosteoporotic controls [64]. Li et al. [65] detected three miRNAs (miR-133a, miR-21, and miR-146) levels in the blood of 120 postmenopausal women and found that plasma miR-21 was decreased and miR-133a was increased in patients with osteoporosis and osteopenia, compared with the normal group according to T-scores of BMD. Weilner et al. [66] aimed to profile whether the expression of circulating miRNAs were variable in patients with newly osteoporotic fracture. Of 175 miRNAs in serum samples, the expression levels of six miRNAs (miR-133b, miR-22-3p, miR-10a-5p, miR-10b-5p, let-7g-5p, and miR-328-3p) were significantly related with bone fracture. These miRNAs were subsequently analyzed and further validated in a cohort with a larger sample size . Panach et al. [78] measured the expression levels of 179 serum miRNAs in osteoporotic women with fractures, and three miRNAs (miR-122-5p, miR-21-5p, and miR-125b-5p) were significantly up-regulated and indicated as valuable biomarkers in bone fracture. MiR-148a was reported to be increased in plasma in postmenopausal women with osteoporosis, and serum miR-148a level was correlated with clinical parameters of bone quality and quantity [67]. In a subsequent analysis, Kocijan et al. [68] assessed circulating miRNA signatures in male and female subjects with idiopathic or postmenopausal osteoporotic fractures and found that eight miRNAs were confirmed to be excellent discriminators of fractures regardless of age and gender. The listed studies reveal an

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important role for circulating miRNAs in osteoporotic patients. As the most abundant RNA species, miRNAs can be easily detected in circulation. It suggested that miRNAs can be utilized as novel biomarkers for early diagnosis and treatment, and it may be a potential target for medicine development. However, more comprehensive studies with larger samples and longer follow-up are warranted to investigate the significance of circulating miRNAs in osteoporosis and bone frature.

MiRNAs and its therapeutic implications for osteoporosis

Generally, it was deemed that the process of epigenetic regulation was static. However, this viewpoint has been altered and epigenetic modifications, including miRNAs, were perceived as dynamic and even reversible. It has been demonstrated that miRNAs are extremely attractive targets for therapeutic regulation in several diseases, such as brain tumors [69], gastrointestinal cancers [70], and cardiovascular diseases [71]. Compounds targeting specific miRNAs are currently utilized for the treatment of lung cancer. More specifically, chemically synthesized miR-34a mimic was administered intratumorally or intravenously by tail vein injections in mice (100 μg), which could restore a loss of function in cancer that drives a therapeutic response of lung cancer [72]. Silencing of miR-103/107 targeting caveolin-1 could lead to improved glucose homeostasis and insulin sensitivity and type II diabetes [73]. Some preliminary studies have showed that miRNAs play a critical role in the treatment of osteoporosis and bone fracture. Resveratrol, as a polyphenolic phytoestrogen with osteoinductive and osteogenic properties, could prevent osteoporosis in ovariectomized rats by suppressing the expression of miR-338-3p and increasing the expression of Runx2 [74]. Recently, it indicated that miR-365 could ameliorate osteogenesis suppression in MC3T3-E1 cells by targeting histone deacetylase 4 (HDAC4) [75]. In a glucocorticoid-induced osteoporosis C57BL/6J mice model, Li et al. [76] showed that curcumin improved bone microarchitecture by activating miRNA-365 targeting matrix metalloproteinase-9 (MMP-9). These two studies both suggest that miR-365 may be an important molecular regulating glucocorticoid-induced osteoporosis. Zhang et al. [77] demonstrated that RANKL was directly regulated by miR-338-3p and reintroduction of RANKL could reverse the inhibitory effects of miR-338-3p on osteoclast formation and bone resorption. However, the studies about therapeutic effects of miRNAs were limited, the specific doses and time point for miRNAs treatment are uncertain, and more preclinical studies are warranted. Since miRNAs are the most abundant RNA species to be found in circulation, quantification of their expression may be used as biomarker for early therapeutic effects and prognostic purposes of miRNAs-based treatment. It is speculated that the uptake of miRNA mimics is safe, which has no effect on normal cells because pathways regulated by the miRNA mimics are already activated by the endogenous miRNA in the cells [72]. However, the promiscuity of miRNAs should be addressed. One certain miRNA may have hundreds of transcription targets, thus the inhibition could lead to unwanted collateral effects. The current predictions by TargetScan, PicTar, EMBL, and ElMMo have a high degree of overlap because they all require stringent seed pairing. However, they are not 100% identical, increasing the difficulties of target predictions. Thus, this is maybe a major limitation to the application and development of therapies of miRNAs.

Conclusions

In summary, miRNA plays a critical role in the process of bone remodeling, including both osteoblast differentiation and osteoblast differentiation. Serum circulating miRNAs were detected and profiled in patients with osteoporosis and bone fracture. Furthermore, increasing evidences show that epigenetic modifications are not static, and are dynamic and even reversible. Thus, a deeper understanding of the role of miRNAs in osteoporosis and bone fracture can inspire critical implications for the early diagnosis and prevention of osteoporosis. It also can provide unique opportunities to develop novel therapeutic approaches of osteoporosis and its related bone fracture.

Author Contribution

Q.Y.F. and S.Z. collected data, synthesized data, and wrote the manuscript. Q.Y.F. and J.Z. reviewed and edited the manuscript. J.Z. contributed to the design of this review.

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Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

ALP, alkaline phosphatase; BMD, bone mineral density; BMM, bone marrow monocyte; BMP-7, bone morphogenetic protein-7; BMPR2, bone morphogenetic protein receptor type II; CCN2, CCN family 2; CpG, cytosines following by guanine; CTGF, connective tissue growth factor; DKK1, Dickkopf-1; HDAC4, histone deacetylase 4; LGR4, leucine-rich repeat-containing G-protein-coupled receptor 4; miRNA, microRNA; MMP-9, matrix metalloproteinase-9; MSC, mesenchymal stem cells; OPG, osteoprotegerin; Pten, phosphatase and tensin homolog; RANK, receptor activator of nuclear factor-kappa B; RANKL, RANK ligand; Runx2, Runt-related transcription factor 2; STAT1, signal transducer and activator of transcription 1; TGFβ1, transforming growth factor β1.

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