microRNA-Mediated Regulation of Bone Remodeling: A Brief Review

Jin Liu,1* Lei Dang,1* Xiaohao Wu,1 Dijie Li,1,2 Qing Ren,1 Aiping Lu,1 and Ge Zhang1 1

Introduction

Bone is a dynamic organ that grows and adapts its shape and structure by modeling in childhood and undergoing constant remodeling in the whole life. Osteoblasts are bone-forming cells that govern new bone formation, whereas osteoclasts are bone resorbing cells capable of removing old bone matrix. The functions of these two types of cells are not only precisely controlled by their distinct intracellular molecular events, but also regulated by the coupling factors during their interaction with each other. The dysregulation of any intracellular event of each cell type or the impairment in their coupling factors will affect bone development and remodeling.

microRNAs (miRNAs) are a class of endogenous, evolutionarily conserved, small non-coding RNAs (generally 20 to 24 nucleotides long) that regulate gene expression at the posttranscriptional level to coordinate a broad spectrum of biological processes. Mechanistically, miRNAs directly bind to the three prime untranslated region (3′UTR) of messenger RNAs (mRNAs) to block their translation or induce mRNA degradation. The genes encoding the miRNAs are initially transcribed as primary miRNAs (pri-miRNAs, ~80 nucleotides long) in the nucleus by RNA polymerase II (Pol II), and further cleaved by the ribonuclease III called Drosha or double-stranded DNA-binding protein Dgcr8 (Di George syndrome critical gene 8), giving rise to precursor miRNAs (pre-miRNAs, ~70 nucleotides long) with hairpin structures. The pre-miRNAs are subsequently exported to the cytoplasm by the nucleocytoplasmic shuttler Exportin-5 in complex with Ran-GTP, and processed by the endoribonuclease Dicer and the co-regulator Ago2 to form small double-stranded miRNAs. Thereafter, the duplex miRNAs are converted into mature single-stranded miRNAs (~22 nucleotides long), and incorporated into the RNA-induced silencing complex (RISC) to target the 3′UTR of mRNAs and mediate gene silencing. The biogenesis of miRNAs is vital for life, because global deletion of either Drosha or Dicer results in early embryonic lethality. Consistently, the conditional knockout of these key miRNA processing factors in skeletal cells, eg, chondrocytes, osteoblasts, and osteoclasts, respectively, leads to skeletal defects highlighting the crucial role of miRNAs in skeleton development and bone remodeling. Therefore, this review summarizes studies in the past decade focusing on miRNA-regulatory mechanisms of osteoblast, osteoclast, and bone remodeling.

miRNA, Osteogenesis, and Bone Formation

Osteoblasts arising from mesenchymal stem cells (MSCs) are responsible for bone matrix synthesis and mineralization during skeletal development and lifelong bone remodeling. The osteogenic differentiation of MSCs and osteoblast-mediated bone formation are not only governed via the master transcription factors, eg, Runx-related transcription factor 2 (Runx2) and Osterix, and their downstream signaling cascades, eg, TGF-β/BMP and Wnt/β-catenin signaling pathways, but also...
posttranscriptionally modulated by various miRNAs. The miRNA-mediated regulatory mechanisms of osteoblast differentiation/functions are summarized in Table 1.

miRNA biogenesis and osteoblast differentiation

Mice with Dicer deletion in Prxl+ mesenchymal osteochondrogenitor cells (Prxl-Cre;Dicer\textsuperscript{lox/lox}) were viable, but exhibited significant skeletal defects including reduced hindlimb size and twisted bone.(15) Interestingly, in later research, Gaur and colleagues(11) reported that conditional excision of the Dicer enzyme in Col1a1+ osteoblast lineage cells (Col1a1-Cre;Dicer\textsuperscript{lox/lox}) is deleterious to fetal survival. Impressively, the embryonic day 14.5 (E14.5) Dicer-mutant fetal pups showed a deformed cartilaginous skeleton with impaired bone formation. Both studies suggest that the Dicer-mediated miRNA processing mechanism is required for the proper hindlimb morphogenesis and skeletal development, whereas the differences in fetal survival between the Prxl-Cre;Dicer\textsuperscript{lox/lox} and Col1a1-Cre;Dicer\textsuperscript{lox/lox} mice could be due to the different Dicer\textsuperscript{lox/lox} mouse strain used. Conversely, a recent study showed that mice with DGC8R conditional deletion in Col1a1+ osteoblast lineage cells (Col1a1-Cre;DGC8R\textsuperscript{lox/lox}) exhibited increased osteoblastic bone formation,(10) suggesting that the DROSHA/DGC8R-mediated miRNA processing pathway could negatively regulate osteoblast activity and bone formation in a Dicer-independent manner.

On the other hand, to overcome the detrimental effect of Dicer inactivation on fetal survival, Bendre and colleagues(9) generated an inducible pre-osteoblast specific Dicer1 knockout model by employing tamoxifen-controllable Cre allele (Sp\textsuperscript{2}P7-Cre;/ErT\textsubscript{2};Dicer\textsuperscript{lox/lox}). They found that tamoxifen-dependent inactivation of Dicer1 in osterix+ preosteoblasts dramatically impaired the bone formation of cortical bone but not trabecular bone in both prepubertal and adult mice, suggesting an important role of Dicer-processed miRNAs in the postnatal regulation of cortical bone homeostasis. Consistently, Liu and colleagues(16) showed that ablation of Dicer in Runx2+ osteoblast lineage cells (Runx2-Cre;Dicer\textsuperscript{lox/lox}) did not induce embryonic lethality, although it could cause remarkable growth retardation, low bone density, and impaired bone formation during postnatal development. Interestingly, they did not find significant difference in the glucocorticoid-induced bone formation reduction between the Runx2-Cre;Dicer\textsuperscript{lox/lox} mice and littermate control mice upon glucocorticoid (GC) treatment. In addition, Gaur and colleagues(11) found that the mice with Dicer deletion in osteocalcin-expressing mature osteoblasts (Ocn-Cre; Dicer\textsuperscript{lox/lox}) were also viable with a perinatal phenotype of delayed bone mineralization, which returned to normal at 1 month of age. Surprisingly, they further observed a second phenotype of significantly increased bone mass developed by 2 months, which continued up to 8 months in long bones and vertebrae.(11) Collectively, these findings indicate that the Dicer-processed miRNAs in early osteoprogenitors are essential for osteogenesis and bone formation, whereas loss of the Dicer-processed miRNAs in mature osteoblasts seem to have anabolic effect on the adult skeleton.

In turn, the miRNA expression and Dicer-mediated miRNA processing mechanism were under control by the osteogenic transcription factor during osteoblast lineage commitment. Zhou and colleagues(12) observed the coincident expression of Dicer and Runx2 during osteogenesis differentiation of mouse MC3T3-E1 preosteoblasts. They further witnessed that Runx2 could directly bind to the Dicer promoter region to enhance Dicer expression.(12) In addition, by comparing the miRNA expression in calvaria of the E18.5 Osx gene knockout embryos with wild-type embryos and verifying in osteoblasts overexpressing Osx, Chen and colleagues(17) identified a group of miRNAs that was downregulated by Osx expression, including miR-133a, miR-204, miR-211, miR-302a, miR-433, miR-501, and miR-544. They also found another group of miRNAs that was upregulated by Osx expression, including miR-141, miR-200a, miR-192, and miR-1194.(17)

Osteoblastic miRNA and osteogenic transcription factor

Runx2 is the master transcription factor for osteoblast differentiation. In a study by Zhang and colleagues,(18) they found that a panel of 11 Runx2-targeting miRNAs (miR-23a, miR-30c, miR-34c, miR-133a, miR-133a, miR-137, miR-204, miR-205, miR-217, miR-218, and miR-338) were inversely expressed relative to Runx2 during osteogenic differentiation of mouse MC3T3 E1 osteoblastic cells and chondrogenic differentiation of mouse ATDC5 prechondrocytes. Specifically, the expression of these miRNAs was remarkably upregulated at a late stage of osteoblast maturation when Runx2 protein expression was decreased and downregulated at late stage of hypertrophic chondrocyte differentiation. They further demonstrated that all these miRNAs could directly target and downregulate the Runx2 protein expression. These results corroborate the previous study mentioned above showing that excision of the miRNA processing enzyme Dicer in mature osteoblasts causes a dramatic high bone mass phenotype,(19) indicating that the Runx2-targeting miRNAs are generally required for attenuating osteoblast maturation. In addition, several independent studies have reported that Runx2 could be directly regulated by other miRNAs, such as miR-30d,(19) miR-467g,(20) and miR-628-3p.(21)

The homeodomain protein Distal-less Homeobox 5 (Dlx5) is an essential activator of Runx2 and Osterix (Osx).(22) A study by Laxman and colleagues(23) reported that miR-203 and miR-320b could negatively regulate BMP-2-stimulated human osteoblast differentiation by inhibiting Dlx5, which in turn suppresses the downstream osteogenic master transcription factor Runx2 and Osx to hamper osteoblast differentiation. The activating transcription factor 4 (ATF4) is another bone-related transcription factor critical for the proliferation, differentiation, and survival of osteoblasts.(24–26) Our laboratory has shown that miR-214 could directly target ATF4 to inhibit osteoblast activity and bone formation.(27) We identified that miR-214-3p, among the most highly expressed miRNAs within bone tissues from aged osteoporotic fracture patients, could downregulate the amount of ATF4 proteins in osteoblasts to contribute to both age-related and hindlimb unloading–induced bone formation reduction. In addition, miR-214 was also reported to posttranscriptionally regulate the expression of Osx, another master transcription factor for osteoblast differentiation expression.(28) Shi and colleagues(29) found that miR-214 could directly target two binding site of Osx 3’UTR to inhibit the Osx protein expression for suppressing the osteogenic differentiation of C2C12 cells.

Osteoblastic miRNA and osteogenic signal

The two crucial osteogenic signals, ie, the Wnt/β-catenin and BMP signaling pathway, are regulated by miRNAs. A previous study found that the negative regulators of Wnt signaling,
### Table 1. Selected miRNAs With Their Targets and Functions in Bone Remodeling

| miRNA(s)                  | Target gene(s)                                      | Models/site of action                                                                 | Function                                                      | Reference                      |
|---------------------------|-----------------------------------------------------|---------------------------------------------------------------------------------------|----------------------------------------------------------------|--------------------------------|
| miR-203                   | Dlx5, BMP-2–stimulated human osteoblasts            | Differentiation ↓                                                                      | Osteoblast differentiation ↓                                  | Laxman and colleagues(23)     |
| miR-320b                  | ATF4, MC3T3-E1 cells; bone tissues from aged osteoporotic fracture patients; OVX and hindlimb‐unloaded mice C2C12 cells | Differentiation ↓                                                                      | Osteoblast activity and bone formation ↓                      | Wang and colleagues(27)       |
| miR-214                   | Osx, Human osteoblast precursor cell line hFOB1.19; primary cultures of human osteoblasts DKK1, Kremen2, sFRP2 | Differentiation ↑                                                                      | Osteoblastic activity and bone formation ↑                     | Shi and colleagues(29)        |
| miR-29a                   | DKK1, Kremen2, sFRP2                                 | Differentiation ↑                                                                      | Osteoblastic activity and bone formation ↑                     | Kapinas and colleagues(30)    |
| miR-355-5p                | DKK1                                               | Differentiation ↑                                                                      | Osteoblastic activity and bone formation ↑                     | Li and colleagues(32)         |
| miR-433-3p                | DKK1                                               | Differentiation ↑                                                                      | Osteoblastic activity and bone formation ↑                     | Tang and colleagues(33)       |
| miR-375-3p                | LRP5, β‐catenin                                     | Differentiation ↑                                                                      | Osteoblastic activity and bone formation ↑                     | Sun and colleagues(34)        |
| miR-135                   | Smad5; BMP-2–induced C2C12 cells                    | Differentiation ↓                                                                      | Osteoblastic activity and bone formation ↑                     | Li and colleagues(35)         |
| miR-106b-5p               | Smad5; BMP-2–induced C2C12 cells                    | Differentiation ↓                                                                      | Osteoblastic activity and bone formation ↑                     | Fang and colleagues(36)       |
| miR-17-5p                 | Smad5                                               | Differentiation ↓                                                                      | Osteoblastic activity and bone formation ↑                     |                                 |
| miRNA (s) and osteoclasts | PDGFR and Dicer knockout BMMs                       | Differentiation ↑                                                                      | RANKL‐induced osteoclastogenesis ↑                             | Sugatani and colleagues(14)   |
| miR-21                    | PDCD4                                               | Differentiation ↑                                                                      | RANKL‐induced osteoclastogenesis ↑                             | Sugatani and Hruska(42)       |
| miR-503                   | FasL                                               | Differentiation ↑                                                                      | Osteoclastic apoptosis ↓                                      | Chen and colleagues(49)       |
| miR-214-3p                | TRAF3; RAW 264.7 cells; primary mouse BMMs; osteoclast‐specific miR‐214‐3p knock out nude mice; osteoclast‐specific miR‐214‐3p knock out mice | Differentiation ↓                                                                      | RANKL‐induced osteoclastogenesis ↓                             | Liu and colleagues(51)        |
| miR-34a                   | PTEN                                               | Differentiation ↑                                                                      | RANKL‐induced osteoclastogenesis ↓                             |                                 |
| miR-182                   | Tgf2                                               | Differentiation ↓                                                                      | RANKL‐induced osteoclastogenesis ↓                             |                                 |
| miR-182                   | Foxo3 and Maml1                                     | Differentiation ↓                                                                      | RANKL‐induced osteoclastogenesis ↓                             |                                 |

(Continues)


| miRNA(s) and osteocytes | Target gene(s) | Models/site of action | Function | Reference |
|------------------------|----------------|-----------------------|----------|-----------|
| miR-27a                | Prdm16         | MC3T3-E1 cells; Col1a1-miR-27a decoy transgenic mice | Osteocyte differentiation ↑ | Zeng and colleagues \(^{(58)}\) |
|                        |                |                       | Enhance TGF-β signaling to accelerate SOST expression |          |
| miR-21                 | PTEN           | Cx43-silenced MLO-Y4 osteocytic cells, miR21\(^{fl/fl}\) mice treated with adenovirus-Cre | Cx43 maintains osteocyte viability by downstream regulation of miR21 to reduce osteocyte apoptosis | Davis and colleagues \(^{(59)}\) |
| miR-199a-3p            | IGF-1 and mTOR | MLO-Y4 osteocytic cells, OVX mice | Osteocytic areas of OVX mice ↑ | Fu and colleagues \(^{(60)}\) |
|                        |                |                       | Estrogen deficiency increases the expression of miR-199a-3p to induce autophagy in osteocytes |          |

**miRNA and osteoblast-osteoclast crosstalk**

- **miR-433-3p (from osteoblasts)**
  - Target gene(s): DKK1 (in osteoclasts)
  - Models/site of action: Human osteoblast precursor cell line hFOB1.19; rat ROS17/2.8 cell line; primary rat MSCs; OVX rat model
  - Function: Relieve the inhibitory effect of DKK1 on osteoblast function
  - Reference: Tang and colleagues \(^{(33)}\)

- **miR-214-3p (from osteoblasts)**
  - Target gene(s): ATF4 (in osteoblasts)
  - Models/site of action: RAW 264.7 cells; OVX mouse; osteoclast-specific miR-214-3p knockout mice; osteoclast-specific miR-214-3p overexpression mice
  - Function: Osteoblast activity and bone formation ↓
  - Reference: Li and colleagues \(^{(65)}\)

- **miR-218 (from osteocytes)**
  - Target gene(s): DKK2 and sFRP2 (in osteoblasts)
  - Models/site of action: Ocy454 osteocytic cells; IDG-SW3 cells; MC3T3-E1 cells
  - Function: Myostatin suppresses osteocyte-derived exosomal miR-218 to inhibit osteoblastic differentiation
  - Reference: Qin and colleagues \(^{(69)}\)

---

OVX = ovariectomized; HG = high glucose.
including Dikkopf-1 (DKK1), Kremen2, and secreted frizzled related protein 2 (sFRP2), were the direct targets of miR-29a. The expression of miR-29a was increased during osteogenic differentiation in the human osteoblast precursor cell line hFOB1.19 as well as in primary cultures of human osteoblasts. Transfection with miR-29a inhibitor increased the endogenous protein levels of the aforementioned Wnt antagonists, whereas transfection with miR-29a mimics mimicked the decreased endogenous protein levels of the aforementioned Wnt antagonists, and therefore, suppressed and potentiated the Wnt signaling. In another study, Zhang and colleagues showed that miR-29a could activate Wnt signaling and promote osteogenic differentiation via directly targeting and downregulating DKK1. Consistently, Li and colleagues observed that overexpression of miR-335-5p could decrease the protein expression levels of DKK1 to inhibit the high-glucose (HG)-induced apoptosis of MC3T3-E1 osteoblasts. In addition, Tang and colleagues observed a positive correlation between the serum DKK1 levels and circulating miR-433-3p levels in ovariectomized (OVX) rats, and further showed that miR-433-3p could target DKK1 to promote osteoblast differentiation in vitro. On the other hand, Sun and colleagues showed that the LRPs, a co-receptor of the Wnt signaling and β-catenin, the downstream signal transducer of the Wnt signaling, were both the targets of miR-375-3p. They found that transfection of miR-375-3p in MC3T3-E1 osteoblasts not only arrested the protein expression of LRPs and β-catenin, but also impaired osteogenesis and induced cell apoptosis.

Li and colleagues found that the expression of miR-135 was decreased during BMP-2–induced osteogenesis of C2C12 cells. They further showed that miR-135 could directly target Smad5, a key transducer of the osteogenic BMP signal, to inhibit the BMP-2–induced osteogenic differentiation. Consistently, Fang and colleagues identified that miR-106b-5p and miR-17-5p could both suppress the osteogenic differentiation of C2C12 and MC3T3-E1 cells by targeting Smad5. Inhibition of miR-106b-5p and miR-17-5p in OVX mice could result in increased bone formation as well as improvement of trabecular microarchitecture.

### miRNA, Osteoclastogenesis, and Bone Resorption

Osteoclasts derived from bone marrow monocyte-macrophage (BMM) precursors are the primary bone-resorbing cells. Osteoclastogenesis involving the fusion of precursors to form multinucleated osteoclasts is regulated by two essential cytokines; i.e., macrophage colony-stimulating factor-1 (M-CSF) and receptor activator of NFκB ligand (RANKL). An increasing line of evidence suggests that miRNAs also play critical roles in regulating osteoclastogenesis and bone resorption. The miRNA-mediated regulatory mechanisms of osteoclast differentiation/functions are summarized in Table 1.

#### miRNA biogenesis and osteoclast differentiation

Sugatani and Hruska found that the RANKL-induced expression of osteoclast transcription factors and their function in osteoclast precursors were inhibited, together with the osteoclastogenesis and bone resorption by small interfering RNA-mediated silencing of either Dgcr8, Dicer, or Ago2. By genetic approach, their CD11b-Cre/Dicer mice lacking Dicer in CD11b+ osteoclast precursors exhibited a mild osteopetrosis phenotype caused by decreased osteoclast formation and impaired bone resorption. Consistently, another study by Mizoguchi and colleagues showed that deletion of Dicer gene in Cathepsin (Ctsk)-expressing osteoclasts at a more mature stage also caused decreased osteoclast formation and bone resorption in vivo, as well as impaired osteoclastic activity in vitro. In line with the bone phenotype in the aforementioned Dicer mutant mice, Sugatani and colleagues found that osteoclast-specific deletion of Dgcr8 (Ctsk-Cre/Dgcr8) resulted in impaired osteoclastic development and bone resorption. Taken together, both the Dgcr8-dependent miRNA biogenesis and Dicer-dependent miRNA processing are indispensable for osteoclastogenesis and osteoclastic bone resorption.

#### Osteoclastic miRNA and osteoclastogenesis

Apart from its critical role in tumor growth and invasion, miR-21 is one of the most commonly studied pro-osteoclastogenic miRNAs identified so far. Sugatani and colleagues had profiled the miRNA expression in RANKL-induced BMM osteoclastogenesis and identified that miR-21, among the 38 upregulated miRNAs, was robustly stimulated by RANKL. They documented that RANKL induced the expression of c-Fos that stimulates miR-21 expression, whereas miR-21 could directly target and downregulate the programmed cell death 4 (PDCD4) to remove the repression from c-Fos. Consistently, they found that BMMs deficient in either the Dgcr8 or Dicer gene possessed significantly decreased miR-21 levels and increased PDCD4 protein levels, but impaired capacity for RANKL-induced osteoclastogenesis. Interestingly, they showed that forced expression of miR-21 could downregulate the PDCD4 protein expression to rescue the osteoclast development in both Dgcr8 and Dicer knockout BMMs. However, it remains elusive whether such a rescue effect of miR-21 overexpression was Dicer-independent in Dicer-deficient BMMs. The same research team reported in a later study that estrogen could enhance the protein expression of FasL, the pro-apoptotic factor in osteoclasts, in concert with mRNAs for the osteoclast differentiation and cell survival. However, in another study by Wang and colleagues, they found that gain of miR-29a function in mice by administering lentivirus-mediated miR-29a precursor not only alleviated the detrimental effects of

---

[mirna-mediated-regulation-of-bone-remodeling]
glucocorticoid treatment on mineral acquisition and ex vivo osteoblast differentiation, but also reduced osteoclast surface, ex vivo osteoclast differentiation, and RANKL expression in bone microenvironments. In turn, miR-29 knockdown in rats by administering lentivirus mediated miR-29a inhibitor accelerated osteoclast resorption, cortical bone porosity and fragility, as well as the loss of ex vivo osteogenic differentiation capacity. Because the mature miR-29a/b/c are highly conserved in human, mouse, and rat, these controversial outcomes between manipulating miR-29a and miR-29 family on osteoclast activity may indicate the independent role of each miR-29 family members in regulating osteoclast.

**Osteoclastic miRNA and osteoporosis**

Li and colleagues detected the upregulated miR-133a in serum isolated from postmenopausal osteoporosis patients, which was negatively correlated with the patients’ lumbar bone mineral density (BMD). They demonstrated that miR-133a knockdown could inhibit the RANKL-induced osteoclastogenesis in vitro and alleviated the bone loss in ovariectomized rats in vivo. Cheng and colleagues identified miR-148a, among the most upregulated miRNAs during the M-CSF and RANKL-stimulated osteoclast differentiation of human circulating CD14+ peripheral blood mononuclear cells (PBMCs), could directly target and downregulate the V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB) to promote osteoclastogenesis. They further showed that CD14+ PBMCs from lupus patients possessed elevated miR-148a levels and enhanced osteoclastogenesis capacity, which may contribute to the lower BMD in lupus patients compared with normal controls. In another study by the same research team, Chen and colleagues showed that miR-503 was markedly downregulated in circulating CD14+ PBMC from postmenopausal osteoporosis patients compared with those from postmenopausal healthy women. Mechanistically, they verified that miR-503 could directly target RANK to dampen the RANKL-induced osteoclastogenesis. These findings from patients with osteoporosis would provide new miRNA-based disease biomarkers and therapeutic targets for developing novel anti-resorption treatment.

**Osteoclastic miRNA and osteolytic bone metastasis**

Ell and colleagues profiled the miRNA expression in osteoclast differentiation induced by conditioned media from highly metastatic breast cancer cells. They identified a series of tumour-suppressed miRNAs, including miR-33a, miR-133a, miR-141, miR-190, and miR-219, that exert inhibitory effect on tumor-induced osteoclastogenesis, and two tumor-induced miRNAs, i.e., miR-378 and miR-16, that are elevated during tumor-induced osteoclastogenesis and correlate with bone metastasis burden. The study has provided experimental and clinical evidence to delineate the role of miRNAs in regulating osteolytic bone metastasis. It is interesting to note that miR-133a was found to inhibit osteoclast differentiation and resorption activity in vitro, in contrast to the aforementioned positive regulatory role of miR-133a in osteoclasts, which may attribute to the different disease mechanism between osteoporosis and cancer bone metastasis. In addition, our laboratory has identified that miR-214-3p was significantly upregulated in bone specimens from breast cancer patients with osteolytic bone metastasis. We showed that miR-214-3p could directly regulate the protein expression TRAF3 rather than phosphatase and tensin homolog (PTEN) the previously verified miR-214-3p target in osteoclasts, to promote osteoclast function in the development of breast cancer osteolytic metastasis. Moreover, the study by Krezsinski and colleagues reported an inhibitory role of miR-34a on osteoclast differentiation and cancer bone metastasis. They identified transforming growth factor-beta-induced factor 2 (Tgfβ2) as a direct target of miR-34a. They proved that ovariectomy-induced osteoporosis, as well as bone metastasis of breast and skin cancers, is almost prevented in osteoclastic miR-34a transgenic mice and can be effectively attenuated by miR-34a nanoparticle treatment.

**Osteoclastic miRNA and inflammatory response**

Tumor necrosis factor alpha (TNF-α), a proinflammatory cytokine involved in the pathogenesis of chronic inflammatory diseases, could stimulate osteoclast differentiation in a Rank-l–dependent mechanism. In a previous study with microarray screening, it was found that miR-378, miR-21, miR-29b, miR-146a, miR-155, and miR-210 were highly expressed, while miR-223 was downregulated during TNF-α–induced osteoclast differentiation of murine BMMs. The expression profile of osteoclast miRNAs with TNF-α stimulation was partly matched with the previous profile outcomes of pro-osteoclastogenic miRNA without TNF-α stimulation. The transcription factor RBP-J is a newly identified osteoclastogenic repressor playing a critical role in inhibiting the TNF-α–induced osteoclast differentiation and bone resorption. Miller and colleagues recently found that miR-182, as the most abundant miRNA in TNF-α–induced osteoclastogenesis, was repressed by RBP-J during osteoclast differentiation. miR-182 could promote the TNF-α–induced osteoclastogenesis via inhibition of Foxo3 and Maml1. Therefore, it proposes an important mechanism by which suppression of miR-182 by RBP-J may restrain TNF-α–induced osteoclastogenesis.

**miRNA and Osteocytes**

Osteocytes are the terminally differentiated cell type of the osteoblastic lineage, accounting for ~98% of the cells comprising the skeleton. They are mechanosensitive cells embedded in the bone matrix that have crucial functions in regulating skeletal homeostasis. However, unlike osteoblasts and osteoclasts, the potential role of miRNA-mediated regulation in osteocytes is just starting to be uncovered. The miRNA-mediated regulatory mechanisms of osteocyte differentiation/functions are summarized in Table 1.

Eguchi and colleagues performed RT-qPCR microarray analysis to examine the miRNA expression profiling in osteocyte formation of murine bone-marrow-derived mesenchymal stem cell line KUSA-A1, by which they identified the upregulated miRNAs, including miR-30d, miR-155, miR-21, miR-16, miR-34c, miR-18ab, miR-19, miR-541, and miR-23a, and the downregulated miRNAs including let-7/miR98, during osteocytic differentiation. Interestingly, miR-30d, miR-155, miR-21, miR-34c, and miR-16, among the upregulated miRNAs were all predicted to repress miRNAs of osteblast stemness-related genes or key osteoblastic factors including several key osteoblastic factors RUNX2, NOTCH1, SMAD1/2/4/7, SOX2/9, TGFB2, BMPR1A, and LRP6 and CCN3. In addition, miR-18ab and miR-19 were predicted to target the osteochondrogenesis
factors CTGF/CCN2. On the other hand, the downregulated miRNAs, eg, let-7/miR-98, were predicted to target and repress mRNA expression of osteocyte-specific dentin matrix protein 1 (DMP1). Consistently, another study conducted by Zeng and colleagues showed that the miR-23a cluster, containing miR23a, miR27a and miR24-2, could promote osteocyte differentiation. By genetic approach, they found that the osteoblast-specific miR-23a cluster gain-of-function mice exhibited low bone mass associated with decreased osteoblast but increased osteocyte numbers, whereas the loss-of-function transgenic mice overexpressing miRNA decoys for either miR-23a or miR-27a showed decreased osteocyte numbers. Moreover, they identified that the upregulated miR-23a cluster could directly target and repress Prdm16 for enhancing the TGF-β signaling to accelerate the expression of sclerostin during osteocytic di
ger signaling to accelerate the expression of sclerostin during osteocytic differentiation. In line with the above microarray data, Davis and colleagues found that the miR-21 expression was markedly downregulated in connexin43 (Cx43)-silenced MLO-Y4 osteocytic cells that undergo spontaneous cell death in culture. Similarly, the bones from Cx43-deficient mice and 24-month-old mice both exhibit reduced levels of the miR-21 and increased levels of the miR-21 target PTEN. They further demonstrated that miR-21 lies downstream of Cx43 to repress PTEN for reducing osteocyte apoptosis. In addition, Fu and colleagues found that miR-199a-3p could mediate the osteocyte autophagy. They observed that miR-199a-3p expression was upregulated in osteocytic areas of OVX mice with estrogen deficiency. Mechanistically, a series of their in vitro data from MLO-Y4 cells documented that estrogen deficiency increased the expression of miR-199a-3p, which could induce autophagy in osteocytes via targeting insulin growth factor-1 (IGF-1) and mammalian target of rapamycin (mTOR) to repress the mTOR-related signaling cascades.

**miRNA and Bone Cell Crosstalk**

Besides their intracellular function, emerging studies have uncovered that miRNAs can traffic in exosomes serving as intercellular signals to mediate cell-cell communications. Evidence of the exosomal miRNA-mediated crosstalk is increasingly witnessed in bone cells and is being extensively investigated. Our laboratory has identified that exosomal miR-214-3p secreted by osteoclasts was transferred to osteoblasts to inhibit osteoblast activity and bone formation. Consistently, another study further demonstrated that ephrinA2 and EphA2 interaction could facilitate the recognition of osteoclast-derived exosome by osteoblasts. On the other hand, a recent study also postulated that the miR-433-3p highly expressed by osteoclasts could be secreted in osteoblast-derived exosomes for targeting DKK1 expression in osteoclasts, which in turn relieves the inhibitory effect of DKK1 on osteoblast function. In another study, Cui and colleagues found that MC3T3 mouse osteoblasts could release exosomes containing osteogenic miRNAs to promote the osteoblast differentiation of the recipient ST2 cells. In addition, a recent study showed that ablation of osteocytes in a transgenic (DMP-1 DTR Tg) mouse with targeted expression of diphtheria toxin receptor (DTR) under the promoter of DMP-1 resulted in the downregulated expression of 12 miRNAs (miR-3473a, miR-3473b, miR-3473e, miR-5128, miR-6244, miR-6239, miR-5132, miR-705, miR-208a, miR-3104, miR-7 of 9

---

**Fig. 1.** Schematic diagram of the key miRNA players in osteoblast differentiation, osteoclast differentiation, and osteoblast-osteoclast crosstalk. Red lines ending with a short perpendicular line indicate that miRNA-mediated regulation upregulates the osteoblast/osteoclast differentiation and activity. Black lines ending with a short perpendicular line indicate that miRNA-mediated regulation downregulates the osteoblast/osteoclast differentiation and activity.
miR-1224, and miR-5621) in serum exosomes, suggesting that osteocyte could also release miRNA-containing exosomes for cell-cell communication. Interestingly, Qin and colleagues found that Myostatin, a myokine secreted by muscles, could suppress miR-218 expression in Ocy454 osteocytes and their exosomes. The Myostatin-treated Ocy454 cell-derived exosomes could inhibit the osteoblastic differentiation of MC3T3 cells, which could be reversed by introduction of miR-218 mimics in Ocy454 exosomes. With this rising interest in this area, it would be so exciting to establish the physiological/pathological role of miRNA-mediated crosstalk among bone cells as well as between bone and other organs, and thereafter, develop new therapeutic agents targeting the adverse crosstalk in bone diseases. The miRNA-mediated mechanisms in bone cell crosstalk are summarized in Table 1.

Summary and Prospective

In summary, miRNA-mediated posttranscriptional regulation is a highly efficient regulatory mechanism for orchestrating the physiological activity of osteoblasts, osteoclasts, and osteocytes (Fig. 1). However, the dysregulation of miRNAs always results in impaired osteoblast, osteoclast, and osteocyte function, leading to abnormal bone remodelling. In addition, miRNA-mediated crosstalk not only represents a novel paracrine-like mechanism for coupling osteoblast and osteoclast function, but also may contribute to pathological uncoupling of bone formation and bone resorption. More in-depth studies are still required to uncover the upstream molecular events conducting the miRNA expression and to build up a miRNA-regulatory network in specific bone cells.

Disclosures

All authors state that they have no conflicts of interest.

Acknowledgments

This work was supported by the Hong Kong General Research Fund (HKBU12102914 to GZ; HKBU12101117 to GZ; and HKBU12136616 to JL); the Natural Science Foundation Council of China (81702189 to JL); and the Interdisciplinary Research Clusters Matching Scheme of Hong Kong Baptist University (RC-IRCs/17-18/02 to GZ).

Authors’ roles: JL and GZ designed the review and wrote most of the manuscript; LD prepared the table and figure and screened the literatures; XHW, DJL and QR did literature searching. APL provided scientific suggestions.

References

1. Zaidi M. Skeletal remodeling in health and disease. Nat Med. 2007;13:791–801.
2. Reppe S, Datta HK, Gautvik KM. Omics analysis of human bone to identify genes and molecular networks regulating skeletal remodeling in health and disease. Bone. 2017;101:88–95.
3. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281–97.
4. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature. 2001;409:363–6.
5. Gregory RI, Yan KP, Amuthan G, et al. The Microprocessor complex mediates the genesis of microRNAs. Nature. 2004;432(7014):235–40.
6. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. Nature. 2004;432(7014):235–5.
7. Kwon SC, Nguyen TA, Choi YG, et al. Structure of human DROSHA. Cell. 2016;164:81–90.
8. Wu Q, Song R, Ortogero N, et al. The RNase III enzyme DROSHA is essential for microRNA production and spermatogenesis. J Biol Chem 2012;287:25173–90.
9. Bendre A, Moritz N, Vaananen V, Maatta JA. Dicer1 ablation in osteoclasts induces bone resorption and osteopenia. Bone. 2018;106:139–47.
10. Choi YJ, Jeong S, Yoon KA, et al. Deficiency of DGC8 increases bone formation through downregulation of miR-22 expression. Bone. 2017;103:287–94.
11. Gaur T, Hussein S, Mudhasani R, et al. 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. 2010;340(1):10–21.
12. Zhou J, Hu Y, Chen Y, et al. Dicer-dependent pathway contribute to the osteogenesis mediated by regulation of Runx2. Am J Transl Res. 2016;8:5354–69.
13. Mizoguchi F, Izu Y, Hayata T, et al. Osteoclast-specific Dicer gene deficiency suppresses osteoclastic bone resorption. J Cell Biol 2010;190:866–75.
14. Sugatani T, Vacher J, Hruska KA. A microRNA expression signature of osteoectostrogenesis. Blood. 2011;117:3648–57.
15. Harfe BD, McManus MT, Mansfield JH, Hornstein E, Tabin CJ. The RNAseIII enzyme Dicer is required for morphogenesis but not patterning of the vertebrate limb. Proc Natl Acad Sci U S A 2005;102:10898–903.
16. Liu P, Baumgart M, Groth M, et al. Dicer ablation in osteoblasts by Runx2 driven cre-loxP recombination affects bone integrity, but not glucocorticoid-induced suppression of bone formation. Sci Rep. 2016;6:32112.
17. Chen Q, Liu W, Sinha KM, Yasuda H, de Crombrugghe B. Identification and characterization of microRNAs controlled by the osteoblast-specific transcription factor Osterix. PLoS One. 2013;8:e58104.
18. Zhang Y, Xie RL, Croce CM, et al. A program of microRNAs controls osteogenic lineage progression by targeting transcription factor Runx2. Proc Natl Acad Sci U S A 2011;108:9863–8.
19. Eguchi T, Watanabe K, Hara ES, Ono M, Kuboki T, Calderwood SK. OstermiR: a novel panel of microRNA biomarkers in osteoblastic and osteocytic differentiation from mesenchymal stem cells. PLoS One. 2013;8:e58796.
20. Kureel J, John AA, Dixit M, Singh D. MicroRNA-467g inhibits new bone regeneration by targeting Ihh/Runx2 signalling. Int J Biochem Cell Biol 2017;85:35–43.
21. Chen H, Ji X, She F, Gao Y, Tang P. miR-628-3p regulates osteoblast differentiation by targeting RUNX2: possible role in atrophic non-union. Int J Mol Med 2017;39:279–86.
22. Miyama K, Yamada G, Yamamoto TS, et al. A BMP-inducible gene, dlx5, regulates osteoblast differentiation and mesoderm induction. Dev Biol. 1999;208:123–33.
23. Laxman N, Mallmin H, Nilsson O, Kindmark A. miR-203 and miR-320 regulate bone morphogenetic protein-2-induced osteoblast differentiation by targeting distal-less homeobox 5 (Dlx5). Genes (Basel) 2016;8:4. DOI: 10.3390/genes8010004
24. Yang X, Matsuda K, Bialek P, et al. ATF4 is a substrate of RSK2 and required for osteoblast differentiation and mesoderm induction. Dev Biol. 2016;8:4. DOI: 10.3390/genes8010004
25. Yang X, Karsenty G. ATF4, the osteoblast accumulation of which is determined post-translationally, can induce osteoblast-specific gene expression in non-osteoblastic cells. J Biol Chem 2004;279:47109–14.
26. Zhang X, Yu S, Galson DL, et al. Activating transcription factor 4 is critical for proliferation and survival in primary bone marrow stromal cells and calvarial osteoblasts. J Cell Biochem 2008;105:885–95.
27. Wang X, Guo B, Li Q, et al. miR-214 targets ATF4 to inhibit bone formation. Nat Med. 2013;19:93–100.
28. Nakashima K, Zhou X, Kunkel G, et al. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell. 2002;108:17–29.

29. Shi K, Lu J, Zhao Y, et al. MicroRNA-214 suppresses osteogenic differentiation of C2C12 myoblast cells by targeting Osterix. Bone. 2013;55:487–94.

30. Kapinas K, Kessler C, Ricks T, Gronowicz G, Delany AM. miR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop. J Biol Chem 2010;285:25221–31.

31. Zhang J, Tu Q. Bonewold LF, et al. Effects of miR-335-5p in modulating osteogenic differentiation by specifically downregulating Wnt antagonist DKK1. J Bone Miner Res 2011;26:1953–63. DOI: 10.1002/jbmr.377

32. Li J, Feng Z, Chen L, Wang X, Deng H. MicroRNA-335-5p inhibits osteoblast apoptosis induced by high glucose. Mol Med Rep 2016;13:4108–12.

33. Tang X, Lin J, Wang G, Lu J. MicroRNA-433-3p promotes osteoblast differentiation through targeting DKK1 expression. PLoS One. 2017;12:e0179860.

34. Sun T, Li CT, Xiong L, et al. miR-375-3p negatively regulates osteogenesis by targeting and decreasing the expression levels of LRPS and beta-catenin. PLoS One. 2017;12:e0171281.

35. Li Z, Hassan MO, Valinina S, et al. A microRNA signature for a BMP2-induced osteoblast lineage commitment program. Proc Natl Acad Sci U S A 2008;105:13906–11.

36. Fang T, Wu Q, Zhou L, Mu S, Fu Q. miR-106b-5p and miR-17-5p suppress osteogenic differentiation by targeting Smad5 and inhibit bone formation. Exp Cell Res 2016;347:74–82.

37. Sugatani T, Hruska KA. Impaired micro-RNA pathways diminish osteoclast differentiation and function. J Biol Chem 2009;284:4667–78.

38. Sugatani T, Hildreth BE 3rd, Toribio RE, Malluche HH, Hruska KA. Expression of DGC8R-dependent microRNAs is dispensable for osteoclastic development and bone-resorbing activity. J Cell Biochem 2014;115:1043–7. DOI: 10.1002/jcb.24759

39. Asangani IA, Rasheed SA, Nikolova DA, et al. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcdd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene. 2008;27:2128–36.

40. Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. J Biol Chem 2008;283:1026–33.

41. Kumarswamy R, Volkmann I, Thum T. Regulation and function of miR-21 in health and disease. RNA Biol. 2011;8:706–13.

42. Sugatani T, Hruska KA. Down-regulation of miR-21 biogenesis by estrogen action contributes to osteoclastic apoptosis. J Cell Biochem 2013;114:1217–22.

43. Hu CH, Sui BD, Du FY, et al. miR-21 deficiency inhibits osteoclast function and prevents bone loss in mice. Sci Rep. 2017;7:43191.

44. Franceschetti T, Kessler CB, Lee SK, Delany AM. miR-29 promotes murine osteoclastogenesis by regulating osteoclast commitment and migration. J Biol Chem 2013;288:33347–60.

45. Wang FS, Chuang PC, Lin CL, et al. MicroRNA-29a protects against glucocorticoid-induced bone loss and fragility in rats by orchestrating bone acquisition and resorption. Arthritis Rheum 2013;65:1530–40. DOI: 10.2002/art.37948

46. Kriegel AJ, Liu Y, Fang Y, Ding X, Liang M. The miR-29 family: genomics, cell biology, and relevance to renal and cardiovascular injury. Physiol Genomics. 2012;44:237–44.

47. Li Z, Zhang W, Huang Y. MiRNA-133a is involved in the regulation of postmenopausal osteoporosis through promoting osteoclast differentiation. Acta Biochim Biophys Sin (Shanghai) 2018;50:273–80. DOI: 10.1093/abbs/gmy006

48. Cheng P, Chen C, He HB, et al. miR-148a regulates osteoclastogenesis by targeting V-raf musculoaponeurotic fibrosarcoma oncogene homolog 2B. J Bone Miner Res 2013;28:1180–90. DOI: 10.1002/jbmr.1845

49. Chen C, Cheng P, Xie H, et al. MiR-503 regulates osteoclastogenesis via targeting RANK. J Bone Miner Res 2014;29:338–47.

50. Eli B, Mercatelli L, Ibrahim T, et al. Tumor-induced osteoclast miRNA changes as regulators and biomarkers of osteolytic bone metastasis. Cancer Cell. 2013;24:542–56.

51. Liu J, Li D, Dang L, et al. Osteoclastic miR-214 targets TRAF3 to contribute to osteolytic bone metastasis of breast cancer. Sci Rep. 2017;7:40487.

52. Zhao C, Sun W, Zhang P, et al. miR-214 promotes osteoclastogenesis by targeting Pten/Pi3k/Akt pathway. RNA Biol. 2015;12:343–53.

53. Krzeszinski JY, Wei W, Huynh H, et al. miR-34a blocks osteoporosis and bone metastasis by inhibiting osteoclastogenesis and Tgf2. Nature. 2014;512(7515):431–5.

54. Kobayashi K, Takahashi N, Jimi E, et al. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. J Exp Med 2000;191:275–86.

55. Zhao B, Grimes SN, Li S, Hu X, Ivashkov LB. TNF-induced osteoclastogenesis and inflammatory bone resorption are inhibited by transcription factor RBPJ. J. Exp Med. 2012;209:319–34.

56. Miller CH, Smith SM, Elgindy M, et al. RBPJ-Regulated miR-182 promotes TNF-alpha-induced osteoclastogenesis. J Immunol. 2016;196: 4977–86.

57. Bonewold LF. The amazing osteocyte. J Bone Miner Res 2011;26:229–38.

58. Zeng HC, Bae Y, Dawson BC, et al. MicroRNA-23a cluster promotes osteocyte differentiation by regulating TGF-beta signaling in osteoblasts. Nat Commun. 2017;8:15000.

59. Davis HM, Pacheco-Costa R, Atkinson EG, et al. Disruption of the Cx43/miR21 pathway leads to osteocyte apoptosis and increased osteoclastogenesis with aging. Aging Cell. 2017;16:551–63.

60. Fu J, Hao L, Tian Y, Liu Y, Gu Y, Wu J. MiR-199a-3p is involved in estrogen-mediated autophagy through the IGF-1/mTOR pathway in osteocyte-like MLO-Y4 cells. J Cell Physiol 2018;233:2292–303.

61. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lottvall JO. Exosome-mediated transfer of miRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007;9:654–9.

62. Braicu I, Tomuleasa C, Monroy P, Cucuianu A, Berindan-Neagoe I, Calin GA. Exosomes as divine messengers: are they the Hermes of modern molecular oncology? Cell Death Differ 2015;22:34–45.

63. Liu J, Li D, Wu X, Dang L, Lu A, Zhang G. Bone-derived exosomes. Curr Opin Pharmacol 2017;34:64–9.

64. Xie Y, Chen Y, Zhang L, Ge W, Tang P. The roles of bone-derived exosomes and exosomal microRNAs in regulating bone remodeling. J Cell Mol Med 2017;21:1033–41.

65. Li D, Liu J, Guo B, et al. Osteoclast-derived exosomal miR-214-3p inhibits osteoblastic bone formation. Nat Commun. 2016;7:10872.

66. Sun W, Zhao C, Li Y, et al. Osteoclast-derived microRNA-containing exosomes selectively inhibit osteoblast activity. Cell Discov. 2016;2:16015.

67. Cui Y, Luan J, Li H, Zhou X, Han J. Exosomes derived from mineralizing osteoblasts promote ST2 cell osteogenic differentiation by alteration of microRNA expression. FEBS Lett. 2016;590:185–92.

68. Sato M, Suzuki T, Kawano M, Tamura M. Circulating osteocyte-derived exosomes contain miRNAs which are enriched in exosomes from MLO-Y4 cells. Biomed Rep. 2017;6:223–31.

69. Qin Y, Peng Y, Zhao W, et al. Myostatin inhibits osteoblastic differentiation by suppressing osteocyte-derived exosomal microRNA-218: a novel mechanism in muscle-bone communication. J Biol Chem 2017;292:11021–33.

70. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008;9:102–14.

71. Lian JB, Stein GS, van Wijnen AJ, et al. MicroRNA control of bone formation and homeostasis. Nat Rev Endocrinol 2012;8:212–27.