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ACTUALIZACIONES / *Review*

UNVEILING THE ROLE OF OSTEOBLASTIC micro RNAs IN THE SKELETON: FROM BIOLOGICAL FUNCTIONS TO THERAPEUTIC POTENTIAL

Farzin Takyar Alyssa Bren, Leslie R Morse, Ricardo A. Battaglino*

Department of Rehabilitation Medicine, University of Minnesota Medical School, EE UU.

Abstract

MicroRNAs (miRNAs) are small non-coding RNA molecules that play critical roles in post-transcriptional gene regulation. They function by binding to target messenger RNA (mRNA) molecules, leading to their degradation or inhibiting their translation into proteins. In the context of skeletal diseases, such as osteoporosis, osteoarthritis, and bone metastasis, there is growing evidence osteoblastic miRNAs, are involved in the regulation of bone formation and maintenance. Osteoblasts are bone-forming cells responsible for synthesizing and depositing the extracellular matrix, which ultimately mineralizes to form bone tissue. Osteoblastic miRNAs modulate various aspects of osteoblast function, including proliferation, differentiation, mineralization, and apoptosis. Dysregulation of these miRNAs can disrupt the balance between bone formation and resorption, leading to skeletal diseases.

The therapeutic implications of targeting osteoblastic miRNAs in skeletal diseases are significant. Modulating the expression levels of specific miRNAs holds promise for developing novel therapeutic strategies to enhance bone formation, prevent bone loss, and promote bone regeneration. Potential therapeutic approaches include the use of synthetic miRNA mimics to restore miRNA expression in diseases associated with miRNA downregulation or the use of anti-miRNA oligonucleotides to inhibit miRNA function in diseases associated with miRNA upregulation. miRNA-based therapies are still in the early stages of development, and further research is needed to fully understand the complexity of miRNA networks. Additionally, the delivery of miRNAs to specific target tissues and cells remains a challenge that needs to be addressed for effective clinical translation. Nonetheless, targeting osteoblastic miRNAs represents a promising avenue for future therapeutic interventions in skeletal diseases.

Key words: *miRNA, osteoblasts, bone deseases.*

*E-mail: rbattagl@umn.edu

Resumen

ROL DE LOS micro-ARN OSTEOBLÁSTICOS EN EL ESQUELETO: DESDE LAS FUNCIONES BIOLÓGICAS AL POTENCIAL TERAPÉUTICO

Los micro-ARNs (miARNss) son pequeños ARN no codificantes que desempeñan un papel fundamental en la regulación génica postranscripcional. Ejercen su función al unirse a moléculas de ARN mensajero (ARNm), promoviendo su degradación e inhibiendo su traducción en proteínas. En el contexto de las enfermedades esqueléticas, como la osteoporosis, la osteoartritis y la metástasis ósea existe evidencia de que los miARNs osteoblásticos están involucrados en la regulación de la formación y del mantenimiento óseo.
Los osteoblastos son células formadoras de hueso responsables de sintetizar y depositar la matriz extracelular, que finalmente se mineraliza para formar el hueso. Los miARNs derivados de osteoblastos modulan varios aspectos de la función de estas células, incluida la proliferación, diferenciación, mineralización y la apoptosis. La desregulación de estos miARNs puede alterar el equilibrio entre la formación y la resorción ósea, lo que lleva a enfermedades óseas.
Las implicaciones terapéuticas de los miARNs osteoblásticos en enfermedades esqueléticas son significativas. La modulación de los niveles de expresión de miARNs específicos es prometedora para desarrollar nuevas estrategias terapéuticas a fin de mejorar la formación, prevenir la pérdida y promover la regeneración ósea. Los enfoques terapéuticos potenciales incluyen el uso de miméticos de miARNs para restaurar la expresión de miARNs o el uso de oligonucleótidos anti-miARNs para inhibir su función.
Las terapias basadas en miARNs aún se encuentran en las primeras etapas de desarrollo. La administración de miARNs a las células y los tejidos específicos sigue siendo un desafío para lograr una aplicación clínica eficaz.
Palabras clave: miRNA, osteoblastos, enfermedades óseas.

Osteoblast differentiation

Osteoblasts are cells of mesenchymal origin specialized in the process of bone formation. This is a tightly controlled process. Dysregulated ossification can cause insufficient or excessive mineralization of bones or mineralization in ectopic sites resulting in a wide range of conditions, such as osteogenesis imperfecta, calcific aortic valve disease, osteoporosis, and fibrodysplasia ossificans progressive. Mesenchymal progenitors generate osteoblasts either directly - as typically observed in intramembranous ossification - or via an osteo-chondroprogenitor - as observed in endochondral ossification. In both pathways, pre-osteoblasts give rise to osteoblasts. Many of the key pathways involved in osteoblast differentiation and signaling have been described. The master molecular switch that drives mesenchymal progenitors to the osteoblastic lineage, *cbfa/runx2,* is a transcription factor with multiple upstream regulators (*Wnt/Notch*, *Sox9*, *Msx2* and *hedgehog*) and downstream targets. Regulation is further executed by the existence of *cbfa/runx2* cofactors like *Osx and Atf4*. Paracrine regulation via bone morphogenetic proteins (BMPs) and parathyroid hormone, adds another layer of regulatory complexity to the differentiation of osteoblasts. Finally, epigenetic regulation can also exert fine-tuned control over the process.

MicroRNAs

MicroRNAs (miRNAs) are short (19-24 nucleotide), non-coding single-stranded RNA molecules that exert post-transcriptional modulation of large sections of the genome by binding to regulatory gene elements and inhibiting the translation of many genes. miRNAs belong to a larger family of non-coding RNA (ncRNA) that also includes transfer RNAs (tRNAs), long non-coding RNAs (lncRNAs), piwi-interacting RNA (piRNAs), small nucleolar RNA (snoRNAs), small nuclear RNA (snRNAs), small Cajal body-specific RNA (scaRNAs) and the more-recently discovered circular RNAs (circRNAs).[1] MiRNAs were first discovered in *C.elegans*, and have since been identified in many species, including humans.[2,3] They act as suppressors of their target genes by binding to their mRNAs, leading to either translational repression or complete degradation of the mRNA transcript.[4] They are thought to regulate at least one-third of the human genome and are involved in many physiological processes.[4] Differential expression of microRNAs cellularly and in the circulation has been implicated in several disease processes, demonstrating their role in pathophysiology and their potential as biomarkers for disease.[5-7] In this review, we will focus on those miRNAs that have been described to be implicated in osteoblast commitment, differentiation, and activity.

Bone formation by osteoblasts involves several steps. The process of regulation of osteoblast differentiation/function is a complex/ multilayered one and is affected by molecular, cellular, organismal, and environmental cues. This review hopes to shed light on the significance of miRNAs and their functional consequences in osteoblast biology, as a whole (as opposed to simply surveying miRNAs that affect different osteoblast functions). Mature miRNAs are generated following sequential cleavage by DROSHA/DGCR8 (in the nucleus) and DICER (in the cytoplasm). To study the global impact of miRNA generation in bone, Choi et al generated mice with a conditional Dgcr8 deletion in osteoprogenitor cells using Col1a1-Cre mice.[8] Dgcr8-cKO mice showed increases in micro-computed tomography (microCT) parameters consistent with increased bone mass. Dynamic morphometric analysis indicated osteoblast activity was increased in Dgcr8-cKO mice. This result illustrates the complexity of the system.

miRNAs processing mechanisms and regulation of osteoblast differentiation

Differentiation of mesenchymal stem cells (MSCs) into osteoblasts is a critical step in bone homeostasis and a phenomenon central to bone remodeling and maintenance. As such, it is a well-orchestrated biological process coordinated by tight and sequential regulation of gene expression. Regulation and synchronization of such gene expression are brought about by various effectors at transcription, post-transcription, translation, and post-translational levels, each of which has its own unique features. At each of these levels, different regulating mechanisms exert their effects within timeframes specific to their actions. For instance, alterations in biological processes that are brought about by hormones and cytokines can persist for extended periods of time. As epigenetic regulators involved in diverse cellular processes, differential expression of miRNAs has been shown to regulate various steps in osteoblast differentiation through alterations in the involved pathways that are short-lived, reversible, and not dramatic in magnitude.

As fine tuners of biological processes, miRNAs can be effective tools in orchestrating the critical steps of the stringently regulated and agile phenomenon of osteoblast differentiation. However, many studies that have investigated the potential roles of miRNAs in osteoblast differentiation have not been organized in a systematic manner consistent with this frame of mind and relevant to the complex concept of osteoblast differentiation. Moreover, these findings are at various stages of development

and a great majority have not been proven to be pertinent to diseases of bone in humans. Most reports have been from experiments performed in osteoblastic cell lines, with very few findings validated in mouse models and almost none carried over to human conditions. As such, a review of various findings regarding miRNAs as an attempt to put these in the context of stages and aspects of osteoblast differentiation seems necessary and required for directing future studies in this field. In this section, we try to address the studies that are available in the literature pertaining to osteoblast differentiation.

MiRNAs biogenesis is a multistep and complicated process that takes place through canonical or non-canonical pathways, involving diverse sets of proteins. Throughout their biogenesis and in preparation for becoming functional, miRNAs go from DNA transcripts to primary miRNAs (pri-miRNAs) to precursor miRNAs (pre-miRNAs) and finally to mature miRNAs.[9] In this process, a microprocessor complex in the nucleus – made up of two main parts, namely Drosha and DiGeorge Syndrome Critical Region 8 (DGCR8)- and the cytoplasmic Dicer complex are critical for pri-miRNAs to pre-miRNA to mature miRNA transitions. Many studies have focused on the associations between their processing mechanisms and osteoblast differentiation from MSCs; however, they have not followed the "primary-to-precursor-to-mature" sequence. On the contrary, the effects of alterations in parts of this process - mostly chosen either randomly or according to findings in studies of phenomena other than miRNAs biogenesis - on bone phenotypes have been assessed.

Tampering with the intra-nuclear portion of miRNAs biogenesis has been reported to have consequences for osteoblast differentiation. Conditional deletion of DGCR8 part of the nuclear miRNAs microprocessor complex in committed osteoblasts in a mouse model using a Cre-loxP system in *Col1a1*-expressing cells resulted in an increase in bone volume and osteoblast activity without significantly altering osteoclast numbers, suggesting that as a result of removing canonical intra-nuclear miRNAs processing mechanism in committed osteoblast lineage cells, differentiation of these cells gets skewed toward a more proliferative and active state.[8] This study identified miR-22 as a target miRNA that potentially mediates this effect, however, to the best of our knowledge further studies that are thoroughly designed to establish such an association are still lacking.

Attempts at understanding potential roles of the extranuclear processing portion of miRNAs biogenesis in osteoblast differentiation and function have mostly focused on Dicer, an RNase III endonuclease that processes pre-miRNAs exported from the nucleus to the cytoplasm. Functions of Dicer have been investigated through the use of multiple Cre-LoxP systems that delete this enzyme at various stages of mesenchymal stem cell differentiation towards osteoblasts. Deletion of Dicer in the limb mesoderm, which gives rise to osteochondral progenitor cells, through a *Prx1*-Cre-LoxP-mediated approach in mice led to viable mice with a morphologically significant decrease in the size of limbs (more prominent in forelimbs), a phenomenon that seems to be a result of lack of miRNA processing before embryonic day 9.5 (E9.5).[10] Interestingly, using a similar approach in targeting osteoprogenitors by *Col1a1*-Cre-LoxP system resulted in mice that were not viable beyond E14.5, whereas mice with deletion of Dicer in mature osteoblasts, achieved through using *Osteocalcin*-Cre-LoxP, were viable with a transient mineralization defect that was resolved by one month of age. The latter mice had an increased bone mass and higher cortical bone width from 2 to 8 months of age, which was seemingly brought about by an increase in osteoblast activity that stayed coupled with that of osteoclasts.[11] Additionally, when Dicer is deleted in Runx2-expressing osteoblasts, a reduction in bone density along with deranged bone formation and slowing of growth in postnatal life ensued.[11]

Moreover, Dicer has also been demonstrated to affect cortical bone homeostasis. As deletion of Dicer was incompatible with fetal survival, a mouse model was designed with inducible *Cre* driven by *Sp7* promoter (Sp7-Cre/ERT2; Dicer flox/flox) to assess postnatal effects of Dicer inactivation. Using this model to delete Dicer in pre-osteoblasts expressing *Osterix* resulted in a significant impairment of cortical bone formation without any effects on trabecular bone, hinting at a potential role for Dicer, and those miRNAs that are processed through Dicer, in postnatal maintenance of cortical bone.[12] It appears, however, that the relationship between Dicer and osteoblast is more complicated, and main players in osteoblast biology have the potential to exert control over this endonuclease. For instance, it has been shown that in the course of the commitment of progenitors to osteoblast lineage, Runx2 can affect miRNA processing mechanisms that involve Dicer.[13] It is unclear, however, whether these back-and-forth regulatory interactions between transcription factors and Dicer mechanisms are orchestrated mainly by the former or the latter.

Interactions with transcriptional regulators of OB differentiation

Osteoblastic transcription factors play significant roles in differentiation and are shown to be modulated by various miRNAs. One such master regulator, Runx2, has been shown in cell-based and animal mechano-transduction models to be modulated by miRNAs, specifically miR-103a, suggesting that miRNAs can play significant roles in transferring mechanical signals to important osteoblastic transcription factors.[14] A correlation between this miRNA and bone formation in bone samples from postmenopausal women has also been reported in a study that is not comprehensive and does not provide compelling evidence but it hints at the potential relevance of miRNAs that act through Runx2 to clinical outcomes.[15] MiR-132–3p, miR-204, and miR-218-5p are among other miRNAs that have been shown to affect Runx2 directly or indirectly.[16-18] Osterix, or Sp7, is a transcription factor that has well-defined roles in osteoblast differentiation by attaching to regions within other osteoblastic genes, such as Osteocalcin, Alkaline Phosphatase and COL1A1, and interacts with important players in the MAPK signaling pathway. Osterix is suggested to be in a regulatory loop with miR-93 that controls the mineralization potential of osteoblasts.[19] Additionally, miR-145 was shown through a bioinformatic analysis to negatively affect osteogenic differentiation.[20] However, none of the interactions of Osterix with miRNAs have been established in robust animal models or in humans.

The Dlx family of transcription factors are shown to be involved in the determination of cell fate and commitment of cells to various cell lineages, including osteoblast and chondrocyte.[21] Dlx5 is shown to mediate the effects of osteogenic signals such as BMP-2 and is involved in temporal control of osteocalcin gene activation.[22,23] It also has been reported to bear the ability to promote the differentiation of cells towards osteogenesis at the expense of inhibiting adipogenic differentiation.[24,25] This evidence suggests that Dlx5 is at constant crosstalk with Runx2 and Osx in mediating a range of stimuli during osteoblast differentiation.

Another major transcription factor that has been shown to have control over osteoblastic collagen production through post-transcriptional mechanisms and to interact with Runx2, is ATF.[26] This transcription factor has also been shown to be under the influence of miRNAs. Data from bone samples of patients with fractures, as well as follow-up experiments in mouse models of ovariectomy and hindlimb unloading, have shown that miR-214 targets ATF4 to suppress bone formation by inhibiting osteoblastic activity.[27] Micro-RNA-214 has also been shown to be involved in M-CSF and RANKL physiology and in osteoblast and osteoclast communication.[28,29] The case of miR-214 and its interaction with

ATF4 and consequent effects on osteoblasts and osteoclasts highlights the complexity of the interplay between transcription factors and micro-RNAs. Moreover, it is evident that many aspects of this complex biology are yet to be clarified.

miRNAs that affect important osteogenic signaling pathways

In addition to the many transcription factors investigated in the process of mesenchymal stem cell differentiation to osteoblasts, a number of signaling pathways have been extensively studied in osteoblast biology. Two of the more important ones are *Wnt* and BMP pathways. Activation of BMP and Wnt signaling, and their constituent factors, is an important event in osteoblast differentiation. Many of the steps in these pathways are regulated by miRNAs. Table 1 summarizes miRNAs that promote or inhibit osteoblast differentiation along with their target genes.

Table 1. miRNAs that promote or inhibit osteoblast differentiation and their target gene(s).

miRNAs	Target gene (s)	Functions	Ref.
		Promoters of Osteoblast Differentiation	
miR-27	APC	Promotes osteoblast differentiation through activation of Wnt	30, 31
miR-335, miR-433	DKK1	Promotes osteoblast differentiation through accumulation of β-catenin and activation of Wnt	30-33
miR-218, miR-29	DKK, sFRP2	Promote osteoblast differentiation	30, 31
miR-210	AcvR1b	Inhibition of TFG-β and promotion of osteoblast differentiation	30
miR-2861	HDAC5	Promotes acetylation of Runx2 and promotes osteoblast differentiation	31, 34
miR-3960	HOXA2	Alleviates Runx2 repression and promotes osteoblast differentiation	31, 34
		Inhibitors of Osteoblast Differentiation	
miR-15b	SMURF11	Prevents ubiquitination of Runx2	31, 35
miR-133, miR-135, miR-204, miR-217, miR-433, miR-30c, miR-338	Runx	Directly target and decrease Runx2 expression	30-32, 36
miR-205, miR23a	Satb2	Prevents complex formation with Runx2 and inhibits osteoblast differentiation	30, 32, 34, 36
miR-31, miR-637, miR-214	Osx	Downregulates Osx expression	30-32 34, 37
miR-141, miR-200a	Dlx5	Inhibit progression of osteoblast differentiation	30
miR-138	FAK	Reduces Runx2, Osx expression	31, 34
miR-206	Cx43	Negative regulator of osteoblast differentiation	30, 31

miRNAs Promoting Osteoblast Differentiation

The Wnt pathway can be divided into canonical, also known as Wnt/β-catenin, and non-canonical. The activation of the Wnt/β-catenin pathway is important in promoting osteoblast differentiation. miR-27 is involved in this promotion through the inhibition of adenomatous polyposis coli (APC) gene expression.[30,31] APC is part of a destruction complex that degrades β-catenin through phosphorylation when Wnt signaling is inactivated.[31] Thus, miR-27 targeting APC results in an accumulation of β-catenin and activation of Wnt signaling leading to the promotion of osteoblast differentiation. Dickkopf-related protein 1 (DKK1) is a known inhibitor of Wnt signaling and is regulated by numerous miRNAs to promote osteoblastogenesis through the Wnt pathway. miR-335 and miR-433 repress DKK1 expression by binding to the 3'UTR of DKKA mRNA.[30-33] DKK1 inhibits Wnt binding to LRP5/6 receptors, which is necessary in the accumulation of non-phosphorylated β-catenin.[31] The repression of DKK1 by miR-335 and miR-433 relieves this inhibition and results in activation of Wnt signaling and accumulation of β-catenin. Additionally, secreted frizzled-related protein (sFRP2) is an inhibitor of the Wnt/β-catenin pathway. Both miR-218 and miR-29 target Wnt inhibitors DKK1 and sFRP2, promoting osteoblast differentiation.[30,31] Further, the expression of miR-218 has been shown to be up-regulated in osteoblasts in response to Wnt signaling.[30]

The other main osteoblastic differentiation pathway is BMP2, which is known to activate transcription factors Runt-related transcription factor 2 (Runx2) and Osterix during osteogenesis. TGF-β signaling pathway inhibits osteoblastic differentiation through BMP2 and is repressed by miR-210.[30] MiR-210 targets AcvR1b (activin A receptor type 1B), an activator of the TFG-β pathway, thus leads to the inhibition of TFG β and promotion of osteoblastic differentiation.[30,31] Runx2 is a bone-specific transcription factor that is required for BMP2 osteoblastic differentiation and is regulated by numerous proteins. Repressors of Runx2 include Homeobox A2 (HOXA2), histone deacetylase 5 (HDAC5), and SMAD Ubiquitination Regulatory Factor 1 (SMURF1). MiR-2861 targets HDAC5 promoting the acetylation of Runx2 and positive regulation of osteoblast differentiation.[34] miR-3960 targets HOXA2 leading to an alleviation of Runx2 repression and promotion of osteoblastogenesis.[34] It has been shown that miR-2861 and miR-3960 belong to the same miR-cluster and are co-expressed, forming an additive effect that greatly increases Runx2-mediated osteoblast differentiation.[34] Further, miR-15b targets SMURF1 preventing the ubiquitination of Runx2, and has been shown to be overexpressed in MSCs during differentiation.[35]

miRNAs Inhibiting Osteoblast Differentiation

MiRNAs negatively regulating osteoblastic differentiation are important control mechanisms and are effectively decreased during osteogenesis. The main pathway regulated by miRNAs inhibition is BMP2, which is responsible for the activation of transcription factors Runx2 and Osterix (Osx). There are numerous miRNAs especially miR-133, miR-135, miR-204, miR-217, miR-433, miR-30c, miR-338 that are thought to target and decrease Runx2 expression.[32-36] This results in an inhibition of osteoblast differentiation as Runx2 is a bone-specific transcription factor that is necessary for the expression of osteoblast differentiation genes. Further, Runx2 forms a coregulatory complex with Satb2 (special AT-rich sequence–binding proteins) which is necessary for the successful differentiation of osteoblasts. miR-205 and miR-23a can target and suppress Satb2, preventing complex formation with Runx2 and inhibiting osteoblast differentiation.[30,31,36] Specifically, miR-205 partly targets Satb2 by preventing the phosphorylation of ERK and p38 MAPK in their respective pathways.[36]

Like Runx2, Osx is a key regulator of osteoblast differentiation and is downregulated by miR-31, miR-637, and miR-214. miR-31 has been found to have an inverse correlation

with Osx expression during osteogenic differentiation and inhibition of miR-31 increases Osx expression.[37] Likewise, miR-637 directly targets Osx and has been shown to promote adipocyte differentiation and inhibit osteoblast differentiation, maintaining the balance between adipocytes and osteoblasts.[38] Additionally, miR-214 expression can reduce Osx levels and also directly targets ATF4, a gene encoding transcription factor required for osteogenesis, resulting in decreased osteoblastogenesis.[30]

Both Runx2 and Osterix can be repressed by miR-141, miR-200a, and miR-138. Distalless homeobox 5 (Dlx5) is a master transcription factor related to osteogenesis and is targeted by miR-141 and miR-200a.[30] Dlx5 plays a role in inducing Runx2 expression and can regulate Osx expression through the BMP-2 pathway.[34,36] By targeting Dlx5, miR-141, and miR-200a inhibit the progression of osteoblast differentiation. Alternatively, miR-138 has been shown to inhibit osteoblast differentiation by targeting FAK (Focal Adhesion Kinase) causing reduced phosphorylation of ERK1/2 and reduced Runx2/Osx expression.[31,34] FAK is an important kinase in osteogenic differentiation and miR-138 greatly inhibits this process.[34] Finally, Connexin 43 (Cx43), is a gap junction protein necessary for osteoblast differentiation and is targeted by miR-206.[30,31] This results in an important negative regulation of osteoblast differentiation.

miRNA roles in the promotion of OC formation by OB

Osteoblast lineage cells can communicate with those in the osteoclast lineage through the production of RANKL, a member of the TNF superfamily, that acts upon its receptor, RANK, on osteoclast precursors and induces the formation of active mature osteoclasts[39]. Initiation of this sequence of events can be triggered by many osteoclastogenic factors - such as PTH and 1,25$(OH)_2$-VitD – that act on osteoblasts and induce the production of RANKL. Moreover, a decoy receptor called osteoprotegerin (OPG), which can be produced by osteoblasts and other cell types present in the bone microenvironment and its production is regulated by many triggering factors, have the ability to block the attachment of RANKL to RANK and regulate the above-mentioned crosstalk between osteoblasts and osteoclasts[40,41]. Therefore, the RANKL/RANK/OPG system maintains homeostasis of osteoblastic support of osteoclast formation. Each of these three players are subject to miRNAs regulation. Those miRNAs that affect RANK are discussed in the context of osteoclast biology[42]. Table 2 provides an overview of miRNAs regulation of RANKL and OPG.

Concluding remarks

In conclusion, the posttranscriptional regulation of gene expression by miRNAs has multiple effects on the differentiation and activity of osteoblasts and its dysregulation can lead to anomalous bone remodeling. Of note, miRNAs may represent a novel mechanism for osteoblast and osteoclast crosstalk which has direct effects on the process of coupling between bone formation and bone resorption. Future studies are required to understand the upstream processes and physiological context that regulate miRNAs expression and to construct an *osteoblast-specific* miRNA-regulatory network.

The elucidation of miRNA-mediated osteoblast gene regulation will aid in the design of mechanism-based therapeutic strategies for the treatment of bone diseases. Indeed, miRNA-based therapeutics have emerged as a very promising approach for the cure of several diseases. Several challenges will have to be sorted out to successfully bring miRNAs to clinical use, among them: the development of a reliable delivery system, the specificity of RNA targets, and the optimization of therapeutically appropriate doses.

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Table 2. microRNAs regulating osteoclast function by targeting RANKL/OPG pathway.

miRNAs	Target Gene	Experimental Model	Functions	Ref.
503	RANK	CD14 + PBMCs	Promote osteoclast differentiation	43
		RAW264.7 cells		44
144-3p	RANK	CD14+ PBMCs	Promote osteoclast differentiation	45
185-5p	RANK	MC3T3-E1 cells	Promote osteoclast differentiation	46
21	OPG	miR-21 deficient mice	Promote osteoclast differentiation	47
		BMSCs, MMCs, and PBMCs	Weakened activity of osteoclasts	48
		Serum in PMOP	Increased number of osteoclasts	49
	PTEN	RAW 264.7 cells		50
17	RANKL	Cholesteatoma keratinocytes, fibroblasts and BMMs	Promote osteoclast differentiation	51
145-5p	OPG	RAW 264.7 cells and CIA mice	Promote osteoclast differentiation	52
320-a	PTEN	RAW264.7 cells	Promote osteoclast differentiation	53
125a-5p	TNFRSF1B	RAW264.7 cells	Promote osteoclast differentiation	54
214	PTEN	BMMS	Promote osteoclast differentiation	55
335	RANKL	SCLC SBC-5 cells	Inhibit osteoclast differentiation	56
106b	RANKL	Chick CAM and OVX mice	Inhibit osteoclast differentiation	57
	OPG/RANKL	CIA mice and BMMs	Inhibit osteoclast differentiation	58
377	RANKL	Mice of calvarial osteolysis and PBMCs from patients undergoing arthroplasty	Relieve particle-induced osteolysis	59
181b-5p	OSM	RAW 264.7 cells and CIA mice	Inhibit osteoclast differentiation	60
26a	EZH2	Rat with osteonecrosis of femoral head	Inhibit osteoclast differentiation	61
	CTGF	BMMs		62
503-3p	Hpse	RAW264.7 cells	Inhibit osteoclast differentiation	63
206-3p	Bmp3 and NFATc1	Neural crest cell knockout mice	Inhibit osteoclast differentiation	64
101-3p	Rap1b	OVX mice treated with bisphosphonates	Inhibit osteoclast differentiation	65
100-5p	FGF21	OVX mice	Inhibit OVX-induced bone resorption	66

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