

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



MicroRNAs in Bone Diseases: Progress and Prospects

Hui-Yi Loh, Yui-Yee Lau, Kok-Song Lai and
Mohd Azuraiddi Osman

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.79275>

Abstract

With 19–25 nucleotides long, microRNAs (miRNAs) are small noncoding RNA molecules which play crucial roles in major cellular functions such as cell cycle control, apoptosis, metabolism, cell proliferation, and cell differentiation. Changes in the expression of miRNAs can cause significant effects to normal and aberrant cells. The dysregulation of miRNAs has been implicated in various human diseases such as brain tumor, osteoarthritis, schizophrenia, and breast cancer. Generally, miRNAs negatively regulate gene expression by binding to their specific mRNAs, thereby blocking their translation of the mRNAs. However, a few studies have reported that miRNAs could also upregulate the translation of certain proteins. This shows the important roles of miRNAs in various cell functions. This chapter will focus on the role of miRNAs in normal osteoblast and osteosarcoma cells. In addition, the great potential of miRNA as a new therapeutic approach to treat human bone diseases will also be discussed.

Keywords: microRNAs, bone diseases, osteoblasts, osteoclasts, bone homeostasis, gene regulation

1. Introduction

MicroRNAs (miRNAs) are short (19–25 nucleotides) single-stranded noncoding RNA molecules that regulate protein expression by complementary binding to mRNA targets with the aid of RNA-induced silencing complex (RISC) [1]. When miRNAs pair perfectly with mRNA targets, mRNAs degradation will occur. Translational repression of gene will happen when miRNAs bind partially complementary to mRNA targets [2]. Since the discovery of the first miRNA, *lin-4* in *Caenorhabditis elegans* in 1993, thousands of miRNAs have been identified in animals and plants [3]. These miRNAs play crucial roles in biological processes such

as cell growth, cell formation and differentiation, apoptosis, and cell metabolism. MiRNAs also regulate bone cells such as osteoblasts, osteoclasts, and osteocytes, which function in the mechanism of bone modeling and bone remodeling [4]. Osteoblasts play important roles in bone formation and osteoclasts function in bone resorption, whereas osteocytes regulate osteoblasts and osteoclasts activities by controlling signaling pathways [4] (**Figure 1**). Expression of many miRNAs has been found to be upregulated or downregulated in bone cancer cells compared to normal bone cells. Some of these miRNAs act as oncogenes such as miR-27, which promote the migration and invasion ability in the osteosarcoma [5]. Some other miRNAs act as tumor repressor genes such as miR-192 and miR-215, which play major roles in cell cycle arrest in cancer cells [6]. Dysregulation of miRNA expression by specific translation regulation such as DNA methylation, which leads to miRNA silencing, has been associated with bone diseases such as osteoporosis, osteogenesis imperfecta, and osteoarthritis [4, 7]. Therefore, understanding the roles of miRNAs in bone cells will provide the opportunity to develop miRNA-based therapy for bone diseases. In this chapter, we highlight the roles of various miRNAs that involve in the formation, resorption, and maintenance of bone in various bone diseases.

2. Regulatory role of microRNAs in normal bone growth and maintenance

Bone is a dynamic tissue that undergoes constant processes of modeling and remodeling throughout life. Bone modeling is the process where bones resculpture or rechange its overall size and shape as an adaptive mechanism against physiological processes or biomechanical influences, customizing or adjusting the skeleton toward the actions in which it encounters [8]. Bones may widen, change axis, or alter curvature by an independent action of osteoblasts and osteoclasts in response to biomechanical forces [9]. Bone modeling aids in the prevention of damage or injury to the bone [10] and regulates growth phase such as facilitating the increase in a child's skull size to accommodate the bigger brain as a child grows and undergoes marked change in the facial features of a child to that as an adult [11].

Meanwhile, bone remodeling is a sequential process, which involves the removal of the old bone (bone resorption) and the deposition of new bone (bone formation) [10, 12]. This process is ultimately important for the maintenance of the bone's strength and integrity by modulating the reshaping or replacement of bone during growth, preventing the accumulation of bone microdamage and regulating mineral homeostasis [8]. Bone remodeling is a lifelong, bone turnover [13] that is tightly regulated by two main population of bone cells: the bone-resorbing osteoclasts of hematopoietic lineage and the bone-forming osteoblasts of mesenchymal lineage [14, 15]. This tightly coupled process requires synchronized activities, balanced by both of these effector cells [8].

MicroRNAs serve as positive and/or negative regulators for various musculoskeletal signaling pathways or mechanisms by regulating bone biology such as in osteoblastic or osteoclastic differentiation, in accordance with the orchestrated balance between bone resorption and

bone formation. MiRNAs are known to be involved in the osteoclast-mediated bone resorption by regulating macrophage colony-stimulating factor (M-CSF) and receptor activator of NF- κ B ligand (RANKL)-induced signaling pathways, which involved in the commitment of osteoclasts from osteoclast progenitors [16].

2.1. MicroRNA roles in normal bone resorption

The receptor activator of NF- κ B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) signaling pathway are the principle operating pathways that regulate osteoclast differentiation and activation in bone remodeling [16]. These signaling pathways are tightly regulated by microRNAs. Hence, the involvement of miRNAs in the process of osteoclasts differentiation is crucial for normal bone resorption.

MiR-31 has been identified to be significantly upregulated in mice bone marrow cells under RANKL-induced osteoclast formation. The suppression of miR-31 by specific antagomirs under receptor activator of NF- κ B ligand (RANKL) treatment decreased the number of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated osteoblastic cells and ring-shaped mature osteoclastic cells. Additionally, less efficient resorption of synthetic calcium phosphate matrix and impaired actin ring formation for the development bone resorption sealing zone were also reported following the miR-31 antagomir transfection. In this situation of impaired osteoclastogenesis, the Ras homolog gene family member A (RhoA), which is the target of miR-31, was upregulated. Interestingly, treatment with RhoA inhibitor, coenzyme C₃ was able to rescue the decrease in number of ring-shaped TRAP-positive multinucleated cells and potentially revert the osteoclastogenesis impairment [17].

During the late stage of osteoclastogenesis, the osteoclasts undergo apoptosis to allow the reversal phase of bone remodeling, which allows the transition of bone resorption to bone formation [8]. At this stage, there is a significant upregulation of miR-26a under RANKL stimulation. Treatment with an miR-26a mimic in preosteoclast cells (pre-OCs) significantly inhibited the formation of osteoclast, peripheral actin ring, and resorption pit, whereas treatment with miR-26a inhibitor dramatically reversed these observations. The study proposed that miR-26a suppressed osteoclasts formation in the late stage of bone remodeling by targeting connective tissue growth factor/CCN family 2 (CTGF/CCN2), which plays an important role in promoting osteoclast formation via upregulation of dendritic cell-specific transmembrane protein (DC-STAMP) [18].

Another miRNA, miR-21 has been shown to be upregulated by RANKL-induced osteoclastogenesis in mouse osteoclast precursor cells' bone marrow-derived macrophages (BMMs). MiR-21 downregulates the expression of programmed cell death 4 (PDCD4), which is a repressor for c-Fos. The activated c-Fos, an important transcription factor for osteoclastogenesis, allows RANKL to induce nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) mRNA expression and stimulates osteoclast-specific markers such as tartrate-resistant acid phosphatase (TRAP) and cathepsin K. The silencing of miR-21 by transduction of BMMs with antisense oligonucleotides of miR-21 inserted in a lentiviral vector increased the expression of PDCD4 and impaired the RANKL-induced osteoclastogenesis [19].

In another study, the overexpression of miR-148a was observed during M-CSF and RANKL-stimulated osteoclast differentiation in CD14⁺ peripheral blood mononuclear cell (PBMCs). The overexpression of miR-148a induced the formation of osteoclast, whereas suppression of miR-148a showed an opposite outcome. *In vivo* study using ovariectomized (OVX) mice that undergoes intravenous injection with specific miR-148a silencing antagomir showed reduction in bone resorption and increase in bone mass density (BMD). Furthermore, osteoclasts number and the levels of osteoclast activity markers such as tryptophan-regulated attenuation protein (TRAP) and nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) mRNA in bone tissue were also decreased following antagomiR-148a treatment. This finding shows that decreased miR-148a levels impaired bone resorption through suppression of osteoclast activity. MiR-148a performs its regulatory role by targeting 3'UTR of V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB), a negative regulator of osteoclastogenesis and resulted in an inhibited expression of MAFB protein [20]. Additionally, MAFB serves as a negative regulator in RANKL-induced osteoclastogenesis by interfering the DNA binding capability of the three major transcription factors; NFATc1, c-Fos, and MITF in osteoclast differentiation [21].

The relative expression of miR-340 was downregulated upon M-CSF and RANKL-induced osteoblast differentiation in BMMs. It has been reported that the overexpression of miR-340 inhibits osteoclast differentiation and reduced the number of osteoclasts cells by targeting 3'UTR of microphthalmia-associated transcription factor (MITF), a transcription factor involved in osteoclast differentiation, leading to the reduced level of MITF mRNA and protein. MITF knockdown will inhibit TRAP, calcitonin receptor, V-ATPase d2, and cathepsin K expression, and thus, suggested that miR-340 may suppress osteoclast differentiation by targeting MITF [22].

2.2. MicroRNAs in normal bone formation

Canonical wnt/ β -catenin signaling pathway is a major pathway required for the commitment of mesenchymal stem cells into osteoblast lineage [15]. The stabilization of β -catenin is important for the expression of wnt-responsive gene [16]. The miR-29 family is one of the well-known miRNA families that regulate osteoblast function, which plays a key role in the positive regulation of osteoblast differentiation by targeting several wnt-signaling pathway inhibitors. The expression of miR-29a is induced by canonical wnt signaling during osteoblast differentiation and has been shown to target dickkopf-1 (Dkk1), Kringle domain-containing transmembrane protein (Kremen2), and secreted frizzled related protein 2 (sFRP2), which acts as inhibitors of wnt receptor complex [23]. Moreover, miR-29b was also found to target several other inhibitors of bone formation such as histone deacetylase 4 (HDAC4), transforming growth factor beta 3 (TGF β 3), activin receptor type-2A (AcvR2A), beta-catenin-interacting protein 1 (CTNNBIP1), and dual specific phosphatase 2 (DUSP2) by binding to their mRNA 3'UTR [24]. Furthermore, the expression level of miR-29 is low during the early phase of osteoblastogenesis and increases at late phase, as miR-29 targets α 1 and α 2(I)collagen, α 1(III)collagen, fibrillin 1, and osteonectin, which are important for the formation of collagen fibril matrix secreted by osteoblasts, and thus allowed for collagen matrix deposition before subsequent mineralization in bone formation process [25]. Therefore, miR-29 family is important in the promotion of osteoblastogenesis by repressing the inhibitors of osteogenesis and in the meanwhile plays crucial regulatory role in the attenuation of collagen synthesis in mineralized bone.

On the other hand, bone morphogenetic protein (BMP)-signaling pathway is crucial for the differentiation of myoblastic cell lines into osteoblast lineage and bone formation [16]. MiR-133 and miR-135 are downregulated in BMP-2-induced osteoblastic differentiation of C2C12 pluripotent mesenchymal cell line. MiR-133 is a negative regulator of Runt-related transcription factor 2 (Runx2), a transcription factor required for osteoblast differentiation, while miR-135 represses the osteoblastic differentiation of C2C12 cells by acting toward mothers against decapentaplegic homolog 5 (Smad5), an intracellular Runx2 co-receptor. Hence, downregulation of miR-133 and miR-135 will increase the expression of Runx2 and Smad5, promoting the BMP-2-induced osteoblast differentiation. Moreover, the overexpression of these miRNAs will suppress the expression of BMP-induced osteoblast-specific protein markers such as alkaline phosphatase (ALP), osteocalcin, and homeobox A10 (HOXA10) [26]. Another miRNA, miR-20 has been shown to involve in the transformation of osteoblast from human MSCs by downregulating the expression of silencing peroxisome proliferator-activated receptor γ (PPAR γ), bone morphogenetic protein and activin membrane-bound inhibitor (Bambi), and cysteine-rich transmembrane BMP regulator 1 (Crim1) and therefore, activated the BMP-2/Runx2 signaling [27].

Another report showed that miR-2861 expression was elevated in primary mice osteoblasts. Overexpression of miR-2861 in mice bone marrow stromal cells (BMSCs) has been reported to promote BMP2-induced osteoblast differentiation. Conversely, the inhibition of miR-2861 expression results in the decrease in osteoblast differentiation. *In vivo* knockdown of miR-2861 in OVX mice resulted in enhanced decrement of bone volume and bone formation rate. Furthermore, histone deacetylase 5 (HDAC5) has been identified as the direct target of miR-2861. HDAC5 deacetylates Runx2 and allow the deacetylated Runx2 to undergo SMAD specific E3 ubiquitin protein ligase 1 (Smurf1)-mediated degradation, decreasing the rate of osteoblast differentiation. Therefore, the abundance of acetylated Runx2 will increase upon HDAC5 suppression by miR-2861 and promote osteoblast differentiation [28]. MiR-3960 is generated

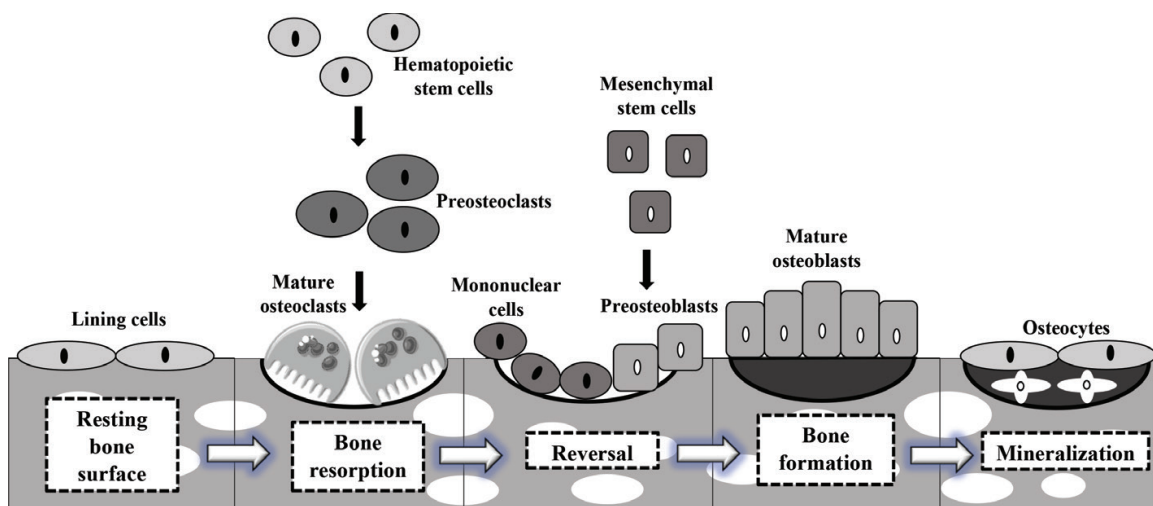


Figure 1. The process of bone remodeling begins with the recruitment of osteoclast progenitor cells to the site of bone remodeling, followed by osteoclast progenitor cells differentiation into mature osteoclasts. Reversal phase allows the transition from bone resorption phase to bone formation phase. In bone formation phase, mesenchymal stem cells differentiate into mature osteoblast and secrete collagenous components for bone formation. Bone remodeling process is completed after the mineralization of collagen fibril matrix and subsequent transformation of osteoblasts into osteocytes.

from the same genetic locus as the miR-2861 due to the transcription from the same primary microRNA (pri-miRNA). MiR-3960 was found to directly target homeobox A2 (HOXA2), a negative regulator of Runx2. Hence, the miR-3960-mediated suppression of HOXA2 by miR-3960 will increase Runx2 expression and osteoblast differentiation [29]. The summary of the MicroRNAs involved in the regulation of normal bone development is shown in **Table 1**.

MicroRNAs	Target gene or protein encoded	Associated event	Reference
<i>MicroRNAs associated with bone resorption</i>			
MiR-31	RhoA	Promotes osteoclast differentiation by targeting RhoA	[17]
MiR-26a	CTGF/CCN2	Inhibits osteoclast differentiation by targeting CTGF/CCN2	[18]
MiR-21	PDCD4	Promotes osteoclast differentiation by targeting PDCD4	[19]
MiR-148a	MAFB	Promotes osteoclast differentiation by targeting MAFB	[20]
MiR-340	MITF	Inhibits osteoclast differentiation by targeting MITF	[22]
<i>MicroRNAs associated with bone formation</i>			
MiR-29	COL1A1, COL3A1, fibrillin 1, osteonectin	Downregulated during the early phase of bone formation and upregulated during the late phase by targets COL1A1, COL3A1, fibrillin 1, and osteonectin to allow the formation of collagen fibril matrix	[25]
MiR-29a	Dkk1, Kremen2, sFRP2	Promotes osteoblasts differentiation by targeting Dkk1, Kremen2, sFRP2 inhibitors of wnt signaling pathway	[23]
MiR-29b	HDAC4, TGFβ3, AcvR2A, CTNNBIP1, DUSP2	Promotes osteoblasts differentiation by inhibitors of bone formation such as HDAC4, TGFβ3, AcvR2A, CTNNBIP1, and DUSP2	[24]
MiR-133	Runx2	Inhibits osteoblast differentiation by targeting Runx2	[26]
MiR-135	Smad5	Inhibits osteoblast differentiation by targeting Smad5	[26]
MiR-20	PPARγ, Bambi, Crim1	Promotes osteoblast differentiation by targeting PPARγ, Bambi and Crim1	[27]
MiR-2861	HDAC5	Promotes osteoblasts differentiation by targeting HDAC5, which represses Runx2	[28]
MiR-3960	Hoxa5	Promotes osteoblasts differentiation by targeting Hoxa5, which represses Runx2	[29]

Table 1. MicroRNAs involved in the regulation of normal bone development.

3. MicroRNAs' expression in various bone diseases

Dysregulation of miRNAs affects critical pathways and biological processes, which lead to various bone diseases. MiRNA profiling studies have revealed that miRNA expression patterns are specific to various types of bone diseases, and it reflects the developmental lineage and pathway that lead to the diseases.

3.1. Benign bone tumor: giant cell tumor

Giant cell tumor of bone (GCTB) is an aggressive benign tumor that is able to metastasize, and up to 6% of GCTB patients grow pulmonary metastases (metastatic spread via blood or lymphatics) [30]. GCTBs are characterized by the presence of numerous multinucleated osteoclast-like giant cells distributed among mononuclear stromal cells [31]. GCTBs are also characterized by extensive bone resorption, which results in regional pain and bone destruction, mostly occurring in distal femur, proximal tibia, distal radius, and sacrum [32, 33]. Histologically, GCTBs can be classified into three main types, which are osteoclast-like multinucleated giant cells, monocytic round cells, and spindle-like stromal cells [34]. Current treatments of GCTBs are ranging from intralesional curettage to wide resection [33]. Since the cause of GCTBs is extensive bone resorption by aggressive lytic process, the repression of osteoclastogenesis becomes a potential approach to cure GCTBs [30].

A study reveals that treatments with miR-16-5p mimic repressed RANKL-induced osteoclastogenesis in GCTBs. However, the formation of RANKL-induced osteoclast was enhanced with miR-16-5p inhibitor. Furthermore, the osteoclastogenesis-related genes like cathepsin K (CK), tartrate-resistant acidic phosphatase (TRAP), and matrix metalloproteinase 9 (MMP9) were also upregulated by miR-16-5p inhibitor. This finding shows that miR-16-5p inhibits osteoclastogenesis; hence, it has the potential to be used as a therapeutic target to control the excessive bone resorption in GCTBs [30]. Another study by Wang et al. found that miR-106b is another microRNA that target RANKL to inhibit osteoclastogenesis and osteolysis in GCTBs [32].

Parathyroid hormone 1 receptor (PTH1R) is a transmembrane receptor that binds to G proteins. The activation of pathways that promote osteoclastogenesis in osteoblasts is induced when PTH binds to parathyroid hormone 1 receptor (PTH1R). Wu et al. reported that miR-125b directly targets the 3'UTR of PTH1R. Overexpression of tumor suppressor miR-125b inhibits the osteoclastogenesis and also PTH1R downstream target such as RANKL and IL-8 [35]. The downregulation of miR-125b in GCTBs revealed that it suppressed the cell growth and proliferation in GCTBs.

3.2. Bone remodeling abnormality: osteoporosis

Osteoporosis is a multifactorial bone disorder characterized by low bone mass, impaired bone quality, and a more susceptibility to fracture [36]. The recent global statistics from the International Osteoporosis Foundation reported that 1 in 3 women and 1 in 5 men above the

age of 50 will suffer from osteoporotic fractures in their lifetime [37]. The primary osteoporosis is generally arising due to the postmenopausal deficiency or loss of sex hormones such as estrogen, while the secondary osteoporosis is due to the presence of underlying diseases and medication of treatments with glucocorticoids, hyperthyroidism, diabetes mellitus, and gastrointestinal disorders [37, 38]. The bone mineral density peaks during adolescence stage of puberty, which then maintained throughout an individual middle age for some decades and subsequently begins to loss upon aging. Bone tissue undergoes continuous process of resorption and formation throughout in an individual lifetime. Osteoporosis occurs when bone resorption rate exceeds the bone formation rate, resulting in a net loss of bone [39]. Studies revealed that osteoporosis incidences may be linked to bone mass-related genetic determinants including low-density lipoprotein receptor-related protein 5 (LRP5), osteoprotegerin (OPG), sclerostin (SOST), estrogen receptor 1, and the receptor activator of RANK/NF- κ B signaling pathway [40].

Receptor activator of nuclear factor kappa-B ligand (RANKL) binds to the receptor activator of nuclear factor κ B (RANK) that is present on the surface of the osteoclast mononuclear precursor cells and facilitates the formation of fully differentiated osteoclasts [41]. The expression of miR-503 is significantly reduced in progenitors of osteoclasts-CD14⁺ peripheral blood mononuclear cells (PBMCs) of postmenopausal osteoporosis patients compared to healthy postmenopausal controls. The overexpression of miR-503 in human PBMCs had dramatically inhibited RANKL-induced osteoclast differentiation in PBMCs of postmenopausal osteoporosis samples. *In vivo* transfection of miR-503 silencing antagomir into a postmenopausal-stimulated ovariectomized (OVX) mice resulted in the increase in RANK protein expression, an increase of bone resorption rate, a decrease in bone mass, and an aggravation of bone loss. Contrastingly, the transfection of the OVX mice with miR-503 overexpressing pre-miR-503 leads to the decrease in RANK protein expression and thus a decrease in bone resorption and an increase in bone mass. Therefore, it is suggested that the low miR-503 expression in postmenopausal osteoporosis patients will promote RANKL-induced osteoclastogenesis, and consequently, bone resorption rate will increase leading to net bone loss [42].

MiR-221 expression is downregulated in postmenopausal osteoporotic bone samples compared to nonosteoporotic bones. In BMP-2-induced osteoblastogenesis, the overexpression of miR-221 resulted in reduced expression of key osteoblast markers, including osteocalcin (OC), alkaline phosphatase (ALP), and collagen type Ia 1 (COL1A1), whereas knockdown of miR-221 promoted the activity of OC, ALP, and COL1A1 [43]. The later study identified Runx2 as a potential target of miR-221. Therefore, this provided an evidence that miR-221 serves as the negative regulator of osteoblast differentiation and contributes to the osteoporosis pathogenesis through the regulation of Runx2 action [44].

Tumor necrosis factor α (TNF- α) inhibits MSC osteogenic differentiation and bone formation in estrogen deficiency-induced osteoporosis with a poorly understood mechanism. A study conducted by Yang et al. showed that the expression of miR-21 is dramatically downregulated in mesenchymal stem cells (MSCs), and this downregulation is due to the suppression by TNF- α during the osteogenesis of MSCs. Moreover, miR-21 has been proved to stimulate the osteoblast differentiation of MSCs by targeting protein sprouty homolog 1 (Spry1), a negative regulator of osteoblast differentiation from MSCs. The later study also demonstrated that the overexpression of miR-21 is able to partially rescue the osteogenic impairment induced by

TNF- α in MSCs. Furthermore, *in vivo* treatment with anti-TNF- α in OVX mice has increased bone formation by upregulating miR-21 expression, suppressing Spry1 expression and remediating the inflammatory conditions. Thus, this study indicated that TNF- α impairs osteoblastic bone formation by suppressing miR-21 expression in estrogen deficiency-induced osteoporosis [45].

A study conducted by Wang et al. showed that glucocorticoid-treated mice experienced low bone mass density (BMD) and bone mass content (BMC). Glucocorticoid treatment also significantly resulted in the decrease of bone matrix COL1A1 expression, an increase in dickkopf-1 (Dkk-1) expression and a reduction in miR-29a expression [46]. MiR-29a plays important role in osteoblast differentiation and bone homeostasis by regulating the expression of Wnt inhibitor Dkk-1 [23]. *In vivo* miR-29a precursor treatment was able to reduce the glucocorticoid-stimulated BMD and BMC, attenuate glucocorticoid-induced loss of trabecular bone volume fraction, decrease the porosity of cortical bone, and rescue the adverse effect of glucocorticoid on peak load of bone tissue. The treatment with miR-29a inhibitor, however, provided opposite effects [46]. Thus, miR-29a is important in protection against glucocorticoid-induced osteopenia, which may lead to osteoporosis by regulating the activity of Wnt signaling and Dkk-1 in osteoblast differentiation and bone mineralization [23, 47].

3.3. Bone collagen matrix retardation: osteogenesis imperfecta

Osteogenesis imperfecta (OI) is a heterogeneous group of inherited connective tissue disorder that occurs in about 1 in 10,000 to 20,000 live births [47]. OI is characterized with clinical features such as susceptibility to bone fractures due to low bone mass, reduced bone strength, or quality and bone deformity [48]. In addition, blue sclerae, short stature, dentinogenesis imperfecta (DI), and hearing loss are other clinical manifestations of OI [49]. The pathogenesis of OI involves the most prevalent autosomal dominant mutation of COL1A1 and COL1A2 genes encoding the alpha1 and alpha2 chains of type I procollagen [50]. Type I procollagen is the major bone structural protein, and therefore, the mutation of COL1A1 and COL1A2 genes may have direct link with serious defects or abnormalities including deformities of collagen primary structure, insufficient bone collagen quantity, deviated posttranslational modification, folding, intracellular transport or matrix incorporation, and bone mineralization. Recessive OI is caused by defects in genes that encode for protein products, which interact with type I collagen [51]. There are four well characterized types (I, II, III, and IV) of COL1A1/COL1A2-linked OI based on different clinical and genetic presentations [52].

Wang et al. performed the preliminary screening of more than 100 bone-related miRNAs in serum of 22 OI patients. The results showed that three miRNAs (miR-26a, miR-30e, and miR-21) were upregulated and eight miRNAs (miR-34c, miR-29a, miR-29b, miR-489, miR-133a, miR-145, miR-210, and miR-1297) were downregulated in OI patients compared to healthy controls. MiR-29a has a universal lower level in the patient group, whereas miR-26a had a universal upper level. This discovery of altered expression of bone-related miRNAs in OI patients' serum profile may become promising miRNA biomarkers for the diagnosis of OI. Although this study did not verify on the relationships of these differentially expressed miRNAs and their potential target genes, the previous studies have showed that these miRNAs may target a range of gene involved in osteogenic signaling pathways such as BMP, Wnt, RANKL, and TGF β /activin [53].

MiR-29b has been shown to modulate osteoblast differentiation by downregulating the activity of COL1A1, COL5A3, and COL4A2 and attenuate the collagen protein accumulation during the mineralization phase of bone formation [24]. Kaneto et al. performed a sequence analysis on the coding region and intron/exon junctions of COL1A1 and COL1A2 genes in five independent patients with type I and type III OI. The sequence analysis has identified eight novel mutations, which may contribute to OI phenotype. Interestingly, Kaneto et al. also determined that the expression levels of COL1A1 and miR-29b are reduced in both type I and type III OI patients. Therefore, it is speculated that miR-29b expression is not an essential for sustaining osteoblastogenesis [54].

3.4. Enlarged, weak bone deformation: Paget's disease of bone

Paget's disease of bone (PDB) is a localized disorder of highly exaggerated bone turnover characterized by excessive bone resorption action by osteoclasts within pagetic lesions, followed by an increase in disorganized new bone formation by osteoblasts [55]. This action will eventually result in marrow fibrosis, highly vascular, weak, enlarged, and disorganized bone deformation [55, 56]. The highly fibrous woven bone with reduced mechanical strength and disorganized structural integrity tends to increase the risk of bone deformity and fracture [56]. Frequently, PDB patients are elderly aged more than 50 years and tend to slightly predominate in males [57]. Mutations in genes encoding for the components that modulate the RANK/NF- κ B signaling pathway are most likely to contribute to the development of PDB. These genes are sequestosome 1 gene (SQSTM1), tumor necrosis factor receptor superfamily member IIA (TNFRSF11A), valosine-containing protein (VCP), and tumor necrosis factor receptor superfamily member IIB (TNFRSF11B) [55].

However, the regulatory roles of miRNAs in PDB remain unknown. Bianciardi et al. performed a serum miRNA expression profile in peripheral blood mononuclear cells (PBMCs) from 20 PDB patients. The results showed that 22 miRNAs were significantly upregulated with a fold change above three (miR-31, miR-32, miR-124a, miR-132, miR-182, miR-221, miR-339, miR-345, miR-410, miR-451, miR-485.3p) or between 2 and 3 (miR-19a, miR-30b, miR-30c, miR-27a, miR-125a, miR-146a, miR-148a, miR-200c, miR-223, miR-301, miR-365) when compared to non-pagetic controls. Among the 22 miRNAs, these 14 miRNAs (miR-19a, miR-miR-27a, miR-30c, miR-32, miR-125a, miR-132, miR-200c, miR-221, miR-223, miR-301, miR-345, miR-365, miR-410, and miR-485-3p) showed significantly higher expression in patients that experienced Q16STM1 mutation [58].

4. Current status and perspectives of microRNA in bone cancer diagnosis and therapy

In 2010, the first microRNA-targeting drug—miravirsen (SPC3649), a locked nucleic acid (LNAs) ribonucleotides antagomir that targets miR-122 had entered clinical trial and is currently in phase II clinical trial to treat chronic hepatitis C (HCV+) patients (ClinicalTrials.gov Identifier: NCT02508090) [59]. The occurrence of the first miRNA-based clinical trial had led to the insight that miRNAs can serve as promising therapeutic tools and perhaps as the next

magic bullet. The side effects arise from current conventional treatments of bone cancer that also lead to the path of translating the bone cancer miRNA-based therapeutic approaches from the benchworks to the clinical settings.

MRX34, a miRNA mimic encapsulated by liposomal nanoparticle developed by Mirna Therapeutics, appeared as the first miRNA mimic that had reached phase 1 clinical study in year 2013 for the treatment of primary liver cancer and other malignancies including multiple myeloma through functional restoration of endogenous miR-34a as an oncosuppressor (ClinicalTrials.gov Identifier: NCT01829971) [60]. MiR-34a is often suppressed or showed to reduce expression in various cancer types, coupled with the loss of p53 function that transcriptionally control its expression [61]. There are a wide varieties of oncogenes such as cyclin-dependent kinase (CDK) 4/6, Wnt 1/3, B-cell lymphoma 2 (BCL2), MYC, cyclin D1 (CCND1), CD44, and histone deacetylase 1 (HDAC1) that are responsible for unregulated cell cycle progression and proliferation, anti-apoptosis, metastasis, chemoresistance, cancer cell self-renewal, and oncogenic transcription, which can be downregulated by miR-34a [62, 63]. In a phase I clinical trial, adult patients with advanced solid tumors refractory to standard conventional treatment were given a standard 3 + 3 dose escalation trial by which MRX34 was infused to the patients twice a week (BIW) for a period of 3 weeks in a four-week-cycle. The phase 1 results showed that MRX34 has a tolerable toxicity or safety profile and supportive evidence of anti-tumor activity in a subset of patients with refractory advanced solid tumors. The patients generally experienced mild adverse effects such as fever, fatigue, back pain, nausea, anorexia, diarrhea, and vomiting after the treatment [64].

To date, there has been no available miRNA-based diagnostic tests or treatments for bone cancers' management. However, miR-34 anti-tumor activity had been demonstrated in numerous cancer types including bone cancer and multiple myeloma, and therefore, providing a fascinating insight into the introduction of miR-34a mimic for the treatment of bone cancers.

The expression of tumor suppressive mir-34 and miR-122 are downregulated in osteosarcoma cells contrasting to healthy normal cells. Xiao et al. has introduced miRNA response elements (MREs) of miR 34 and miR 122 in osteosarcoma cells through the employment of adenovirus to enable the selective expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). This study reported that the adenovirus (Ad) TRAIL-34-122 resulted in higher apoptotic and cytotoxicity levels in the osteosarcoma cells, compared to the normal cells by selectively expressing TRAIL in miR-34 and miR-122 modulated fashion. The following *in vivo* study in BALB/c nude mice further indicated that Ad-TRAIL-34-122 is able to reduce osteosarcoma xenografts' growth without causing significant liver toxicity [65]. Additionally, Gaur et al. reported that chitosan nanoparticle-mediated delivery of miR-34a mimic preserves bone integrity and reduces tumor growth in a tumor established, intrafemoral nude mice model that represented prostate cancer bone metastasis [66].

Furthermore, Martino et al. has evaluated the activity of synthetic miR-34a in multiple myeloma cells. This study demonstrated that transfection with miR-34a mimic tends to inactivate the early expression of prosurvival and proliferative kinases Erk-2 and Akt. The reduced expression of Erk-2 and Akt is followed by the downregulation of caspase-6/3 expression, which can next induce apoptosis in multiple myeloma cells. Martino et al. subsequently tested the efficiency of miR-34a mimic delivery by encapsulating the mimic in stable nucleic acid

lipid particles (SNALPs). SNALP-encapsulated miR-34a mimic is highly efficient with its anti-tumor activity in both multiple myeloma cells and in *in vivo* SCID mice bearing human multiple myeloma xenografts by showing reduced expression of miR-34a target notch 1 homolog (NOTCH1) and the absence of cytotoxicity effect [67].

5. Future prospects of microRNAs in the treatment of bone disorders and its potential

Although the publication of research findings on microRNAs in bone disorders are still limited, the fast-growing list of literatures indicates the significance of miRNAs in the regulation of bone biology and bone disorders. This has led to the advancement of research to explore potential relevance of miRNAs as diagnostic biomarkers and therapeutics. In this section, the potential of miRNAs as the biomarkers and therapeutic agents will be focused on cancer-related bone disorder (osteosarcoma) and noncancer-related bone disorder (osteoporosis).

5.1. MicroRNAs as diagnostic biomarkers

The comprehensive expression profile of key microRNAs in different bone disorders has the potential to increase the accuracy of the prognosis and diagnosis of bone disorders in combination with other conventional diagnostic approaches.

Hu et al. reported that a total of 268 miRNAs were dramatically dysregulated between human osteosarcoma cell line, MG-63, and human osteoblast HOB cell line. Five miRNAs (miR-9, miR-99, miR-195, miR-148a, and miR-181a) were validated to be overexpressed and four of these miRNAs (miR-143, miR-145, miR-335, and miR-539) were validated to be downregulated in the human OS MG-63 cell lines compared to osteoblast HOB cell lines. The bioinformatics analysis showed that the target genes of these nine miRNAs are associated with multiple cancer-related events including cell proliferation, differentiation, cell cycle, apoptosis, signaling, migration, and invasion [68].

Another study by Jones et al. using pretreatment biopsy samples from conventional (osteoblastic/fibroblastic) osteosarcoma patients and control samples of healthy bone tissue showed that 34 miRNAs were significantly dysregulated with 11 having higher expression and 23 having lower expression among the osteosarcoma group. MiR-181a and miR-181b were the most upregulated miRNAs in osteosarcoma group while miR-29b, miR-451, and miR-16 were among the most downregulated. The miRNA signature profile in the sample of metastatic osteosarcoma group compared to nonmetastatic osteosarcoma group showed that higher expression of miR-27a and miR-181c* was found in patients with metastatic tumor. Additionally, higher expression of miR-451 and miR-15b was associated with chemosensitive patients compared to chemoresistant samples. *In vitro* and *in vivo* functional validation in osteosarcoma cell lines confirmed the tumor suppressive role of miR-16 and the pro-metastatic role of miR-27a. The analysis of target genes of these miRNAs indicated that these miRNAs may target several known osteosarcoma-related genes that regulate transcription, cell cycle control, and cancer signaling pathways [69].

Li et al. identified potential miRNA biomarkers for the early diagnosis and relapse prediction of osteosarcoma by developing a serum-based miRNA profile. All the putative miRNAs were verified through RT-qPCR, and the expression of seven miRNAs (miR-106a-5p, miR-16-5p, miR-20a-5p, miR-425-5p, miR-451a, miR-25-3p, and miR-139-5p) was found to be downregulated in the serum of OS patients compared to the healthy control. These miRNAs are also correlated with other type of cancer pathogenesises such as lung carcinoma, colorectal carcinoma, breast carcinoma, nasopharyngeal carcinoma, etc. [70].

Yuan et al. demonstrated that miR-21 expression was significantly higher in serum from osteosarcoma patients compared to healthy controls as measured by RT-qPCR. The high expression of miR-21 is associated with aggressive Enneking tumor staging, neoadjuvant chemotherapeutic resistance, and reduced overall survival rate [71]. Previous studies indicated that miR-21 has influences on the cell proliferation, cell cycle progression, tumor metastatic behavior, and susceptibility to chemotherapeutic treatment [43, 72–74]. These tumor-promoting behaviors of miR-21 was due to its targeting regulatory roles on a vast number of tumor suppressive genes such as phosphatase and tensin homolog protein (PTEN) [72], myristoylated alanine-rich protein kinase C substrate protein (MARCKS) [43], programmed cell death 4 protein (PDCD4) [73], and cell division cycle 25 homolog A protein (CDC25A) [74].

Dong et al. showed that expression of miR-223 was significantly reduced in the serum of osteosarcoma patients and osteosarcoma cell lines compared to healthy controls as measured by RT-qPCR. Osteosarcoma patients with lower expression of serum miR-223 tend to have distant metastasis, more advanced clinical stages, and shorter survival time [75]. Furthermore, it has been demonstrated that miR-223 may play an important role in the regulation of epithelial cell transforming sequence 2 (Ect2) signaling, an important pathway for osteosarcoma pathogenesis in terms of cell cycle progression, proliferation, recurrence, and poor chemotherapeutic responses [76].

Lian et al. performed TaqMan low-density array (TLDA) and RT-qPCR on plasma samples derived from osteosarcoma patients before surgery, patients after 1 month of surgery and healthy individuals. The results showed that four plasma miRNAs (miR-195-5p, miR-199a-3p, miR-320a, and miR-374a-5p) were significantly upregulated in the presurgical osteosarcoma patients. The expression level of these four plasma miRNAs were decreased after surgical removal of the tumors, suggesting the potential of these miRNAs as the biomarkers for osteosarcoma. Additionally, circulating miR-195-5p and miR-199a-3p were correlated with metastasis status whereas miR-199a-3p and miR-320a were correlated with histological subtype [77]. Besides, it has been discovered that miR-195-5p involved in the inhibition of osteosarcoma cell migration and invasion by targeting fatty acid synthase (FASN) [78], while miR-199a-3p regulated the p53 signaling pathway and inhibits osteosarcoma cell growth, migration, and induce apoptosis [79, 80].

5.2. MicroRNAs as therapeutic agents or targets

Growing lists of *in vitro* and *in vivo* studies on the regulatory roles of microRNAs in bone disorders, which conducted by various research teams, have supported miRNAs as the potential therapeutics candidates. However, specific, efficient, and safe delivery of miRNA to its target

sites is crucial for the translation of miRNA-based therapeutics strategies. Effective delivery systems in various bone disorder models had been observed by the application of biomaterial constructs, viral vectors, nanoparticles, and polymers with the potential to restore the normal functions of bone homeostasis and carcinogenesis.

The expression of miR-199a-3p, which may inhibit tumor cell growth, is reduced in osteosarcoma cells. Zhang et al. developed a lipid-modified dextran-based polymeric nanoparticle platform for encapsulation of miR-199a-3p and another potent tumor suppressive miRNA, let-7a, and transfected into osteosarcoma cells lines, KHOS and U-2OS. Western blot analysis and 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay showed that dextran nanoparticles loaded with miRNAs could efficiently downregulate the expression of mechanistic target of rapamycin (mTOR) and Met proteins and effectively inhibit the growth and proliferation of osteosarcoma cells [81].

MiR-143 expression is downregulated in 143B human osteosarcoma cell line, an osteosarcoma cell line with high metastatic tendency to the lung. Osaki et al. inoculated the 143B osteosarcoma cells transfected with firefly luciferase gene (143B-luc) into athymic mice in order to develop a primary tumor and spontaneous lung metastasis. Then, systemic administration of miR-143 mimic and miR-negative control 1 (NC1) mixed with atelocollagen was performed on the osteosarcoma mice model to study the therapeutic potential of miR-143 against spontaneous lung metastasis of osteosarcoma. After 1 week, the luciferase signal was detected only at the right knee primary lesion where 143B-luc cells were inoculated. After 2 weeks, one out of four mice administered with miR-NC1 was detected with luciferase signal at the pulmonary area indicating lung metastasis, whereas no luciferase signal was observed in mice injected with miR-143 mimic. After days 19–20, two out of 10 mice injected with the miR-NC1 control died due to lung metastasis. At third week, six of the eight live mice administered with miR-NC1 control were identified with lung metastasis, while contrastingly, only two out of 10 mice injected with miR-143 mimic displayed lung metastasis. Furthermore, the tumor weight and the expression of proliferative cell nuclear antigen in primary tumor showed no significant difference between both groups (miR-143 mimic and miR-NC1 control). Therefore, all these data showed that miR-143 mimic suppresses lung metastasis from a primary tumor but did not have effect on the primary tumor cell proliferation. Additionally, it is speculated that the downregulation of miR-143 may promote lung metastasis of human osteosarcoma cells by promoting MMP-13 upregulation [82]. Shimbo et al. introduced synthetic miR-143 into MSC cells and increased the amount of exosome-formed miR-143 in the conditioned medium. The transfection of 143B osteosarcoma cell lines with extracellular miR-143 in the conditioned medium from MSCs (exosome-formed miR-143) reduced the migration ability of osteosarcoma cells compared to the control. In addition, Shimbo et al. also showed that the transfection efficiency of exosome-formed miR-143 was less than that attained with the lipofection. Nevertheless, migration assay performed on the 143B osteosarcoma cells showed that the inhibitory effect on cell migration was similar between exosome and lipofection method [83].

Jiang et al. constructed lentiviral vectors overexpressing and silencing miR-126. Both of the miR-126 overexpressing and silencing lentiviral vectors were then transfected into MG63 and U-2 OS osteosarcoma cell lines. This study aimed to determine the interlink between cisplatin (DDP) and methotrexate (MTX) osteosarcoma chemotherapeutic drugs and miR-126 on the effect to

inhibit osteosarcoma cell proliferation and apoptosis. The results showed that DDP and MTX induce apoptosis and inhibit the cell cycle of osteosarcoma cell lines at a greater efficiency in miR-126 overexpressing manner. Nonetheless, DDP and MTX did not significantly impact the apoptosis and cell proliferation in the miR-126 silenced group. On that account, it is suggested that miR-126 may strengthen the sensitivity of osteosarcoma cell to DDP and MTX. However, the regulatory mechanisms behind this process still remain to be discovered [84].

Cai et al. developed polyurethane (PU) nanomicelles drug carrier modified with Asp8 acidic peptide (Asp8-PU-anti-miR214) for targeted delivery of anti-miR-214. Polyurethane (PU) is a linear polymer composed of organic units molecularly linked by carbamate (urethane) group [85]. Besides, it is known that PU tends to have high compatibility in living system by not being toxic or reactive and have high mechanical flexibility [85–88]. The highly negatively charged peptide Asp8 has also been accounted as an excellent targeting tool of bone resorption area [89]. In this study, miR-214 was chosen due to its regulatory role in bone remodeling by which the elevated expression *in vivo* was associated with reduced bone formation in aged patients. This is due to the direct targeting action of miR-214 on activating transcription factor 4 (ATF4), which enable the inhibition of osteoblast activity [90]. Apart from that, miR-214 also modulates osteoclast differentiation by targeting the PTEN-PI3k-Akt pathway [91]. Asp8-PU-anti-miR214 delivery system to osteoclasts at the bone resorption surface of ovariectomized (OVX) osteoporosis mice model was able to improve the bone microarchitecture, increased bone mass, and decreased osteoclast number. Above and beyond, a number of osteoclast-related genes including tryptophan RNA-binding attenuation protein (TRAP) and cathepsin K (CTSK) were successfully downregulated by the anti-miR-214. Interestingly, Asp8-PU-anti-miR214 was also proven as a potential drug delivery candidate that does not overt toxicity or elicit an immune response. Therefore, Asp8-PU serves as a potential bone-resorption surface-targeting delivery system for the treatment of postmenopausal osteoporosis and osteoclast-stimulated bone disorders [85].

Zhang et al. designed a hyperbranched polymer (HP) and miR-26a (HP/miRNA) nanosized polyplexes, which were encapsulated in biodegradable microspheres to overcome problems with uncontrolled release and achieved the controllable two-stage delivery strategy (microspheres and polyplexes). Microspheres attach to cell-free nanofibrous polymer 3D scaffolds to prevent off-target effects of the miRNA delivery. The 3D scaffolds were implanted into osteoporotic mice model, and the results showed that this technology was able to regenerate critical-sized bone with low cytotoxicity effect by targeting glycogen synthase kinase 3 beta (Gsk-3 β) to activate the osteoblastic activity of endogenous stem cells [92].

Overexpression of miR-140* and miR-214 was detected in bone marrow-derived MSCs isolated from ovariectomized rats (OVX-BMSCs). Li et al. demonstrated that engineered OVX-BMSCs expressing the hybrid baculovirus-mediated miRNA sponges can continuously antagonize cellular miR-140* and miR-214 levels *in vitro*. At the same time, the attenuation of miR-140* and miR-214 expression can also efficiently support the osteogenesis of OVX-BMSCs and intensify the capability of OVX-BMSCs to suppress osteoclast maturation. Remarkably, the osteoinductive effect of suppressing miR-214 was more potent compared to miR-140* suppression. This study also discovered that the allotransplantation of miR-214 sponges-expressing OVX-BMSCs in osteoporotic rat models with a femoral metaphysis found with critical-size bone defect was able to improve the likelihood of bone healing, remodeling,

and bone quality at 4 weeks postimplantation. Moreover, co-expression of bone morphogenic protein 2 (BMP2) and miR-214 sponges in OVX-BMSCs can synergistically enhance the bone formation and healing in osteoporotic rats [93].

6. Challenges

Although recent studies reveal that microRNA has the potential to become diagnostic biomarker and effective therapeutic agents for bone diseases, there are still challenges for developing miRNA-based treatment. Since each miRNA may regulate many different mRNA targets and the expression of target genes might be controlled by different miRNAs, it became an obstacle to identify all targets and miRNAs involved in bone diseases [94]. Moreover, miRNAs are cancer type specific, they may perform as oncogene or tumor suppressor in different cell types, and thus result in off-target effects of miRNAs [95]. Garzon et al. reveals that miR-29 mimics serve as anticancer agents and regulate in bone growth; meanwhile, they target several tumorigenesis pathways like proliferation (CDK6), methylation (DNMT1, DNMT3a and b), and apoptosis (MCL-1) [96].

Currently, one of the major challenges facing by the researches is the mechanism of *in vivo* delivery. There are lots of mechanical and biological barriers to cope with for success transferring of miRNA into the target genes. The first barrier is the abnormal tumor vessels in leaky structure, which cause the poor blood perfusion and affect the delivery of naked miRNA. In addition, the extracellular matrix is very complex, consisting of tumor-associated macrophages and monocytes, which can trap miRNA in capsule and have the ability to hinder the miRNA to target the cancer cells. MiRNA is also susceptible to nucleases such as serum RNase A-type nucleases, which break phosphodiester bonds between nucleotides [97]. Furthermore, the small-sized miRNA is easily filtered by kidney and cleared in the blood circulation [98]. Hence, the instability of miRNAs needs to be overcome in order for the miRNAs to reach the target genes. Even if miRNAs are successfully transferred into the target tissue, the uptake of miRNAs into the cells is not guaranteed. The miRNA oligonucleotides consist of negative charges, and it prevents them from passing through the plasma membranes of the target cells [96]. Strategy to improve endosomal escape should also be taken in consideration since the endocytosis mechanism that capsulated miRNA causing degradation might be happened [97].

Besides delivery considerations, the autoimmunological pathways are necessary to be emphasized. MiRNAs are recognized as foreign particles by immune system in the body, which will trigger the adaptive or innate immune responses causing unpredictable toxicities [99]. Chen et al. reported that miRNA duplexes can trigger toll-like receptors (TLRs) to secrete the inflammatory cytokines and type I interferons. Activation of TLRs 3, 7, and 8 by single- or double-stranded RNAs promotes innate and adaptive immune systems and also prepare the surrounding immune cells, for instance, natural killer cells, dendritic cells, monocytes, B cells, etc., to increase the sensitivity to RNA stimulation [97]. The immune responses toward the miRNA still required further studies.

Numerous findings of miRNA are based on *in vitro* studies using cell lines and are not fully validated in *in vivo*. In addition, the major methods used to measure miRNA levels are

quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) and microarray analysis, and there is no standardized techniques to measure the miRNA expression levels [38]. Further techniques need to be optimized for better miRNA detection and analysis.

Despite there are many challenges, the potentials of miRNA as a diagnostic tool and treatment for bone diseases look promising. We believe that in the next few years, researches will be able to develop efficient delivery methods of the miRNA to its specific target site with minimum or no side effect.

7. Conclusions

As a conclusion, microRNA plays important roles in bone development and maintenance. MiRNA dysregulation leads to the pathogenesis of various bone diseases. Nowadays, miRNAs are being excavated as new directions for diagnostic biomarkers and drug targets to cure bone diseases. However, there are still many limitations and barriers for the development of miRNA-based biomarkers and therapeutics. Further investigations are needed to understand the miRNA gene regulation in bone and to overcome the challenges faced in miRNA delivery systems. MiRNA studies not only provide new eras of basic bone biology researches, but also contribute to new diagnostic and therapeutic methods into clinical practice to various bone diseases.

Acknowledgements

The authors thank Fundamental Research Grant Scheme (FRGS/1/2015/ST03/UPM/02/5) from Ministry of Higher Education, Malaysia for the support.

Author details

Hui-Yi Loh, Yui-Yee Lau, Kok-Song Lai and Mohd Azuraiddi Osman*

*Address all correspondence to: azuraiddi@upm.edu.my

Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Selangor, Malaysia

References

- [1] Nugent M. MicroRNA function and dysregulation in bone tumors: The evidence to date. *Cancer Management and Research*. 2014;**6**(1):15-25
- [2] Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? *Nature Reviews. Genetics*. 2008;**9**: 102-114

- [3] Paranjape T, Slack FJ, Weidhaas JB. MicroRNAs: Tools for cancer diagnostics. *Gut*. 2009; **58**(11):1546-1554
- [4] Moore BT, Xiao P. MiRNAs in bone diseases. *MicroRNA*. 2013;**2**(1):20-31
- [5] Lin T, Ma QP, Zhang YF, Zhang HF, Yan JP, Gao CH. MicroRNA-27a functions as an oncogene in human osteosarcoma by targeting CCNG1. *Oncology Letters*. 2018;**15**(1): 1067-1071
- [6] Braun CJ, Zhang X, Savelyeva I, Wolff S, Moll UM, Schepeler T, et al. p53-responsive microRNAs 192 and 215 are capable of inducing cell cycle arrest. *Cancer Research*. 2008; **68**(24):10094-10104
- [7] Sun MG, Zhou XY, Chen LL, Huang SS, Leung V, Wu N, et al. The regulatory roles of microRNAs in bone remodeling and perspectives as biomarkers in osteoporosis. *BioMed Research International*. 2016;**2016**:1652417
- [8] Clarke B. Normal bone anatomy and physiology. *Clinical Journal of the American Society of Nephrology*. 2008;**3**(Suppl 3):131-139
- [9] Pathria MN, Chung CB, Resnick DL. Acute and stress-related injuries of bone and cartilage: Pertinent anatomy, basic biomechanics, and imaging perspective. *Radiology*. 2016; **280**(1):21-38
- [10] Martin TJ, Seeman E. Bone remodelling: Its local regulation and the emergence of bone fragility. *Best Practice & Research. Clinical Endocrinology & Metabolism*. 2008;**22**(5): 701-722
- [11] Martin RB, Burr DB, Sharkey NA, Fyhrie DP. Growth, modelling and remodelling of bone. In: Martin RB, Burr DB, Sharkey NA, Fyhrie DP, editors. *Skeletal Tissue Mechanics*. 2nd ed. New York: Springer; 2015. pp. 95-173
- [12] Raggatt LJ, Partridge NC. Cellular and molecular mechanisms of bone remodeling. *The Journal of Biological Chemistry*. 2010;**285**(33):25103-25108
- [13] Langdahl B, Ferrari S, Dempster DW. Bone modeling and remodeling: Potential as therapeutic targets for the treatment of osteoporosis. *Therapeutic Advances in Musculoskeletal Disease*. 2016;**8**(6):225-235
- [14] Feng X, McDonald JM. Disorders of bone remodelling. *Annual Review of Pathology*. 2011;**6**:121-145
- [15] Eriksen EF. Cellular mechanisms of bone remodeling. *Reviews in Endocrine & Metabolic Disorders*. 2010;**11**(4):219-227
- [16] Proff P, Römer P. The molecular mechanism behind bone remodelling: A review. *Clinical Oral Investigations*. 2009;**13**(4):355-362
- [17] Mizoguchi F, Murakami Y, Saito T, Miyasaka N, Kohsaka H. MiR-31 controls osteoclast formation and bone resorption by targeting RhoA. *Arthritis Research & Therapy*. 2013;**15**(5):R102

- [18] Kim KS, Kim JH, Kim IY, Lee JW, Seong SM, Park YW, et al. MicroRNA-26a regulates RANKL-induced osteoclast formation. *Molecules and Cells*. 2015;**38**(1):75-80
- [19] Sugatani T, Vacher J, Hruska KA. A microRNA expression signature of osteoclastogenesis. *Blood*. 2012;**117**(13):3648-3657
- [20] Cheng P, Chen C, He HB, Hu R, Zhou HD, Xie H, et al. MiR-148a regulates osteoclastogenesis by targeting V-maf musculoaponeurotic fibrosarcoma oncogene homolog B. *Journal of Bone and Mineral Research*. 2013;**28**(5):1180-1190
- [21] Kim KS, Kim JH, Lee JW, Jin HM, Kook H, Kim KK, et al. MafB negatively regulates RANKL-mediated osteoclast differentiation. *Blood*. 2007;**109**(8):3253-3259
- [22] Zhao HY, Zhang J, Shao HY, Liu JW, Jin MR, Chen JP, et al. MiRNA-340 inhibits osteoclast differentiation via repression of MITF. *Bioscience Reports*. 2017;**37**(4):BSR20170302
- [23] Kapinas K, Kessler C, Ricks T, Gronowicz G, Delany AM. MiR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop. *The Journal of Biological Chemistry*. 2010;**285**(33):25221-25231
- [24] Li ZY, Hassan MQ, Jafferji M, Aqeilan RI, Garzon R, Croce CM, et al. Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. *The Journal of Biological Chemistry*. 2009;**284**(23):15676-15684
- [25] Kapinas K, Kessler CB, Delany AM. MiR-29 suppression of osteonectin in osteoblasts: Regulation during differentiation and by canonical Wnt tumour. *Journal of Cellular Biochemistry*. 2010;**108**(1):216-224
- [26] Li ZY, Hassan MQ, Volinia S, van Wijnen AJ, Stein JL, Croce CM, et al. A microRNA signature for a BMP2-induced osteoblast lineage commitment program. *Proceedings of the National Academy of Sciences*. 2008;**105**(37):13906-13911
- [27] Zhang JF, Fu WM, He ML, Xie WD, Lv Q, Wan G, et al. MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. *RNA Biology*. 2011;**8**(5):829-838
- [28] Li H, Xie H, Liu W, Hu R, Huang B, Tan YF, et al. A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. *The Journal of Clinical Investigation*. 2009;**119**(12):3666-3677
- [29] Hu R, Liu W, Li H, Yang L, Chen C, Xia ZY, et al. A Runx2/miR-3960/miR-2861 regulatory feedback loop during mouse osteoblast differentiation. *The Journal of Biological Chemistry*. 2011;**286**(14):12328-12339
- [30] Sang S, Zhang ZC, Qin S, Li CW, Dong Y. MicroRNA-16-5p inhibits osteoclastogenesis in giant cell tumor of bone. *BioMed Research International*. 2017;**2017**:1-6
- [31] Cowan RW, Singh G. Giant cell tumor of bone: A basic science perspective. *Bone*. 2013;**52**(1):238-246

- [32] Wang T, Yin HB, Wang J, Li ZX, Wei HF, Liu Z, Wu ZP, Yan WJ, et al. MicroRNA-106b inhibits osteoclastogenesis and osteolysis by targeting RANKL in giant cell tumor of bone. *Oncotarget*. 2015;**6**(22):18980-18996
- [33] Sobti A, Agrawal P, Agarwala S, Agarwal M. Giant cell tumor of bone—An overview. *Archives of Bone and Joint Surgery*. 2016;**4**(1):2-9
- [34] Zhang J, Xiao XJ, Liu J. The role of circulating miRNAs in multiple myeloma. *Science China. Life Sciences*. 2015;**58**(12):1262-1269
- [35] Wu PF, Liang JY, Yu F, Zhou ZB, Tang JY, Li KH. MiR-125b inhibits stromal cell proliferation in giant cell tumor of bone by targeting parathyroid hormone 1 receptor. *Iranian Journal of Basic Medical Sciences*. 2015;**18**:705-709
- [36] Johnell O, Kanis J. Epidemiology of osteoporotic fractures. *Osteoporosis International*. 2005;**16**(Suppl 2):6-10
- [37] Sozen T, Ozisik L, Basaran NC. An overview and management of osteoporosis. *European Journal of Rheumatology*. 2017;**4**(1):46-56
- [38] Mirza F, Canalis E. Management of endocrine disease: Secondary osteoporosis: Pathophysiology and management. *European Journal of Endocrinology*. 2015;**173**(3):R131-R151
- [39] Zaheer S, LeBoff MS. Osteoporosis: Prevention and treatment. [Updated 2016 Aug 3]. In: De Groot LJ, Chrousos G, Dungan K, et al., editors. *Endotext* [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK279073/>
- [40] Ji MX, Yu Q. Primary osteoporosis in postmenopausal women. *Chronic Diseases and Translational Medicine*. 2015;**1**(1):9-13
- [41] Lian JB, Stein GS, Van Wijnen AJ, Stein JL, Hassan MQ, Gaur T, et al. MicroRNA control of bone formation and homeostasis. *Nature Reviews. Endocrinology*. 2012;**8**(4):212-227
- [42] Chen C, Cheng P, Xie H, Zhou HD, Wu XP, Liao EY, et al. MiR-503 regulates osteoclastogenesis via targeting RANK. *Journal of Bone and Mineral Research*. 2014;**29**(2):338-347
- [43] Li T, Li D, Sha JJ, Sun P, Huang YR. MicroRNA-21 directly targets MARCKS and promotes apoptosis resistance and invasion in prostate cancer cells. *Biochemical and Biophysical Research Communications*. 2009;**383**(3):280-285
- [44] Zhang YQ, Gao YL, Cai LJ, Li FN, Lou Y, Xu N, et al. MicroRNA-221 is involved in the regulation of osteoporosis through regulates RUNX2 protein expression and osteoblast differentiation. *American Journal of Translational Research*. 2017;**9**(1):126-135
- [45] Yang N, Wang G, Hu CH, Shi YY, Liao L, Shi ST, et al. Tumor necrosis factor α suppresses the mesenchymal stem cell osteogenesis promoter miR-21 in estrogen deficiency-induced osteoporosis. *Journal of Bone and Mineral Research*. 2013;**28**(3):559-573

- [46] Wang FS, Chung PC, Lin CL, Chen MW, Ke HJ, Chang YH, et al. MicroRNA-29a protects against glucocorticoid-induced bone loss and fragility in rats by orchestrating bone acquisition and resorption. *Arthritis and Rheumatism*. 2013;**65**(6):1530-1540
- [47] Monti E, Mottes M, Fraschini P, Brunelli PC, Forlino A, Giacomo V, et al. Current and emerging treatments for the management of osteogenesis imperfecta. *Therapeutics and Clinical Risk Management*. 2010;**6**:367-381
- [48] Shaker JL, Albert C, Fritz J, Harris G. Recent developments in osteogenesis imperfecta. *F1000Research*. 2015;**4**:2-11
- [49] Abukabbos H, Al-Sineedi F. Clinical manifestations and dental management of dentinogenesis imperfecta associated with osteogenesis imperfecta: Case report. *Saudi Dental Journal*. 2013;**25**(4):159-165
- [50] Marini JC, Forlino A, Cabral WA, Barnes AM, Antonio JDS, Milgrom S, et al. Consortium for osteogenesis imperfecta mutations in the helical domain of type I collagen: Regions rich in lethal mutations align with collagen binding sites for integrins and proteoglycans. *Human Mutation*. 2007;**28**:209-221
- [51] Forlino A, Cabral WA, Barnes AM, Marini JC. New perspectives on osteogenesis imperfecta. *Nature Reviews. Endocrinology*. 2011;**7**(9):540-557
- [52] Van Dijk FS, Sillence DO. Osteogenesis imperfecta: Clinical diagnosis, nomenclature and severity assessment. *American Journal of Medical Genetics Part A*. 2014;**164**(6):1470-1481
- [53] Wang ZQ, Lu YQ, Zhang XM, Ren XZ, Wang YZ, Li ZL, et al. Serum microRNA is a promising biomarker for osteogenesis imperfecta. *Intractable & Rare Diseases Research*. 2012;**1**(2):81-85
- [54] Kaneto CM, Lima PSP, Zanette DL, Prata KL, Pina Neto JM, de Paula FJ, et al. COL1A1 and miR-29b show lower expression levels during osteoblast differentiation of bone marrow stromal cells from Osteogenesis Imperfecta patients. *BMC Medical Genetics*. 2014;**15**(1):45
- [55] Sabharwal R, Gupta S, Sepolia S, Panigrahi R, Mohanty S, Subudhi SK, et al. An insight in to paget's disease of bone. *Nigerian Journal of Surgery: Official Publication of the Nigerian Surgical Research Society*. 2014;**20**(1):9-15
- [56] Ralston SH, Layfield R. Pathogenesis of paget disease of bone. *Calcified Tissue International*. 2012;**91**(2):97-113
- [57] Roodman G, Windle J. Science in medicine-paget disease of bone. *The Journal of Clinical Investigation*. 2005;**115**(2):200-207
- [58] Bianciardi S, Merlotti D, Sebastiani G, Valentini M, Gonnelli S, Caffarelli C, et al. MicroRNA expression profiling in paget's disease of bone. *Bone Abstracts*. 2016;**5**:452
- [59] Gebert LFR, Rebhan MAE, Crivelli SEM, Denzler R, Stoffel M, Hall J. Miravirsin (SPC3649) can inhibit the biogenesis of miR-122. *Nucleic Acids Research*. 2014;**42**(1):609-621

- [60] Shah MY, Ferrajoli A, Sood AK, Lopez-Berestein G, Calin GA. microRNA therapeutics in cancer — An emerging concept. *eBioMedicine*. 2016;**12**:34-42
- [61] Slabáková E, Culig Z, Remšík J, Souček K. Alternative mechanisms of MiR-34a regulation in cancer. *Cell Death & Disease*. 2017;**8**(10):1-10
- [62] Bouchie A. First microRNA mimic enters clinic. *Nature Biotechnology*. 2013;**31**(7):577-577
- [63] Bader AG. MiR-34 — A microRNA replacement therapy is headed to the clinic. *Frontiers in Genetics*. 2012;**3**(120):1-9
- [64] Beg MS, Brenner AJ, Sachdev J, Borad M, Kang YK, Stoudemire J, et al. Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. *Investigational New Drugs*. 2017;**35**(2):180-188
- [65] Xiao F, Chen JW, Lian CJ, Han PC, Zhang CY. Tumor necrosis factor-related apoptosis-inducing ligand induces cytotoxicity specific to osteosarcoma by microRNA response elements. *Molecular Medicine Reports*. 2015;**11**(1):739-745
- [66] Gaur S, Wen YF, Song JH, Parikh NU, Mangala LS, Blessing AM, et al. Chitosan nanoparticle-mediated delivery of miRNA-34a decreases prostate tumor growth in the bone and its expression induces non-canonical autophagy. *Oncotarget*. 2015;**6**(30):29161-29177
- [67] Di Martino MT, Campani V, Misso G, Gallo Cantafio ME, Gullà A, Foresta U, et al. In vivo activity of MiR-34a mimics delivered by stable nucleic acid lipid particles (SNALPs) against multiple myeloma. *PLoS One*. 2014;**9**(2):1-10
- [68] Hu H, Zhang Y, Cai XH, Huang JF, Cai L. Changes in microRNA expression in the MG-63 osteosarcoma cell line compared with osteoblasts. *Oncology Letters*. 2012;**4**(5):1037-1042
- [69] Jones KB, Salah Z, Sara DM, Galasso M, Gaudio E, Nuovo GJ, et al. MicroRNA signatures associate with pathogenesis and progression of osteosarcoma. *Cancer research*. 2013;**72**(7):1865-1877
- [70] Li H, Zhang K, Liu LH, Ouyang Y, Guo HB, Zhang H, et al. MicroRNA screening identifies circulating microRNAs as potential biomarkers for osteosarcoma. *Oncology Letters*. 2015;**10**(3):1662-1668
- [71] Yuan J, Chen L, Chen X, Sun W, Zhou X. Identification of serum microRNA-21 as a biomarker for chemosensitivity and prognosis in human osteosarcoma. *The Journal of International Medical Research*. 2012;**40**(6):2090-2097
- [72] Lou YH, Yang XS, Wang FL, Cui ZM, Huang Y. MicroRNA-21 promotes the cell proliferation, invasion and migration abilities in ovarian epithelial carcinomas through inhibiting the expression of PTEN protein. *International Journal of Molecular Medicine*. 2010;**26**:819-827
- [73] Fassan M, Pizzi M, Giacomelli L, Mescoli C, Ludwig K, Pucciarelli S, et al. PDCD4 nuclear loss inversely correlates with miR-21 levels in colon carcinogenesis. *Virchows Archiv*. 2011;**458**(4):413-419

- [74] Wang P, Zou FD, Zhang XD, Li H, Dulak A, Robert J, et al. MiR21 negatively regulates CDC25A and cell cycle progression in colon cancer cells. *Cancer Research*. 2009;**69**(20):8157-8165
- [75] Dong JB, Liu YL, Liao WS, Liu R, Shi P, Wang LM. MiRNA-223 is a potential diagnostic and prognostic marker for osteosarcoma. *Journal of Bone Oncology*. 2016;**5**(2):74-79
- [76] Xu JL, Yao Q, Hou Y, Xu M, Liu S, Yang LQ, et al. MiR-223/Ect2/p21 signaling regulates osteosarcoma cell cycle progression and proliferation. *Biomedicine & Pharmacotherapy*. 2013;**67**(5):381-386
- [77] Lian F, Cui Y, Zhou CL, Gao KW, Wu LW. Identification of a plasma four-microRNA panel as potential noninvasive biomarker for osteosarcoma. *PLoS One*. 2015;**10**(3):1-12
- [78] Mao JH, Zhou RP, Peng AF, Liu ZL, Huang SH, Long XH, et al. MicroRNA-195 suppresses osteosarcoma cell invasion and migration in vitro by targeting FASN. *Oncology Letters*. 2012;**4**(5):1125-1129
- [79] Tian Y, Zhang YZ, Chen W. MicroRNA-199a-3p and microRNA-34a regulate apoptosis in human osteosarcoma cells. *Bioscience Reports*. 2014;**34**(4). DOI: 10.1042/BSR20140084
- [80] Duan ZF, Choy E, Harmon D, Liu XZ, Susa M, Mankin H, et al. MicroRNA-199a-3p is down regulated in human osteosarcoma and regulates cell proliferation and migration. *Molecular Cancer Therapeutics*. 2011;**10**(8):1337-1345
- [81] Zhang LL, Iyer AK, Yang XQ, Kobayashi E, Guo YQ, Mankin H, et al. Polymeric nanoparticle-based delivery of microRNA-199a-3p inhibits proliferation and growth of osteosarcoma cells. *International Journal of Nanomedicine*. 2015;**10**:2913-2924
- [82] Osaki M, Takeshita F, Sugimoto Y, Kosaka N, Yamamoto Y, Yoshioka Y, et al. MicroRNA-143 regulates human osteosarcoma metastasis by regulating matrix metalloproteinase-13 expression. *Molecular Therapy*. 2011;**19**(6):1123-1130
- [83] Shimbo K, Miyaki S, Ishitobi H, Kato Y, Kubo T, Shimose S, et al. Exosome-formed synthetic microRNA-143 is transferred to osteosarcoma cells and inhibits their migration. *Biochemical and Biophysical Research Communications*. 2014;**445**(2):381-387
- [84] Jiang LD, He AY, He XJ, Tao C. MicroRNA-126 enhances the sensitivity of osteosarcoma cells to cisplatin and methotrexate. *Oncology Letters*. 2015;**10**(6):3769-3778
- [85] Cai MX, Yang L, Zhang SF, Liu JF, Sun Y, Wang XG. A bone-resorption surface-targeting nanoparticle to deliver anti-miR214 for osteoporosis therapy. *International Journal of Nanomedicine*. 2017;**12**:7469-7482
- [86] Yu SJ, Ding JX, He CL, Cao Y, Xu WG, Chen XS. Disulfide cross-linked polyurethane micelles as a reduction-triggered drug delivery system for cancer therapy. *Advanced Healthcare Materials*. 2014;**3**(5):752-760
- [87] Gencturk A, Kahraman E, Güngör S, Ozhan G, Ozsoy Y, Sarac AS. Polyurethane/hydroxypropyl cellulose electrospun nanofiber mats as potential transdermal drug delivery

- system: Characterization studies and in vitro assays. *Artificial Cells, Nanomedicine, and Biotechnology*. 2017;**45**(3):655-664
- [88] Akduman C, Ozgüney I, Kumbasar EP. Preparation and characterization of naproxen-loaded electrospun thermoplastic polyurethane nanofibers as a drug delivery system. *Materials Science & Engineering. C, Materials for Biological Applications*. 2016;**64**:383-390
- [89] Carinci F. Restoration of incisor area using one-piece implants: Evaluation of crestal bone resorption. *Dental Research Journal*. 2012;**9**(Suppl 2):S151-S154
- [90] Wang XG, Guo BS, Li Q, Peng J, Yang ZJ, Wang AY, et al. MiR-214 targets ATF4 to inhibit bone formation. *Nature Medicine*. 2013;**19**(1):93-100
- [91] Zhao CY, Sun WJ, Zhang PF, Ling SK, Li YH, Zhao DS, et al. MiR-214 promotes osteoclastogenesis by targeting Pten/PI3k/Akt pathway. *RNA Biology*. 2015;**12**(3):343-353
- [92] Zhang XJ, Li Y, Chen YE, Chen JH, Ma PX. Cell-free 3D scaffold with two-stage delivery of miRNA-26a to regenerate critical-sized bone defects. *Nature Communications*. 2016;**7**:1-15
- [93] Li KC, Chang YH, Yeh CL, Hu YC. Healing of osteoporotic bone defects by baculovirus-engineered bone marrow-derived MSCs expressing MicroRNA sponges. *Biomaterials*. 2016;**74**:155-166
- [94] Kumar RMR, Boro A, Fuchs B. Involvement and clinical aspects of microRNA in osteosarcoma. *International Journal of Molecular Sciences*. 2016;**17**(6):1-5
- [95] Sampson VB, Yoo SM, Kumar A, Vetter NS, Kolb EA. MicroRNAs and potential targets in osteosarcoma: Review. *Frontiers in Pediatrics*. 2015;**3**(August):1-9
- [96] Garzon R, Marcucci G, Croce CM. Targeting microRNAs in cancer: Rationale, strategies and challenges. *Nature Reviews. Drug Discovery*. 2010;**9**(10):775-789
- [97] Chen YC, Gao DY, Huang L. In vivo delivery of miRNAs for cancer therapy: Challenges and strategies. *Advanced Drug Delivery Reviews*. 2015;**81**:128-141
- [98] Sand M, Gambichler T, Sand D, Skrygan M, Altmeyer P, Bechara FG. MicroRNAs and the skin: Tiny players in the body's largest organ. *Journal of Dermatological Science*. 2009;**53**(3):169-175
- [99] Greco SJ, Munoz JL, Rameshwar P. MicroRNA cancer therapeutics and the challenge of drug delivery. In: Singh SR, Rameshwar P, editors. *MicroRNA in Development and in the Progression of Cancer*. New York: Springer; 2014. pp. 349-358