**The role of MicroRNAs in Osteoclasts and Osteoporosis**

Peifu Tang1,3, Qi Xiong1,2, Wei Ge2,*, and Lihai Zhang1,*

1Department of Orthopedics; General Hospital of Chinese PLA; Beijing, China; 2National Key Laboratory of Medical Molecular Biology & Department of Immunology; Institute of Basic Medical Sciences; School of Basic Medicine; Peking Union Medical College; Beijing, China

© Peifu Tang, Qi Xiong, Wei Ge, and Lihai Zhang
*Correspondence to: Wei Ge; Email: wei.ge@chem.ox.ac.uk; Lihai Zhang; Email: zhanglihai@gmail.com
Submitted: 11/06/2014; Accepted: 11/07/2014
http://dx.doi.org/10.1080/15476286.2014.996462
This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

**Keywords:** MicroRNA, osteoclast, osteoporosis

**Abbreviations:** GM-CSF, Granulocyte macrophage colony-stimulating factor; MiRNA, microRNA; 3'UTR, 3' untranslated region; BMMs, bone marrow macrophages; PDCD4, programmed cell death 4; Fasl, Fas ligand; PLO, particle-induced osteolysis; Calcr, calcitonin receptor; RDX, radixin; M-RIP, myosin phosphatase-Rho interacting protein; ITGA5, integrin α5; Fzd3, frizzled 3; ALP, alkaline phosphatase; TRAP, tartrate-resistant acid phosphatase; CXCL11, chemokine (C-X-C motif) ligand 11; CXCR3, chemokine (C-X-C motif) receptor 3; SLC39A1, solute carrier family (zinc transporter) member 1; TRAF6, TNF receptor-associated factor 6; OVX, ovariectomy; MAFB, V-maf musculoaponeurotic fibrosarcoma oncogene homolog B; CBL, Casitas B-lineage lymphoma proto-oncogene; PAG1, phosphoprotein associated with glycosphingolipid microdomains; TOB2, transducer of ERBB2; sICAM1, soluble intracellular adhesion molecule.

Osteoclasts are the exclusive cells of bone resorption. Abnormally activating osteoclasts can lead to low bone mineral density, which will cause osteopenia, osteoporosis, and other bone disorders. To date, the mechanism of how osteoclast precursors differentiate into mature osteoclasts remains elusive. MicroRNAs (miRNAs) are novel regulatory factors that play an important role in numerous cellular processes, including cell differentiation and apoptosis, by post-transcriptional regulation of genes. Recently, a number of studies have revealed that miRNAs participate in bone homeostasis, including osteoclastic bone resorption, which sheds light on the mechanisms underlying osteoclast differentiation. In this review, we highlight the miRNAs involved in regulating osteoclast differentiation and bone resorption, and their roles in osteoporosis.

**Introduction**

Osteoporosis is caused by an imbalance between osteoblastic bone formation and osteoclastic bone resorption.1 Osteoclasts, the sole cell type capable of bone resorption in the human body, originate from haematopoietic progenitors which can also differentiate into monocytes and macrophages.2 The process of osteoclast differentiation is regulated by a variety of cytokines, growth factors and hormones, including M-CSF, RANKL, sex hormones, and parathyroid hormone (PTH). Of these signaling molecules, RANKL binds the membrane receptor RANK on osteoclast precursors, activates downstream signaling pathways, such as the MAPK, PI3K and NF-κB pathways, and regulates the expression of various transcription factors, including c-Fos, PU.1, Fra-1 and NFATc1.3-5 These pathways and transcription factors modulate osteoclast differentiation, maturation and survival. Investigation of the molecular mechanisms that mediate osteoclastic differentiation as well as the regulation of their pathological function will contribute to a better understanding of the pathogenesis of osteoporosis and the development of new treatment strategies for this disease.

MicroRNAs (MiRNAs), a class of noncoding RNAs 19–25 nucleotides in length, control gene expression at the post-transcriptional level.6 Since 1993, when the first miRNA, named lin-4, was discovered in *Caenorhabditis elegans*,7 numerous miRNAs have been identified in different organisms. The latest miRBase release (v20, June 2013) contained 24,521 miRNA loci from 206 species that can produce 30,424 mature miRNA products.8 A number of miRNAs regulate various pathophysiological events, including organ development, haematopoietic function, organogenesis, apoptosis, proliferation and tumorigenesis. MiRNAs are also involved in bone cell differentiation and function, bone development and diseases. MiR-223, which is almost exclusively expressed in mouse bone marrow, is regulated by the enhancer modulators PU.1 and CAAT.9,10 In addition, miR-125b,11 miR-26a,12 miR-133 and miR-135 13 have all been implicated in the differentiation of osteoblasts. The expression pattern of miRNAs throughout the differentiation of osteoclast precursors into mature osteoclasts has also been explored, and the expression of 44 miRNAs increased by more than 2-fold.14 Moreover, the
| **miRNAs** | **species** | **Target gene(s)** | **Endogenous miRNA expression** | **miRNA function** | **Related disease(s)** | **References** |
|------------|-------------|--------------------|---------------------------------|-------------------|----------------------|---------------|
| miR-21     | mouse       | Fasl, PDCD4        | Upregulated in RANKL-induced osteoclastogenesis | Enhances osteoclast differentiation, inhibits osteoclast apoptosis | Postmenopausal osteoporosis; osteoporosis | 23, 25 |
| miR-29a    | rat         | Not mentioned      | Reduced expression in glucocorticoid-induced bone loss | Inhibits GC-induced osteoclast differentiation | Glucocorticoid-induced bone loss | 33 |
| miR-29b    | human       | c-Fos, MMP2        | Downregulated in RANKL induced osteoclastogenesis | Inhibits osteoclast differentiation | Multiple myeloma-related bone disease | 34 |
| miR-29     | mouse       | CDC42, SRGA, NR, NFIA, CD93, CALC | Increased during osteoclast differentiation | miR-29 family member that sustains migration and commitment of precursors to osteoclastogenesis | Possibly related to increased osteoclast formation with aging | 35 |
| miR-223    | mouse       | Not mentioned      | Downregulated in osteoclast differentiation | Overexpression completely blocks osteoclast formation | Might be related to bone metabolic disorders | 14, 52 |
| miR-378    | human       | NFIA               | Downregulated in osteoclast differentiation | Inhibits osteoclast differentiation | Bone destruction in rheumatoid arthritis | 53 |
| miR-146a   | mouse       | Not mentioned      | Upregulated in osteoclast differentiation | Not mentioned | Osteolytic bone metastasis | 14, 64 |
| miR-29b    | human       | TRAF6              | Not mentioned | Inhibits PBMC differentiation into osteoclast | Joint destruction in arthritis | 49 |
| miR-133a   | human       | CXCL11, CXCR3, SLC39A1 | Up-regulated in low BMD postmenopausal women | Not mentioned | Postmenopausal osteoporosis | 46 |
| miR-503    | human       | RANK               | Markedly reduced in postmenopausal osteoporosis women | Inhibits RANKL-induced osteoclast differentiation | Postmenopausal osteoporosis | 59 |
| miR-31     | mouse       | RhoA               | Highly upregulated during osteoclast development upon RANKL stimulation | Promote ring-shaped mature osteoclasts formation, attributed to cytoskeleton organization | Not mentioned | 38 |
| miR-124    | mouse       | NFATc1, RhoA, Roc1 | Decreased rapidly upon stimulation of BMMs with RANKL | Negatively regulates osteoclastogenesis, reduces the proliferation and motility of osteoclast precursors | Not mentioned | 42 |
| miR-125a   | human       | TRAF6              | Dramatically downregulated during osteoclastogenesis | Inhibit osteoclastogenesis | May be involved in metabolic disease | 67, 68 |
| miR-148a   | human       | MAFB               | Upregulated during osteoclast differentiation | Promote osteoclastogenesis | Osteoporosis, contributes to low BMD in lupus patients | 67 |
| miR-155    | mouse       | SOCS1, MITF, SHIP  | RANKL treatment suppress miR-155 levels in BMMs from Dicer-deficient mice | Suppress osteoclast differentiation | Osteoclast-mediated diseases | 15, 76, 77 |
| miR-422a   | human       | CBL, CD226, IGF1, PAG1, TOR2 | Upregulated with marginal significance in the low BMD postmenopausal women | Not mentioned | Postmenopausal osteoporosis | 80 |

*Italics: potential targets*
involvement of Dicer (part of the Rnase III family, cleaves double-stranded RNA or pre-microRNA into short double-stranded RNA fragments) in regulating osteoclastic bone resorption indicates that miRNAs are novel regulatory factors of osteoclastogenesis. Therefore, miRNAs may represent new therapeutic targets for the pharmacological control of bone diseases. Recently, some researchers reviewed the importance of miRNAs in post-transcriptional regulation of skeletal development. The functions of miR-21, miR-155 and miR-223 in osteoclast differentiation have been summarized in previous reviews. In this paper, we reviewed the role of all relevant miRNAs in osteoclasts and related bone diseases, such as osteoporosis (Table 1).

**MiR-21**

Mature miR-21, which is encoded by a single gene, is conserved in mammals. The mouse miR-21 includes miR-21a, miR-21b and miR-21c, of which the genes are mapped to chromosome 11, chromosome 3 and chromosome 8 respectively. As to human miR-21, of which the gene is located in chromosome 17 between the stop codon and poly A signal of the 3′-UTR (3′ untranslated region) of transmembrane protein 49 (TMEM49). MiR-21 is involved in the RANKL-induced differentiation of osteoclasts derived from mouse bone-marrow macrophages (BMMs). Transcription factors for osteoclastogenesis, such as c-Fos and PU.1, trigger miR-21 transcription via the AP-1 and PU.1 binding sites in the miR-21 promoter. Sugatani et al. reported that miR-21 regulates osteoclast formation through a positive feedback loop that involves c-Fos/miR-21/PDCD4 (programmed cell death 4). In this process, the osteoclastogenesis transcription factor c-Fos upregulates the expression of miR-21. miR-21 then downregulates PDCD4 protein expression, which in turn represses the PDCD4-induced inhibition of c-Fos and thereby promotes RANKL-induced osteoclastogenesis. Sugatani and colleagues also discovered that miR-21 plays a critical role in estrogen-controlled osteoclastogenesis. Notably, estrogen inhibits osteoclastogenesis and induces osteoclastic apoptosis. Estrogen downregulates miR-21 biogenesis so that protein levels of FasL (Fas ligand), a target of miR-21, are elevated post-transcriptionally and thus induce osteoclastic apoptosis. Wear particles induce aggressive osteoclastic bone resorption after joint replacement, which causes aseptic loosening. Zhou et al. found that miR-21 was markedly upregulated in particle-induced osteolysis (PIO) model animals and knockout miR-21 in particle-stimulated tissue could ameliorate the symptoms of osteolysis. In short, the upregulation of miR-21 plays an important role in the pathological processes underlying osteoporosis and osteolysis during aseptic implant loosening in joint replacement patients.

---

**Figure 1.** Schematic regulatory networks of miRNAs regulating osteoclast differentiation. MiRNAs can directly (solid lines) or indirectly (dotted lines) repress transcriptional factors, and affect osteoclast differentiation, apoptosis, or phenotype. Bold dotted lines indicate the promotion of cellular processes, dotted boxes indicate osteoclast specific genes, and dotted ovals indicate cellular processes or phenotypes.
MiR-29

The miR-29 family includes miR-29a, miR-29b and miR-29c. MiR-29b is represented by 2 members, miR-29b-1 and miR-29b-2. MiR-29a was first discovered in cervical cancer cells (HeLa). Based on seed sequence target prediction, miR-29 family members may regulate >6,000 gene expressions. An earlier study by Mott et al. has suggested that NF-κB activation suppresses miR-29b-1 and miR-29a promoter, and the expression of mature miR-29b is mediated via the recognition of NF-κB binding sites. As NF-κB is an important regulator of osteoclast differentiation, researchers have started to focus on whether miR-29a is active in osteoclasts. Several studies confirmed that miR-29 is a key mediator of osteoclast differentiation. Gong et al. reported that miR-29a was down expressed in SBC-5 small-cell lung cancer cells in bone metastases. However, the downregulation of miR-29a had no correlation with osteolytic lesions. Wang et al. discovered that osteoporosis caused by glucocorticoids was associated with reduced expression of miR-29a, and a gain of miR-29a function reduced glucocorticoid-induced osteoclast differentiation in vitro. Pre-miR-29a precursor treatment in rats significantly alleviated glucocorticoid-induced bone loss, while knockout miR-29a accelerated bone resorption by osteoclasts, cortical bone porosity and bone fragility. Moreover, they demonstrated that miR-29a might be involved in the Wnt and Dkk-1 signaling pathways to promote osteoblast differentiation and mineral deposition in bone. Consistent with this finding, Rossi et al. showed that miR-29b expression decreased progressively in the differentiation of CD14+ hematopoietic stem cells to osteoclasts stimulated by RANKL and M-CSF in vitro. Furthermore, the overexpression of miR-29b has pronounced negative effects on the TRAP expression, collagen degradation and lacunae generation, which are all characteristics of osteoclast activity. However, others have reported contradictory results. Franceschetti et al. demonstrated that miR-29 family members are positive regulators of osteoclast formation by targeting mRNAs encoding NFI-A and the calcitonin receptor (Calcr), both of which are important for cytoskeletal organization, commitment, and osteoclast resorption and survival. The expression of miR-29 family increases during osteoclast differentiation, while the inhibition of miR-29a, miR-29b and miR-29c suppresses the formation of TRAP-positive multinucleated osteoclasts. MiR-29a knockdown generates smaller osteoclasts from both primary bone marrow cells and the monocytic cell line RAW264.7. MiR-29 knockdown also impaired commitment and migration of osteoclast precursors. However, miR-29 knockdown does not influence cell viability, actin ring formation, or apoptosis in mature osteoclasts. NFI-A is a negative regulator of the M-CSF receptor in osteoclasts and downregulation of the NFI-A mRNA by miR-29 may play a role in promoting osteoclastogenesis. In short, the miR-29 family plays a critical role in osteoclast differentiation, although there are conflicting reports as to their mechanisms of action.

MiR-31

MiR-31 is encoded by a single locus and is expressed in a wide variety of tissues and cells in humans and mice. In addition, miR-31 is the only member of a broadly conserved miRNA ‘seed family’ that is present in vertebrates and Drosophila. Corresponding target predications using bioinformatic analyses showed that miR-31 targeted >200 different mRNAs in animal cells. Genes encoding proteins that are active in cell motility, including cell polarity, cytoskeletal dynamics and cell adhesion, were significantly enriched in these predicted targets. In in vitro assays, miR-31 directly inhibited at least 6 mRNAs that are important for the progression of tumors, including RhoA, DDX (radixin), MMP16 (matrix metallopeptidase 16), M-RIP (myosin phosphatase-Rho interacting protein), ITGA5 (integrin α5) and Fzd3 (frizzled 3). Mizoguchi et al. found that RANKL treatment enhanced miR-31 expression of differentiation and the migration of osteoclast precursors. Therefore, cell fusion was not affected by miR-31. Therefore, they suggested that cytoskeleton disorganization caused impaired bone resorption and the formation of osteoclasts resulting from the inhibition of miR-31. The same research group also demonstrated that RhoA, a molecular switch that transduces extracellular signals to actin and the microtubule cytoskeleton, might be a target of miR-31 in osteoclasts. Therefore, miR-31 inhibits osteoclastic bone resorption by the repression of osteoclast formation.

MiR-124

Many overexpression and loss-of-function studies in vertebrates suggest that miR-124, a highly abundant brain-enriched miRNA, is a progenitor self-renewal inhibitor and enhancer of neuronal differentiation. MiR-124 has also been shown to regulate the myogenic and adipogenic differentiation of bone marrow-derived mesenchymal stem cells. Lee et al. demonstrated that osteoclastogenesis from mouse BMMs is regulated by miR-124 via the suppression of differentiation and the migration of osteoclast precursors. In summary, miR-124 inhibits osteoclast formation through the suppression of differentiation and the migration of osteoclast precursors.

MiR-133a

MiR-133a was initially considered to be a muscle-specific miRNA involved in the regulation of muscle-cell differentiation and the pathogenesis of myogenic disease and heart disease. A recent study confirmed that miR-133a plays a key role in skeletal system regulation. Mesenchymal cells have been shown to express miR-133a in a lineage-related pattern. This miRNA was shown to...
directly target the Runx2 gene 3′-UTR when overexpressed in MC3T3, an osteoblast cell line, and suppress ALP (alkaline phosphatase, a marker of osteoblast formation) production and osteoblast differentiation. 44 Ji et al. determined that miR-133a is downregulated in primary human osteosarcoma tissues and osteosarcoma cell lines and these changes significantly correlated with tumor progression. 45 In addition, miR-133a has also been shown to be involved in osteoclast regulation. Wang et al. compared miR-133a expression in PBMCs (peripheral blood monocytes) between postmenopausal female Caucasians with normal or low bone mineral density (BMD) and found that miR-133a expression in the low BMD group was significantly higher than in the high BMD group. Bioinformatics target gene analysis demonstrated that miR-133a negatively regulates 3 potential osteoclast-related target genes, CXCL11 [chemokine (C-X-C motif) ligand 11], CXCR3 [chemokine (C-X-C motif) receptor 3] and SLC39A1 [solute carrier family (zinc transporter), member 1], although it appears that all the 3 genes do not have significant roles in osteoclast differentiation. 46 Therefore, miR-133a is an important regulator of circulating monocytes in postmenopausal women and may serve as a biomarker for postmenopausal osteoporosis.

**MiR-146a**

MiR-146a was initially found in a human acute monocytic leukemia cell line called THP-1. In THP-1, the elevation of miR-146a expression by LPS (lipopolysaccharides) stimulation depends on NF-κB, which controls Toll-like receptor and cytokine signaling via a negative feedback loop and suppression of the protein levels of TRAF6 (TNF receptor-associated factor 6) and IL-1 receptor-associated kinase 1. 47,48 Both TRAF6 and NF-κB are important molecules in osteoclastogenesis. RANKL treatment causes the time-dependent downregulation of miR-146a expression, while miR-146a is highly expressed during osteoclast differentiation in TNF-α/RANKL-treated cells relative to RANKL-treated cells. 14 Nakasa et al. transfected PBMCs from healthy volunteers with double-stranded miR-146a, cultured them in the presence of M-CSF and either TNF-α or RANKL, and found that miR-146a inhibits the differentiation of PBMCs into osteoclasts in a dose-dependent manner in both osteoclastogenesis culture stimulation systems. At the same time, c-Jun, NF-ATc1, PU.1, and TRAP expression levels were also downregulated. The intravenous injection of miR-146a prevented bone erosion in mice with collagen-induced arthritis, although this treatment did not completely ameliorate the associated inflammation. 49 These investigations suggest that miR-146a expression induced by TNF-α/RANKL treatment may serve as a negative regulator of osteoclastogenesis.

**MiR-223**

MiR-223 is almost exclusively expressed in bone marrow and is specifically expressed in CD11b+ myeloid cell lineages. 9 Although a preliminary study could not detect an increase in myeloid output from the bone marrow upon miR-223 expression, subsequent studies have suggested a profound role for miR-223 in myelopoiesis. 50 Moreover, IKK-α, an important regulator of the NF-κB pathway, has been confirmed as a regulated target of miR-223. 51 In vitro studies showed that the differentiation of human monocytes into macrophages with GM-CSF was accompanied by a reduction in the expression of miR-223, which led to a substantial increase in IKK-α protein expression levels and eventually inhibited the NF-κB signaling pathway. 51 These results indicated that miR-223 influences the differentiation of monocytes/macrophages. As osteoclasts differentiate from monocytes/macrophages, osteoclast differentiation would be likely to be a target of miR-223 regulation. Further investigations by Sugatani et al. using RAW264.7, a mouse osteoclast precursor cell line, demonstrated that miR-223 plays a critical role in osteoclast differentiation. Overexpression of miR-223 completely inhibited TRAP+ multinucleated cell formation. However, these researchers were unable to identify the miR-223 target mRNA during RAW264.7 osteoclast precursor differentiation. 52 In a subsequent study, Kagiya et al. used microarrays to screen for the expression of mature miRNAs in RAW264.7 cells treated with a combination of TNF-α and RANKL and found that miR-223 was downregulated during osteoclast differentiation using qRT-PCR. 14 When miR-223 was overexpressed in PBMCs, the number of TRAP + multinucleated osteoclasts decreased. NFI-A is confirmed as a validated miR-223 target gene, and thus the expression of this gene was not detected when miR-223 was overexpressed. Although no significant difference was observed in the expression of NFI-A mRNA between the ds-miR-223 and non-specific dsRNA groups, Western blotting demonstrated that NFI-A protein was still downregulated by miR-223, which suggested a mechanism by which miR-223 suppresses osteoclastogenesis. 53 In summary, miR-223 is downregulated during osteoclast differentiation, and therefore it is possible to inhibit osteoclast differentiation and maturation and ultimately reduce bone erosion through the upregulation of miR-223 expression.

**MiR-503**

MiR-503 was first identified in human retinoblastoma tissues using the miRNA microarray technique. 54 These methods demonstrated that miR-503 expression was upregulated in human parathyroid and adrenocortical carcinomas. 54-56 MiR-503 influences the proliferation, migration, adhesion, and network formation of endothelial cells. 57 MiR-503 is transcribed as a polycistronic message when monocytes differentiate into macrophages and targets an overlapping set of cell cycle regulators to induce G1 arrest. 58 Chen et al. reported that, when compared with healthy postmenopausal women, miR-503 expression was significantly reduced in the circulating progenitors of osteoclast-CD14 + PBMCs from postmenopausal women with osteoporosis. When miR-503 was overexpressed in human PBMCs, RANKL-induced osteoclast differentiation was significantly inhibited, while the silencing of miR-503 promoted osteoclastogenesis. In the same study, RANK was shown to be a target of miR-503. In the OVX (ovariectomy) mouse, a miR-503 antagonist was able to increase the expression of the RANK protein, which led to enhanced bone resorption and
a reduction in BMD. Therefore, mimicking overexpression of miR-503 by using agomir inhibited bone resorption and prevented osteoporosis in OVX mice.\(^{59}\) Taken together, these findings suggest that miR-503 may play an important role in the pathogenesis of postmenopausal osteoporosis.

**MiR-378**

MiR-378 is generally considered to be a tumor suppressor and is downregulated in gastric, colon and throat cancer tissues.\(^{60,61}\) In a test of bone marrow from patients with myelodysplastic syndrome, Dostalova et al. determined that miR-378 expression was downregulated in patients with a deletion of the long arm of chromosome 5, which indicated that miR-378 may play a role in the regulation of bone marrow cells.\(^{62}\) Furthermore, in a study by Kahai et al., miR-378 was shown to affect osteoblast differentiation by regulating the expression of nephronectin.\(^{63}\) MiR-378 expression increased by 59-fold in TNF-\(\alpha\)/RANKL-treated RAW264.7 cells.\(^{14}\) EII et al. reported that miR-378 was associated with bone metastases in cancer. MiR-378 expression was elevated during osteoclast differentiation in the serum of mice with bone metastases. Moreover, miR-378 regulated the expression of sICAM1 in serum, while sICAM1 was secreted by bone metastatic cancer cells and activated the NF-\(\kappa\)B signaling pathway.\(^{64}\) Therefore, the upregulation of miR-378 appears to play a regulatory role in the erosion of tumor bone metastases by affecting the NF-\(\kappa\)B signaling pathway.

**MiR-125a**

In human cells, miR-125 consists of 3 homologs, miR-125a, miR-125b-1, and miR-125b-2. A number of genes, including matrix-metalloproteases, transcription factors, members of Bcl-2 family and others are targets of miR-125, and changes in these genes could lead to enhanced metastasis, proliferation, and invasiveness. Moreover, the ectopic expression of miR-125a was shown to inhibit the proliferation and metastasis of hepatocellular carcinoma by targeting MMP11 and VEGF.\(^{65,66}\) In addition, miR-125a is downregulated during osteoclast differentiation stimulated by M-CSF and RANKL.\(^{67}\) Guo et al. discovered that CD14\(^+\) PBMCs were prevented from differentiating into osteoclasts by miR-125a overexpression, which then suppressed the levels of TRAP and NFATc1 mRNA. They also observed that transfecting antagonists of miR-125a into PBMCs promoted osteoclast formation. TRAF6 is a target gene of miR-125a, and NFATc1, a downstream target of TRAF6, binds to the promoter of miR-125a to modulate the expression of miR-125a.\(^{68}\) Thus, miR-125a forms a negative feedback loop with TRAF6 and NFATc1 to modulate osteoclast differentiation and maturation.

**MiR-148a**

MiR-148a is expressed aberrantly in normal tissue, and the expression of miR-148a is significantly altered in some neoplastic and non-neoplastic diseases. To elaborate, miR-148a was found to be significantly upregulated in plasma of patients with multiple myeloma, and high levels of miR-148a have been related to shorter relapse-free survival durations.\(^{69}\) Cheng et al. found that miR-148a was dramatically upregulated during M-CSF- and RANKL-induced osteoclast differentiation from CD14\(^+\) PBMCs. They also showed that patients with lupus experienced significantly elevated levels of miR-148a in CD14\(^+\) PBMCs, a process that resulted in enhanced osteoclastogenesis and lower BMD. Overexpression of miR-148a promotes the formation of osteoclasts, whereas the inhibition of miR-148a attenuates their formation. OVX mice that were intravenously injected with antagonirs of miR-148a decreased bone resorption and increased bone mass. These regulatory functions of miR-148a were achieved by binding the 3'-UTR of MAFB (V-maf musculoaponeurotic fibrosarcoma oncogene homolog B) mRNA and inhibiting MAFB protein expression.\(^{67}\) Furthermore, MAFB negatively regulates RANKL-induced osteoclastogenesis by attenuating DNA binding of 3 key regulators, NFATc1, c-Fos, and Mitf.\(^{70}\) Therefore, the miR-148a-induced modulation of MAFB regulates NFATc1, c-Fos, and other regulatory factors that ultimately modulate osteoclast differentiation.

**MiR-155**

MiR-155, which is highly specifically expressed by haematopoietic cells, plays a regulatory role in a variety of cancers, including leukemia, lymphoma, liver cancer, lung cancer, and breast cancer.\(^{67}\) MiR-155 also regulates various inflammatory cytokines in rheumatoid arthritis (RA). The expression of miR-155 is elevated in the PBMCs, synovial tissue, synovial fluid, and fibroblasts of patients with RA and thus plays an important role in bone destruction.\(^{72-74}\) In a model of K/BxN serum-transfer arthritis that requires only innate effector mechanisms, a significant reduction of localized bone destruction was observed in miR-155\(^{-/-}\) mice, and this observation was linked to reduced osteoclast generation.\(^{75}\) Compared to WT (wild type) mice, osteoclast-specific Dicer deficient mice had increased bone mass and trabecular thickness, decreased osteoclast formation, and reduced expressions of TRAP and NFATc1. MiR-155 levels in BMMs from Dicer-deficient mice were inhibited by RANKL treatment. In contrast, miR-155 levels in WT cells were not significantly changed by RANKL treatment. When bone marrow-derived macrophages from osteoclast-specific Dicer-deficient mice were stimulated with RANKL, TRAP\(^+\)-cells grown in Dicer-deficient bone-marrow cell culture were typically smaller in size than WT cells. Determining the number of multinucleated TRAP\(^+\)-cells showed that Dicer-deficient osteoclasts inhibited the RANKL induction of TRAP\(^+\)-cell development. This study also showed that Dicer-deficient osteoclasts inhibited Trap mRNA expression and slightly suppressed the level of Nfatc1 mRNA. These researchers speculated that miR-155 was suppressed by Dicer deficiency upon RANKL treatment, which may then cause the upregulation of SHIP, an osteoclast inhibitor.\(^{15}\) Mann et al. found that miR-155 expression increased rapidly during the
process of RAW264.7 differentiating into macrophages, and miR-155 inhibited the osteoclast differentiation of RAW264.7 cells by inhibiting MITF (microphthalmia-associated transcription factor), which is a transcription factor essential for osteoclast differentiation. Zhang et al. reported that IFN-β induced miR-155 and mediated IFN-β-induced suppression of osteoclast differentiation by interacting with MITF and SOCS1, positive regulators of osteoclastogenesis. Taken together, these findings demonstrate that miR-155 regulates osteoclastogenesis via targeting several essential transcriptional factors.

**MiR-422a**

MiR-422a plays an important role in human diseases, such as colon cancer and multiple sclerosis and has been shown to inhibit pathways that stimulate tumor cell proliferation in osteosarcoma. In the field of skeletal research, one study has shown that miR-422a was down-regulated by peptide-15 (analog of the sequence GTGPQQGIAQGRGVV) in the collagen α1 (I) chain, which is known to increase bone formation. In osteoclasts, Cao et al. discovered that miR-422a is significantly upregulated in a low BMD group of postmenopausal women relative to a high BMD group. Moreover, several target genes were predicted to be related to osteoclastogenesis, including CBL (Casitas B-lineage lymphoma proto-oncogene), CD226 (cluster of differentiation 226), IGF1 (insulin-like growth factor 1), PAG1 (phosphoprotein associated with glycosphingolipid microdomains), and TOB2 (transducer of ERBB2). This group also demonstrated an apparent negative correlation between each of these 5 genes and miR-422a, although none of the correlations reached statistical significance. These studies suggest that miR-422a is possibly involved in the regulation of postmenopausal osteoporosis and might be a potential biomarker of osteoporosis.

**Others**

During the differentiation and maturation of osteoclasts, there are other miRNA expression changes in addition to the ones discussed above. Kaiya et al. found 52 mature miRNAs with expression changes when RAW264.7 cells were treated with RANKL during osteoclast formation. In addition to the miRNAs already discussed, expression levels of miR-483, miR-680, miR-689, miR-714, and miR-721 were shown to be elevated in a time-dependent fashion, while miR-23b and miR-342-3p expression levels decreased. Notably, miR-26a, miR-199a-3p, and miR-1224 expression levels declined at 24 h after treatment and then increased 82 h after treatment with RANKL. EII et al. cultured the RAW264.7 cells with tumor-conditioned media, and detected increased expression levels of 42 miRNAs that were upregulated by more than 2-fold; they also found that 45 miRNAs were downregulated. However, the molecular mechanism and function of these miRNAs in osteoclasts remain unclear.

**Conclusions**

Bone homeostasis is maintained by a balance between osteoblastic bone formation and osteoclastic bone resorption. Metabolic bone disorders, such as osteopenia, osteoporosis, and RA-related bone destruction, occur when osteoclastic bone resorption exceeds osteoblastic bone formation. The treatment of osteoporosis is typically focused on inhibiting the excessive activation of osteoclasts. Therefore, understanding the mechanisms of osteoclast differentiation and maturation will assist in developing treatments for osteoporosis. The discovery of the OPG/RANK/RANKL pathway in osteoclast differentiation and maturation is a major landmark in osteoporosis research. However, more studies are necessary regarding the molecular mechanism underlying osteoclast differentiation. MiRNAs have been shown to be important regulators of many biological functions. The studies reviewed here show that miRNAs are deeply involved in the regulation of osteoclast differentiation and bone resorption (Fig. 1). The overexpression or inhibition of specific miRNAs effectively inhibits osteoclast differentiation and bone resorption. MiRNA-based therapeutics have shown some promise for the treatment of osteoporosis. However, further studies are required if miR-based therapeutics are to be used in the clinic, and many more questions are raised. For example, what other important miRNAs can regulate osteoclasts? What specific pathways are regulated by miRNAs in osteoclasts? What side effects would occur with the regulation of osteoclasts by miRNAs in clinic? How can miRNAs be effectively delivered in vivo? All of these questions will need to be answered in the future.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Funding**

P-F Tang, Q. Xiong and L-H Zhang are supported by the National Natural Science Foundation of China (31370947). W. Ge is supported by the National Natural Science Foundation of China (81373150).

---

**References**

1. Teitelbaum SL. Bone resorption by osteoclasts. Science 2000; 289:1504-8; PMID:10968780
2. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003; 423:337-42; PMID:12748650; http://dx.doi.org/10.1038/nature01658
3. Asagiri M, Takayanagi H. MiR-422a: a regulator of osteoclast differentiation. Bone 2007; 40:251-64; PMID:17098490; http://dx.doi.org/10.1016/j.bone.2006.09.023
4. Leibbrandt A, Penninger JM. RANK/RANKL: regulators of immune responses and bone physiology. Ann N Y Acad Sci 2008; 1143:123-50; PMID:19765348; http://dx.doi.org/10.1196/annals.1443.016
5. Negishi-Koga T, Takayanagi H. Ca2+-NFATc1 signaling is an essential axis of osteoclast differentiation. Immunol Rev 2009; 229:1401-14; PMID:19754911; http://dx.doi.org/10.1111/j.1600-065X.2009.00821.x
6. Landgraf P, Raus M, Sheridan R, Sever A, Iovino N, Aravin A, Pfeffer S, Rice A, Kampani AO, Landthaler M, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 2007; 129:1401-14; PMID:17684727; http://dx.doi.org/10.1016/j.cell.2007.04.040
7. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75:843-54; PMID:8252621; http://dx.doi.org/10.1016/0092-8674(93)90529-Y

www.landesbioscience.com RNA Biology 1361
17. Gamez B, Rodriguez-Carballo E, Ventura F. Micro-RNAs in RAW264.7 cells treated with a combination of Zn and TNF-alpha. J. Immunol. 2008; 181:2677-95; PMID:19002059; http://dx.doi.org/10.4049/jimmunol.181.4.2677

18. Lian J, Stein GS, Stein JS. Expression profiling of micro-RNAs in RAW264.7 cells treated with a combination of Zn and TNF-alpha. J. Cell Biochem. 2010; 111:866-75; PMID:20039311

19. van der Eerden BC. Micro-RNAs in the skeleton: cell-restricted or potent intercellular communicators? Arch Biochem Biophys 2014; 541C:46-55; PMID:24832391; http://dx.doi.org/10.1016/j.abb.2014.04.016

20. Chaiken S, Carbone B, Ventura F. Micro-RNAs and post-transcriptional regulation of skeletal development. J Mol Endocrinol 2014; 52:R197-9; PMID:24253214; http://dx.doi.org/10.1530/JME-13-0294

21. Lian JB, Stein GS, van Wijnen AJ, Stein JS, Hassan MK, Tato K, Yasuda K, DeMayo F. Micro-RNAs in postnatal bone development. J Bone Miner Res 2013; 28:15479-50; PMID:24149532; http://dx.doi.org/10.1002/jbmr.845

22. Fujita T, Takakura T, Minoguchi S, Koyama Y, Sakurai K. miR-21 Gene expression triggered by particle-induced osteolysis pathogenesis. J Orthop Res 2012; 30:1837-42; PMID:22508653; http://dx.doi.org/10.1002/jor.22176

23. Suganita T, Vacher J, Hruska KA. A micro-RNA expression signature of osteoclastogenesis. Blood 2011; 117:3648-57; PMID:21275303; http://dx.doi.org/10.1182/blood-2010-10-314115

24. Garcia-Falcón V, Robinson LJ, Borysenko CW, Lehmann T, Kalla S, Blai HC. Negative regulation of RANKL-induced osteoclastic differentiation in RAW264.7 Cells by estrogen and phytoestrogens. J Bio Chem 2005; 280:13720-7; PMID:15644335; http://dx.doi.org/10.1074/jbc.M410219200

25. Suganita T, Hruska KA. Down-regulation of miR-21 biogenesis by estrogen action contributes to osteoclastogenesis. J Cell Biochem 2013; 114:1217-22; PMID:23238785; http://dx.doi.org/10.1002/jcb.24471

26. Holt G, Marum T, Reilly J, Mork R. The biology of aseptic loosening. Clin Orthop Relat Res 2007; 460:240-52; PMID:17620815

27. Zhou Y, Liu Y, Cheng L. miR-21 expression is related to particle-induced osteolysis pathogenesis. J Orthop Res 2012; 30:1837-42; PMID:22508940; http://dx.doi.org/10.1002/jor.22128

28. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel gene coding for small expressed RNAs. Science 2001; 294:853-6; PMID:11567960; http://dx.doi.org/10.1126/science.1064921

29. Wang Y, Zhang X, Li H, Yu Y, Ren X. The role of miR-21 family in cancer. Eur J Cell Biol 2013; 92:123-8; PMID:23357522; http://dx.doi.org/10.1007/s10095-013-1062-x

30. Liston A, Papadopoulos AS, Ans-Daemel B, Dooley P. J. Micro-RNA-29 in the adaptive immune system: setting the stage. Cell 2012; 150:387-400; PMID:22441842; http://dx.doi.org/10.1016/j.abb.2014.04.016

31. Cai B, Li J, Wang J, Luo X, Xu A, JI, Liu Y, Wang N, Liang H, Zhang M, Chen N, et al. miR-124 regulates cardiomyocyte differentiation of bone marrow-derived mesenchymal stem cells via targeting STAT3 and p38 signaling. Stem Cells 2012; 30:1746-55; PMID:22662953; http://dx.doi.org/10.1002/stem.1154

32. Lee Y, Kim HH, Park CK, Kim YG, Lee HJ, Kim JY, Kim HH. Micro-RNAs regulate osteoclast differentiation. J. Bone Miner Res 2008; 23:2339-46; PMID:18384814; http://dx.doi.org/10.1001/jbmr.2008.003.015

33. Fujita T, Inoue M, Sato M, Iwakiri M, Ohno Y. Alteration of miR-21 expression in osteoclasts and osteoblasts, and its role in osteoclastogenesis. J Cell Physiol 2010; 225:395-402; PMID:20385656; http://dx.doi.org/10.1002/jcp.225463
