

Review

MicroRNA Regulation of Bone Marrow Mesenchymal Stem Cells in the Development of Osteoporosis in Obesity

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Abstract

Obesity and osteoporosis are global health problems characterized by high rates of prevalence and mortality due to complications. As people with visceral obesity age, the adipogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) increases, and adipocytes become the predominant stromal cells in the bone marrow microenvironment, which hinders the physiological regeneration and mineralization of bone tissue. Primary and secondary osteoporosis remain severe progressive diseases. Both osteoporosis and obesity are associated with microRNAs (miRNAs) that induce adipogenesis and osteoresorption. This review presents analyses of the roles and clinical potential of miRNAs in the epigenetic control of BMSC differentiation and the formation and function of osteoclasts in osteoporosis with and without obesity. Understanding the fine-tuned regulation of the expression of genes critical for the balance of osteogenesis/osteolysis processes may provide hope for the development of effective and safe osteoporosis therapies in the future.

Keywords: osteoporosis; obesity; MSC-BM; microRNA; osteoblast

1. Introduction

Obesity and osteoporosis are characterized by high prevalence and mortality rates due to associated complications; therefore, they represent global health problems. Osteoporosis is a systemic, multifactorial disease characterized by the loss of bone mass, impaired metabolism, and disrupted bone tissue microarchitecture, which are accompanied by an increase in skeletal fragility and fracture injuries caused by minimal trauma [1].

The effects of obesity on bone health are very controversial. Increased body weight due to obesity increases the mechanical load on the bones, stimulating bone growth. In subcutaneous and visceral adipose tissue, aromatase synthesizes estrogen, which inhibits osteoclast activity and increases bone mass [2]. Therefore, an increased body mass index (BMI) value in postmenopausal women is associated with estrogen production and increased osteoblast activity, which generally reduces the risk of fractures. However, a critical increase in adipose tissue has been shown to exert a negative effect on bone mass [3]. Thus, osteoporosis is exacerbated by proinflammatory cytokines with proresorptive properties (tumor necrosis factor (TNF)- α , interleukin (IL)-15, IL-6, IL-7, IL-8, IL-17, and receptor activator of nuclear factor kappa-B ligand (RANKL)) and some adipokines, the levels of which increase in obesity [4]. The influences of

these mediators on signaling pathways (in preosteoblasts, osteoblasts, and stromal cells) lead to a shift in bone remodeling processes toward increased osteoclastogenesis, resulting in bone resorption.

With increasing age, bone marrow mesenchymal stem cells (BMSCs) exhibit a decreased capacity for differentiation into osteoblasts, and adipogenic differentiation increases with an increase in the volume of yellow bone marrow. Furthermore, under visceral obesity conditions, BMSC differentiation into adipocytes is promoted, which has a negative effect on bone strength [5–8]. Thus, with increasing age against a background of morbid obesity, adipocytes become the predominant stromal cells in bone marrow [9,10].

The modern view of bone formation and resorption is based on epigenetic regulation mechanisms [11]. The involvement of microRNAs is considered key to the control of BMSC gene expression [12,13].

MicroRNAs are small noncoding RNAs with lengths of 19–22 nucleotides, and they control biological and pathological processes, including organogenesis, apoptosis, cell proliferation, and differentiation [14]. MicroRNAs bind to specific sequences in the 3' and 5' untranslated regions (UTRs) of their target mRNAs to induce translational repression and mRNA deadenylation and decapping [11,14].



Studies have shown that microRNAs are stable outside the cell and do not degrade when held at room temperature for up to 24 hours or at -80°C for up to 9 months or after thawing in plasma/serum eight times [15]. Over the past 20 years, the number of publications in the PubMed database (<https://pubmed.ncbi.nlm.nih.gov>) retrieved with the search keyword “microRNA and cell differentiation” has increased by 780 fold (reaching 1557 articles in 2021). However, the roles of microRNAs in the autocrine and paracrine regulation of BMSC differentiation, including the development of osteoporosis in obesity, have not been adequately studied.

This review is dedicated to analyzing the roles and clinical potential of microRNAs in the epigenetic control of BMSCs and osteoclast differentiation in osteoporosis with obesity.

2. Key Moments in the Induction of Osteogenesis and Adipogenesis in MSCs

In the differentiation of mesenchymal stem cells, a cascade of events causes phenotypic and metabolic changes. Decreased expression of stem cell genes and the activation of genes associated with mature phenotypic function are the first steps in the cascade, and they lead to cellular morphological changes. Several studies have shown that factors and signaling pathways that stimulate adipogenesis simultaneously inhibit osteogenesis [16]. The reciprocal balance of the transcriptome between adipogenesis, osteogenesis, and chondrogenesis is essential for skeletal tissue development and the maintenance of homeostasis in a mature organism [17].

In healthy bones, the continuous resorption of old bone mainly by osteoclasts and the formation of new tissue by osteoblasts occur. Osteoclasts originate from the hematopoietic lineage [18] and monocyte/macrophage-derived osteoclast precursors [19]. Osteoblasts originate from BMSCs and can differentiate into adipogenic clones [20]. The microenvironment strictly controls BMSC differentiation, and imbalanced differentiation leads to a higher rate of adipogenic MSC differentiation, which is detrimental to osteogenesis [17,20,21].

An increase in adipose tissue mass leads to serious effects on the bone marrow microenvironment due to secreted factors. That is, the secretion of proinflammatory cytokines, adipokines, free fatty acids, exosomes, and microRNAs by adipocytes stimulates osteoclasts and promotes increased bone resorption [22]. In addition, obesity contributes to a disturbance/alteration in adipokine secretion; therefore, the secretion of adiponectin decreases, and the production of leptin and proinflammatory factors increases. Studies of transgenic mice harboring the human adiponectin gene demonstrated the stimulatory effect of adiponectin on bone formation through the activation of the Wingless-type MMTV integration site (WNT)/ β -catenin pathway. Adiponectin action is mediated through

receptors (AdipoR1 and AdipoR2) located on BMSCs [23]. In addition, the effects of leptin on osteogenesis are multidirectional. Leptin may directly or indirectly affect bone metabolism through central hypothalamic and peripheral signaling pathways [24]. A study of mice deficient in leptin and its receptors showed a reduced bone formation rate, which increased significantly after the subcutaneous administration of leptin [25]. Leptin receptors were found on red bone marrow MSCs, osteoclasts, and osteoblasts [26]. The administration of leptin by intraventricular infusion resulted in bone loss in both leptin-deficient and wild-type mice [27].

Increased TNF- α production by adipocytes inhibits osteoblastogenesis and is a potent trigger of bone resorption [28]. IL-15 stimulates the secretion of TNF- α , which further enhances the osteoclastogenic effect [28]. In pre-osteoblasts, TNF- α upregulates nuclear factor- κ B (NF- κ B) activity by inhibiting the bone morphogenic protein (BMP) 2-induced production of runt-related transcription factor (RUNX) 2.

Simultaneously, TNF- α initiates the expression of RANK [28] and blocks the Wnt pathway by activating the *SOST* and *DKK-1* genes [9]. Razny *et al.* (2019) [29] showed that patients with obesity and insulin resistance have defects in osteoblastogenesis associated with decreased expression of β -catenin and microRNAs involved in the regulation of osteoblast differentiation and increased expression of Wnt signaling pathway inhibitor genes (*DKK-1*, *DKK-2*, *SOST*, and *SFRP1*), leading to a predominance of osteoclastogenesis.

Obesity is associated with increased osteoblast production of RANKL, which regulates osteoclast differentiation, proliferation, survival, and apoptosis. Thus, adipose tissue secretes M-CSF, which induces RANKL-mediated bone resorption by triggering the PI3K/AKT/NF κ B signaling cascade, activating NFATC1, or upregulating the expression of the transcription factors HIF-1 α and PPAR γ by activating the PI3K/AKT/mTOR pathway [4,9]. IL-1, IL-6, IL-11, IL-15, and IL-17 are proresorptive cytokines produced by activated T lymphocytes that stimulate RANKL production [30].

Osteoprotegerin (OPG), which is a member of the TNF receptor superfamily (TNFRSF11B), is secreted by osteoblasts. It is a RANKL decoy receptor that blocks the RANKL/RANK interaction [31,32]. An increased OPG level with age is considered a homeostatic response to limit bone resorption [33]. It should be considered that high insulin levels in obesity interact with insulin receptors on osteoblasts, blocking the production of OPG and increasing the secretion of osteocalcin [34]. The presence of obesity and type 2 diabetes is associated with the active production of oxidized lipids and carbohydrates in addition to damaged proteins, leading to the formation of enhanced glycation end products (AGEs). AGEs interact with the AGE receptor (RAGE) and trigger a cascade of intracellular re-

sponses (NFkB, AP-1, CREB, STAT3, and NFAT), leading to the development of inflammatory responses [35]. The accumulation of AGEs in bone tissue leads to the development of pathological conditions associated with low bone density and osteoporosis [36]. Considering these findings, the triggering of inflammatory responses is a link between metabolic disorders, osteogenesis and bone resorption.

The intracellular mechanisms that determine MSC fate in the context of adipogenic and osteogenic differentiation are under active investigation [17]. The processes of MSC differentiation into adipocytes and osteocytes *in vitro* can be conditionally classified into two stages: (1) clone predetermination (the commitment stage) from an MSC to a committed progenitor and (2) maturation (the terminal differentiation stage) from the progenitor to a cell with a mature phenotype [16,21].

Numerous studies have shown that the major signaling pathways involved in regulating MSC lineage commitment are the transforming growth factor-beta (TGF β)/BMP/specific E3 ubiquitin protein ligase (Smads), WNT, Hedgehog (Hh), Notch, fibroblast growth factor (FGF), Janus kinase/signal transducer and activator of transcription (JAK/STAT), mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K/Akt), and extracellular signal-regulated kinase (ERK1/2) pathways [21,37].

Adipogenic differentiation is associated with increased production of cyclic AMP, leading to the phosphorylation of the transcription factor CCAAT/enhancer-binding protein (C/EBP). Upon activation, C/EBP binds to regulatory elements and stimulates the transcription of C/EBP α and peroxisome proliferator activated receptor gamma (PPAR γ). When C/EBP synthesis is inhibited, the transcription of C/EBP α and PPARG is continuously promoted because C/EBP α binds to regulatory C/EBP elements. PPAR γ transcription is also controlled by sterol regulatory element-binding protein (SREBP1) transcription factors and the Kruppel-like factor family [21]. The later stages of adipogenic differentiation are associated with the expression of fatty acid synthase, glycerophosphate dehydrogenase, acetyl-CoA carboxylase, glucose transporter type 4, insulin receptor, and acetyl selective fatty acid binding protein (aP2) [17].

Thus, adipogenesis and osteogenesis are regulated by complex factor cascades, and the role of RNA-dependent epigenetic regulation is extremely important. MicroRNAs form interaction networks that provide additional control over biological processes. In regenerative medicine, MSCs are used in alternative therapies for the treatment of obesity [38,39] and bone regeneration [17]. Understanding the regulatory pathways involved in cell commitment and investigating the factors that regulate the balance between osteogenic and adipogenic differentiation in MSCs are of great importance for the theoretical understanding of the pathogenesis of osteoporosis in obesity and will enable the

identification of molecular targets for the treatment of osteoporosis in the future.

3. MicroRNAs and the Development of Osteoporosis in Obesity

3.1 MicroRNAs that Regulate Adipogenesis in Bone Marrow

MicroRNAs may control the transition processes between the osteogenic and adipogenic differentiation of MSC-BMs [17]. An analysis of data reported in the scientific literature showed increases in the levels of miR-222, miR-140-5p and miR-142-5p, miR-142-3p [40], miR-143-3p [41], miR-217 [42], and miR-16 [43] in serum/plasma and an increase in miR-221/222 only in women [44].

In an animal model of obesity, the levels of miR-211, miR-130b, miR-188, and miR-128 were increased [45–48] (Table 1, Ref. [49–63]). Several microRNAs are known to induce BMSC adipogenesis by inhibiting the activity of the transcription factor *RUNX2*, which is involved in osteoblastogenesis. These microRNAs include miR-204, miR-211, miR-217, miR-221, and miR-222.

Notably, high miR-204 and miR-211 expression in BMSCs induces adipogenesis by decreasing the activity of the transcription factor *RUNX2* (Table 1). *RUNX2* deficiency leads to increased adipogenesis. As a result, researchers have observed increases in the expression of *PPAR γ* , *aP2*, and *adipsin* genes in ST2 cell cultures with miR-204 and miR-211 [49] (Table 1).

Notably, miR-130a can regulate BMSC differentiation in both the adipogenic or osteogenic directions. Bioinformatics analysis revealed that *Smurf2* and *PPAR γ* are targets of miR-130a [52]. The age-related decline in miR-130 expression leads to the differentiation of BMSCs in the adipogenic direction and is associated with age-related bone loss.

Zhu *et al.* (2017) [50] showed that miR-217 expression was negatively associated with bone formation and the osteogenic differentiation of BMSCs in rats because this miRNA inhibited *RUNX2* activity. This molecular mechanism was mediated by the ERK and p38 MAPK signaling pathways (Fig. 1).

The ERK signaling pathway is involved in the insulin-like growth factor receptor (IGF)-1-mediated osteogenic differentiation of BMSCs. Therefore, increased expression levels of miR-221-3p and miR-222-5p against a high-glucose concentration background were associated with the suppression of BMSC osteogenic differentiation via the IGF-1/ERK pathway [51] (Table 1) (Fig. 1).

In patients with osteoporosis, the miR-128 level was shown to be significantly increased compared to that in patients without osteoporosis [53] (Table 1). The authors suggested that miR-128 inhibits osteoblast differentiation by suppressing *sirtuin (SIRT) 6* expression, thus accelerating osteoporosis development (Fig. 1). Studies of C2C12 cells transfected with short interfering RNA against *SIRT6* (si-

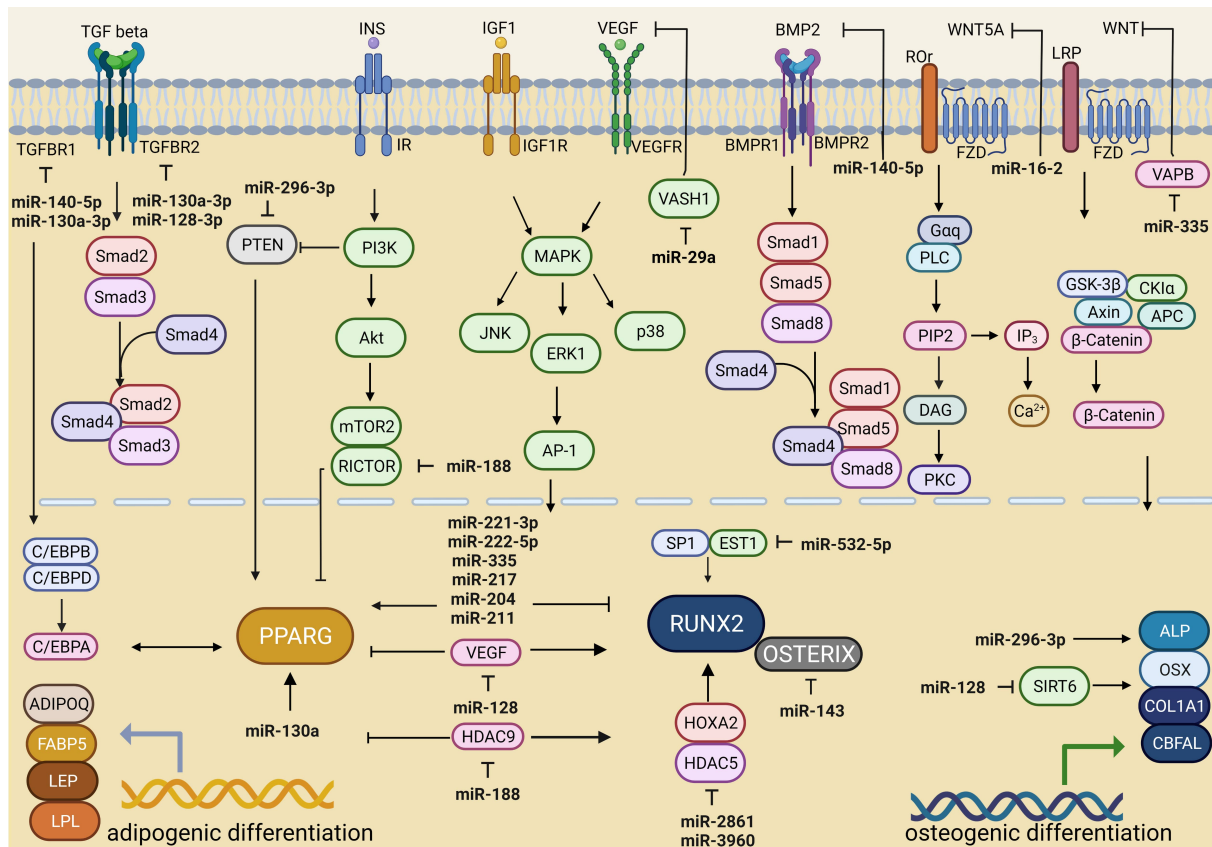


Fig. 1. miRNA-dependent regulation of signaling pathways associated with adipogenic and osteogenic differentiation. Created with BioRender.com.

SIRT6) showed decreases in osteocalcin mRNA and protein expression, alkaline phosphatase (ALP) activity, and collagen I type- $\alpha 1$ (COL1A1) secretion during osteoblast differentiation, indicating the involvement of SIRT6 in osteogenesis. Zhang *et al.* [54] showed that the addition of miR-128 mimetics promoted adipogenic differentiation and inhibited osteogenic differentiation in human MSCs (hMSCs) by suppressing vascular endothelial growth factor (VEGF) signaling pathway activation (Table 1) (Fig. 1).

TGF- β signaling inhibits adipogenesis *in vitro* and *in vivo* [64] (Fig. 1). Adipogenesis/osteogenesis is controlled by the direct association of *transforming growth factor receptor 1* (TGFR1) and *WNT11* with miR-140-5p [55] (Fig. 1). Culturing primary bone marrow stromal cells for 72 hours in adipogenic medium (AIM; α -MEM with 10% fetal bovine serum (FBS), 0.5 μ M dexamethasone, 0.25 μ M methylisobutylxanthine, 5 μ g/mL insulin, and 50 μ M indomethacin) induced higher miR-140-5p expression than culturing in osteogenic medium (OIM; α -MEM with 10% FBS, 50 μ g/mL ascorbic acid, and 5 mmol/L β -glycerophosphate). Moreover, miR-140-5p promoted adipocyte differentiation by increasing C/EBP mRNA levels via TGFR repression [55]. MiR-130a-3p can inhibit TGF- β /Smad signaling, which occurs partially via TGFR1 and TGFR2 [65].

In another study, miR-140-5p was shown to reduce the activity of molecules involved in the BMP signaling pathway, including *BMPR2*, *BMPR1B*, and *SMAD5*, and prevent the osteogenic differentiation of undifferentiated hMSCs [56] (Table 1) (Fig. 1).

In patients with osteoporosis, the expression level of miR-16-2 was significantly increased in bone tissue and was associated with bone resorption factors and osteoblast differentiation process inhibition [57] (Table 1). The *WNT5A* gene is a target of miR-16-2-3p (miR-16-2*), and this mRNA-miRNA interaction blocks WNT signaling and leads to adipogenesis induction mediated through *PPAR* γ activation [66] (Table 1).

High expression of miR-143 has been found in BMSCs during osteoporosis and has been associated with adipogenesis [58,67]. A common target between miR-143 and miR-145 is the *OSX* gene, which is critical for BMSC osteogenic differentiation [59,60,67] (Table 1).

An increase in the level of miR-188 in BMSC mediates bone loss and promotes adipocyte accumulation during aging [61]. *In vitro* studies showed that miR-188 inhibited histone deacetylase 9 (*HDAC9*) and *rapamycin-insensitive companion of mammalian target of rapamycin* (*RICTOR*) expression and upregulated *PPAR* γ expression in MSCs (Fig. 1). *HDAC9* inactivates *PPAR* γ , suppresses adipog-

Table 1. miRNAs that regulate the switching of adipogenic and osteogenic differentiation and their target genes and signaling pathways.

№	miRNAs and target cells	Target gene	Signaling pathway	Cell effect	miRNA levels in bone tissue	References
1	↑miR-204 miR-211 rat BMSCs, ST2 cells	<i>RUNX2</i>	activation of PPAR pathway (gene <i>PPARγ</i>)	enhancement of adipogenic differentiation	-	[49]
2	↑miR-217 rat BMSCs	<i>RUNX2</i>	downregulation of MAPK/ERK pathway	suppression of osteogenic differentiation	-	[50]
3	↑miR-221-3p miR-222-5p rat BMSCs with high glucose	<i>RUNX2</i>	activation IGF-1/ERK pathway	suppression of osteogenic differentiation	↑mandibles of diabetic rats (diabetic rat model)	[51]
4	↓miR-130a mouse and human BMSCs		activation of PPAR γ pathway	enhancement of adipogenic differentiation	↓old mice	[52]
5	↑miR-128 C2C12 cell line	<i>SIRT6</i>	-	enhancement of adipogenic differentiation and suppression of osteogenic differentiation	↑patients with osteoporosis	[53]
6	↑miR-128 human BMSCs	<i>VEGF</i>	downregulation of VEGF pathway			[54]
7	↑miR-140-5p mouse BMSCs in adipogenic medium <i>in vitro</i>	<i>TGFBR1</i>	increased mRNA gene <i>C/EBPs</i>	enhancement of adipogenic differentiation	-	[55]
8	↑miR-140-5p human BMSCs	<i>BMP2</i>	downregulation of BMP2-mediated pathway	inhibition of osteogenic commitment	-	[56]
9	↑miR-16-2 human BMSCs	<i>WNT5A</i>	downregulation of WNT pathway activation of pathway PPAR γ	enhancement of adipogenic differentiation	↑patients with osteoporosis	[57]
10	↑miR-143, miR-145 human BMSCs MC3T3-E1 cell line	<i>Osx</i>	-	suppression of osteogenic differentiation	↑postmenopausal osteoporosis	[58–60]
11	↑miR-188 <i>in vitro</i>	<i>HDAC9</i> <i>RICTOR</i>	increased mRNA of gene <i>PPARγ</i>	age-related bone loss enhancement of adipogenic differentiation	↑aged mice (18 months old)	[61]
12	↑miR-532-5p MC3T3-E1 cell line	<i>ETS1</i>	downregulation of <i>RUNX2</i> pathway	suppression of osteogenic differentiation	↑patients with osteoporosis	[62]
13	↓miR-29a <i>in vitro/in vivo</i>	-	downregulation p21 pathway (gene <i>Oxr1</i>) downregulation of FOXO pathway (gene <i>FoxO3</i>)	enhancement of adipogenic differentiation and suppression of osteogenic differentiation	↓patients with osteoporosis animal model	[63]

RUNX2, runt-related transcription factor 2; SIRT6, sirtuin 6; VEGF- vascular endothelial growth factor; TGFBR1- transforming growth factor beta receptor 1; BMP2, bone morphogenetic protein 2; WNT5A, Wnt family member 5A; OSX, osterix; HDAC9, histone deacetylase 9; RICTOR, RPTOR-independent companion of MTOR complex 2; ETS1, ETS proto-oncogene 1, transcription factor; FoxO3, forkhead box O; IGF-1, insulin-like growth factor 1; ERK, extracellular signal-regulated kinase.

enesis, and stimulates MSC osteogenesis [68] (Fig. 1). The RICTOR component of mammalian target of rapamycin (mTOR) complex 2, which is a member of the mTOR complex, suppresses PPAR γ activity and inhibits the adipogenic differentiation of MSCs [69] (Fig. 1).

The development of obesity was associated with elevated levels of miR-143 in serum and adipose tissue [70]. Moreover, miR-204 [71], miR-296 [52], and miR-128 [72] were increased in visceral adipose tissue in the context of obesity. Specifically, the authors showed that in obesity, miRNA-21 expression was increased in visceral and subcutaneous adipose tissue but was decreased in serum [73,74] (Table 1).

However, in women, miR-130 [52] and miR-145-5p levels were found to be decreased in subcutaneous adipose tissue [75,76], indicating obesity (Table 1). The level of miR-145 was found to be suppressed against the background of chronic subclinical inflammation in the adipose tissue of the greater omentum in obese patients with/without type 2 diabetes [77] and in serum in the contexts of obesity [76] and osteoporosis [78] (Table 1).

In addition, low miR-532-5p expression was associated with obesity [79]; in contrast, high expression was found in individuals with low bone mineral density [62,80]. The target of miR-532-3p is the transcription factor *ETS proto-oncogene 1 (ETS1)* (Fig. 1). During osteoblast differentiation, synergistic interaction between the transcription factors ETS1 and transcription factor specificity protein 1 (SP1) promotes *RUNX2* expression [81] (Table 1). The overexpression of miR-532-3p in MC3T3-E1 cells indirectly reduced the expression of osteogenic markers (collagen 1, *RUNX2*, ALP, osteopontin, and osteocalcin) and resulted in the loss of bone mineral density [62] (Table 1). Therefore, considering these recent studies, we propose that a decrease in miR-532-5p expression against the background of obesity may contribute to the maintenance of bone integrity (Table 1).

These studies indicated that the aforementioned microRNAs (miRNA-204, miRNA-211, miRNA-217, miRNA-221, miRNA-222, miRNA-130/130a, miRNA-296, miRNA-140, miRNA-16-2/16-3p, miRNA-21, miRNA-143, miRNA-188, and miRNA-128) may control osteoresorption processes by activating BMSC adipogenesis, inhibiting key gene/transcription factor expression against a background of obesity and dysregulating normal osteogenesis (Table 1).

3.2 MicroRNAs that Promote the Osteoblastogenesis of BMSCs

MicroRNAs may exert direct impacts on osteoblast formation by regulating transcription factors and maintaining bone tissue homeostasis.

An increase in miR-296 expression can trigger the osteogenic differentiation of MSCs (osteoblastogenesis) and decrease the activity of the transcription factor *Pten*, which

is critical for BMSC differentiation into adipocytes [82] (Table 2, Ref. [82–89]). In a human osteoblast cell line (hFOB 1.19 cells), the overexpression of miR-296 contributed to a significant increase in matrix mineralization, ALP activity, osteocalcin expression, and increased *core-binding factor al (Cbfa1)* expression [83] (Table 2).

The mechanism of action of miR-2861 and miR-3960 in the same locus is related to increased *RUNX2* transcription factor expression and repressed activity of its inhibitors *histone deacetylase 5 (HDAC5)* and *homeobox A2 (HOXA2)*, leading to osteoblast differentiation [84,85] (Table 2).

Low levels of miR-2861 have been found in the bone tissue of patients with osteoporosis [85] (Table 2) and in the subcutaneous adipose tissue of obese patients [90].

A recent study by Abazari *et al.* (2020) [91] showed that miR-2861-transduced induced pluripotent stem cells (iPSCs) exhibited high viability, mineralization levels, alkaline phosphatase activity, calcium contents, and expression of genes related to bone formation compared with untransduced iPSCs. The combined use of miR-2861-transduced iPSCs with a poly(lactic-co-glycolic acid) (PLGA) nanofiber scaffold and an artificial extracellular matrix had a positive effect on the osteogenic differentiation potential of iPSCs; therefore, this strategy can be used in bone tissue engineering.

No information was found on the dynamics of changed miR-3960 levels against the background of obesity. However, in obese patients with type 2 diabetes, the exosomal level of miR-3960 was significantly higher than those in patients with type 2 diabetes and healthy donors [92]. Previously, Ding *et al.* (2015) [93] demonstrated a decrease in hsa-miR-3960 in the serum of patients with type 2 diabetes.

miR-335 is the most highly expressed microRNA in BMSCs [94], but its expression decreases during the osteogenic differentiation of MSCs [95]. Hu *et al.* (2021) [86] demonstrated that the introduction of extracellular vesicles containing miRNA-335 derived from BMSCs promoted osteoblast differentiation in cell models and recovery after fractures in an animal model by suppressing the expression of the *vesicle-associated membrane protein-associated protein B (VapB)* gene (Table 2). The overexpression of *VapB* inactivates the WNT/ β -catenin signaling pathway and inhibits osteogenic differentiation [86] (Fig. 1). Previously, Tomé *et al.* (2011) [88] found that the overexpression of miR-335 impaired the proliferation and migration abilities of primary hBMSCs and inhibited both adipogenic and osteogenic differentiation, suggesting that miR-335 is part of a common regulatory pathway involved in the proliferation, migration, and differentiation of MSCs (Table 2).

Associations have been established between the loss of microRNA-29a and markers of oxidative stress (8-hydroxydeoxyguanosine (8-OHdG), DNA hypermethylation (5-methylcytosine (5mC)) and osteoblast aging in human osteoporosis because these processes are regulated by

Table 2. miRNAs that regulate enhanced osteogenic differentiation and their target genes and signaling pathways.

No	miRNAs and target cells	Target gene	Signaling pathway	Cell effect	miRNA levels in bone tissue	References
1	↑miR-296-3p BMSCs	<i>PTEN</i>	negative regulation of PPAR γ pathway	enhancement of osteogenic differentiation		[82]
2	↑miR-296-3p hFOB 1.19 cell line	-	increased mRNA for genes <i>CBFAL</i> <i>OSX</i> <i>COL-1</i>	positive effect on differentiation, migration, invasion and proliferation of osteoblasts, negative effect on cell apoptosis	↓osteoporotic mice model	[83]
3	↑miR-2861 miR-3960 ST2 cell line	<i>HDAC5</i> <i>HOXA2</i>	activation of transcription factor <i>RUNX2</i>	enhancement of osteogenic differentiation	↓patients with osteoporosis	[84,85]
4	↑ex-miR-335 <i>in vitro</i> <i>in vivo</i>	<i>VAPB</i>	activation of Wnt/ β -catenin pathway	enhancement of osteogenic differentiation, bone fracture repair	-	[86,87]
5	↓miR-335 human BMSCs	<i>RUNX2</i>	-	enhancement adipogenicof osteogenic differentiation	↓bone marrow aspirates from patients with osteoarthritis	[86,88]
6	↑ ex-miR-29a BMSCs <i>in vitro</i> <i>in vivo</i>	<i>VASH1</i>	-	stimulation of angiogenesis and osteogenesis	-	[89]

RUNX2, runt-related transcription factor 2; OSX, osterix; PTEN, phosphatase and tensin homolog deleted on chromosome 10; HDAC5, histone deacetylase 5; HOXA2, homeobox A2; VapB, vesicle-associated membrane protein-associated protein B/C; VASH1, vasohibin 1; CBFAL, core-binding factor al; Col-1, collagen type I.

oxidation resistance protein-1 (Oxr1) and *forkhead box O (FoxO3)* gene products [63] (Table 1).

miR-29a in exosomes derived from MSC-BMs promoted angiogenesis and osteogenesis *in vitro* and *in vivo*. The *vasohibin 1 (VASH1)* gene, which is considered a negative regulator of angiogenesis, was identified as a direct target of exosomal miR-29a [89] (Table 2). Another study showed that miR-29a overexpression disrupted adipogenesis [96]. However, miR-335, miR-218, and miR-29a levels increased with obesity [97–99].

Thus, increasing the levels of miR-296-3p, miR-2861, miR-3960, miR-335-5p, and miR-29a in the MSC-BM microenvironment may normalize/activate osteogenesis processes and prevent bone loss in osteoporosis.

3.3 MicroRNAs that Regulate Osteoclastogenesis and Osteoresorption

Osteoporosis is characterized by an increase in osteoclastogenesis associated with bone resorption. Against a background of obesity, a significant fraction of miRNA influences the rate of osteoclastogenesis, which leads to excessive osteolysis. Inflammation plays an important role in the induction of osteoclast differentiation [28].

Key microRNAs involved in osteoclast differentiation have been identified in RAW264.7 and 293T mouse monocyte/macrophage lines. The expression of miR-199b-5p, miR-10a-5p, miR-10b-5p, miR-24-1-5p, miR-223-3p, and miR-338-3p was suppressed, whereas miR-291a-5p, miR-125b-1-3p, and miR-291b-5p expression was enhanced

(Table 3, Ref. [100–108]) (Fig. 2).

Further studies showed that the overexpression of miR-338-3 restricted osteoclast formation by regulating *IKK β* expression. Thus, miR-338-3p might regulate osteoclast formation because the inhibitor of nuclear factor kappa-B kinase genes (*IKBB*, *IKK β* , *IKKBK* and *IKK2*) are key in the NF- κ B signaling pathway (Table 3) (Fig. 2).

The level of miR-338-3p is increased in the serum of women with postmenopausal osteoporosis; however, it is decreased in obese patients [100,101] (Table 3).

Numerous studies have shown that miR-128 is involved in the regulation of signaling pathways associated with osteoclastogenesis [102,109] and with reactive oxygen species, inflammation, and fibrosis in many tissue types [109]. In HK2 cells, miR-128-3p was shown to inhibit apoptosis and inflammation by targeting the *TGFBR2* gene [110]. Another target of miR-128-3p is *MyD88*, which activates NF- κ B [111]. According to the results of the associated study, the overexpression of miR-128 suppressed the inflammatory response induced by *S. aureus* in RAW264.7 macrophages by reducing *MyD88*.

In contrast, Shen (2020) [112] showed that the miR-128/SIRT1/NF- κ B signaling axis may regulate osteoclast differentiation. The authors of one study showed that *SIRT1* was a target of miR-128. Increased *SIRT1* levels inactivated NF- κ B signaling by decreasing the acetylation of lysine 310 acetyl-NF- κ B p65 (Lys310), thereby inhibiting osteoclastogenesis (Table 3). In bone samples from women with postmenopausal osteoporosis and mouse bone mar-

Table 3. MiRNAs that regulate osteoclast differentiation and their target genes and signaling pathways.

No	miRNAs and target cells	Target gene	Signaling pathway	miRNA levels in bone tissue	References
1	↑miR-338-3p RAW264.7, 293T cell line	<i>IKKβ</i>	activation of NF-κB pathway	↑osteoporosis in OVX mice	[100,101]
2	↑miR-128 bone marrow macrophages	<i>SIRT1</i>	activation of NF-κB pathway	↑bone samples from postmenopausal osteoporosis osteoporosis-model mice	[102]
3	↑miR-483-5p CD14+PBMC cell line	<i>IGF2</i>	activation of osteoclastogenesis	↑patients with osteoporosis	[103]
4	↑miR-133a RAW264.7, THP-1 cell line	-	activation of osteoclastogenesis	-	[104]
5	↑miR-151a-3p RAW264.7, THP-1 cell line	-	increased <i>NFATc1</i> , <i>c-Fos</i> , <i>TRAP</i> mRNA	-	[105]
6	↓miR-125a-5p RAW 264.7 cell line	<i>TNFRSF1B</i>	activation of NF-κB pathway activation of osteoclastogenesis	↑patients with osteoporosis	[106,107]
7	↑ex-miR-143 ex-miR-145 mice BMSCs	<i>Cd226</i> <i>Srgap2</i>	activation of osteoclastogenesis	-	[108]

IkBβ, inhibitor of nuclear factor kappa B; *SIRT1*, sirtuin 1; *IGF2*, insulin-like growth factor 2; *TNFRSF1B*, TNF receptor superfamily member 1B; *Cd226/Dnam*, 1DNAX accessory molecule-1; *Srgap2*, SLIT ROBO Rho GTPase-activating protein 2; *NFATc1*, nuclear factor of activated T cells 1; *c-Fos/AP-1*, activating protein-1; *TRAP*, triiodothyronine receptor auxiliary protein.

row macrophages (BMMs), miR-128 levels were increased [112] (Table 3) (Fig. 2). Thus, an increase in miR-128 is associated with osteoclast activation. Given the multidirectional effect of miR-128 on the regulation of inflammation, further studies are needed to evaluate the possibility of using microRNAs in the treatment of osteoporosis.

High expression of miR-483-5p and low levels of *IGF2* were found in blood serum and bone tissue in patients with osteoporosis. Moreover, an increase in miR-483-5p promoted the differentiation of CD14+ PBMCs into osteoclasts [103] (Table 3) (Fig. 2). In recent studies, miR-483-5p was positively correlated with the BMI value and associated with a poor prognosis and an elevated risk of developing diabetes mellitus and cardiovascular disease [113,114].

In women with postmenopausal osteoporosis, the levels of miR-133a and miR-151a-3p were increased in blood serum and negatively correlated with bone mineral density [104,105] (Table 3). The overexpression of miR-133a and miRNA-151a-3p activated the RANKL-induced differentiation of RAW264.7 and THP-1 cells (murine monocyte/macrophage lines) into osteoclasts (Fig. 2).

In subcutaneous adipose tissue, an increase in miR-133a levels was detected against a background of obesity [115]. However, Oliveira *et al.* (2020) [116] showed that miR-133a levels were reduced in subcutaneous adipose tissue. Specifically, the microRNA-151a-3p level was reduced in the subcutaneous adipose tissue of obese women [74] and in the serum of obese patients [117] (Fig. 2).

The target of miR-125a-5p is the *tumor necrosis factor ligand superfamily member 1B* (*TNFRSF1B*) gene. The overexpression of miR-125a-5p indirectly inhibited osteoclast differentiation and functional activity by decreasing

the expression of *TNFRSF1B* [107] (Table 3). MiR-125a-5p was found in serum MSCs derived from the bone marrow of patients with osteoporosis [118,119] and was actively increased in osteoblasts and osteoclasts [106] in subcutaneous adipose tissue in obesity and type 2 diabetes [120], and these findings were associated with bone fragility in women [121] (Fig. 2).

The expression of miR-143-145 was significantly increased in the MSCs of postmenopausal women compared with that in the MSCs of premenopausal women. The authors demonstrated that miR-143/miR-145 migrated to osteoclasts through extracellular vesicle transport and that these miRNAs triggered osteoclast activity by targeting *Cd226* and *Srgap2* [108] (Table 3) (Fig. 2).

An increase in TNF-α levels in obesity negatively affects the expression of miR-143, which is induced during adipogenesis [58]. miR-143 is involved in signal transduction through extracellular *signal-regulated kinase 5* (*ERK5*) and functions as a positive regulator of human adipocyte differentiation [122].

The overexpression of miR-145 contributes to the activation of the NF-κB pathway and to increases in the proinflammatory factors TNF-α, IL-1, CCL-2, CCL-4, and CCL-7 [123] by activating NF-κB, p-IκBα, p-STAT3, and ac-p65 *in vivo* and *in vitro*.

The anti-inflammatory role of miR-145 was demonstrated by Li *et al.* (2018) [77]. The authors found that in human adipose tissue, the expression of *ADP ribosylation factor 6* (*Arf6*) is increased and that the amount of miR-145 is reduced in patients with obesity and type 2 diabetes. Studies in the THP-1 cell line have shown that miR-145 inhibits the secretion of proinflammatory factors by reduc-

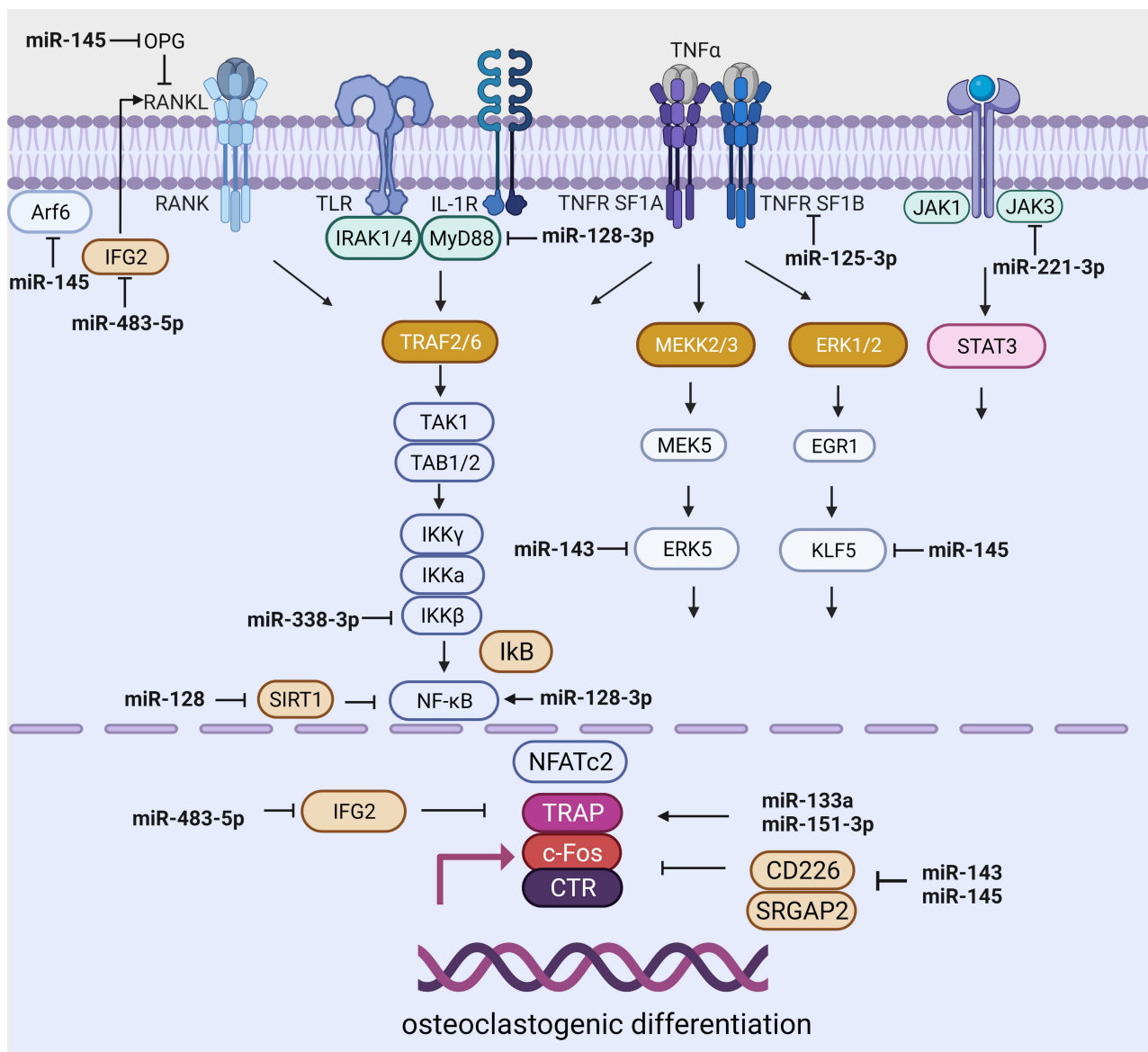


Fig. 2. miRNA-dependent regulation of signaling pathways related to osteoclast differentiation. Created with BioRender.com.

ing *Arf6* expression. He *et al.* (2020) [124] showed that miR-145-based therapy was associated with a significant reduction in inflammatory status. Accordingly, the overexpression of miR-145 reduces inflammation by targeting the *OPG* and *Kruppel-like factor 5 (KLF5)* genes.

miR-222 is a component of the miR221/222 cluster, whose members are located on the X chromosome (XP113) [125]. An increase in miR-222 levels has been associated with inflammation [126,127].

Thus, an increase in blood serum miR-222 levels was positively correlated with the production of proinflammatory cytokines and the concentration of high-sensitivity CRP in patients with calcified aortic valves [126].

Significantly increased levels of miR-221-3p in the synovial membrane of patients with rheumatoid arthritis and osteoarthritis lead to a decrease in the anti-inflammatory response by directly targeting the *Janus ki-*

nase 3 (JAK3) gene, transforming M2 macrophages to the pro-inflammatory M1 type [128]. In a mouse model of rheumatoid arthritis, the TNF- α -induced secretion of miR-221-3p in synovial fibroblast exosomes was shown to negatively affect osteoblast differentiation and promote bone loss [129].

The aforementioned microRNAs (miR-338-3p, miR-483-5p, miR-133a, miR-151a-3p and miR-125a-5p) can enhance osteoresorption, leading to the disruption of bone tissue homeostasis by inhibiting osteogenesis through various molecular mechanisms (Fig. 2). The roles of miR-128 and miR-145 in regulating osteoclastogenesis are unclear and require further research.

Table 4. miRNAs as diagnostic markers for osteoporosis.

No	miRNAs	Material studied	Diagnosis	References
Decreased microRNA levels				
1	miR-30b-5b, miR-103-3p, miR-142-3p, miR-328-3p	serum	osteoporosis in postmenopausal women, osteopenia, osteoporosis	[135]
2	miR-27	serum	osteoporosis in postmenopausal women	[136]
3	miR-491-5p, miR-485-3p	plasma	osteoporosis in postmenopausal women, vertebral fractures	[137]
4	miR-328-3p, miR-22-3p, let-7g-5p	serum	osteoporosis in postmenopausal women, fractures	[138]
5	miR-29a-3p, miR-23a-3p, miR-21-5p	serum		[139]
6	miR-144-3p	serum		[140]
7	miR-21-5p, miR-122-5p, miR-125b-5p	serum	osteoporosis, acute hip fractures	[141]
8	miR-181c-5p, miR-497-5p	serum	osteoporosis in postmenopausal women	[142]
9	miR-550a-3p, miR-532-5p, miR-378a-5p miR-365a-3p, miR-324-3p, miR-215-5p, miR-186-5p miR-140-5p, miR-93-5p, miR-30e-5p, miR-29b-3p miR-19a-3p, miR-19b-3p, miR-16-5p, miR-7-5p let-7b-5p	serum	secondary osteoporosis (idiopathic osteoporosis, osteoporosis in postmenopausal women), fractures	[87]
10	miR-4516, miR-122-5p	serum	secondary osteoporosis, osteopenia	[78]
11	miR-203	serum		[143,144]
12	miR-17, miR-20a, miR-21, miR-29a	serum	osteoporosis	[145]
13	miR-19a-3p	serum		[146]
14	miR-122-5p, miR-4516	serum		[78]
15	miR-122-5p	serum	osteoporosis in postmenopausal women	[147]
Elevated microRNA levels				
1	miR-133a	serum	osteoporosis in postmenopausal women	[136]
2	miR-223-3p, miR-148a-3p, miR-125b-5b, miR-124-3p miR-122-5p, miR-100-5p, miR-27a-3p, miR-25-3p miR-24-3p, miR-23-3p, miR-21-5p	serum	osteoporosis	[119]
3	miR-148a-3p, miR-125b-5p, miR-124-3p, miR-122-5p miR-100-5p, miR-93-5p, miR-24-3p, miR-23a-3p, miR-21-5p	serum		[106]
4	miR-125b-5p, miR-122-5p, miR-21-5p	serum		[121]
5	miR-125, miR-30, miR-5914	serum	osteoporosis in postmenopausal women, fractures	[148]
6	miR-125	serum		[119]
7	miR-2861, miR-124-3p	serum		[139]
8	miR-145, miR-125b, miR-122a, miR-100, miR-27a-3p miR-24-3p	serum		[140]
9	miR-133a	serum		[104]
10	miR-532-3p, miR-375, miR-335-5p, miR-152-3p miR-133b, miR-127-3p, miR-23a-3p, miR-21-5p miR-19b-3p	serum	fractures, low bone mineral density, patients receiving treatment for osteoporosis	[149]
11	miR-23b-3p, miR-140-3p	serum	osteoporosis, acute hip fractures	[141]
12	miR-148a	serum	osteoporosis in postmenopausal women	[147]
13	miR-335-5p, miR-320a, miR-152-3p	serum	secondary osteoporosis (idiopathic osteoporosis, osteoporosis in postmenopausal women), fractures	[87]
14	miR-579-3p	serum	osteoporosis	[13]
15	miR-410	serum	osteoporosis in postmenopausal women	[150]
16	miR-100	plasma		[151]
17	miR-375	serum	osteoporosis	[152]
18	miR-208a-3p, miR-155-5p, miR-637	serum		[153]

4. Clinical Potential of microRNAs for the Treatment of Osteoporosis in Obesity

The long-term use of drugs for the treatment of osteoporosis is challenging because of a number of limita-

tions, including severe gastrointestinal side effects, thromboembolism, and increased risk of sarcoma [130]. In this regard, new and safe therapeutic approaches are needed to correct this pathology. MicroRNAs are antagonists of

gene expression and can be used to treat osteoporosis and osteoporotic fractures [130]. Notably, existing drugs for the treatment of osteoporosis (e.g., teriparatide and denosumab) may alter the microRNA expression profile. For example, in women with osteoporosis, teriparatide decreased the expression levels of miR-133a and miR-33, and after treatment with denosumab, increases in the expression levels of miR-503 and miR-222 as well as decreases in the activity of the genes related to osteoclastogenesis, namely, *RANK* and *cathepsin K* (*CTSK*), were observed [131].

In the classical approach, microRNAs are used to restore the expression of a missing microRNA. This outcome is often achieved through the use of expression constructs delivered through viral vectors [132].

On the other hand, gene expression can be controlled in other ways. Certain approaches inhibit pathological microRNA expression. The loss of miRNA function can be achieved by using microRNA sponges, microRNA masks, and anti-microRNA oligonucleotides (anti-miRs) [130].

Different types of chemical modifications are used to introduce anti-miR effects [130]. For example, miR-31, miR-106a, and miR-148a inhibit MSC-BM differentiation in osteoblasts by directly targeting *RUNX2* [133] (Table 1). After *in vitro* transfection of anti-microRNA into cells, osteogenic gene expression and ALP activity were increased [133]. Chorionic cells and placental cells were used as alternative sources of MSCs in this study [134]. In addition, inhibition of the expression of specific microRNAs increased the ability of MSCs to undergo osteogenic differentiation culture *in vitro*, and this strategy can be used to stimulate bone regeneration.

Targeting osteoporosis marker genes for the development of microRNA-based therapies seems to be a promising clinical direction. In addition, curbing excessive osteoclastogenesis can be achieved through the search for molecular switches that direct BMSC differentiation from favoring adipogenesis to favoring osteogenesis [130]. However, no microRNA-based therapy that can be successfully used in the clinic has been found. We analyzed the literature to identify microRNAs with high potential for use as diagnostic markers of osteoporosis, which may also serve as a basis for the development of oligonucleotide therapeutic approaches in the future (Table 4, Ref. [13,78,87,104,106,119,121,135–153]).

Clinical trials for the use of microRNAs have been performed to study osteoporosis in women [154], men [155], hyperparathyroid patients with and without osteoporosis, and osteoporosis patients undergoing thyroidectomy [156]. MicroRNAs have been studied as potential biomarkers of coronary artery calcification (CAC), and their relationships with bone mineral density and use as markers of bone metabolism have been investigated [157]. Biomarkers (including microRNAs) in orthopedic and maxillofacial surgery patients have been sought to identify risks associated with bisphosphonate use [158]. A study on the

relationships between the gut microbiota and bone microarchitecture has also been conducted [159].

In addition, a study on bone-specific microRNAs in the blood serum of postmenopausal women with osteoporosis against a background of antiresorptive or osteoanabolic therapy has been performed. The results are expected to be reported after the end of the study in 2022 [160].

A number of clinical trials have been performed to test oligonucleotide therapies, including treatment with antisense oligonucleotides [161–164].

However, despite the many approaches used to stabilize oligonucleotides in cells, oligonucleotide bioavailability is still low, and their efficient delivery to target organs other than the liver remains a challenge [165]. Increasing the concentration of administered oligonucleotides can lead to toxic side effects [166]. Therefore, methods for the delivery of oligonucleotides via exosomes are under development for future application [167].

5. Conclusions

Osteoporosis remains an incurable and serious multifactorial disease. The relationship between osteoporosis and obesity and the regulation of osteoclast activity, in particular, is detailed in a review by Roy *et al.* (2016) [9]. Recently discovered miRNAs involved in the epigenetic control of mRNA play important roles in regulating the adipogenic/osteogenic differentiation of BMSCs and osteoclastogenesis [168–170].

In the present review, we summarize recent findings on the regulatory interactions between mRNAs and miRNAs in the regulation of BMSC differentiation and the activation of osteoclastogenesis in obesity and osteoporosis. We hypothesize that miR-122, miR-21, miR-125, miR-23-3p, miR-140, miR-2861, and miR-133 have high potential for use in the diagnosis of osteoporosis. However, in view of the large body of work devoted to the search for biomarkers of pathologies, this finding remains to be confirmed because the changes in miRNA levels in blood serum/plasma have multidirectional dynamics.

Many studies show the promising possibility of using short-stranded noncoding RNAs for therapeutic purposes [171,172]. The complex regulation of the expression of genes important for the balance between osteogenesis and osteolysis gives us hope for the development of a safe therapy for osteoporosis. To reduce the severity of osteoporosis, treatment should ideally target the following: the hypoactivation of osteoclasts, the restoration of osteoblast function, the prevention of adipogenic differentiation in BMSCs, and the restoration of bone perfusion. In this regard, to reduce the severity of adipogenesis in BMSCs, we suggest therapy with inhibitors, such as miR-204, miR-211, miR-217, miR-221, miR-222, miR-128, miR-140, miR-16, miR-188, miR-143, miR-145, and miR-532. The positive regulation of osteoblastogenesis in BMSCs can be achieved by increasing the levels of miR-296-3p, miR-2861, miR-3960, miR-335, and miR-29a mimetics in the BMSC microenvironment.

Perhaps, to inhibit osteoclast hyperactivation, therapy with miR-483 inhibitors or miR-338, miR-133a, miR-151, and miR-125 mimetics should be considered. The ambiguous roles of miR-128 and miR-145 in the regulation of osteoclasts require further research.

The relevance of the search for targeted miRNAs stems from the fact that promoting the reparative regeneration of bone tissue (osteosynthesis) under its pathological remodeling conditions in osteoporosis is an unsolved clinical problem. Among the numerous available drugs, BMPs have direct bone tissue regenerative properties [173]. However, when administered systemically, they cause numerous side effects (including bone resorption) in 20–70% of cases, which negates the benefit of their specific osteogenic activity [174]. An obstacle to the widespread use of BMPs is also their high price (due to high costs in the research and development phase), which is disproportionate to the therapeutic efficacy of the drugs.

In this context, the therapeutic use of miRNAs, which modulate the (post)transcriptional activity of genes at the mRNA level, is considered a promising new biotechnological direction in regenerative medicine. However, a number of unresolved issues remain, especially regarding the potential side effects caused by (multiple) crossover effects of microRNAs on intracellular signaling pathways, which may lead to unpredictable responses in cells of nontarget tissues and organs in the long term.

Therefore, the development of local systems for the targeted delivery and release of microRNAs (capsules of different sizes, nanoparticles, scaffolds, etc.) that overcome tissue barriers independently [175] or with the help of carrier cells (e.g., MSCs) [176] is an urgent task to be addressed in the near future.

Abbreviations

BMSCs, bone marrow mesenchymal stem cells; MSCs, mesenchymal stem cells; BMI, body mass index; TNF, tumor necrosis factor; IL, interleukin; UTRs, untranslated regions; RANKL, receptor activator of nuclear factor kappa-B ligand; WNT, Wingless-type MMTV integration site; BMP, bone morphogenic protein; RUNX, runt-related transcription factor; NF- κ B, nuclear factor- κ B; TGF, transforming growth factor; Smads, specific E3 ubiquitin protein ligase; JAK/STAT, Janus kinase/signal transducer and activator of transcription; MAPK, mitogen activated protein kinase; ERK, extracellular signal-regulated kinase; C/EBP β , CCAAT-enhancer-binding protein; PPAR γ , peroxisome proliferator activated receptor gamma; α 2, fatty acid binding protein; SIRT, sirtuin; ALP, alkaline phosphatase; VEGF, vascular endothelial growth factor; TGFBR1, transforming growth factor receptor 1; RICTOR, rapamycin-insensitive companion of mammalian target of rapamycin; mTOR, target of rapamycin; ETS, ETS proto-oncogene; Cbfa1, core-binding factor α 1; HDAC5, histone deacetylase 5; HOXA2, homeobox A2;

iPSCs, pluripotent stem cells; I κ B β , inhibitor of nuclear factor kappa-B kinase; TNFRSF1B, tumor necrosis factor ligand superfamily member 1B; anti-miR, anti-microRNA oligonucleotides.

Author Contributions

MV, NT, KY (Author A, C and D)—original draft preparation; IKh, LL, IK (Author B, F and J)—editing; MV, NT, KY, VM, DS, AK, OKh (Author A, B, C, D, E, G, H)—gathered information and performed material systematization; MV (Author A) visualization. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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