Cell. Mol. Life Sci. (2014) 71:4747–4761
DOI 10.1007/s00018-014-1700-6

Cellular and Molecular Life Sciences

REVIEW

MicroRNAs involved in bone formation

Garyfallia Papaioannou · Fatemeh Mirzamohammadi · Tatsuya Kobayashi

Received: 27 May 2014 / Revised: 31 July 2014 / Accepted: 4 August 2014 / Published online: 10 August 2014

Abstract During skeletal development, mesenchymal progenitor cells undergo a multistage differentiation process in which they proliferate and become bone- and cartilage-forming cells. This process is tightly regulated by multiple levels of regulatory systems. The small non-coding RNAs, microRNAs (miRNAs), post-transcriptionally regulate gene expression. Recent studies have demonstrated that miRNAs play significant roles in all stages of bone formation, suggesting the possibility that miRNAs can be novel therapeutic targets for skeletal diseases. Here, we review the role and mechanism of action of miRNAs in bone formation. We discuss roles of specific miRNAs in major types of bone cells, osteoblasts, chondrocytes, osteoclasts, and their progenitors. Except a few, the current knowledge about miRNAs in bone formation has been obtained mainly by in vitro studies; further validation of these findings in vivo is awaited. We also discuss about several miRNAs of particular interest in the light of future therapies of bone diseases.

Keywords Bone development · Cartilage · Mesenchymal stem cells · Osteoporosis · Dicer

G. Papaioannou · F. Mirzamohammadi · T. Kobayashi (✉)
Endocrine Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Thier 1101, 50 Blossom Street, Boston, MA 02114, USA
e-mail: kobayash@helix.mgh.harvard.edu

G. Papaioannou
e-mail: gpapaioannou@mgh.harvard.edu

F. Mirzamohammadi
e-mail: fmirzamohammadi@mgh.harvard.edu

Abbreviations

BMP	Bone morphogenetic protein
MAPK	Mitogen-activated protein kinase
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
CSF	Colony-stimulating factor
RANK	Receptor activator of NFκB
RANKL	Ligand to receptor activator of NFκB
TRAP	Tartrate-resistant acid phosphatase
TGF-β	Transforming growth factor beta
IGF	Insulin-like growth factor
HDAC	Histone deacetylase
MEF	Myocyte enhancer factor
MSC	Mesenchymal stem cells
BMSC	Bone marrow stromal cells
ADSC	Adipose-derived stem cells
BMD	Bone mineral density
ALP	Alkaline phosphatase
TNF-α	Tumor necrosis factor alpha
USSC	Unrestricted somatic stem cells

Introduction

Bone formation requires tightly coordinated proliferation and differentiation of various types of cells. This is achieved through multiple levels of regulatory systems. Cues for proliferation and differentiation of bone cells are usually provided by extracellular signaling molecules, which eventually control gene expression to regulate cellular functions. Gene expression can be regulated by several different mechanisms. Relatively recently, small non-coding RNAs, microRNAs (miRNAs), discovered in diverse organisms, have been shown to play important

regulatory roles in gene expression mainly at the post-transcriptional level. The discovery of this novel regulatory system prompted vigorous investigations into the roles of miRNAs in skeletal cells.

Bone- and cartilage-forming cells are originated from mesenchymal cells of the neural crest and lateral and paraxial mesoderm [1]. Bone formation starts with mesenchymal condensation in which mesenchymal cells form clusters at regions where future bones are to develop. In the mesenchymal condensation, cells differentiate to progenitors for chondrocytes that form cartilage and osteoblasts that produce mineralized bone. Mineralized bones are generated through two different processes: membranous and endochondral ossification. In membranous ossification, mesenchymal cells differentiate directly into bone-forming osteoblasts, whereas in endochondral ossification, mesenchymal cells first differentiate to chondrocytes that produce extracellular matrix proteins, such as type II collagen and aggrecan, to form a cartilage template. This cartilage template (the growth plate) is subsequently replaced by mineralized bone.

The growth plate cartilage drives longitudinal bone growth. Proliferation and differentiation of growth plate chondrocytes need to be tightly coordinated for proper growth of long bones. The growth plate consists of three groups of chondrocytes: resting (or periarticular in embryos), proliferating, and hypertrophic chondrocytes. Resting chondrocytes proliferate slowly, and differentiate into proliferating chondrocytes that form orderly columns. Proliferating chondrocytes further differentiate into post-mitotic hypertrophic chondrocytes that express type X collagen. Hypertrophic chondrocytes undergo mineralization and then are replaced by mineralized bone. This continuous proliferation and differentiation of chondrocytes, followed by their replacement by mineralized bone, leads to the longitudinal bone growth.

Signaling molecules play important roles in regulation of chondrocyte differentiation. Indian hedgehog (Ihh), secreted by prehypertrophic and hypertrophic chondrocytes, regulates chondrocyte proliferation and Ihh acts on resting chondrocytes to regulate their proliferation and differentiation, and expression of parathyroid hormone-related peptide (PTHrP, Pthlh), a signaling molecule that inhibits hypertrophic differentiation. Ihh also induces osteoblast differentiation of perichondrial cells, coupling chondrogenesis and osteogenesis [2]. Bone morphogenetic protein (**BMP**) signaling is essential for chondrocyte differentiation in vivo [3]. BMP signaling in prechondrogenic cells stimulates their differentiation into chondrocytes [4]. BMP signaling also promotes differentiation of proliferating chondrocytes into hypertrophic chondrocytes. BMPs bind to type I and type II BMP receptors and signal through Smads and mitogen-activated protein kinase (MAPK) pathways [5]. Fibroblast growth factors (**FGFs**) bind to the cell surface tyrosine/kinase receptors, fibroblast growth factor receptors (**FGFR1-4** in humans and mice) that activate multiple intracellular signaling pathways, including MAPK, phosphoinositide 3-kinase (PI3K), and JAK/STAT1 (Janus kinase/signal transducer and activator of transcription) to regulate chondrocyte differentiation [6].

Osteoblasts are differentiated from local mesenchymal progenitor cells. Osteoblasts produce mineralized bone matrix whose major component is type I collagen, then die or further differentiate into bone-lining cells or osteocytes that are embedded in the bone matrix. Osteoblast differentiation is regulated by multiple signaling systems. During the endochondral bone formation, Ihh, produced by hypertrophic chondrocytes, induces osteoblast differentiation of mesenchymal progenitor cells in the perichondrium of endochondral bones [7]. **Wnt** signaling is essential for osteoblast differentiation in both membranous and endochondral bone formation [8–11]. Wnt ligands bind to frizzled receptors and their co-receptor, low-density lipoprotein receptor-related protein 5 (LRP5) or LRP6, and signal through multiple intracellular signaling pathways. In the canonical pathway, Wnt signaling stabilizes β-catenin and promotes its nuclear localization [12]. β-catenin forms a transcriptional complex containing lymphoid enhancer-binding factor 1(Lef1). β-catenin-independent, non-canonical Wnt signaling pathways also regulate functions and differentiation of cells of the osteoblast-lineage [13]. **Notch** signaling [14], mediated by Notch receptors (Notch 1–4) and the ligands, Jagged 1 and 2, and Delta-like 1, 3, and 4, suppresses differentiation of mesenchymal cells into pre-osteoblastic cells, whereas it promotes differentiation of pre-osteoblasts into osteoblasts.

Mineralized bone is constantly remodeled though coupled bone formation and bone resorption. Bone resorption is mediated by osteoclasts that are differentiated from precursor cells of the monocyte/macrophage lineage. Osteoclast differentiation requires two essential signaling molecules; colony-stimulating factor 1 (CSF1) and ligand to receptor activator of NFκB (RANKL) [15]. These signaling molecules sequentially induce differentiation of osteoclasts that express specific markers including tartrate-resistant acid phosphatase (TRAP; Acp5) and cathepsin K (Ctsk). As bone is resorbed by osteoclasts, growth factors [e.g., transforming growth factor beta (TGF-β), insulin-like growth factor 1 (IGF-1)] are released from the bone matrix; these growth factors, in turn, regulate the function and differentiation of osteoblasts.

Signals from extracellular space are eventually conveyed to the nucleus where transcription factors primarily control gene expression. Sex determining region Y (SRY) box 9 (**Sox9**) is an essential transcription factor for chondrocyte differentiation. It is expressed in common progenitors for chondrocytes and osteoblasts. Upon differentiation, its

expression becomes restricted to chondrocytes. Sox9 is also important for chondrocyte function; it regulates expression of collagen type II, collagen XI, and aggrecan [16, 17]. Sox9-related molecules, Sox 5 and Sox 6, enhance the activity of Sox9 [18]. Myocyte enhancer factor 2 (**MEF2**) family transcription factors promote hypertrophic differentiation of chondrocytes. Histone deacetylase 4 (**HDAC4**) is a major negative regulator of MEF2s and thereby suppresses hypertrophic differentiation [19]. **Runx2**, a member of the Runt transcription factor family, stimulates chondrocyte hypertrophy [20, 21]. Runx2 is essential for osteoblast differentiation [22, 23]. **Osterix** (Osx, Sp7) that acts at a level genetically downstream of Runx2 is the other transcription factor necessary for osteoblast differentiation [24]. Transcription factors also regulate osteoclast differentiation. The transcription factor, PU.1, is expressed in monocytes and required for differentiation into early osteoclast precursors. In later stages, Fos, Mitf (microphthalmia-associated transcription factor), Nfatc1 (Nuclear factor for activated T cell c1), and NFkB are individually essential for differentiation and function of osteoclasts [25].

Gene expression is also controlled at the post-transcriptional level where miRNAs play significant roles. MiRNAs, ~22 nucleotide-long, non-coding RNAs, directly bind to RNAs in a sequence-complimentary manner to inhibit translation and facilitate degradation of their target transcripts. MiRNAs are encoded in the genome, and are transcribed as long primary transcripts (pri-miRNAs). Pri-miRNAs are subsequently processed by the Drosha/DGCR8 microprocessor complex into small hairpin precursor miRNAs (pre-miRNAs). Pre-miRNAs are transported by exportin-5 to the cytoplasm and are cleaved by Dicer into mature and functional miRNAs [26].

The importance of miRNAs in skeletal development was initially demonstrated by studies deleting *Dicer* in skeletal cells in vivo. The global reduction in miRNAs caused significant defects in multiple types of bone cells. However, understanding roles of individual miRNAs in bone formation in vivo is still rudimentary; most of our current knowledge about roles of miRNAs is based on in vitro studies using various experimental systems.

In this review, we discuss the role of miRNAs in three major cell types of bone: chondrocytes, osteoblasts, and osteoclasts. For each cell type, we first discuss about in vivo studies and then review major findings from in vitro studies.

The role of miRNAs in chondrocytes

In vivo studies

The effect of a global reduction of miRNAs in cartilage and bone development was shown in mice, in which the Dicer gene was conditionally deleted in the limb bud mesenchymal cells [27] and in chondrocytes [28]. Chondrocyte-specific *Dicer* deletion resulted in significant growth defects and premature death by the time of weaning. A reduction in proliferating chondrocytes and expansion of the hypertrophic region were observed, suggesting miRNAs regulate both proliferation and differentiation of growth plate chondrocytes.

The RNA, *Dnm3os,* is transcribed from an intron of the mouse Dynamin 3 (*Dnm3*) gene and encodes three miRNAs, **miR-199a**, **miR-199a*,** and **miR-214**. It is expressed in multiple types of cells including mesenchymal cells as well as perichondrial cells and periarticular chondrocytes. Its function was studied in mutant mice in which the 5′ region of *Dnm3os* was replaced by insertion of a lacZ gene [29]. This modification caused a reduction in all three miRNAs. The mutant mice showed growth retardation, craniofacial hypoplasia, dorsal vertebral hypoplasia, and osteopenia, strongly suggesting the essential role of these three miRNAs in normal mouse skeletal development. In human and mouse mesenchymal stem cells (MSCs), miR-199 is upregulated upon chondrocyte differentiation [30, 31].

The role of **miR-140,** which is expressed in chondrocytes in a relatively specific manner, has been studied in zebrafish [32] and mice [33–35]. In mice, miR-140 expression is mostly found in the cartilage of developing limbs, ribs, vertebrae, sternum, and the skull [36]. MiR-140 is encoded in an intronic sequence of the Wwp2 gene. MiR-140 expression shows an overlapping pattern with those of *Wwp2*, *Sox9,* and *Col2a1* (encoding type II collagen) [37]. In chondrogenic ATDC5 cells, Sox9 increases miR-140 expression and cell proliferation [37]. In this paper, Sp1 transcription factor, a cell cycle inhibitor, was found upregulated upon miR-140 inhibition in vitro and was suggested as a miR-140 target. Nakamura et al. [35] found a similar regulatory role of Sox9 in miR-140 expression in vivo using *Sox9* mutant zebrafish. A study in mice also confirmed this relationship of Sox9 and miR-140 [18]. This study also showed that a proximal upstream region of the pri-miR-140 gene possessed a chondrocyte-specific promoter activity, which was directly regulated by Sox9.

MiR-140-null mice show a mild skeletal growth defect with short endochondral bones and reduced longitudinal growth of the skull [33]. Miyaki et al. [33], found that these mice also showed age-related osteoarthritic changes in the articular cartilage presumably due to aberrant expression of the matrix-degrading enzyme, Adamts-5, a direct target of miR-140. The growth plate phenotype was further analyzed in a study using independently generated miR-140-null mice, [34]. The reduction in endochondral growth was attributed to the premature chondrocyte differentiation

toward post-mitotic chondrocytes and the delayed differentiation of resting to proliferating chondrocytes. By comparing profiles of Argonaute2-associated RNA between control and miR-140-null mice, *Dnpep* that encodes for an aspartyl aminopeptidase was found a direct target of miR-140. In addition to *Dnpep,* multiple possible miR-140 target genes, including *Pdgfra*, *HDAC4*, *Smad3*, and *Rala*, have been identified in various experimental systems [32, 36, 38, 39]; however the role of possible regulation of these genes by miR-140 in cartilage and bone in vivo is unclear.

In a recent in vivo study, we have provided evidence that **let-7** miRNAs regulate chondrocyte proliferation in the growth plate [40]. Suppression of let-7 miRNAs through overexpression of the let-7 inhibitor, Lin28a, in mouse cartilage caused mild growth impairment because of reduced chondrocyte proliferation. Lin28a-overexpressing chondrocytes showed upregulation of predicted let-7 target genes including the cycle regulators, cell division cycle 34 (*Cdc34*), and E2F transcription factor 5 (*E2f5*). Although the phenotype of Lin28 transgenic mice was mild, when the let-7 suppression, via Lin28a overexpression, was combined with miR-140 deficiency, the mice showed a dramatic growth defect. This finding suggests that let-7 miRNAs and miR-140 coordinately regulate skeletal development by regulating chondrocyte proliferation and differentiation, respectively.

In vitro studies

H19 is a non-protein-coding, maternally expressed gene, abundant in embryonic tissues, and known to play a key role in imprinting of the *Igf2* gene. In addition to the role of imprinting, *H19* also produces **miR-675** from its transcripts. MiR-675 is highly expressed in murine and human articular cartilage. Its expression levels in chondrocytes correlate with the differentiation state, and *Sox9* and *Col2a1* expression. MiR-675 positively regulates *Col2a1* expression in human articular chondrocytes [41]. **MiR-1247** is another highly expressed miRNA in mouse cartilage. Mir-1247 targets *Sox9* whereas Sox9 negatively regulates miR-1247 expression. This reciprocal regulation appears to create a negative feedback loop [42].

Yang et al. [43] found that **miR-145** was downregulated during chondrocyte differentiation of murine embryonic mesenchymal cells (C3H10T1/2 cell line). MiR-145 targeted and suppressed expression of *Sox9*. Overexpression of miR-145 decreased mRNA levels of chondrocyte marker genes, including *Col2a1, Acan* (encoding aggrecan), *Col9a1, Col11a1,* and *Comp* (encoding cartilage oligomeric matrix protein). On the contrary, miR-145 inhibition increased their expression. Thus, miR-145 negatively regulates chondrocyte differentiation, and its downregulation appears to be an important regulatory mechanism for chondrocyte differentiation.

Ohgawara et al. [44] focused on miRNAs that regulated CCN family protein 2/connective tissue growth factor (Ccn2/Ctgf), a molecule that plays an important role in endochondral bone formation. Initially they performed computational analysis and found binding sites for five miRNAs in the 3′-untranslated region of *Ccn2* (miR-26, miR-199, miR-375, miR-19, and miR-18). Then, they compared miRNA expression between non-chondrocytic cells (HeLa cells) and chondrocytic cells (HCS-2/8), and found that **miR-18a** was most differentially expressed in the chondrocytic cells. Subsequently they performed a luciferase assay and showed that miR-18a suppressed *Ccn2* expression. Thus, suppression of miR-18a appears to be necessary for *Ccn2* expression upon chondrocyte differentiation.

Sumiyoshi et al. [45] found that **miR-1** was repressed upon hypertrophic differentiation of chondrocytes. MiR-1 overexpression in a human chondrocytic cell line (HCS-2/8) and chicken primary chondrocytes reduced aggrecan expression, demonstrating the inhibitory role of miR-1 in chondrogenesis.

Zhong et al. [46] compared miRNA expression profiles in rat articular cartilage at post-natal day 0, 21 and 42, and found that miR-146a and miR-195 were upregulated, whereas miR-337 was downregulated as rats grew. **MiR-337** showed the greatest change; it was expressed at a high level at birth but quickly downregulated to an undetectable level as rats reached adulthood. Among the potential miR-337 targets predicted by the computational program, TargetScanS, *Tgfbr2,* encoding type II TGF-β receptor, was confirmed as a target by in vitro reporter experiments. Overexpression of miR-337 in a human chondrocytic cell line, caused downregulation of Tgfbr2 protein, and inhibited chondrocyte differentiation.

Chicken chondrocytes abundantly express **miR-181a**. Mir-181a represses expression of *Ccna2* (encoding for cyclin A2) and *Acan*. The regulation of these chondrocyte genes by miR-181a may act as a negative feedback for cartilage homeostasis [47]. Another study in chicken cells found that **miR-221** was increased upon inhibition of chondrocyte differentiation [48]. Blockade of miR-221 increased proliferation of limb mesenchymal cells. MiR-221 appeared to regulate chondrocyte proliferation by targeting *Mdm2* that encodes an inhibitor of the tumor suppressor, p53.

The expression level of **miR-375** in chicken limb mesenchymal cells is decreased upon differentiation into chondrocytes. MiR-375 inhibition increased chondrogenesis in cultured chicken limb mesenchymal cells. In wound-healing assay, miR-375 inhibition facilitated formation of cellular condensation by increasing cell

migration, and subsequently stimulated chondrocyte differentiation. This study demonstrated that cadherin-7 was a direct miR-375 target that mediated these actions of miR-375 [49].

MiRNAs in the chondrocyte differentiation of stem cells

Using large scale miRNA screening, Guérit et al. [50] identified **miR-574-3p** as one of the four most upregulated miRNAs during chondrogenesis in MSCs. MiR-574-3p exhibited a similar expression pattern to that of miR-140. MiR-574-3p expression increased at early stages of chondrogenesis and was maintained at an elevated level throughout differentiation. Retinoid X receptor alpha (Rxra), whose expression progressively decreased during the differentiation process, was validated as a direct target of miR-574-3p. It was also shown that Sox9 binds to the promoter region of the miR-574 gene and positively regulates its transcription during chondrogenesis. Since Rxra was previously demonstrated to inhibit Sox9 activity, miR-574-3p may be a constituent of a positive feedback loop that regulates chondrogenesis.

In mouse MSCs, **miR-24** and **miR-199b** were upregulated by more than fivefold upon differentiation into chondrocytes and osteoblasts [30]. MiR-101, miR-124a, and miR-199a were also upregulated, while miR-18 and miR -96 were downregulated. In human MSCs, miR-199a was upregulated by 4 fold after chondrocyte differentiation [31]. These miRNAs may regulate lineage determination during MSC differentiation.

MiR-449 negatively regulates chondrocyte differentiation of MSCs [51]. In human bone marrow MSCs and in human chondrosarcoma cell lines, miR-449 suppresses Lef1, a critical component of the Wnt signaling pathway. This suppression leads to downregulation of chondrocyte phenotypes, including *Col2a1* and *Sox9* expression, and reduced proteoglycan production.

MiR-335, discussed earlier about its role in osteogenesis, was also studied in chondrocytes [52]. Upon chondrocyte differentiation of murine MSCs, the abundance of miR-335 increased significantly. MiR-335 overexpression in murine MSCs promoted chondrogenesis. *Rock1* and *Daam1*, whose products were reported to negatively regulate *Sox9*, were found to be miR-335 targets. MiR-335 is co-expressed with its host gene, *Mest.* Sox9 downregulates miR-29a and miR-29b that suppress *Mest* expression. Thus, these miRNAs and their target genes may form a positive feedback loop; miR-335 decreases *Rock1* and *Daam1* to increase Sox9, which in turn increases *Mest* and miR-335 transcription by suppressing miR-29a and miR-29b.

The role of miRNAs in osteoblasts

In vivo studies

The role of miRNAs in bone was first revealed when ***Dicer*** was deleted in an osteoblast-specific manner. Deletion of *Dicer* in bone marrow stromal cells (BMSCs) caused defective osteoblast differentiation without significant effects on the cell growth or survival. During the growth phase (up to day 19 of culture), *Runx2* and *Col1a1* were expressed normally but then failed to increase further [53]. Mature osteoblast markers also failed to express. This finding suggests that miRNAs are necessary for normal differentiation of osteoprogenitors into mature osteoblasts. This notion is consistent with an in vivo study in which *Dicer* was deleted in osteoblast progenitors using *Osx-Cre* transgenic mice. These mice showed reduced mature osteoblasts expressing osteocalcin [54].

When *Dicer* was deleted in mature osteoblasts and osteocytes using a transgenic expressing Cre recombinase under the control of an osteocalcin (*bglap*) promoter, mice showed no obvious skeletal defects at birth except a mild delay in bone formation of some bones [53]. However, at 1 month of age the *Dicer*-deficient bone showed a greater bone mass with increased *Col1a1, Sp7* (*Osterix, Osx),* and *bglap* expression compared with control. There were no overt changes in osteoclasts. These findings suggest that miRNAs are required for normal differentiation of pre-osteoblasts into mature osteoblasts, whereas they suppress functions of mature osteoblasts.

Li et al. [55] found that **miR-2861** was expressed primarily in mouse osteoblasts. Using a stromal cell line and bone marrow stromal cells, they showed that miR-2861 promoted osteoblast differentiation induced by BMP-2 stimulation, whereas suppression of this miRNA inhibited it. Histone deacetylase 5 (HDAC5) was identified as a miR-2861 target. In addition to inhibiting MEF2 transcription factors through direct physical binding, HDAC5 also deacetylates lysine residues of various proteins, including Runx2, to regulate protein functions. It has been reported that deacetylation of Runx2 facilitates its ubiquitination and degradation [56]. It appears that miR-2861 reduces *HDAC5* expression, and thereby increases Runx2 levels to promote osteoblast differentiation. In the same study, in vivo treatment of mice with antisense miR-2861 caused reductions in bone mineral density (BMD) and histological parameters of bone formation. They also found a mutation in the miR-2861 gene in patients with adolescent osteoporosis. This study suggests that a single miRNA can significantly influence bone mass. However, since miR-2861 is located in a promoter region of *Cdk9*, a gene encodes a cell cycle regulator; further investigation is required to determine the causal role of the miR-2861

dysfunction in adolescent osteoporosis. The primary transcript encoding miR-2861 also generates another miRNA, **miR-3960**. MiR-3960 also induces osteoblast differentiation, whereas its inhibition reduces osteogenesis [57]. MiR-3960 appears to target and suppress *Hoxa2* which inhibits *Runx2* expression. Another interesting finding in this study is that Runx2 binds to the common promoter of the miR-3960/miR-2861 gene and increases its expression. These regulations may create a positive feedback loop that maintains Runx2 levels upon osteoblast differentiation.

In a study that aimed at identifying specific miRNAs associated with osteoporosis, **miR-214** was found to inhibit bone formation [58]. MiR-214 was expressed at higher levels in elderly individuals with history of bone fractures than those without fractures. The miR-214 level was inversely correlated with bone formation markers. The inhibitory effect of miR-214 in bone formation was confirmed in mouse models and by in vitro experiments. They found that miR-214 suppressed activating transcription factor 4 (*Atf4*), a transcription factor that promotes the expression of osteoblast-specific genes such as osteocalcin [59]. Transgenic mice overexpressing miR-214 in osteocalcin-expressing cells (mature osteoblasts), showed reduced bone formation, which was rescued by miR-214 inhibition. This miRNA may play a significant role in postmenopausal osteoporosis because its inhibition in ovariectomized mice rescued their osteopenic phenotype. In an in vitro study, miR-214 was found to also target *Osx*. MiR-214 overexpression in osteoblast cell lines (Saos-2 and U2OS cells) reduced *Osx* expression, whereas osteoblastic trans-differentiation of C2C12 cells was enhanced upon miR-214 inhibition [60].

MiR-206 is one of the muscle-specific miRNAs. In a study by Inose et al. [61], ectopic expression of miR-206 in mouse osteoblasts in vivo reduced bone mass without any effect in bone resorption. MiR-206 appears to inhibit osteoblast differentiation by downregulating connexin-43 (Gja1), a gap junction protein that plays an important role in osteoblast and osteocyte functions. Knockdown of miR-206 promoted osteoblast differentiation. The biological significance of this finding is not clear because endogenous miR-206 expression in osteoblasts is low.

Another miRNA that was studied in vivo is **miR-34c.** MiR-34 family miRNAs are direct transcriptional targets of p53 and mediate part of p53′s function [62]. MiR-34c was upregulated during osteoblast differentiation of myoblastic C2C12 cells and BMSCs. Transgenic mice overexpressing miR-34c in osteoblasts displayed age-related osteoporosis. This effect is due to reduced osteoblast proliferation and differentiation as well as increased bone resorption. Transcriptome profiling and reporter assays identified several molecules involved in the Notch signaling pathway as miR-34c targets [63]. MiR-34c likely regulates osteoclastogenesis indirectly via regulation of Notch signaling in osteoblasts. Mir-34c overexpression also caused downregulation of *Runx2* and *Satb2* (special AT-rich sequence binding protein 2) that facilitate osteoblast differentiation [63].

MiR-182, expressed in osteoblasts, negatively regulates osteoblast proliferation and differentiation [64]. MiR-182 appears to suppress the expression of *Foxo1*, a transcription factor that regulates the reduction and oxidation balance in osteoblasts. Foxo1 regulates bone mass, as its depletion results in decreased osteoblast proliferation and bone formation [65, 66]. Furthermore, *Foxo1* overexpression in vitro rescues the antiproliferative and pro-apoptotic effects of miR-182. In vivo experiments using zebrafish confirmed the inhibitory effect of miR-182 on bone formation [64].

The **miR-17-92** cluster is located on chromosome 13 in humans and encodes six individual miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a). This cluster has two paralogs: the miR-106b-25 and the miR-106a-363 clusters. The miR-106b-25 cluster is located on human chromosome 7 and mouse chromosome 5 and comprises three miRNAs: miR-106b, miR-93, and miR-25, while the miR-106a-363 is located on chromosome X and encodes six miRNAs: miR-106a, miR-18b, miR-20b, miR-19b-2, miR-92a-2, and miR-363. The 15 miRNAs encoded by the miR-17-92 and its two paralogous genes form four functionally distinct "seed" family groups (miR-17, miR-18, miR-19, and miR-92) [67]. The miR-17-92 cluster miRNA gene is the first miRNA gene whose mutations were found to cause skeletal developmental defects in humans. Deletion of these genes causes a brachy-syndactyly syndrome (Feingold syndrome type II) [68, 69]. On the other hand, duplication of this gene causes variable skeletal phenotypes; one family shows skeletal overgrowth with polydactyly [70], whereas the other shows short stature and brachydactyly [71]. Mice hemizygous or homozygous for *miR17-92* deletion showed reduced skeletal growth with delayed ossification of digital and skull bones [68]. The miR-17-92 cluster miRNAs are expressed abundantly in osteoprogenitor cells and embryonic stem (ES) cells, but decrease upon osteoblast differentiation [72]. This study also found that haploinsufficiency of the miR-17-92 cluster gene in mice resulted in reduced bone mass likely due to impaired osteoblast proliferation and differentiation.

In vitro studies

In addition to these in vivo studies discussed so far, there are a significant number of in vitro studies that have investigated roles of specific miRNAs in osteoblasts.

As suggested by in vivo models, miR-17-92 miRNAs appear to regulate both proliferation and differentiation of

osteoprogenitors. A study using human periodontal ligament tissue-derived mesenchymal stem cells (PDLSCs) found that suppression of miR-17 promoted osteoblast differentiation, whereas its overexpression inhibited it [73]. Another recent study showed that expression levels of miRNA-17 family miRNAs, including miR-17, -106a, and -20a, were decreased in human adipose-derived mesenchymal stem cells (hADSCs) during differentiation toward the osteogenic lineage, whereas they were increased during adipocyte differentiation [74]. Overexpression of miR-17 and miR-106a promoted adipocyte differentiation and inhibited osteogenesis by suppressing *Bmp2* expression. Another study using mouse palatal mesenchymal cells showed that miR-17-92 miRNAs targeted multiple molecules involved in the TGF-β pathway to inhibit TGF-β signaling to promote proliferation [75].

MiR-17-92 cluster miRNAs may inhibit apoptosis in osteoblasts by targeting the pro-apoptotic protein, Bim (bcl-2 interacting mediator of cell death) [76]. This study found that miR-17, miR-20a, and miR-92a were significantly reduced in osteoblasts undergoing apoptosis. Overexpression of miR-17-92a miRNAs reduced dexamethasone-induced apoptosis and enhanced the anti-apoptotic effect of estrogen in osteoblasts.

MiR-34a was found to be one of the most significantly upregulated miRNAs upon osteoblast differentiation in human MSCs, whereas miR-34b exhibited modest upregulation and miR-34c, which was discussed earlier, was not detectable [77]. MiR-34a overexpression in vitro seemed to regulate the expression of *Jag1* that encodes a Notch ligand. In addition, miR-34a regulated osteoblast proliferation, likely through targeting the cell cycle regulators, *Ccnd1* (cyclin D1), *Cdk4* (cyclin-dependent kinase 4), *Cdk6* (cyclin-dependent kinase 6), *E2f3* (E2F transcription factor 3), and *Cdc25a* (cell division cycle 25A). Pre-miR-34a transfection inhibited heterotopic bone formation of subcutaneously implanted MSCs in immune deficient mice, whereas anti-miR-34a stimulated it [77]. In another study conducted in C2C12 cells, **miR-34b** as well as miR-34c were upregulated upon activation of Wnt signaling and upon osteoblast differentiation in a mouse osteoblast cell line [78].

MiR-29 regulates osteoblast differentiation by directly and indirectly modulating Wnt signaling [79, 80]. Three miR-29 family miRNAs are transcribed from two separate genomic loci. Mir-29c and miR-29b-2 are generated from the same primary miRNA that is transcribed from a locus located on the chromosome 1; miR-29a and miR-29b1 are produced from another primary miRNA encoded on chromosome 7 in humans [81]. MiR-29 binding sites are found in the RNA encoding osteonectin, the most abundant non-collagen extracellular matrix protein in bone. In primary cultures of mouse osteoblast cells, the osteonectin level decreases upon cell maturation and mineralization. This decrease coincides with an increase in levels of miR-29a and miR-29c, suggesting that these miRNAs might negatively regulate osteonectin expression. Reporter experiments confirmed their interaction. Inhibition of miR-29a/c using antagomirs caused an increase in osteonectin levels. Based on these findings, a model was proposed that during osteoblast differentiation Wnt signaling induces osteoblast differentiation and upregulates miR-29a/c, which in turn, reduces osteonectin protein levels at the mineralization stage [79]. MiR-29a stimulates osteoblast differentiation in primary human osteoblast cultures and suppresses expression of multiple Wnt signaling inhibitors, such as Dkk1 (Dickkopf-related protein 1), to enhance Wnt signaling. This may create a positive feedback loop that facilitates osteoblast differentiation [80]. MiR-29a expression is suppressed by glucocorticoid treatment. Rats treated with glucocorticoids for 2–4 weeks showed reduced BMD and decreased miR-29a expression [82]. In these mice, expression of *Col1a1* and osteocalcin was decreased, whereas *Dkk1* expression was increased. Overexpression of miR-29a in vivo, via a lentivirus-based gene transfer system, significantly ameliorated these changes caused by glucocorticoid treatment. Furthermore, when rats were treated with a miR-29a inhibitor, they displayed significant reductions in bone mass, mineral apposition, and histomorphometric parameters for osteoblast functions. MiR-29a inhibitor-treated osteoblasts also showed a differentiation defect ex vivo. Additionally miR-29a reduced osteoclast activity possibly by downregulating RANKL expression. This study suggests that miR-29a could be exploited to reduce the negative effects of glucocorticoids on bone. Another in vitro study confirmed the role of miR-29 miRNAs in osteoblast differentiation [83]. This study found that miR-29b targeted multiple negative regulators of osteoblast differentiation, such as *HDAC4, Tgfb3* (TGF-β3), *Acvr2a* (activin receptor IIA), *Ctnnb1* (β-catenin), and *Dusp2* (dual specificity phosphatase 2). It was also suggested that miR-29b influenced extracellular matrix production by targeting collagen genes, including *Col1a1,* and might prevent fibrosis of bone tissue.

Li et al. [84] reported that **miR-143** suppressed osteogenic differentiation by targeting *Osx* in osteoblastic MC3T3-E1 cells. MiR-143-mimic transfection upregulated *Osx,* alkaline phosphatase (ALP) and osteocalcin levels, whereas miR-143 inhibitor reduced their expression. Furthermore, *Osx* overexpression reversed the negative effect of miR-143 on osteogenic differentiation.

Bhushan et al. [85] provided evidence that **miR-181** miRNAs (**miR-181a, b, c,** and **d**) promote osteoblast differentiation by downregulating TGF-β signaling. They showed that miR-181a was upregulated upon osteoblast differentiation in C2C12 cells, MC3T3-E1 cells, and

primary calvarial osteoblasts. Expression of other miR-181 miRNAs also increased during mouse calvarial and tibial development. Analysis of the mRNA expression profile in MC3T3-E1 cells transfected with miR-181a revealed that *Tgfbr1* (TGF-β receptor I) and *Tgfbi* (TGF-β, induced) that encode molecules in the TGF-β signaling pathway that negatively regulates osteoblastic differentiation were significantly downregulated. Luciferase reporter assay confirmed that these genes were targeted by miR-181.

Gámez et al. [86] reported that **miR-322** increased expression of osteogenic genes including *Osx, Runx2, Msx2*, and *Ibsp* (integrin binding sialoprotein) in C2C12 cells in response to BMP-2. MiR-322 appears to increase *Osx* expression by targeting of *Tob2*. Tob2 facilitates deadenylation and degradation of mRNAs. *Tob2* overexpression reduced Osx mRNA, which was inhibited by overexpression of miR-322.

MiR-335-5p was found to be expressed in osteoblasts and hypertrophic chondrocytes in mouse embryos. MiR-335 acts as a suppressor of Dkk1, to enhance Wnt signaling and promote osteogenesis [87].

In expression profiling of miRNAs in MC3T3-E1 cells, **miR-141** and **miR-200a** were found to be downregulated by BMP-2 treatment [88]. Their overexpression in this cell line reduced osteogenesis, as shown by decreased ALP activity. Conversely, inhibition of these miRNAs stimulated osteoblast differentiation. These miRNAs appear to target the osteogenic transcription factor, Dlx5, to inhibit osteoblast differentiation.

MiR-23a, **miR-27a**, and **miR-24-2**, transcribed from a miRNA cluster gene, were shown to suppress the osteogenic gene, *Satb2,* in primary rat osteoblasts [89]. Satb2 facilitates bone formation by enhancing the function of Runx2. Transcription of these miRNAs is negatively regulated by *Runx2* through a Runx binding element in the promoter region. The Runx2-mediated suppression of these miRNAs is expected to increase *Satb2* expression and enhance osteoblast phenotypes during differentiation. On the other hand, in terminally differentiated osteocytes, miR-23a suppressed *Runx2*, creating a feedback mechanism to control osteoblast maturation. In another study, miR-23a was overexpressed in a murine pre-osteoblast cell line [90]. MiR-23a overexpression reduced tumor necrosis factor-α (TNF-α)-induced cell apoptosis by inhibiting expression of the apoptosis-inducing gene, *Fas*. Since TNF-α is one of the inflammatory cytokines that are elevated in estrogen deficiency, miR-23a expression in osteoblasts may be an endogenous counter-mechanism against post-menopausal osteoporosis.

The search for miRNAs targeting *Runx2* led to the identification of **miR-23a**, **miR-30c**, **miR-34c**, **miR-133a**, **miR135a**, **miR137**, **miR204**, **miR-205**, **miR-217**, **miR218**, and **miR-338**. These miRNAs downregulated Runx2 to inhibit osteoblast and chondrocyte differentiation [91].

Lei et al. [92] investigated the association between genetic polymorphisms in predicted miRNA binding sites in the 3′-UTR of mRNAs and osteoporosis. This study identified three polymorphisms in miRNA target sites of the Fgf2 gene that were significantly associated with femoral neck BMD. These sites were targeted by 9 miRNAs, **miR-146a/b**, **miR-549**, **miR-563**, **miR-212**, **miR-132**, **miR-505**, **miR-421**, **miR-361**, **miR-582**, and **miR-302b.** *Fgf2* expression was decreased in patients of the high BMD group compared with those in the low BMD group.

Lisse et al. [93] investigated regulation of osteoblastic miRNAs by vitamin D. **MiR-637** and **miR-1228** were upregulated upon vitamin D treatment and were shown to target *Col4a1* and *Bmp2k* (bone morphogenetic protein 2 kinase). MiR-637 was also shown to target *Osx* to suppress osteoblast differentiation in early differentiation stages [94].

Chen et al. [95] compared miRNAs expressed in calvariae of E18.5 *Osx*-null embryos with those in wild-type embryos to identify Osx-regulated miRNAs. Ten miRNAs, including **miR-133a,** and its two paralogous miRNAs, **miR-204** and **miR-211,** were downregulated in the absence of *Osx*. These miRNAs target *Runx2* as discussed earlier. Additionally, miR-204/211 targets *Sost* that encodes the Wnt antagonist, Sclerostin. Thirty miRNAs were upregulated in *Osx* deficiency, including miR-141 and miR-220a that target *Dlx5*.

An et al. [96] performed miRNA and mRNA profiling in bones of ovariectomized mice. MiRNAs -127, -133a, -133*a, -133b, -136, -206, -378, and -378* were upregulated after ovariectomy, whereas miR-204 was downregulated. This study also demonstrated that miR-127 suppressed and miR-136 enhanced osteoblast differentiation, whereas both miRNAs promoted cell death in osteocytic cells.

A recent study aimed to identify specific miRNAs that can be used as biomarkers for osteoporosis [97]. MiR-21, miR-23a, miR-24, miR-100, and miR-125b were upregulated both in the serum and in bone tissues of patients with osteoporosis. This finding is in line with the previous finding that miR-23a, miR-24, and miR-125b suppress osteogenesis in vitro, as discussed earlier. This study presents the possibility of using miRNAs as diagnostic markers in bone diseases.

MiRNAs in mesenchymal stem cells

Mesenchymal stem cells can differentiate into multiple cell lineages including osteoblasts, chondrocytes and adipocytes. Manipulation of MSCs to generate desired types of

cells has been intensively studied in fields of tissue engineering and regenerative medicine in the hope of developing cell-based therapies. MiRNAs have drawn attention as a tool to manipulate MSCs to expand and differentiate them into bone cells.

Several miRNAs change their expression during osteoblast differentiation of MSCs. Laine et al. [31] found that **MiR-96** and **miR-199a** were increased by 16- and 2-fold, respectively, in human MSCs during osteoblast differentiation; their exact roles in osteogenesis is currently unclear. **MiR-346** is also increased during osteoblast differentiation of MSCs. Mir-346 overexpression promoted osteogenesis via targeting RNA encoding glucogen synthase kinase-3β (Gsk3b) that inhibits canonical Wnt signaling by facilitating β-catenin degradation [98]. **Mir-31** was found to be upregulated in MSCs differentiating into mineralizing osteoblasts [99]. In this study, miR-31 was shown to negatively regulate *Osx*. However, this result is somewhat contradicting to that of a previous study in which miR-31 was downregulated upon hMSC differentiation toward osteoblasts and suppressed osteogenesis by targeting *Runx2* and *Bmpr2* [100]. Another study also reported downregulation of miR-31 during osteoblast differentiation [101]. MiR-31 inhibition enhanced osteoblast differentiation by de-repressing *Satb2* that cooperated with *Runx2*. It is possible that miR-31 mainly suppresses osteoblast differentiation in early stages, but promotes it during late stages.

Eskildsen et al. [102] found that **miR-138** was downregulated upon osteoblast differentiation. MiR-138 overexpression and knockdown in hMSCs decreased and increased bone formation, respectively, after subcutaneous implantation of hMSCs into immuno-compromised mice. MiR-138 was found to target *Ptk2*, a gene encoding focal adhesion kinase (FAK) that activates the extracellular signal-regulated kinase (ERK) signaling pathway. MiR-138 reduced ERK signaling, inhibited Runx2 phosphorylation, and reduced *Osx* expression. Zhang et al. [94] showed that **miR-637 expression** in hMSCs decreased upon osteoblast differentiation and increased during adipogenic differentiation. Since MiR-637 targeted *Osx,* miR-637 might regulate lineage switching between osteoblasts and adipocytes. In addition, **miR-204** and **miR-211** appear to be important negative regulators of *Runx2*. These miRNAs both inhibited osteogenesis, whereas miR-204 promoted adipogenesis in MSCs [103].

MiR-370 was found to be downregulated upon osteoblast differentiation of mesenchymal cells (C3H10T1/2 cells) treated with BMP-2 [104]. MiR-370 overexpression reduced ALP activity and resulted in a mild decrease in mineralization, demonstrating that miR-370 inhibits osteoblast differentiation. *Bmp2* and *Ets1*, a proto-oncogene, were predicted as potential targets of miR-370. MiR-370 overexpression reduced expression of these genes as well as the osteoblast genes including *Spp1* that encodes osteopontin and *Runx*2.

The possible shift of MSC differentiation tendency from the osteoblast to adipocyte lineage has been implicated in osteoporosis. MiRNA profiling in MSCs from ovariectomized mice demonstrated a significant upregulation of **miR-705** and **miR-3077-5p** [105]. After estrogen supplementation, expression of these miRNAs returned to normal levels. In vitro experiments also found that expression levels of these miRNAs and osteoblastic phenotypes were inversely correlated. Furthermore, overexpression of these miRNAs increased adipogenic differentiation, whereas their inhibition decreased it. In this study, it was shown that miR-705 and miR-3077-5p targeted *Hoxa10* and *Runx2*, respectively.

Gong et al. [106] attempted to identify miRNAs regulated by the osteo-promotive gene, *Satb2*, in murine MSCs. Among ten downregulated miRNAs after *Satb2* overexpression, miR-27a was selected for further analysis. MiR-27a was found to target *Bmp2*, *Bmpr1a*, and *Smad9*, and miR-27a overexpression inhibited osteoblast differentiation.

M**iR-210** is highly expressed in ST2 mouse mesenchymal cells. Mizuno et al. [107] found that miR-210 was upregulated upon BMP-4-induced osteoblast differentiation. Overexpression of miR-210 in these cells promoted osteoblast differentiation, as assessed by ALP activity and osteocalcin expression. This is likely through targeting activin A receptor type 1B (*Acvr1b*) that mediates TGF-β/activin signaling. Another miRNA found to be upregulated in ST2 cells during osteoblast differentiation was **miR-125b** [108]. MiR-125b inhibition promoted osteoblast differentiation. MiR-125b might target *ErbB2,* which encodes for a protein tyrosine kinase that regulates proliferation of osteoblasts and chondrocytes [109].

MiR-124a and **miR-181a** were among six downregulated miRNAs upon BMP-4-induced osteoblast differentiation of mouse-induced pluripotent stem (iPS) cells [110] Their downregulation appears to facilitate osteoblast differentiation; suppression of these six miRNAs induced expression of osteoblastic marker genes, such as *Runx2* and *Spp1*, in iPS cells. These miRNAs appear to suppress osteoblast differentiation at early differentiation stages.

MiRNA expression profiling in unrestricted somatic stem cells (USSCs), derived from the human umbilical cord blood, identified **miR-135b** as the most significantly downregulated miRNA upon osteogenic differentiation [111]. Overexpression of miR-135b reduced expression of bone markers including *Ibsp* and *Osx*. Another study using USSCs to define a miRNA signature associated with osteoblast-lineage commitment identified several miRNAs;

miR-146a, **miR-181a**, **miR-199b-5p**, and **miR-30b** were upregulated, and **miR-1274a**, **miR-139-3p**, **miR-221**, **miR-376a**, and **miR-376c** were downregulated upon osteoblast differentiation [112]. Further study revealed that inhibition of miR-1274a, miR-221, and miR-376a increased osteoblast differentiation.

Human adipose tissue-derived stem cells (hADSCs), isolated from adipose tissues, are capable of differentiating into osteoblastic cells. In hADSCs, **miR-26a** was found to target Smad1 transcription factor and inhibit osteoblast differentiation [113]**. MiR-196a** enhanced osteoblast differentiation of hADSCs by targeting *Hoxc8* [114]. **MiR-218** decreased expression of the Wnt antagonists, *Dkk2* and *Sfrp2*, and thereby promoted osteogenesis in hADSCs. Additionally, Wnt signaling enhanced miR-218 expression, creating a signal-amplification circuit [115]. Like in MSCs discussed earlier, **miR-31** knockdown in rat ADSCs enhanced osteogenesis by targeting *Satb2*. MiR-31 knockdown also promoted repair of "critical-sized calvarial defects" after engraftment of cells treated with a miR-31 inhibitor and synthetic scaffolds [116].

Mesenchymal stem cells from patients with post-menopausal osteoporosis showed decreased osteogenic potential ex vivo [117]. Deregulation of miRNAs in estrogen deficiency may account for the reduced osteogenic potential. Yang et al. [118] found that **miR-21** was downregulated in MSCs isolated from ovarectomized mice. MiR-21 was suppressed by TNF-α, an inflammatory cytokine that plays a pathogenic role in post-menopausal osteoporosis. Furthermore, miR-21 overexpression increased the osteoblast differentiation of MSCs. Treatment of ovariectomized mice with anti-TNF-α increased miR-21 expression and bone formation. These data suggest that miR-21 suppression in estrogen deficiency may decrease osteoblast differentiation of MSCs and contribute to the bone loss. *Spry1,* a gene encoding for a negative regulator of the FGF and ERK-MAPK signaling pathways, was identified as a miR-21 target that mediated the above effects; overexpression of *Spry-1* in hMSCs from healthy donors, reduced osteoblast differentiation.

The role of miRNAs in osteoclasts

The significance of miRNAs in osteoclasts was first shown by in vitro experiments in which miRNAs were globally reduced in osteoclast precursors by deleting *Dgcr8, Dicer,* or *Ago2*, genes individually necessary for miRNA biogenesis or function. The global reduction in miRNAs inhibited osteoclast differentiation and function. Reduced levels of transcription factors necessary for the osteoclastogenesis, such as PU.1, Mitf, Fos, and Nfatc1, were observed in miRNA-deficient osteoclast precursors [119]. In this study, *Dicer* deletion in macrophages and osteoclasts in vivo using the *CD11b-Cre* transgenic mice decreased osteoclast number and increased trabecular bone mass. **MiR-223** expressed in cells of the osteoclast lineage targeted NF1A, a negative regulator of macrophage colony-stimulating factor receptor (Csf1r), and facilitated osteoclast differentiation. Osteoclast-specific deletion of the Dicer gene using Cathepsin-Cre transgenic mice confirmed that miRNA deficiency in osteoclasts causes a mild increase in bone mass with a decrease in osteoclast number [120]. Additionally, these mice also showed decreased osteoblast activity and reductions in mineral apposition and bone formation rates. In a recent study, miRNAs were reduced in osteoclasts by conditionally deleting the *Dgcr8* gene, which encodes a molecule essential for processing pri-miRNAs into pre-miRNAs, using cathepsin-Cre transgenic mice [121]. Mice missing *Dgcr8* in osteoclasts showed increased trabecular and cortical bone with impaired osteoclastogenesis confirming the role of miRNAs in osteoclast differentiation and function.

Chen et al. [122] showed a reduced level of **miR-503** in the circulating osteoclast precursor cells, CD14+ peripheral blood mononuclear cells (PBMCs), in patients with post-menopausal osteoporosis. Overexpression of miR-503 decreased *TRAP* and *Nfatc1* expression, and inhibited osteoclastogenesis in CD14+ PBMCs obtained from post-menopausal women. MiR-503 was shown to directly target mRNA encoding receptor activator of NFκB (RANK). Mice treated with miR-503 or antagomir-503 showed an increase or decrease in BMD, respectively. In addition, it was shown that 17β-estradiol (E2) increased miR-503 expression, further supporting the notion that the reduction in miR-503 caused by estrogen deficiency contributes to development of post-menopausal osteoporosis.

A study, in which human CD14+ PBMCs was used to investigate changes in miRNA expression during osteoclastogenesis, found **miR-148a** as the most dramatically upregulated miRNA [123]. Overexpression of miR-148a in human PBMCs or mouse bone marrow cells increased osteoclast differentiation. V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (Mafb), a negative regulator of osteoclastogenesis, was identified as a target of miR-148a. Ovariectomized mice as well as control mice treated with antagomir-148 showed a significant increase in BMD, accompanied by reduced osteoclast activity and bone resorption. In this study, miR-148 was also found to be increased in patients with systemic lupus erythymatosus (SLE) who had never received corticosteroid therapy. The miR-148 level was correlated with increased osteoclastogenesis, suggesting that miR-148 may play a role in the increased risk for bone loss in these patients.

A miRNA that was significantly downregulated during osteoclastogenesis in human PBMCs was **miR-125a** [124].

MiR-125a overexpression inhibited osteoclastogenesis, while its inhibition promoted it by upregulating the direct target, TNF receptor-associated factor 6 (Traf6), an osteoclastogenesis-promoting factor. In addition, the osteoclast-promotive transcription factor, Nfatc1, was found to bind to the promoter of the miR-125a gene to inhibit its transcription, potentially creating a positive feedback loop.

MiRNA profiling in bone marrow-derived monocyte/macrophage precursors after RANKL stimulation showed downregulation of 33 miRNAs and upregulation of 38 miRNAs upon osteoclast differentiation [125]. **MiR-21** was strongly upregulated by RANKL treatment, and also by transcription factors that regulate differentiation and function of osteoclasts, including Fos. Inhibition of miR-21 reduced osteoclastogenesis in vitro with concomitant upregulation of its target gene *Pdcd4* that encodes a negative regulator of Fos. MiR-21 appears to suppress *Pdcd4* to augment Fos function, and thereby, facilitate osteoclastogenesis. Another miRNA that was upregulated during osteoclast differentiation of mouse bone marrow cells was **miR-31** [126]. Inhibition of miR-31 in mouse bone marrow macrophages using antagomirs compromised the cytoskeleton function and suppressed osteoclast formation. Upregulation of the GTPase, RhoA, a target of miR-31 appears to be responsible for this effect because treatment with a RhoA inhibitor rescued the defect in osteoclastogenesis of miR-31 antagomir-treated cells.

Osteoclast differentiation is inhibited by interferon-β (IFN-β). **MiR-155** appears to partly mediate this inhibitory effect of IFN-β on osteoclastogenesis [127]. MiR-155 was strongly induced by IFN-β in bone marrow-derived macrophages in culture. Overexpression of miR-155 suppressed osteoclast differentiation by targeting *Socs1* and *Mitf*, positive regulators of osteoclastogenesis.

The role of miR-29 was studied in human and mouse cells by different groups, and somewhat contradicting results were obtained. In human cells, **miR-29b**, was found to be decreased during osteoclast differentiation in vitro [128]. MiR-29b overexpression in osteoclast precursors reduced the expression of *Nfatc1*, *Ctsk* (Cathepsin K), *Mmp9*, *TRAP,* and *RANK*. It also reduced bone resorption activity, assessed by the lacunae generation (pit formation) assay, and collagen degradation. This effect appeared to be due to the suppression of *Fos* and *Mmp2* expression by miR-29b. In contrast, in mouse cells, all miR-29 family members (a, b, and c) were increased upon osteoclast differentiation in primary bone marrow-derived macrophages as well as in a monocytic cell line [129]. Inhibition of miR-29 significantly reduced osteoclastogenesis in these cells. This study identified several groups of miR-29 target genes that have coherent functions. Cell division control protein 42 (Cdc42) and SLIT-ROBO Rho GTPase-activating protein 2 (Srgap2) are both important for osteoclast function and migration by regulating the cytoskeleton. G protein-coupled receptor 85 (Gpr85) and CD93 potentially regulate macrophage differentiation. Nf1A inhibits macrophage and osteoclast maturation. The reason of the discrepancy between human and mouse cells is currently unclear. It is possible that miR-29 promotes the initial osteoclast lineage commitment while it inhibits osteoclast maturation, since more differentiated cells (peripheral blood osteoclast precursors) were used in the human study whereas bone marrow progenitors were used in the mouse study.

Conclusions

These studies have demonstrated that miRNAs regulate many aspects of bone development, and play significant roles in bone physiology and pathophysiology. The majority of these findings have been obtained from studies using in vitro experimental systems; toward further understanding of physiological roles of individual miRNAs at tissue and organ levels, and toward the ultimate goal of translating these findings into clinical applications, studies using in vivo models are required.

Acknowledgments This work is supported by the NIH grant, AR056645, to T.K.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kronenberg HM (2003) Developmental regulation of the growth plate. Nature 423:332–336
2. Long F, Chung UI, Ohba S, McMahon J, Kronenberg HM, McMahon AP (2004) Ihh signaling is directly required for the osteoblast lineage in the endochondral skeleton. Development 131:1309–1318
3. Yoon BS, Ovchinnikov DA, Yoshii I, Mishina Y, Behringer RR, Lyons KM (2005) Bmpr1a and Bmpr1b have overlapping functions and are essential for chondrogenesis in vivo. Proc Natl Acad Sci USA 102:5062–5067
4. Kobayashi T, Lyons KM, McMahon AP, Kronenberg HM (2005) BMP signaling stimulates cellular differentiation at multiple steps during cartilage development. Proc Natl Acad Sci USA 102:18023–18027
5. Pogue R, Lyons K (2006) BMP signaling in the cartilage growth plate. Curr Top Dev Biol 76:1–48
6. Itoh N, Ornitz DM (2011) Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. J Biochem 149:121–130
7. Long F (2012) Building strong bones: molecular regulation of the osteoblast lineage. Nat Rev Mol Cell Biol 13:27–38
8. Hu H, Hilton MJ, Tu X, Yu K, Ornitz DM, Long F (2005) Sequential roles of Hedgehog and Wnt signaling in osteoblast development. Development 132:49–60

9. Day TF, Guo X, Garrett-Beal L, Yang Y (2005) Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell 8:739–750
10. Hill TP, Spater D, Taketo MM, Birchmeier W, Hartmann C (2005) Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Dev Cell 8:727–738
11. Rodda SJ, McMahon AP (2006) Distinct roles for hedgehog and canonical Wnt signaling in specification, differentiation and maintenance of osteoblast progenitors. Development 133:3231–3244
12. Canalis E (2013) Wnt signalling in osteoporosis: mechanisms and novel therapeutic approaches. Nat Rev Endocrinol 9:575–583
13. van Amerongen R (2012) Alternative Wnt pathways and receptors. Cold Spring Harb Perspect Biol 4
14. Rebay I, Fleming RJ, Fehon RG, Cherbas L, Cherbas P, Artavanis-Tsakonas S (1991) Specific EGF repeats of Notch mediate interactions with delta and serrate: implications for Notch as a multifunctional receptor. Cell 67:687–699
15. Boyce BF (2013) Advances in the regulation of osteoclasts and osteoclast functions. J Dent Res 92:860–867
16. Bi W, Deng JM, Zhang Z, Behringer RR, de Crombrugghe B (1999) Sox9 is required for cartilage formation. Nat Genet 22:85–89
17. Lefebvre V, Huang W, Harley VR, Goodfellow PN, de Crombrugghe B (1997) SOX9 is a potent activator of the chondrocyte-specific enhancer of the pro alpha1(II) collagen gene. Mol Cell Biol 17:2336–2346
18. Yamashita S, Miyaki S, Kato Y, Yokoyama S, Sato T, Barrionuevo F et al (2012) L-Sox5 and Sox6 proteins enhance chondrogenic miR-140 microRNA expression by strengthening dimeric Sox9 activity. J Biol Chem 287:22206–22215
19. Arnold MA, Kim Y, Czubryt MP, Phan D, McAnally J, Qi X et al (2007) MEF2C transcription factor controls chondrocyte hypertrophy and bone development. Dev Cell 12:377–389
20. Yoshida CA, Yamamoto H, Fujita T, Furuichi T, Ito K, Inoue K et al (2004) Runx2 and Runx3 are essential for chondrocyte maturation, and Runx2 regulates limb growth through induction of Indian hedgehog. Genes Dev 18:952–963
21. Takeda S, Bonnamy JP, Owen MJ, Ducy P, Karsenty G (2001) Continuous expression of Cbfa1 in nonhypertrophic chondrocytes uncovers its ability to induce hypertrophic chondrocyte differentiation and partially rescues Cbfa1-deficient mice. Genes Dev 15:467–481
22. Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR et al (1997) Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 89:765–771
23. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K et al (1997) Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 89:755–764
24. Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR et al (2002) The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 108:17–29
25. Roodman GD (2006) Regulation of osteoclast differentiation. Ann N Y Acad Sci 1068:100–109
26. Tetreault N, De Guire V (2013) miRNAs: their discovery, biogenesis and mechanism of action. Clin Biochem 46:842–845
27. Harfe BD, McManus MT, Mansfield JH, Hornstein E, Tabin CJ (2005) The RNaseIII enzyme dicer is required for morphogenesis but not patterning of the vertebrate limb. Proc Natl Acad Sci USA 102:10898–10903
28. Kobayashi T, Lu J, Cobb BS, Rodda SJ, McMahon AP, Schipani E et al (2008) Dicer-dependent pathways regulate chondrocyte proliferation and differentiation. Proc Natl Acad Sci USA 105:1949–1954
29. Watanabe T, Sato T, Amano T, Kawamura Y, Kawamura N, Kawaguchi H et al (2008) Dnm3os, a non-coding RNA, is required for normal growth and skeletal development in mice. Dev Dyn 237:3738–3748
30. Suomi S, Taipaleenmaki H, Seppanen A, Ripatti T, Vaananen K, Hentunen T et al (2008) MicroRNAs regulate osteogenesis and chondrogenesis of mouse bone marrow stromal cells. Gene Regul Syst Bio 2:177–191
31. Laine SK, Alm JJ, Virtanen SP, Aro HT, Laitala-Leinonen TK (2012) MicroRNAs miR-96, miR-124, and miR-199a regulate gene expression in human bone marrow-derived mesenchymal stem cells. J Cell Biochem 113:2687–2695
32. Eberhart JK, He X, Swartz ME, Yan YL, Song H, Boling TC et al (2008) MicroRNA Mirn140 modulates Pdgf signaling during palatogenesis. Nat Genet 40:290–298
33. Miyaki S, Sato T, Inoue A, Otsuki S, Ito Y, Yokoyama S et al (2010) MicroRNA-140 plays dual roles in both cartilage development and homeostasis. Genes Dev 24:1173–1185
34. Nakamura Y, Inloes JB, Katagiri T, Kobayashi T (2011) Chondrocyte-specific microRNA-140 regulates endochondral bone development and targets Dnpep to modulate bone morphogenetic protein signaling. Mol Cell Biol 31:3019–3028
35. Nakamura Y, He X, Kato H, Wakitani S, Kobayashi T, Watanabe S et al (2012) Sox9 is upstream of microRNA-140 in cartilage. Appl Biochem Biotechnol 166:64–71
36. Tuddenham L, Wheeler G, Ntounia-Fousara S, Waters J, Hajihosseini MK, Clark I et al (2006) The cartilage specific microRNA-140 targets histone deacetylase 4 in mouse cells. FEBS Lett 580:4214–4217
37. Yang J, Qin S, Yi C, Ma G, Zhu H, Zhou W et al (2011) MiR-140 is co-expressed with Wwp2-C transcript and activated by Sox9 to target Sp1 in maintaining the chondrocyte proliferation. FEBS Lett 585:2992–2997
38. Pais H, Nicolas FE, Soond SM, Swingler TE, Clark IM, Chantry A et al (2010) Analyzing mRNA expression identifies Smad3 as a microRNA-140 target regulated only at protein level. RNA 16:489–494
39. Karlsen TA, Jakobsen RB, Mikkelsen TS, Brinchmann JE (2014) microRNA-140 targets RALA and regulates chondrogenic differentiation of human mesenchymal stem cells by translational enhancement of SOX9 and ACAN. Stem Cells Dev 23:290–304
40. Papaioannou G, Inloes JB, Nakamura Y, Paltrinieri E, Kobayashi T (2013) let-7 and miR-140 microRNAs coordinately regulate skeletal development. Proc Natl Acad Sci USA 110:E3291–E3300
41. Dudek KA, Lafont JE, Martinez-Sanchez A, Murphy CL (2010) Type II collagen expression is regulated by tissue-specific miR-675 in human articular chondrocytes. J Biol Chem 285:24381–24387
42. Martinez-Sanchez A, Murphy CL (2013) miR-1247 functions by targeting cartilage transcription factor SOX9. J Biol Chem 288:30802–30814
43. Yang B, Guo H, Zhang Y, Chen L, Ying D, Dong S (2011) MicroRNA-145 regulates chondrogenic differentiation of mesenchymal stem cells by targeting Sox9. PLoS ONE 6:e21679
44. Ohgawara T, Kubota S, Kawaki H, Kondo S, Eguchi T, Kurio N et al (2009) Regulation of chondrocytic phenotype by micro RNA 18a: involvement of Ccn2/Ctgf as a major target gene. FEBS Lett 583:1006–1010
45. Sumiyoshi K, Kubota S, Ohgawara T, Kawata K, Nishida T, Shimo T et al (2010) Identification of miR-1 as a micro RNA

that supports late-stage differentiation of growth cartilage cells. Biochem Biophys Res Commun 402:286–290
46. Zhong N, Sun J, Min Z, Zhao W, Zhang R, Wang W et al (2012) MicroRNA-337 is associated with chondrogenesis through regulating TGFBR2 expression. Osteoarthritis Cartilage 20:593–602
47. Sumiyoshi K, Kubota S, Ohgawara T, Kawata K, Abd El Kader T, Nishida T et al (2013) Novel role of miR-181a in cartilage metabolism. J Cell Biochem 114:2094–2100
48. Kim D, Song J, Jin EJ (2010) MicroRNA-221 regulates chondrogenic differentiation through promoting proteosomal degradation of slug by targeting Mdm2. J Biol Chem 285:26900–26907
49. Song J, Kim D, Chun CH, Jin EJ (2013) MicroRNA-375, a new regulator of cadherin-7, suppresses the migration of chondrogenic progenitors. Cell Signal 25:698–706
50. Guerit D, Philipot D, Chuchana P, Toupet K, Brondello JM, Mathieu M et al (2013) Sox9-regulated miRNA-574-3p inhibits chondrogenic differentiation of mesenchymal stem cells. PLoS ONE 8:e62582
51. Paik S, Jung HS, Lee S, Yoon DS, Park MS, Lee JW (2012) miR-449a regulates the chondrogenesis of human mesenchymal stem cells through direct targeting of lymphoid enhancer-binding factor-1. Stem Cells Dev 21:3298–3308
52. Lin X, Wu L, Zhang Z, Yang R, Guan Q, Hou X et al (2013) MiR-335-5p Promotes Chondrogenesis in Mouse Mesenchymal Stem Cells and is Regulated Through Two Positive Feedback Loops. J Bone Miner Res 29:15751585
53. Gaur T, Hussain S, Mudhasani R, Parulkar I, Colby JL, Frederick D et al (2010) Dicer inactivation in osteoprogenitor cells compromises fetal survival and bone formation, while excision in differentiated osteoblasts increases bone mass in the adult mouse. Dev Biol 340:10–21
54. Raaijmakers MH, Mukherjee S, Guo S, Zhang S, Kobayashi T, Schoonmaker JA et al (2010) Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. Nature 464:852–857
55. Li H, Xie H, Liu W, Hu R, Huang B, Tan YF et al (2009) A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. J Clin Invest 119:3666–3677
56. Jeon EJ, Lee KY, Choi NS, Lee MH, Kim HN, Jin YH et al (2006) Bone morphogenetic protein-2 stimulates Runx2 acetylation. J Biol Chem 281:16502–16511
57. Hu R, Liu W, Li H, Yang L, Chen C, Xia ZY et al (2011) A Runx2/miR-3960/miR-2861 regulatory feedback loop during mouse osteoblast differentiation. J Biol Chem 286:12328–12339
58. Wang X, Guo B, Li Q, Peng J, Yang Z, Wang A et al (2013) miR-214 targets ATF4 to inhibit bone formation. Nat Med 19:93–100
59. Yang X, Matsuda K, Bialek P, Jacquot S, Masuoka HC, Schinke T et al (2004) ATF4 is a substrate of RSK2 and an essential regulator of osteoblast biology; implication for Coffin-Lowry Syndrome. Cell 117:387–398
60. Shi K, Lu J, Zhao Y, Wang L, Li J, Qi B et al (2013) MicroRNA-214 suppresses osteogenic differentiation of C2C12 myoblast cells by targeting Osterix. Bone 55:487–494
61. Inose H, Ochi H, Kimura A, Fujita K, Xu R, Sato S et al (2009) A microRNA regulatory mechanism of osteoblast differentiation. Proc Natl Acad Sci USA 106:20794–20799
62. He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y et al (2007) A microRNA component of the p53 tumour suppressor network. Nature 447:1130–1134
63. Bae Y, Yang T, Zeng HC, Campeau PM, Chen Y, Bertin T et al (2012) miRNA-34c regulates Notch signaling during bone development. Hum Mol Genet 21:2991–3000
64. Kim KM, Park SJ, Jung SH, Kim EJ, Jogeswar G, Ajita J et al (2012) miR-182 is a negative regulator of osteoblast proliferation, differentiation, and skeletogenesis through targeting FoxO1. J Bone Miner Res 27:1669–1679
65. Rached MT, Kode A, Xu L, Yoshikawa Y, Paik JH, Depinho RA et al (2010) FoxO1 is a positive regulator of bone formation by favoring protein synthesis and resistance to oxidative stress in osteoblasts. Cell Metab 11:147–160
66. Ambrogini E, Almeida M, Martin-Millan M, Paik JH, Depinho RA, Han L et al (2010) FoxO-mediated defense against oxidative stress in osteoblasts is indispensable for skeletal homeostasis in mice. Cell Metab 11:136–146
67. Concepcion CP, Bonetti C, Ventura A (2012) The microRNA-17-92 family of microRNA clusters in development and disease. Cancer J 18:262–267
68. de Pontual L, Yao E, Callier P, Faivre L, Drouin V, Cariou S et al (2011) Germline deletion of the miR-17 approximately 92 cluster causes skeletal and growth defects in humans. Nat Genet 43:1026–1030
69. Tassano E, Di Rocco M, Signa S, Gimelli G (2013) De novo 13q31.1-q32.1 interstitial deletion encompassing the miR-17-92 cluster in a patient with Feingold syndrome-2. Am J Med Genet A 161A:894–896
70. Kannu P, Campos-Xavier AB, Hull D, Martinet D, Ballhausen D, Bonafe L (2013) Post-axial polydactyly type A2, overgrowth and autistic traits associated with a chromosome 13q31.3 microduplication encompassing miR-17-92 and GPC5. Eur J Med Genet 56:452–457
71. Hemmat M, Rumple MJ, Mahon LW, Strom CM, Anguiano A, Talai M et al (2014) Short stature, digit anomalies and dysmorphic facial features are associated with the duplication of miR-17 $\sim$ 92 cluster. Mol Cytogenet 7:27
72. Zhou M, Ma J, Chen S, Chen X, Yu X (2013) MicroRNA-17-92 cluster regulates osteoblast proliferation and differentiation. Endocrine 45:302–310
73. Liu Y, Liu W, Hu C, Xue Z, Wang G, Ding B et al (2011) MiR-17 modulates osteogenic differentiation through a coherent feedforward loop in mesenchymal stem cells isolated from periodontal ligaments of patients with periodontitis. Stem Cells 29:1804–1816
74. Li H, Li T, Wang S, Wei J, Fan J, Li J et al (2013) miR-17-5p and miR-106a are involved in the balance between osteogenic and adipogenic differentiation of adipose-derived mesenchymal stem cells. Stem Cell Res 10:313–324
75. Li L, Shi JY, Zhu GQ, Shi B (2012) MiR-17-92 cluster regulates cell proliferation and collagen synthesis by targeting TGFB pathway in mouse palatal mesenchymal cells. J Cell Biochem 113:1235–1244
76. Guo L, Xu J, Qi J, Zhang L, Wang J, Liang J et al (2013) MicroRNA-17-92a upregulation by estrogen leads to Bim targeting and inhibition of osteoblast apoptosis. J Cell Sci 126:978–988
77. Chen L, Holmstrom K, Qiu W, Ditzel N, Shi K, Hokland LB et al (2013) MicroRNA-34a Inhibits osteoblast differentiation and in vivo bone formation of human stromal stem cells. Stem Cells 32:902–912
78. Tamura M, Uyama M, Sugiyama Y, Sato M (2013) Canonical Wnt signaling activates miR-34 expression during osteoblastic differentiation. Mol Med Rep 8:1807–1811
79. Kapinas K, Kessler CB, Delany AM (2009) miR-29 suppression of osteonectin in osteoblasts: regulation during differentiation and by canonical Wnt signaling. J Cell Biochem 108:216–224
80. Kapinas K, Kessler C, Ricks T, Gronowicz G, Delany AM (2010) miR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop. J Biol Chem 285:25221–25231

81. Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM et al (2008) Widespread microRNA repression by Myc contributes to tumorigenesis. Nat Genet 40:43–50
82. Wang FS, Chuang PC, Lin CL, Chen MW, Ke HJ, Chang YH et al (2013) MicroRNA-29a protects against glucocorticoid-induced bone loss and fragility in rats by orchestrating bone acquisition and resorption. Arthritis Rheum 65:1530–1540
83. Li Z, Hassan MQ, Jafferji M, Aqeilan RI, Garzon R, Croce CM et al (2009) Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. J Biol Chem 284:15676–15684
84. Li E, Zhang J, Yuan T, Ma B (2014) miR-143 suppresses osteogenic differentiation by targeting Osterix. Mol Cell Biochem 390:69–74
85. Bhushan R, Grunhagen J, Becker J, Robinson PN, Ott CE, Knaus P (2013) miR-181a promotes osteoblastic differentiation through repression of TGF-beta signaling molecules. Int J Biochem Cell Biol 45:696–705
86. Gamez B, Rodriguez-Carballo E, Bartrons R, Rosa JL, Ventura F (2013) MicroRNA-322 (miR-322) and its target protein Tob2 modulate Osterix (Osx) mRNA stability. J Biol Chem 288:14264–14275
87. Zhang J, Tu Q, Bonewald LF, He X, Stein G, Lian J et al (2011) Effects of miR-335-5p in modulating osteogenic differentiation by specifically downregulating Wnt antagonist DKK1. J Bone Miner Res 26:1953–1963
88. Itoh T, Nozawa Y, Akao Y (2009) MicroRNA-141 and -200a are involved in bone morphogenetic protein-2-induced mouse pre-osteoblast differentiation by targeting distal-less homeobox 5. J Biol Chem 284:19272–19279
89. Hassan MQ, Gordon JA, Beloti MM, Croce CM, van Wijnen AJ, Stein JL et al (2010) A network connecting Runx2, SATB2, and the miR-23a $\sim$ 27a $\sim$ 24-2 cluster regulates the osteoblast differentiation program. Proc Natl Acad Sci USA 107:19879–19884
90. Dong J, Cui X, Jiang Z, Sun J (2013) MicroRNA-23a modulates tumor necrosis factor-alpha-induced osteoblasts apoptosis by directly targeting Fas. J Cell Biochem 114:2738–2745
91. Zhang Y, Xie RL, Croce CM, Stein JL, Lian JB, van Wijnen AJ et al (2011) A program of microRNAs controls osteogenic lineage progression by targeting transcription factor Runx2. Proc Natl Acad Sci USA 108:9863–9868
92. Lei SF, Papasian CJ, Deng HW (2011) Polymorphisms in predicted miRNA binding sites and osteoporosis. J Bone Miner Res 26:72–78
93. Lisse TS, Chun RF, Rieger S, Adams JS, Hewison M (2013) Vitamin D activation of functionally distinct regulatory miRNAs in primary human osteoblasts. J Bone Miner Res 28:1478–1488
94. Zhang JF, Fu WM, He ML, Wang H, Wang WM, Yu SC et al (2011) MiR-637 maintains the balance between adipocytes and osteoblasts by directly targeting Osterix. Mol Biol Cell 22:3955–3961
95. Chen Q, Liu W, Sinha KM, Yasuda H, de Crombrugghe B (2013) Identification and characterization of microRNAs controlled by the osteoblast-specific transcription factor Osterix. PLoS ONE 8:e58104
96. An JH, Ohn JH, Song JA, Yang JY, Park H, Choi HJ et al (2013) Changes of microRNA profile and microRNA-mRNA regulatory network in bones of ovariectomized mice. J Bone Miner Res 29:644–656
97. Seeliger C, Karpinski K, Haug A, Vester H, Schmitt A, Bauer J et al (2014) Five freely circulating miRNAs and bone tissue miRNAs are associated with osteoporotic fractures. J Bone Miner Res 29(1718):1728
98. Wang Q, Cai J, Cai XH, Chen L (2013) miR-346 regulates osteogenic differentiation of human bone marrow-derived mesenchymal stem cells by targeting the Wnt/beta-catenin pathway. PLoS ONE 8:e72266
99. Baglio SR, Devescovi V, Granchi D, Baldini N (2013) MicroRNA expression profiling of human bone marrow mesenchymal stem cells during osteogenic differentiation reveals Osterix regulation by miR-31. Gene 527:321–331
100. Gao J, Yang T, Han J, Yan K, Qiu X, Zhou Y et al (2011) MicroRNA expression during osteogenic differentiation of human multipotent mesenchymal stromal cells from bone marrow. J Cell Biochem 112:1844–1856
101. Deng Y, Wu S, Zhou H, Bi X, Wang Y, Hu Y et al (2013) Effects of a miR-31, Runx2, and Satb2 regulatory loop on the osteogenic differentiation of bone mesenchymal stem cells. Stem Cells Dev 22:2278–2286
102. Eskildsen T, Taipaleenmaki H, Stenvang J, Abdallah BM, Ditzel N, Nossent AY et al (2011) MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells in vivo. Proc Natl Acad Sci USA 108:6139–6144
103. Huang J, Zhao L, Xing L, Chen D (2010) MicroRNA-204 regulates Runx2 protein expression and mesenchymal progenitor cell differentiation. Stem Cells 28:357–364
104. Itoh T, Ando M, Tsukamasa Y, Akao Y (2012) Expression of BMP-2 and Ets1 in BMP-2-stimulated mouse pre-osteoblast differentiation is regulated by microRNA-370. FEBS Lett 586:1693–1701
105. Liao L, Yang X, Su X, Hu C, Zhu X, Yang N et al (2013) Redundant miR-3077-5p and miR-705 mediate the shift of mesenchymal stem cell lineage commitment to adipocyte in osteoporosis bone marrow. Cell Death Dis 4:e600
106. Gong Y, Xu F, Zhang L, Qian Y, Chen J, Huang H et al (2014) MicroRNA expression signature for Satb2-induced osteogenic differentiation in bone marrow stromal cells. Mol Cell Biochem 387:227–239
107. Mizuno Y, Tokuzawa Y, Ninomiya Y, Yagi K, Yatsuka-Kanesaki Y, Suda T et al (2009) miR-210 promotes osteoblastic differentiation through inhibition of AcvR1b. FEBS Lett 583:2263–2268
108. Mizuno Y, Yagi K, Tokuzawa Y, Kanesaki-Yatsuka Y, Suda T, Katagiri T et al (2008) miR-125b inhibits osteoblastic differentiation by down-regulation of cell proliferation. Biochem Biophys Res Commun 368:267–272
109. Fisher MC, Clinton GM, Maihle NJ, Dealy CN (2007) Requirement for ErbB2/ErbB signaling in developing cartilage and bone. Dev Growth Differ 49:503–513
110. Okamoto H, Matsumi Y, Hoshikawa Y, Takubo K, Ryoke K, Shiota G (2012) Involvement of microRNAs in regulation of osteoblastic differentiation in mouse induced pluripotent stem cells. PLoS ONE 7:e43800
111. Schaap-Oziemlak AM, Raymakers RA, Bergevoet SM, Gilissen C, Jansen BJ, Adema GJ et al (2010) MicroRNA hsa-miR-135b regulates mineralization in osteogenic differentiation of human unrestricted somatic stem cells. Stem Cells Dev 19:877–885
112. Bakhshandeh B, Soleimani M, Hafizi M, Paylakhi SH, Ghaemi N (2012) MicroRNA signature associated with osteogenic lineage commitment. Mol Biol Rep 39:7569–7581
113. Luzi E, Marini F, Sala SC, Tognarini I, Galli G, Brandi ML (2008) Osteogenic differentiation of human adipose tissue-derived stem cells is modulated by the miR-26a targeting of the SMAD1 transcription factor. J Bone Miner Res 23:287–295
114. Kim YJ, Bae SW, Yu SS, Bae YC, Jung JS (2009) miR-196a regulates proliferation and osteogenic differentiation in mesenchymal stem cells derived from human adipose tissue. J Bone Miner Res 24:816–825
115. Zhang WB, Zhong WJ, Wang L (2014) A signal-amplification circuit between miR-218 and Wnt/beta-catenin signal promotes

human adipose tissue-derived stem cells osteogenic differentiation. Bone 58:59–66
116. Deng Y, Zhou H, Zou D, Xie Q, Bi X, Gu P et al (2013) The role of miR-31-modified adipose tissue-derived stem cells in repairing rat critical-sized calvarial defects. Biomaterials 34:6717–6728
117. Dalle Carbonare L, Valenti MT, Zanatta M, Donatelli L, Lo Cascio V (2009) Circulating mesenchymal stem cells with abnormal osteogenic differentiation in patients with osteoporosis. Arthritis Rheum 60:3356–3365
118. Yang N, Wang G, Hu C, Shi Y, Liao L, Shi S et al (2013) Tumor necrosis factor alpha suppresses the mesenchymal stem cell osteogenesis promoter miR-21 in estrogen deficiency-induced osteoporosis. J Bone Miner Res 28:559–573
119. Sugatani T, Hruska KA (2009) Impaired micro-RNA pathways diminish osteoclast differentiation and function. J Biol Chem 284:4667–4678
120. Mizoguchi F, Izu Y, Hayata T, Hemmi H, Nakashima K, Nakamura T et al (2010) Osteoclast-specific dicer gene deficiency suppresses osteoclastic bone resorption. J Cell Biochem 109:866–875
121. Sugatani T, Hildreth BE 3rd, Toribio RE, Malluche HH, Hruska KA (2014) Expression of DGCR8-Dependent MicroRNAs Is Indispensable for Osteoclastic Development and Bone-Resorbing Activity. J Cell Biochem 115:1043–1047
122. Chen C, Cheng P, Xie H, Zhou HD, Wu XP, Liao EY et al (2014) MiR-503 regulates osteoclastogenesis via targeting RANK. J Bone Miner Res 29:338–347
123. Cheng P, Chen C, He HB, Hu R, Zhou HD, Xie H et al (2013) miR-148a regulates osteoclastogenesis by targeting V-maf musculoaponeurotic fibrosarcoma oncogene homolog B. J Bone Miner Res 28:1180–1190
124. Guo LJ, Liao L, Yang L, Li Y, Jiang TJ (2014) MiR-125a TNF receptor-associated factor 6 to inhibit osteoclastogenesis. Exp Cell Res 321:142–152
125. Sugatani T, Vacher J, Hruska KA (2011) A microRNA expression signature of osteoclastogenesis. Blood 117:3648–3657
126. Mizoguchi F, Murakami Y, Saito T, Miyasaka N, Kohsaka H (2013) miR-31 controls osteoclast formation and bone resorption by targeting RhoA. Arthritis Res Ther 15:R102
127. Zhang J, Zhao H, Chen J, Xia B, Jin Y, Wei W et al (2012) Interferon-beta-induced miR-155 inhibits osteoclast differentiation by targeting SOCS1 and MITF. FEBS Lett 586:3255–3262
128. Rossi M, Pitari MR, Amodio N, Di Martino MT, Conforti F, Leone E et al (2013) miR-29b negatively regulates human osteoclastic cell differentiation and function: implications for the treatment of multiple myeloma-related bone disease. J Cell Physiol 228:1506–1515
129. Franceschetti T, Kessler CB, Lee SK, Delany AM (2013) miR-29 promotes murine osteoclastogenesis by regulating osteoclast commitment and migration. J Biol Chem 288:33347–33360