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Review Article

The regulatory roles of long noncoding RNAs in osteoporosis

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Abstract: Osteoporosis is a common metabolic bone disease characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue, which leads to decreased bone strength and increased fracture risk. Osteoporosis mainly results from a disruption of the balance between bone formation mediated by osteoblasts and bone resorption mediated by osteoclasts. At present, the molecular mechanisms underlying osteoporosis are still not fully understood. Long noncoding RNAs (lncRNAs) are RNA molecules that exceed 200 nucleotides (nt) in length and have limited or no protein-coding capacity. Over the past decade, numerous lncRNAs have been demonstrated to participate in multiple biological processes and to play essential roles in the pathogenesis of various diseases. In this review, we summarize recent progress in research on lncRNAs in osteoporosis and mainly focus on their regulatory roles in osteogenesis and osteoclastogenesis. Moreover, we briefly discuss the potential clinical applications of lncRNAs in osteoporosis.

Keywords: Long non-coding RNA, osteogenesis, osteoclastogenesis, osteoporosis, review

Introduction

Osteoporosis is a systemic and progressive disease characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue, resulting in a decrease in bone strength and an increase in fracture risk (referred to as osteoporotic fractures). The incidence rate of osteoporosis is remarkably correlated with age, especially in people aged >50 years [1]. At present, with a larger proportion of the population reaching an advanced age, osteoporosis is gradually considered one of the most important health problems of an aging society. According to statistics, almost one-fifth of men and one-third of women over the age of 50 years will experience osteoporotic fractures in their lives, and the most common sites of osteoporotic fractures are the forearm, hip, and lumbar spine [2]. Furthermore, it is estimated that more than 8.9 million fractures are caused by osteoporosis worldwide per year, which is associated with high morbidity and mortality [3, 4].

Bone remodeling is a continuous and dynamic process whereby old bone is removed by osteoclasts (bone-resorbing cells), and subsequently new bone is formed by osteoblasts (bone-forming cells), ultimately resulting in the renewal of the bone approximately every ten years [5]. Bone remodeling can repair microdamage to maintain a stable bone mass and optimize the shape and structure of the bone to allow it to better respond to mechanical loading. In people with osteoporosis, bone loss is the consequence of increased osteoclast activity and/or decreased osteoblast activity [6]. Many factors contribute to an individual's risk of primary osteoporosis, such as advanced age, hormone deficiency, increased oxidative stress, and genetic disorders [7-10]. Additionally, impaired kidney function, chronic obstructive pulmonary disease (COPD), some digestive system diseases, and the use of certain medications such as glucocorticoids can predispose individuals to secondary osteoporosis [7, 11-13]. At present, most existing drugs for osteoporosis can be classified into two types according to

their mechanisms of action: antiresorptive drugs such as bisphosphonates and denosumab, which slow down bone resorption and protect bone from further deterioration, and anabolic drugs such as teriparatide and romosozumab, which enhance bone formation and increase bone density and strength [14]. These drugs have been confirmed to provide certain benefits for patients with osteoporosis. However, patients receiving these drugs often experience various serious side effects. For instance, bisphosphonates are effective medications for metabolic bone diseases, but they are also responsible for potentially severe side effects, which include multiple myeloma, renal toxicity, atypical femoral fracture, and osteonecrosis of the jaw [15]. In addition, patients treated with bisphosphonates are at increased risk of esophageal cancer [16]. Romosozumab, a humanized sclerostin-targeting antibody that enhances bone formation and inhibits bone resorption, is the approved anabolic drug for osteoporosis [17]. However, many common side effects, such as wrist fracture, noncardiac chest pain, stroke, and heart attack, have been reported [18]. To date, all the available drugs for osteoporosis have serious side effects; therefore, finding novel therapeutic targets for osteoporosis is an urgent need.

Long noncoding RNAs (lncRNAs) are a novel class of noncoding RNAs (ncRNAs) that are more than 200 nucleotides (nt) in length and have limited or no protein-coding capacity [19]. They were initially considered to be "transcriptional noise" since they usually exhibit lower evolutionary conservation and lower expression levels than protein-coding transcripts (or mRNAs) [20, 21]. In recent years, with the development of RNA deep sequencing technology, numerous lncRNAs have been identified to be abundant in various species and involved in multiple biological processes, thus participating in the development and progression of many diseases, such as tumors, cardiovascular diseases, ocular diseases and metabolic diseases [22-26]. Importantly, some lncRNAs have great potential to act as biomarkers for disease diagnosis [27, 28]. In this review, we first introduce the classification, properties, functions, and mechanisms of lncRNAs. Then, we summarize up-to-date knowledge regarding the molecular regulation by lncRNAs in osteoblasts and osteoclasts. Finally, we also briefly discuss the potential applications of lncRNAs as biomarkers and therapeutic targets for osteoporosis.

Properties and classification of lncRNAs

Although approximately 80% of the human genome is transcribed, only 2% of the human genome is transcribed into mRNAs, suggesting that the vast majority of the human genome is transcribed into ncRNAs [21, 29]. According to the GENCODE database, one of the most comprehensive lncRNA annotation databases, approximately 15,779 lncRNAs in the human genome have been identified (https://www.gencodegenes.org/) [21]. Another annotation database, which is termed NONCODEV5, includes approximately 548,640 lncRNAs across 17 different species [30]. LncRNAs are defined as a wide variety of RNA transcripts longer than 200 nt and have limited or no protein-coding capacity [31]. Current studies have indicated that most lncRNAs are located in the nucleus, where they generally regulate gene expression by mediating epigenetic changes, such as DNA methylation, histone modification, and chromatin remodeling. In addition, a small percentage of lncRNAs are located in the cytoplasm, where they modulate mRNA stability and translation and interfere with posttranscriptional regulation [32]. LncRNAs have been called mRNA-like long-chain RNAs because there are several common properties between lncRNAs and mRNAs. For instance, lncRNAs are mostly transcribed by RNA polymerase II, which is similar to the mechanism of generation of mRNAs, but some non-polyadenylated lncRNAs can also be transcribed by RNA polymerase III [33, 34]. In addition, most lncRNAs have a 5'-cap and 3' poly(A) tail, which is similar to the structure of mRNAs [35]. Generally, in comparison to mRNAs, lncRNAs are less evolutionarily conserved and less abundant but exhibit more tissue-specific expression patterns [21, 36]. At present, there is not a unified criterion to classify lncRNAs. LncRNAs can be divided into different classifications according to diverse criteria, such as their genomic location, transcript length, mechanism of action, and subcellular localization [37]. A common classification of lncRNAs is based on their genomic location in relation to their adjacent protein-coding genes [37-39]. In this way, lncRNAs can be divided into the following five categories: (A) sense lncRNAs; (B) antisense lncRNAs; (C) intronic lnc-

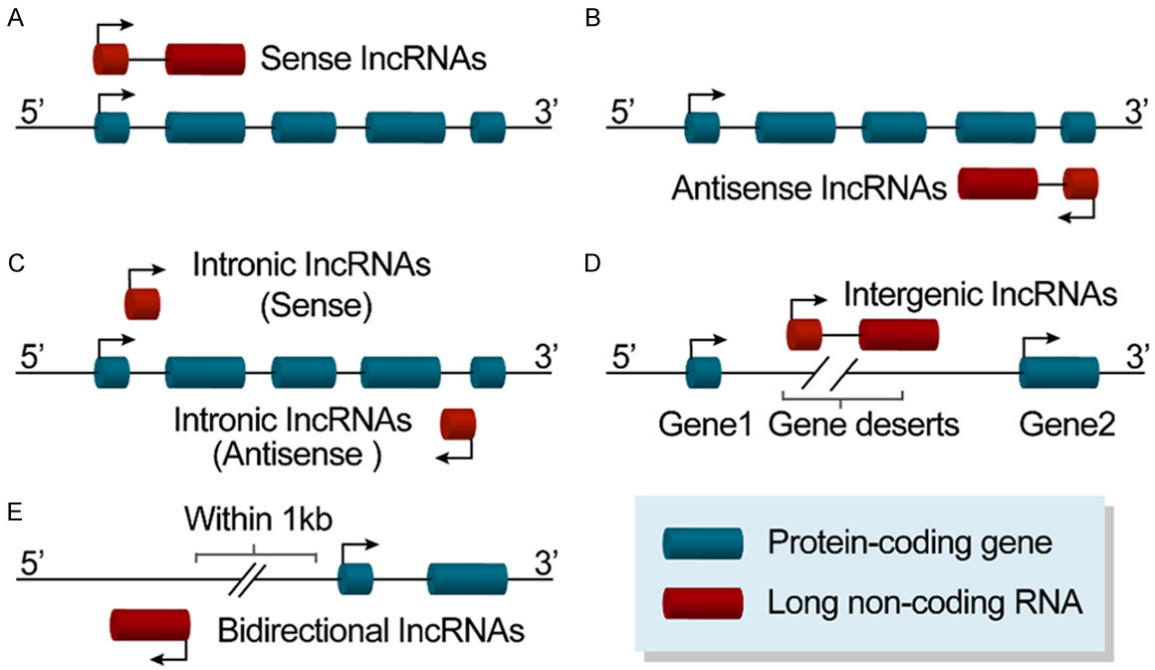

Figure 1. Classification of lncRNAs based on their genomic location in relation to their adjacent protein-coding genes. (A) Sense lncRNAs, which are transcribed from the sense genomic strand of protein-coding genes; (B) antisense lncRNAs, which are transcribed from the antisense genomic strand of protein-coding genes; (C) intronic lncRNAs, which are entirely transcribed from introns of protein-coding genes; (D) intergenic lncRNAs (or lincRNAs), which are transcribed from the interval between two independent protein-coding genes (also referred to as "gene deserts"); and (E) bidirectional lncRNAs, which are transcribed in the opposite direction from their adjacent protein-coding genes (within 1 kb).

RNAs; (D) intergenic lncRNAs (or lincRNAs); and (E) bidirectional lncRNAs (**Figure 1**).

Functions and mechanisms of lncRNAs

LncRNAs can modulate gene expression at the pretranscriptional, transcriptional, and posttranscriptional levels (**Figure 2**). The molecular mechanisms involved in the pretranscriptional regulatory effect of lncRNAs mainly include the following aspects. (A) LncRNAs can directly bind to DNA methyltransferase (DNMT) for either activation or inhibition of DNA methylation. For instance, LINC00628 directly binds to DNMT1, DNMT3A, and DNMT3B, thereby promoting the DNA methylation of the LAMA3 promoter and inhibiting the expression of LAMA3 [40]. In contrast, lncRNA DBCCR1-003 can prevent the methylation of the tumor suppressor gene DBCCR1 by binding to DNMT1, thereby promoting the expression of DBCCR1 [41]. (B) LncRNAs can regulate gene transcription by recruiting histone-modifying enzymes to modulate histone modifications such as histone methylation, acetylation, and ubiquitination [19]. (C) LncRNAs can recruit chromatin-modifying complexes to specific genomic loci, thereby inhibiting or promoting the expression of downstream genes [42, 43]. For instance, polycomb repressive complex-1 (PRC1) and complex-2 (PRC2) are important chromatin regulatory factors because of their ability to regulate epigenetic cellular memory. LncRNA ANRIL can recruit PRC1 and PRC2 to the INK4b-ARF-INK4a locus, leading to its repression [44, 45]. Regarding regulation at the transcriptional level by lncRNAs, the mechanisms mainly include the following aspects. (D) LncRNAs can interact with transcription factors and then prevent them from binding chromatin, thereby regulating gene transcription. For instance, lncRNA Evf2 can recruit the transcription factor Dlx2 and then form a stable complex, thereby increasing the transcriptional activity of the Dlx-5/6 enhancer [46]. (E) LncRNAs can directly interact with RNA polymerase II, thus affecting the expression of downstream genes. For instance, human Alu RNAs, transcribed from short interspersed elements (SINEs), have the ability to inhibit transcription by binding to Pol II [47]. Regarding

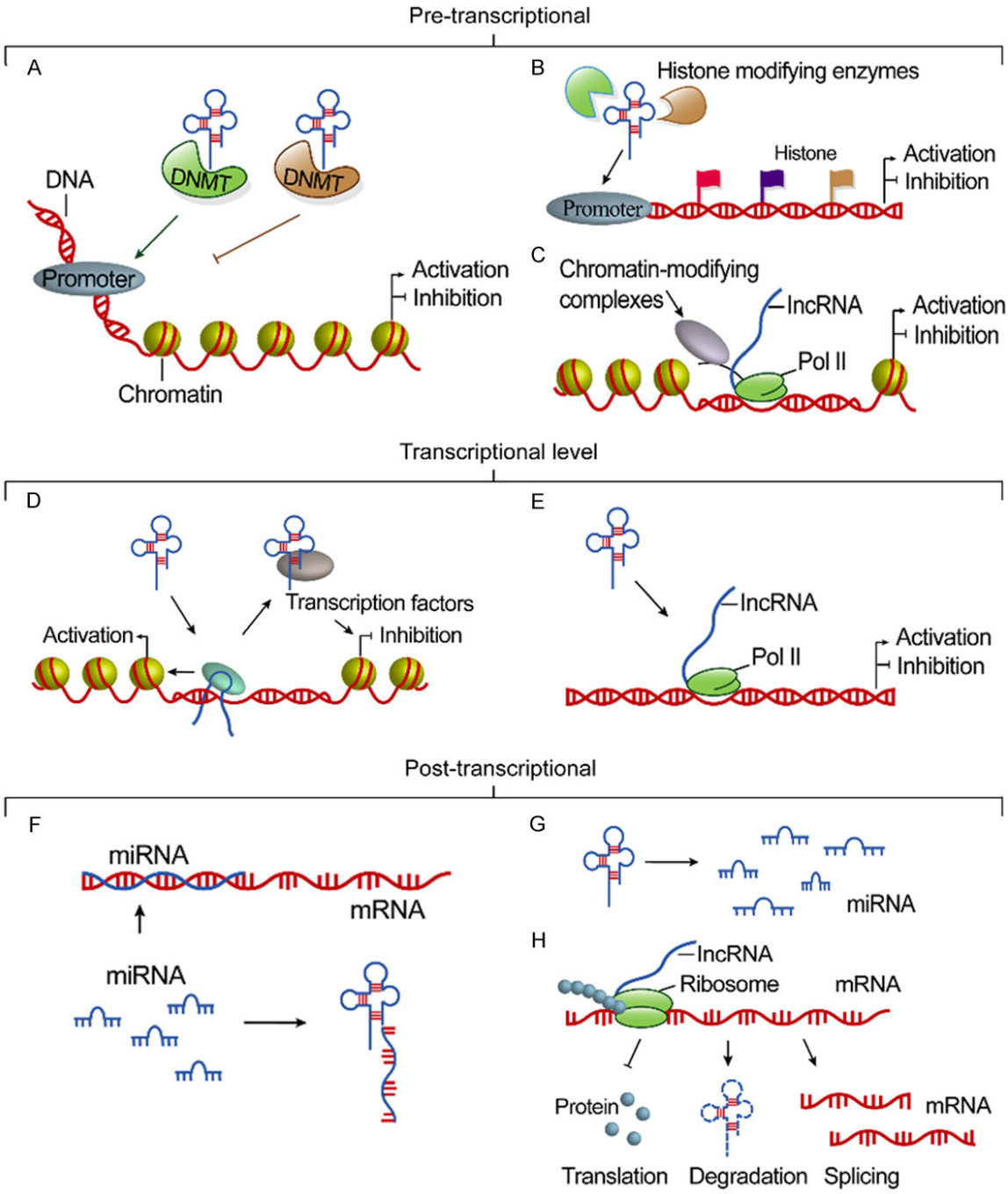

Figure 2. Functions and mechanisms of lncRNAs. A. LncRNAs can regulate the DNA methylation of target genes by binding to DNA methyltransferase, thus inhibiting or promoting target gene expression. B. LncRNAs can recruit histone-modifying enzymes to modulate histone modification, thus regulating gene transcription. C. LncRNAs can recruit chromatin-modifying complexes to specific genomic loci, thereby inhibiting or promoting the expression of downstream genes. D. LncRNAs can interact with transcription factors and then prevent them from binding chromatin, thereby regulating gene transcription. E. LncRNAs can directly interact with Pol II, thus affecting the expression of downstream genes. F. LncRNAs can function as endogenous miRNA sponges by competitively binding to miRNAs. G. LncRNAs can function as precursor molecules to generate diverse miRNAs. H. LncRNAs can directly bind to mRNAs and then regulate their degradation, translation, and splicing.

regulation at the posttranscriptional level by lncRNAs, the mechanisms are diverse and mainly include the following aspects. (F) LncRNAs can function as endogenous miRNA sponges by

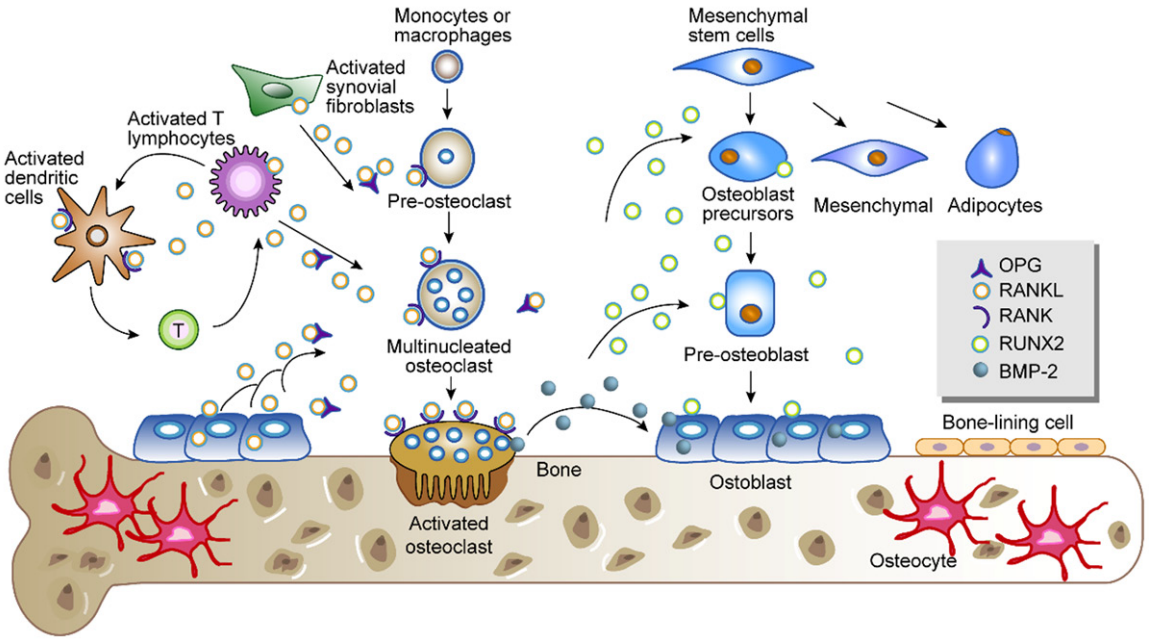

Figure 3. The process of osteoclastic differentiation and osteoblastic differentiation. RANKL can be produced and secreted by osteoblasts, activated T cells, and activated synovial fibroblasts and then binds to RANK, thus promoting osteoclastic differentiation and bone resorption. Conversely, OPG can inhibit RANKL binding to its receptor RANK, thereby blocking the differentiation of osteoclast precursors into activated osteoclasts. In addition, both RUNX2 and BMP-2 are powerful inducers that can promote the differentiation of MSCs into osteoblasts, thereby enhancing bone formation.

competitively binding to miRNAs. For instance, in breast cancer, upregulated LINC00665 competitively binds to miR-379-5p, thus reducing the ability of miR-379-5p to repress its downstream target gene LIN28B [48]. (G) LncRNAs can function as precursor molecules to generate diverse miRNAs. For instance, H19 can produce a 23-nt miRNA precursor for miRNA-675, and miRNA675 can target corresponding mRNAs and then inhibit their expression [49]. (H) LncRNAs can directly bind to mRNAs and then regulate their degradation, translation, and splicing [19, 50].

Molecular mechanism of osteoporosis

Osteoporosis results from a disruption of the balance between bone formation mediated by osteoblasts and bone resorption mediated by osteoclasts. Osteoclasts are multinucleated cells that originate from monocyte/macrophage precursor cells and secrete hydrochloric and proteolytic enzymes, thereby dissolving bone minerals and digesting the bone matrix (**Figure 3**) [51]. The differentiation from monocyte/macrophage precursor cells to osteoclasts mainly depends on receptor activator of NF-κB ligand (RANKL) and macrophage-colony stimulating factor (M-CSF) [51]. RANKL is a member of the tumor necrosis factor (TNF) family that can be expressed by multiple cells, such as osteoblasts, activated T cells, and activated synovial fibroblasts [52]. RANKL can promote osteoclastic differentiation by binding and activating its receptor, RANK, a member of the TNF receptor superfamily that is located on the surface of osteoclasts [52]. Osteoprotegerin (OPG) is a naturally occurring antagonist of RANKL and can inhibit RANKL binding to its receptor RANK, thereby blocking the differentiation of osteoclast precursors into mature osteoclasts [53]. In addition, M-CSF plays a permissive role in the response of osteoclasts to RANKL because it can enhance the activity and survival of osteoclasts [54, 55]. Many conditions, such as estrogen deficiency, immunological diseases, and malignant bone disorders, have been reported to activate the RANKL/RANK pathway and thus promote osteoclastic differentiation, ultimately leading to the occurrence of osteoporosis [56-58].

Unlike osteoclasts, osteoblasts mainly originate from mesenchymal stem cells (MSCs) and

synthesize a complex extracellular matrix (mainly type I collagen), which is subsequently deposited and mineralized (**Figure 3**). At the molecular level, the Wnt/β-catenin signaling pathway plays a central role in regulating osteoblastic differentiation and bone formation. On the one hand, the activation of the Wnt/β-catenin signaling pathway promotes the differentiation of MSCs to osteoblasts by blocking adipogenesis through inhibition of the expression of relevant adipogenic transcription factors [59]. On the other hand, activation of the Wnt/β-catenin signaling pathway inhibits osteoclastic differentiation by promoting the expression of OPG [60]. This pathway is negatively regulated by several Wnt antagonists, including Dickkopf-1 (Dkk-1), SOST, and sclerostin [61]. RUNX2 (Cbfa1) is another important transcription factor for the differentiation of MSCs into osteoblasts [62, 63]. RUNX2 deletion can block osteoblastic production, which subsequently leads to the inhibition of bone formation [63]. In addition, osteoclasts can produce and secrete a powerful inducer for osteoblastic differentiation, which is termed bone morphogenetic protein-2 (BMP-2) [64].

The expression profile of lncRNAs in osteoporosis

Through high-throughput sequencing and bioinformatics analysis, accumulating evidence has shown that various lncRNAs are aberrantly expressed in osteoporosis patients compared with healthy people, which indicates that lncRNAs probably participate in the occurrence and development of osteoporosis. According to the RNAsequencing (RNA-seq) data in 3 blood samples from postmenopausal osteoporosis (PMOP) patients and 2 blood samples from healthy women, Fei et al. identified 51 differentially expressed lncRNAs, of which 25 were significantly upregulated and 26 were significantly downregulated in blood samples from PMOP patients [65]. Another study using RNA-seq data in peripheral blood lymphocytes of patients with PMOP identified 70 differentially expressed lncRNAs, 475 differentially expressed mRNAs, 260 differentially expressed circRNAs and 13 differentially expressed miRNAs compared with those in healthy people [66]. Zhou et al. analyzed the transcriptome-wide lncRNA expression profile in peripheral blood monocytes between high and low hip BMD subjects by reannotating the Affymetrix Exon 1.0 ST Array, and 575 lncRNAs were finally identified to be differentially expressed, of which 309 were upregulated and 266 were downregulated in the high BMD group [67].

Importantly, functional prediction results suggested that most of the above dysregulated lncRNAs were involved in multiple biological processes related to osteogenesis and osteoclastogenesis, such as hematopoiesis, inflammatory response, osteoclast differentiation, metabolism, and regulation of signaling pathway. However, the exact mechanisms of these dysregulated lncRNAs in osteoporosis need to be further investigated in future studies.

Regulatory roles of lncRNAs in osteoblasts

The differentiation from MSCs to osteoblasts is a precise process regulated by multiple signaling pathways [68-70]. Many studies have shown that the expression profile of lncRNAs is dynamically altered during the process of osteogenic differentiation. For instance, Song et al. identified 574 differentially expressed lncRNAs and 2597 differentially expressed mRNAs in immortalized MSCs cultured in differentiation medium compared with those cultured in growth medium [71]. Another study using lncRNA microarray data on bone marrow-derived mesenchymal stem cells (BMSCs) identified 1206 differentially expressed lncRNAs in osteogenic differentiated BMSCs compared with undifferentiated BMSCs, of which 687 were upregulated and 519 were downregulated [72]. Zhang et al. identified 1408 differentially expressed lncRNAs (785 upregulated and 623 downregulated) in BMSCs at 7 days after osteogenic induction compared with uninduced BMSCs [73]. In addition, by analyzing the RNA-seq data at different osteoblastic differentiation stages, Qiu et al. determined that 3948 lncRNAs and 4058 mRNAs were dynamically altered during the process of osteoblastic differentiation [74]. In this section, the molecular regulation by lncRNAs in osteoblasts will be broadly discussed in terms of lncRNAs acting as promoters and inhibitors of osteogenic differentiation (**Table 1**).

LncRNAs act as promoters of osteogenic differentiation

LncRNA H19 is one of the most abundant and conserved ncRNAs in mammalian development

Table 1. The molecular regulation exerted by lncRNAs in osteogenic differentiation

LncRNAs name	Functional role	Molecular Mechanism	References
H19	Promotion	(1): H19 inhibits the RUNX2 expression via miR-675/TGF-β1/Smad3/HDAC axis (2): H19 acts as a miRNA sponge for miR-141 and miR-22, both of which are negative regulators for β-catenin, thus activating the Wnt/β-catenin signaling pathway (3): Low expression of H19 promotes the expression of Dkk4, which is a Wnt antagonist, thus inhibiting the Wnt/β-catenin signaling pathway	[75, 78, 79]
MALAT	Promotion	(1): MALAT1 increases the expression of Smad4 by serving as a miRNA sponge for miR-204, thereby increasing osteogenic marker expression (2): MALAT1 increases ATF4 expression by serving as a miRNA sponge for miR-214, thereby promoting osteogenic differentiation of BMSCs (3): BMSC-derived exosomal MALAT1 acts as a miR-34c sponge to upregulate the expression of SATB2, which is a protein that can increase the activity of RUNX2 and ATF4	[85, 89, 91]
Lnc-ob1	Promotion	Lnc-ob1 promotes the expression of OSX via inhibition of histone H3K27me3 at its promoter	[92]
LOC103691336	Promotion	LOC103691336 activates the SMADs and p38 signaling pathway via miR-138-5p/BMPR2 axis	[95]
FER1L4	Promotion	FER1L4 acts as a miRNA sponge for miR-874-3p to promote the expression of VEGFA, which is an angiogenic/vasculogenic factor that can promote angiogenesis and osteogenesis	[97]
LINC00707	Promotion	LINC00707 sponges miR-145 to promote the expression of LRP5, which is the receptor of the Wnt/β-catenin signaling pathway, thus activating the Wnt/β-catenin signaling pathway	[100]
PGC1β-OT1	Promotion	PGC1β-OT1 sponges miR-148a-3p to inhibit KDM6B expression, thus regulating Hox gene expression	[102]
TER	Promotion	TERC acts as a miRNA-217 sponge to upregulate RUNX2 expression	[104]
MEG3	Not clear	(1): MEG3 overexpression blocks the interaction between BMP4 and transcription factor SOX2, a negative regulator of BMP4, thereby enhancing the transcription of BMP4 and then promoting the osteogenic differentiation of MSCs (2): MEG3 acts as a miR-27a-3p sponge to upregulate IGF1 expression, thus activating the PI3k/Akt signaling pathway and then promoting PDLSC osteogenic differentiation (3): MEG3 acts as a miRNA sponge for miR-133a-3p to inhibit SLC39A1 expression	[105, 107, 108]
MSC-AS1	Promotion	MSC-AS1 acts a miRNA sponge for microRNA-140-5p to upregulate the expression of RUNX2	[109]
GAS5	Promotion	GAS5 binds to microRNA-498 to promote the expression of RUNX2, thereby promoting the osteogenic differentiation of BMSCs and alleviating the progression of osteoporosis	[110]
TUG1	Promotion	TUG1 acts a miRNA sponge for miR-204-5p to promote the expression of RUNX2 and then promote osteogenic differentiation	[111]
Linc-ROR	Promotion	Linc-ROR sponges miR-138 and miR-145 to promote ZEB2 expression, which is a positive regulator of the expression of β-catenin, thereby activating the Wnt/β-catenin signaling pathway	[112]
HOTTIP	Promotion	HOTTIP interacts with WDR5, a positive regulator for the expression of β-catenin, thus activating the Wnt/β-catenin signaling pathway and then promoting osteogenic differentiation	[115]
NKILA	Promotion	NKILA promotes osteogenesis through increasing the activity of the RXFP1/AKT signalling pathway and inhibiting the NF-κB signalling pathway	[115]
ODSM	Promotion	ODSM sponges miR-139-3p to increase the expression of its target gene ELK1, which can promote osteoblast differentiation and inhibit osteoblast apoptosis	[118]
ANCR	Inhibition	(1): ANCR inhibits RUNX2 expression by recruiting EZH2, which catalyzes H3K27me3 at the RUNX2 gene promoter (2): ANCR sponges miR-758 to upregulate the expression of Notch2, thus activating the Notch2-Wnt/β-catenin signaling pathway and then suppressing osteogenic differentiation	[121, 125]

HOTAIR	Inhibition	(1): HOTAIR inhibits miR-17-5p expression, a pro-osteogenic miRNA that targets SMAD7, via increasing the DNA methylation level of miR-17-5p promoter (2): HOTAIR inhibits ALPL expression via decreasing the histone H3K4 methylation levels at the ALPL promoter region	[126, 129]
MIR31HG	Inhibition	MIR31HG directly binds to IκBα (an NF-κB inhibitor) to result in NF-κB activation, thus inhibiting osteogenic differentiation of human ADSCs	[134]
ORLNC1	Inhibition	ORLNC1 binds to miR-296 to upregulate Pten expression, a negative regulator for osteoblastic differentiation	[135]
ODIR1	Inhibition	ODIR1 inhibits OSX expression, via recruiting E3 ubiquitin ligase CUL3, which promotes FBXO25 degradation and then decreases the enrichment of H2BK120ub and H3K4me3 at OSX promoter region	[136]
AK016739	Inhibition	AK016739 inhibits osteogenic differentiation through negatively regulating RUNX2, TCF7, and LEF1	[139]
AK045490	Inhibition	Knockdown of AK045490 enhances osteoblast differentiation through promoting the nuclear translocation of β-catenin and upregulating the expression of RUNX2, TCF1, and LEF1	[140]
HoxA-AS3	Inhibition	Downregulation of HOXA-AS3 blocks the recruitment of EZH2 to the RUNX2 gene promoter, resulting in the decreased enrichment of H3K27me3 at the RUNX2 promoter and then inducing its expression	[141, 142]
SNHG	Inhibition	SNHG1 inhibits the p38 MAPK signaling pathway through ubiquitination mediated by Nedd4	[143]
MIAT	Inhibition	MIAT inhibits osteogenic differentiation through promoting TNFa expression	[144]

and has been demonstrated to promote osteogenic differentiation of MSCs through multiple regulatory mechanisms. H19 is a primary precursor of miR-675, and Huang et al. found that the expression levels of both H19 and miR-675 were upregulated during osteogenic differentiation of human MSCs [75]. Overexpression of H19 promotes osteogenic differentiation in vitro and enhances heterotopic bone formation in vivo, whereas its knockdown causes the opposite effects [75]. The pro-osteogenic effect of H19 is partially mediated by miR-675. On the one hand, miR-675 directly targets TGF-β1 to inhibit Smad3 phosphorylation. Then, the inhibition of Smad3 phosphorylation blocks the recruitment of histone deacetylase (HDAC) 4/5 to RUNX2, resulting in the repression of RUNX2 [75-77]. On the other hand, miR-675 has been shown to be able to reduce the mRNA and protein levels of HDAC4/5 via unclear regulatory mechanisms [75]. Moreover, Liang et al. reported that H19 could act as a miRNA sponge for miR-141 and miR-22, both of which are negative regulators for β-catenin, ultimately activating the Wnt/β-catenin signaling pathway and then promoting osteogenic differentiation of human MSCs [78]. In another study by Li et al., H19 was found to be expressed at low levels in disuse osteoporosis (DOP). Further experiments indicated that low expression of H19 can promote the expression of Dickkopf 4 (also termed Dkk4, which is a Wnt antagonist) and subsequently inhibit the Wnt/β-catenin signaling pathway, thereby leading to the inhibition of osteogenic differentiation [79]. Recently, Wu et al. reported that H19 could also act as a miRNA sponge for miR-185-5p, which could alleviate the repression of IGF1, resulting in the enhancement of matrix mineralization of osteoblasts [80]. MiR-19b-3p expression was shown to be upregulated during osteoblast differentiation in BMP-2-induced BMSCs and postmenopausal osteoporosis patients, while H19 expression was shown to be downregulated [81]. Upregulating the expression of H19 inhibits miR-19b-3p expression and significantly promotes the cell proliferation and osteogenic differentiation of BMSCs, suggesting that H19 can regulate osteogenic differentiation by interacting with miR-19b-3p [81]. However, the specific regulatory mechanism of the H19/miR-19b-3p axis in the process of osteogenic differentiation needs to be further investigated. Finally, it has been reported that H19 can also regulate cartilage differentiation and osteogenic differentiation of human adipose-derived stem cells (ADSCs) [82, 83].

LncRNA MALAT has also been identified as a pro-osteogenic lncRNA. Che et al. first reported that RANKL could induce the expression of MALAT, and MALAT could regulate OPG expression in a normal human fetal osteoblastic cell line [84]. Subsequently, several studies have reported that MALAT can promote osteogenic differentiation by acting as a miRNA sponge. For instance, Xiao et al. demonstrated that MALAT1 increases the expression of Smad4 by acting as a miRNA sponge for miR-204, thereby increasing the expression of osteogenic markers such as alkaline phosphatase (ALP), RUNX2, and osteocalcin (OCN), and then promoting osteogenic differentiation of human aortic valve interstitial cells [85]. Gao et al. reported that MALAT1 acts as a miR-143 sponge to upregulate the expression of osterix (OSX), which is an essential transcription factor for osteoblast differentiation and bone formation [86], thereby promoting osteogenic differentiation of human MSCs [87]. Yi et al. demonstrated that MALAT1 acts as a miR-30 sponge to upregulate the expression of RUNX2, resulting in the enhancement of osteogenic differentiation of ADSCs [88]. In another study by Huang et al., the expression of MALAT1 was shown to be downregulated, while the expression of miR-214 was shown to be upregulated in steroid-induced avascular necrosis of the femoral head tissues. Mechanistically, MALAT1 increases ATF4 expression by acting as a miRNA sponge for miR-214, thereby promoting osteogenic differentiation of BMSCs [89]. Moreover, Zheng et al. observed significantly downregulated expression of MALAT1 in osteoporotic rats in comparison to normal rats [90]. Inhibition of MALAT1 significantly reduces the expression levels of osteogenic markers such as ALP, RUNX2, and OCN and inhibits osteogenic differentiation of BMSCs. This effect is likely mediated by the mitogen-activated protein kinase (MAPK) signaling pathway since the expression levels of MAPK signaling pathway-related proteins such as ERK1/2 and P38 are significantly upregulated in BMSCs after MALAT1 inhibition [90]. In another recent study, Yang et al. found that BMSC-derived exosomes could promote the osteogenic ability of osteoblasts, and MALAT1 was shown to be significantly enriched in

BMSC-derived exosomes [91]. Further experiments demonstrated that BMSC-derived exosomal MALAT1 acts as a miR-34c sponge to upregulate the expression of SATB2, a protein that can increase the activity of RUNX2 and ATF4, thereby enhancing the activity of human osteoblasts and alleviating the symptoms of osteoporosis in OVX rats [91].

Another lncRNA identified as pro-osteogenic is lnc-ob1 [92]. By analyzing high-throughput RNA-seq data in human and mouse undifferentiated BMSCs and osteoblastic differentiated BMSCs, Sun et al. identified 2028 differentially expressed lncRNAs during human osteoblast differentiation and 1436 differentially expressed lncRNAs during mouse osteoblast differentiation [92]. Further tissue expression analysis indicated that the expression of lnc-ob1 was significantly higher in bone tissues than in other tested tissues [92]. The authors also found that the expression of lnc-ob1 was significantly upregulated in osteoblastic differentiated BMSCs, ADSCs and embryonic stem cells, while its expression was downregulated in osteoblasts from aged osteoporotic patients [92]. Overexpression of lnc-ob1 significantly enhances osteoblast differentiation in vitro and increases the bone formation rate in vivo, whereas knockdown of lnc-ob1 causes the opposite effects. More importantly, osteoblast-targeted delivery of lnc-ob1 can reverse bone loss in OVX rats, suggesting that lnc-ob1 could be a potential therapeutic target for osteoporosis [92]. Mechanistically, lnc-ob1 upregulates the expression of OSX in mouse and human osteoblasts, probably via inhibition of histone H3 trimethylation at lysine 27 (H3K27me3) at its promoter, ultimately enhancing the activity of osteoblasts and bone formation rate [92].

Magnesium (Mg) and its alloys have good biodegradability and biocompatibility; therefore, they are widely used in orthopedic surgery [93]. It has been reported that Mg has the ability to promote osteogenic differentiation in vitro and induce new bone formation in vivo [93, 94]. By performing a lncRNA expression microarray using Mg-implanted callus tissues and stainless-steel-implanted callus tissues, Li et al. finally identified 91 significantly upregulated lncRNAs in Mg-implanted callus tissues [95]. Among them, LOC103691336 was subsequently demonstrated to be the most upregulated and was associated with the expression of BMPR2, which is the kinase receptor of BMPs [95]. Further experiments indicated that LOC103691336 plays a pro-osteogenic role in Mg-mediated osteogenic differentiation. This effect is mediated by miR-138-5p, which has been demonstrated to inhibit osteogenic differentiation and has binding sites for BMPR2 and LOC103691336 [95, 96]. Specifically, LOC103691336 acts as a miR-138-5p sponge to upregulate BMPR2 expression, thus activating the Smad and p38 signaling pathways and promoting Mg-mediated osteogenic differentiation [95].

LncRNA FER1L4 was identified as a pro-osteogenic lncRNA, with increased expression during consecutive osteogenic induction of periodontal ligament stromal cells (PDLSCs) [97]. FER1L4 overexpression increases the expression of osteogenic markers such as ALP, RUNX2, and OCN, whereas knockdown of FER1L4 reduces their expression [97]. This effect is likely mediated by miR-874-3p, which plays an essential role in the chemotherapeutic resistance of colorectal cancer [98]. The expression of miR-874-3p is negatively correlated with the expression of FER1L4 in PDLSCs during osteogenic differentiation. Mechanistic investigations revealed that FER1L4 might contribute to enhanced osteogenic differentiation by acting as a miR-874-3p sponge to upregulate the expression of VEGFA, which is an angiogenic/vasculogenic factor that can promote angiogenesis and osteogenesis [97, 99]. In addition, Cai et al. recently described a pro-osteogenic lncRNA termed LINC00707 in human BMSCs [100]. LINC00707 sponges miR-145 to upregulate the expression of LRP5, which is the receptor of the Wnt/β-catenin signaling pathway, thereby promoting the osteogenic differentiation of human BMSCs [100, 101]. A miRNA sponge function has also been reported for lncRNA PGC1β-OT1, which is 1759 nt in full length and located in both the nucleus and cytoplasm [102]. PGC1β-OT1 overexpression increases osteogenic marker expression while decreasing the expression of adipogenic markers, including C/EBPα, PPARγ, and aP2. Mechanistic investigations have revealed that GC1β-OT1 sponges miR-148a-3p to inhibit the expression of lysine-specific demethylase 6b (KDM6B), a histone demethylase that can regulate Hox gene expression by removing H3-

K27me3 at its promoter, thereby regulating adipocyte and osteoblast differentiation [102, 103]. Furthermore, Gao et al. reported that lncRNA TER has a pro-osteogenic role during the osteogenic process [104]. The serum level of TERC has been shown to be downregulated in osteoporosis patients compared with healthy controls, and knockdown of TERC in vitro inhibits osteogenesis in human MSCs, as evidenced by a decrease in ALP activity [104]. Specifically, TERC acts as a miRNA-217 sponge to upregulate RUNX2 expression, thus promoting human MSC osteogenesis and alleviating the progression of osteoporosis [104].

LncRNA MEG3 has also been identified as a pro-osteogenic lncRNA, with upregulated expression in MSCs from multiple myeloma patients [105]. MEG3 knockdown significantly decreases the expression of osteogenic markers, including RUNX2, OSX, and OCN, whereas MEG3 overexpression increases their expression [105]. In addition, MEG3 knockdown inhibits the transcription of BMP4, a secreted signaling molecule that plays a crucial role in the regulation of osteogenic differentiation and embryonic development [105]. Specifically, MEG3 overexpression blocks the interaction between BMP4 and the transcription factor SOX2, a negative regulator of BMP4, thereby enhancing the transcription of BMP4 and promoting the osteogenic differentiation of MSCs [105]. Another study by Li et al. found that MEG3 could positively regulate osteogenesis and negatively regulate adipogenesis in human ADSCs by targeting miR-140-5p [106]. Recently, Liu et al. reported that MEG3 could also participate in the osteogenic differentiation process of PDLSCs [107]. Bioinformatics analysis showed that in comparison to healthy periodontal tissues, in periodontitis periodontal tissues the PI3K/Akt signaling pathway is dysregulated and the expression of IGF1 is downregulated. Moreover, MEG3 expression has also been shown to be downregulated in periodontitis periodontal tissues. Mechanistic investigations indicated that MEG3 acts as a miR-27a-3p sponge to upregulate IGF1 expression, thus activating the PI3k/Akt signaling pathway and promoting PDLSC osteogenic differentiation in periodontitis [107]. However, another study by Wang et al. reported that MEG3 plays an anti-osteogenic role during osteogenic differentiation [108]. The authors found that MEG3 expression was significantly higher in BMSCs derived from OVX rats and patients with postmenopausal osteoporosis than in those from healthy people [108]. Molecular analysis showed that MEG3 inhibits osteogenic differentiation of BMSCs by upregulating miR-133a-3p expression to reduce the expression of SLC39A1, which can promote osteogenic differentiation of human MSCs [108].

LncRNA MSC-AS1 has been shown to be highly expressed in BMSCs during osteogenic differentiation [109]. Inhibition of MSC-AS1 results in reduced osteoblast differentiation in BMSCs at 14 days of osteogenic induction [109]. Further mechanistic investigations have revealed that MSC-AS1 acts as a miRNA sponge for microRNA-140-5p to upregulate the expression of RUNX2, thus promoting the osteogenic differentiation of BMSCs [109]. LncRNA GAS5 has also been identified as a pro-osteogenic lncRNA, with decreased expression in human BMSCs isolated from osteoporosis patients [110]. Overexpression of GAS5 in human MSCs promotes osteogenic differentiation and reduces the expression of microRNA-498, while knockdown of microRNA-498 downregulates both the mRNA and protein levels of RUNX2 [110]. Specifically, GAS5 sponges microRNA-498 to promote the expression of RUNX2, thereby promoting the osteogenic differentiation of BMSCs and alleviating the progression of osteoporosis [110]. In another study by Gao et al., the expression of lncRNA TUG1 was higher in calcified aortic valve tissues than in adjacent normal tissues [111]. Knockdown of TUG1 significantly inhibits osteogenic differentiation in vitro, whereas its overexpression has the opposite effects [111]. Bioinformatics analysis indicated that TUG1 has putative binding sites with miR-30b-5p, miR-125b-5p, miR-148a-3p miR-204-5p, and miR-214-3p. A biotin-labeled pulldown assay was subsequently conducted to identify the miRNAs that could interact with TUG1. Finally, the expression of miR-204-5p was shown to be significantly increased in the TUG1 pulldown fraction. Further experiments indicated that TUG1 could increase the expression of RUNX2 by absorbing miR-204-5p, thereby promoting osteogenic differentiation in aortic valve calcification [111].

Another lncRNA identified as pro-osteogenic is linc-ROR, which has been previously reported

to be highly expressed in embryonic stem cells and in induced pluripotent stem cells [112]. Feng et al. found that the expression of linc-ROR was upregulated during osteogenic differentiation of human BMSCs [113]. Overexpression of linc-ROR significantly enhances osteogenesis in human MSCs, as evidenced by the upregulated expression of several osteogenic markers, including ALP, OCN, and OSX. However, knockdown of linc-ROR greatly reversed these effects. Mechanistic investigations revealed that linc-ROR sponges miR-138 and miR-145 to promote the expression of ZEB2, a positive regulator of the expression of β-catenin, thus activating the Wnt/β-catenin signaling pathway and promoting osteogenic differentiation of human BMSCs [113]. A similar function was also reported in a recent study by Jiang et al. [114]. Through microarray analysis of human BMSCs during osteogenesis, the authors identified 164 differentially expressed lncRNAs with at least a two-fold change, including lncRNA HOTTIP, which had highest fold change and was subsequently selected as a candidate lncRNA for further study. Knockdown of HOTTIP greatly inhibits the osteogenic differentiation of human BMSCs cultured in osteogenic induction medium, as evidenced by decreased calcium deposition. However, overexpression of HOTTIP in human BMSCs via lentiviral transfection upregulates the expression levels of ALP, RUNX2, and OSX. These results suggest that HOTTIP has a pro-osteogenic role during osteogenesis of BMSCs [114]. Specifically, HOTTIP interacts with WDR5, a positive regulator of the expression of β-catenin, thus activating the Wnt/β-catenin signaling pathway and promoting the osteogenic differentiation of human BMSCs [114]. Furthermore, by loading human BMSCs expressing HOTTIP on collagen scaffolds and then implanting them in the subcutaneous tissue of nude mice, the authors observed an increase in bone volume and higher BMD in nude mice via micro-CT [114].

LncRNA NKILA has also been identified as a positive regulator of MSC osteogenesis [115]. Its expression is significantly increased during osteogenic induction of menstrual blood-derived mesenchymal stem cells (MenSCs) and umbilical cord mesenchymal stem cells (UC-MSCs). Knockdown of NKILA in MenSCs via lentiviral infection decreases ALP activity, inhibits osteogenic marker expression, and markedly inhibits calcium deposition in MenSCs. However, overexpression of NKILA in UCMSCs via lentiviral infection greatly reverses these effects. Further experiments suggested that NKILA positively regulates the osteogenesis of MSCs by regulating two distinct signaling pathways [115]. On the one hand, overexpression of NKILA promotes osteogenic differentiation of MSCs by increasing the activity of the RXFP1/AKT signaling pathway, which is an essential positive regulator of osteogenesis. On the other hand, overexpression of NKILA can inhibit the expression of NF-κB via an HDAC2-mediated deacetylation process, thus increasing the expression of RUNX2 and then promoting osteogenic differentiation of MSCs [115].

Hindlimb unloading (HU) mouse models are often used to simulate bone loss under microgravity (MG) unloading conditions [116, 117]. In a recent study by Wang et al., the expression levels of lncRNA ODSM were found to be significantly upregulated in the femurs of mice compared with other tissues and organs. Moreover, they also observed that ODSM expression was significantly decreased in the femurs of HU mice [118]. Knockdown of ODSM in MC3T3-E1 cells significantly promotes osteoblast apoptosis and inhibits osteoblast mineralization, as evidenced by increased levels of apoptosis-related proteins such as Bax and cleaved caspase-3 and decreased levels of Bcl-2 protein [118]. However, MC3T3-E1 cells transfected with pEX-ODSM and then cultured in an MG unloading environment showed a significantly decreased ratio of apoptotic osteoblasts. These effects were partially mediated by miR-139-3p. Specifically, ODSM can act as a miRNA sponge for miR-139-3p and then increase the expression of its target gene ELK1 [118]. Previously, the same team led by Wang demonstrated that ELK1 could promote osteoblast differentiation and inhibit osteoblast apoptosis in MC3T3-E1 cells [119]. In addition, using the (AspSerSer) 6-liposome system to overexpress ODSM in vivo can partially reverse the decreased osteoblast activity and bone formation and improve the bone architecture and mechanical properties in HU mice [118].

LncRNAs act as inhibitors of osteogenic differentiation

LncRNA ANCR (DANCR) has been demonstrated to play an anti-osteogenic role during osteogenic differentiation. Zhu et al. first reported

that the expression of ANCR is significantly downregulated in hFOB1.19 cells cultured in differentiation medium [120]. Silencing of ANCR promotes osteogenic differentiation, as evidenced by increased activity of ALP, and overexpression of ANCR inhibits osteoblast proliferation in vitro [120]. Mechanistically, upregulation of ANCR represses RUNX2 gene expression by recruiting zeste homolog 2 (EZH2), which catalyzes H3K27me3 at the RUNX2 gene promoter, thereby causing the inhibition of osteogenic differentiation [120]. Another study conducted by Cai et al. also confirmed the above results [121]. They found that the expression of ANCR was significantly increased in OVX rats compared with the control group (without any operation) and sham group (open/close abdomen operation without ovaries removed) [121]. Knockdown of ANCR via small interfering RNA (siRNA) enhances the ability of osteoblast cells and inhibits their apoptosis [121]. The results of Western blotting showed that EZH2 expression was significantly downregulated in osteoblast cells in the si-ANCR group, while the expression of RUNX2 and OSX was significantly upregulated. Further RNA pulldown assays indicated that ANCR could specifically bind to EZH2, suggesting that ANCR could regulate osteogenic differentiation via the EZH2/RUNX2 axis [121]. Moreover, Jia et al. reported that ANCR expression is negatively correlated with Wnt/β-catenin signaling pathway activation, and this correlation results in the inhibition of osteogenic differentiation of PDLSCs [122]. Similar results were also reported in another study. Jiang et al. found that ANCR was significantly highly expressed in the serum of fracture patients compared with healthy people [123]. Further investigation indicated that ANCR knockdown activates the Wnt/β-catenin signaling pathway, which induces RUNX2 expression and then promotes the proliferation and differentiation of osteoblasts [123]. A miRNA sponge function has also been described for ANCR in regulating osteogenic differentiation [124]. Peng et al. reported that ANCR acts as a miR-758 sponge to upregulate the expression of Notch2, thus activating the Notch2-Wnt/β-catenin signaling pathway and suppressing osteogenic differentiation of PDLSCs [124]. In addition, Zhang et al. reported that ANCR inhibits the proliferation and osteogenic differentiation of human BMSCs by inactivating the p38 MAPK signaling pathway [125].

LncRNA HOTAIR was also identified as an anti-osteogenic lncRNA, with significantly increased expression in MSCs of patients with nontraumatic osteonecrosis of the femoral head but decreased expression in BMP-2-induced osteogenic differentiation [126]. Knockdown of HOTAIR significantly increases the expression of osteogenic markers such as ALP, RUNX2, and COL1A1, whereas overexpression of HOTAIR decreases the expression of these osteogenic markers [126]. Bioinformatics analysis indicated that HOTAIR contains sequences complementary to miR-17-5p, which has been demonstrated to play a pro-osteogenic role in osteogenic differentiation by targeting Smad7 [127]. Moreover, miR-17-5p expression has been shown to be downregulated during osteogenic differentiation, while it is increased during adipocyte differentiation [126, 128]. Specifically, HOTAIR downregulation decreases the DNA methylation level of the miR-17-5p promoter and then increases miR-17-5p expression, thereby promoting osteogenic differentiation [126]. Moreover, Misawa et al. proposed that HOTAIR could inhibit mineralization in osteoblastic osteosarcoma cells via epigenetic mechanisms [129]. HOTAIR knockdown via siRNA increases the histone H3K4 methylation levels at the ALPL promoter region, resulting in the upregulation of ALPL, which plays a critical role in the mineralization of bone matrix [129].

Multiple signaling pathways related to bone remodeling are dysregulated under an inflamed microenvironment induced by inflammatory cytokines [130-132]. It has been reported that NFκB activation can suppress osteogenic differentiation, while its inhibition can enhance osteogenic differentiation [133]. Jin et al. found that knockdown of lncRNA MIR31HG in human ADSCs not only promotes osteogenic differentiation but also reverses the inhibition of osteogenic differentiation induced by inflammation [134]. Further investigation indicated that the expression of MIR31HG is upregulated by inflammatory cytokines via NF-κB signaling. In turn, MIR31HG directly binds to IκBα (an NF-κB inhibitor) to result in NF-κB activation, thus inhibiting osteogenic differentiation of human ADSCs [134].

By analyzing the differential expression profile of lncRNAs between OVX rats and sham-operated controls, Yang et al. found that the expr-

ession of lncRNA ORLNC1 was much higher in serum, bone tissues, and BMSCs of OVX rats [135]. Overexpression of ORLNC1 in vitro significantly inhibits osteogenic differentiation and promotes adipogenic differentiation of BMSCs. However, knockdown of ORLNC1 in vitro can reverse these effects by inducing osteoblast-specific gene expression and repressing adipogenic differentiation-related gene expression. Through further study, ORLNC1 was demonstrated to directly bind to miR-296, and miR-296 was shown to directly bind to the 3'-UTR of Pten and then repress the expression of Pten, which is a negative regulator of osteoblastic differentiation. In summary, ORLNC1 inhibits osteogenesis and promotes adipogenesis of BMSCs by specifically sponging miR-296 to upregulate Pten expression [135]. LncRNA ODIR1 has also been identified as a negative regulator of osteogenic differentiation [136]. The expression of ODIR1 has been shown to be significantly decreased during osteogenic differentiation of human umbilical cord-derived (hUC)-MSCs [136]. Overexpression of ODIR1 significantly inhibits osteogenic differentiation in vitro and in vivo, whereas its knockdown has the opposite effects [136]. Mechanistically, ODIR1 downregulates the expression of OSX in hUC-MSCs by recruiting the E3 ubiquitin ligase CUL3, which promotes FBXO25 degradation and then decreases the enrichment of H2BK-120ub and H3K4me3 at the OSX promoter region, ultimately inhibiting osteogenic differentiation [136].

Microtubule-actin crosslinking factor 1 (MACF1) is a large spectraplakin protein that can promote osteoblast differentiation via the β-catenin/TCF1-Runx2 axis [137, 138]. Yin et al. identified a downregulated lncRNA during osteoblast differentiation, AK016739, from microarray analyses of an MACF1-knockdown pre-osteoblast cell line. The expression of AK016-739 was subsequently demonstrated to be negatively correlated with the expression of several osteogenic transcription factors, such as RUNX2, TCF7, and LEF1 [139]. AK016739 overexpression significantly inhibits osteoblast differentiation, whereas inhibition of AK0167-39 rescues this effect in OVX rats [139]. In another study by Li et al., the expression of lncRNA AK045490 was higher in bone tissues of mice and negatively correlated with osteogenic differentiation [140]. Knockdown of AK045490 in vitro enhances osteoblast differentiation by promoting the nuclear translocation of β-catenin and upregulating the expression of Runx2, TCF1, and LEF1 [140]. LncRNA HoxA-AS3 has also been identified as an anti-osteogenic lncRNA [141]. Knockdown of HoxA-AS3 in MSCs inhibits adipogenic differentiation while promoting osteogenic differentiation [141]. Specifically, downregulation of HOXA-AS3 blocks the recruitment of EZH2 to the RUNX2 gene promoter, resulting in decreased enrichment of H3K27me3 at the RUNX2 promoter and its induced expression [141]. Moreover, it has been reported that HOXC-AS3 is the natural antisense transcript of HOXC10 [142]. HOXC-AS3 can inhibit the osteogenesis of MSCs derived from multiple myeloma patients by regulating HOXC10 transcription [142].

In another recent study, Jiang et al. found that the expression levels of lncRNA SNHG were downregulated in osteogenic inducer-stimulated BMSCs compared with the control group [143]. Moreover, both p-p38 protein levels and ALP activity are upregulated in osteogenic inducer-stimulated BMSCs. However, SNHG1 overexpression can greatly reverse these effects. Further experiments indicate that SNHG1 represses the p38 MAPK signaling pathway through ubiquitination mediated by Nedd4, therefore resulting in the inhibition of the osteogenic differentiation of BMSCs [143]. Moreover, in vivo knockdown of SNHG1 via lenti-siRNA-SNHG1 injection can increase the BMD in OVX rats [143]. Another negative regulator of the osteogenic differentiation of human ADSCs is lncRNA MIAT [144]. Jin et al. found that the expression of MIAT is significantly downregulated during the process of human ADSC osteogenic differentiation. Knockdown of MIAT in vitro significantly promotes osteogenic differentiation of human ADSCs, as evidenced by the upregulated expression of RUNX2, ALP, and OCN [144]. Moreover, knockdown of MIAT in vivo can promote new bone formation [144]. Importantly, the authors found that the expression of MIAT was closely related to the expression of TNFa, which can inhibit osteogenic differentiation. Knockdown of MIAT can rescue the inhibitory effects of TNFa on osteogenic differentiation, suggesting that MIAT could affect osteogenic differentiation via TNFa [144].

Regulatory roles of lncRNAs in osteoclasts

Osteoclasts are multinucleated cells that originate from monocyte/macrophage precursor

Table 2. The molecular regulation exerted by lncRNAs in osteoclastogenesis

LncRNAs name	Functional role	Molecular Mechanism	References
AK077216	Promotion	AK077216 inhibits NIP45 expression and then promotes the expression of NFATc1, a crucial positive regulator for RANKL-induced osteoclast differentiation	[148]
LINC00311	Promotion	LINC00311 regulates the Notch signaling pathway by inhibiting DLL3 expression	[149]
MALAT1	Promotion	EPCs-derived exosomal MALAT1 promotes osteoclastogenesis via miR-124/ITGB1/IGFBP1 axis	[152]
TUG1	Promotion	TUG1 promotes osteoclastogenesis through inhibiting PTEN expression	[155]
MIRG	Promotion	MIRG binds to miR-1897 and then enhance NFATc1 expression, thus promoting osteoclastogenesis	[156]
Bmncr	Inhibition	Not clear	[157]
NONMMUT037835.2	Inhibition	NONMMUT037835.2 promotes osteoclast formation and fusion through downregulating the RANK expression and inhibits the NF-κB/MAPK signaling pathway	[147]

cells and are responsible for bone resorption [145]. The regulatory roles of lncRNAs in osteoclasts have been less studied than those in osteoblasts. The first study that systematically analyzed the expression profile of lncRNAs at different stages of osteoclastogenesis was conducted by Dou et al. in 2016 [146]. In that study, RAW264.7 cells were used as osteoclast precursors and induced with RANKL (100 ng/ml) and M-CSF (50 ng/ml) for 24, 72 and 96 hours to represent preosteoclasts, mature osteoclasts and activated osteoclasts, respectively. Further microarray analysis was conducted to analyze the differential expression profile of lncRNAs between osteoclasts at different stages and undifferentiated osteoclast precursors. Finally, the authors identified 4348 differentially expressed lncRNAs in preosteoclasts, of which 1643 were upregulated and 2705 were downregulated. Of 4602 differentially expressed lncRNAs in mature osteoclasts, 1896 were upregulated and 2706 were downregulated. Of 5840 differentially expressed lncRNAs in activated osteoclasts, 2716 were upregulated and 3124 were downregulated [146]. They further identified a total of 554 lncRNAs that were differentially expressed at all stages of osteoclastogenesis, of which 170 were upregulated and 348 were downregulated [146]. All the differentially expressed lncRNAs showed a greater than two-fold change [146]. In another recent study by Chang et al., bone marrow-derived macrophages (BMMs) were used as osteoclast precursors and induced with RANKL (50 ng/ml) and M-CSF (10 ng/ml) for 2 and 6 days to represent preosteoclasts and mature osteoclasts, respectively [147]. They subsequently analyzed the differential expression profile of lncRNAs between osteoclasts at the two different stages and undifferentiated osteoclast precursors using a mouse lncRNA microarray [147]. Finally, the authors identified 837 differentially expressed lncRNAs in preosteoclasts, of which 471 were upregulated and 366 were downregulated. Of 1614 differentially expressed lncRNAs in mature osteoclasts, 639 were upregulated and 975 were downregulated [147]. They further identified a total of 641 lncRNAs that were differentially expressed at both early and later stages of osteoclastogenesis, of which 382 were upregulated and 295 were downregulated [147]. All the differentially expressed lncRNAs showed a greater than two-fold change [147]. In this section, the molecular regulation by lncRNAs in osteoclasts will be broadly discussed in terms of lncRNAs acting as promoters and inhibitors of osteoclastogenesis (**Table 2**).

LncRNAs act as promoters of osteoclastogenesis

The expression of lncRNA AK077216 was shown to be significantly upregulated during RANKL-induced osteoclast differentiation and in the bone marrow and spleen tissues of OVX rats [148]. Overexpression of AK077216 in vitro promotes osteoclast differentiation and enhances the bone resorption capacity of osteoclasts, suggesting that AK077216 is a positive regulator of osteoclastogenesis. Moreover, the authors also found that actin ring formation and fusion were enhanced in AK077216-overexpressing cells, while they were inhibited in AK077216-silenced cells [148]. Mechanistically, upregulation of AK077216 inhibits NIP45 expression and then promotes the expression of NFATc1, which is a crucial positive

regulator of RANKL-induced osteoclast differentiation, thereby enhancing osteoclastogenesis and bone resorption [148]. As mentioned earlier, bisphosphonates are antiresorptive drugs for osteoporosis that can inhibit osteoclast activity and then slow bone resorption. By analyzing the chip GSE63009, Wang et al. found that the expression of LINC00311 was significantly downregulated in osteoclasts treated with bisphosphonates compared with osteoclasts not treated with bisphosphonates [149]. Overexpression of LINC00311 in vitro increases the number of TRAP-positive osteoclasts, accelerates osteoclast proliferation and growth, and inhibits osteoclast apoptosis. In addition, in comparison to that in sham-operated controls, overexpression of LINC00311 in OVX rats significantly reduces the BMDs in the lumbar spine, femur, and tibia [149]. Further experiments have indicated that overexpression of LINC00311 in vitro and in vivo increases the mRNA and protein levels of Notch2 and TRAP while decreasing DLL3, Notch1, Jagged1, and Hes-1 mRNA and protein levels [149]. Mechanistic investigations have revealed that LINC00311 regulates the Notch signaling pathway by inhibiting DLL3 expression, thus promoting the proliferation and differentiation of osteoclasts [149].

Endothelial progenitor cells (EPCs) are hematologic precursor cells that are able to indirectly promote bone repair by stimulating neovascularization [150]. It has been reported that EPC-derived exosomes can promote osteoblastic differentiation of BMSCs by mediating the cell-tocell communication between EPCs and BMSCs [151]. In a recent study by Cui et al., EPC-derived exosomes could also promote osteoclastogenesis of BMMs, and MALAT1 was subsequently detected to be significantly enriched in EPC-derived exosomes compared with EPCs. Knockdown of MALAT1 in EPC-derived exosomes via siRNA significantly inhibits the migration and osteoclastic differentiation of BMMs in vitro [152]. In addition, using a mouse femur fracture model, the authors observed that EPC-derived exosomes promote the homing and osteoclastic differentiation of transplanted BMMs and further promote bone repair, while inhibition of MALAT1 in EPC-derived exosomes has opposite effects [152]. Specifically, EPC-derived exosomal MALAT1 regulates osteoclastogenesis by acting as a miR-124 sponge to upregulate the expression of integrin beta 1 (ITGB1), an essential receptor for insulin-like growth factor-binding protein 1 (IGFBP1), which has the ability to promote osteoclastogenesis and bone resorption [152, 153]. Furthermore, MALAT1 is also capable of promoting MSC angiogenesis by inducing the expression of VEGF and IDO [154].

LncRNA TUG1 has also been identified as a positive regulator of osteoclastogenesis, with significantly upregulated plasma levels in osteoporosis patients compared with healthy people [155]. Overexpression of TUG1 in vitro decreases the mRNA levels of PTEN and promotes the proliferation of mouse osteoclasts. However, knockdown of TUG1 via siRNA has the opposite effects [155]. In another recent study by Li et al., the expression of lncRNA MIRG was reported to be significantly increased during osteoclastogenesis. Inhibition of MIRG via lentiviral transfection significantly represses the expression of osteoclast differentiation-associated genes, including TRAP, NFATc1, and c-FOS [156]. Mechanistic investigations have revealed that MIRG sponges miR-1897 to increase the expression of its target gene NFATc1, thus promoting osteoclastogenesis and bone resorption of BMMs derived from mouse femur [156].

LncRNAs act as inhibitors of osteoclastogenesis

At present, studies investigating lncRNAs as inhibitors of osteoclastogenesis are scarce. Previously, Chen et al. found that the expression of Bmncr is significantly decreased during RANKL-induced osteoclast differentiation and in the bone marrow and spleen tissues of OVX rats [157]. Overexpression of Bmncr in RAW 264.7 cells via lentivirus transfection inhibits osteoclast differentiation and bone resorption capacity, and it also inhibits the expression of osteoclast differentiation-associated genes, such as Atp6v0d2, Acp5, Ctr, and Mmp9, suggesting that Bmncr plays a negative role in regulating osteoclastogenesis [157]. In another recent study, Chang et al. found that overexpression of lncRNA NONMMUT037835.2 suppresses osteoclast differentiation, while inhibition of NONMMUT037835.2 promotes osteoclast formation and fusion [147]. Further in-

vestigation showed that NONMMUT037835.2 might inhibit osteoclastogenesis by downregulating the expression of RANK and inhibiting the NF-κB/MAPK signaling pathway [147].

Clinical application of lncRNAs in osteoporosis

Currently, many studies have reported that lncRNAs are able to act as potential diagnostic and prognostic biomarkers for multiple diseases, especially cancers [158-161]. As we have described, many lncRNAs are aberrantly expressed in patients with osteoporosis compared with healthy people, which suggests that lncRNAs could also be potential diagnostic and prognostic biomarkers for osteoporosis. According to official clinical trial registries (www.clinicaltrials.gov), several clinical trials involving lncRNAs acting as diagnostic and prognostic biomarkers have been registered. However, none of them are evaluating the diagnostic and prognostic value of lncRNAs in osteoporosis. In fact, at present, only a few studies with small sample sizes have assessed the diagnostic and prognostic value of lncRNAs in osteoporosis. Previously, Yu et al. reported that plasma levels of lncRNA CASC11 are upregulated in postmenopausal osteoporosis patients compared with healthy people [162]. Subsequently, ROC curve analysis was performed to assess the diagnostic capacity of plasma CASC11 for osteoporosis. Generally, an area under the ROC curve (AUC) with a value from 0.75 to 0.92 is acceptable for a diagnostic test, and a value ranging between 0.93 and 0.96 is considered to be excellent [163]. The ROC curve analysis results indicated that plasma CASC11 has an excellent diagnostic value with an AUC reaching 0.96 [162]. In addition, the authors also observed that patients with high CASC11 plasma levels have a longer treatment course and higher recurrence rate, suggesting that high CASC11 plasma levels are associated with a poor prognosis [162]. Another lncRNA, TUG1, is also highly expressed in the plasma of osteoporosis patients compared with healthy people [155]. ROC curve analysis results indicated that plasma TUG1 has a good diagnostic value for osteoporosis with an AUC reaching 0.90. Moreover, the plasma levels of TUG1 are significantly upregulated with an increase in the osteoporosis stage [155]. The expression of lncRNA-NEF has been shown to be significantly downregulated in plasma of postmenopausal osteoporosis patients compared with healthy people [164]. Plasma lncRNA-NEF is sufficient to distinguish postmenopausal osteoporosis patients from healthy people, with an AUC reaching 0.8919. Moreover, in comparison to that in patients with high lncRNA-NEF plasma levels, the recurrence rate is significantly higher and the treatment course is significantly longer in patients with low lncRNA-NEF plasma levels, suggesting that low lncRNA-NEF plasma levels are associated with a poor prognosis [164]. Another recent study by Huang et al. reported that the plasma levels of lncRNA SNHG1 were significantly downregulated in postmenopausal osteoporosis patients compared with healthy postmenopausal females and healthy premenopausal females [165]. The results of the ROC curve analysis indicated that plasma SNHG1 has a good diagnostic value for osteoporosis, with an AUC reaching 0.79 [165].

As the regulatory roles of lncRNAs in osteoporosis are gradually being revealed, lncRNAs might be developed as novel therapeutic targets for osteoporosis. According to the functions of lncRNAs involved in osteoporosis, treatment strategies can be roughly divided into two categories. One is to upregulate lncRNAs that can promote osteogenic differentiation or inhibit osteoclastogenesis. Another is to inhibit lncRNAs that can suppress osteogenic differentiation or promote osteoclastogenesis. However, a detailed discussion about how to upregulate or downregulate the expression of specific lncRNAs is beyond the scope of this review.

Conclusion and prospective

Over the past decade, the lncRNA field has made significant progress. Numerous novel lncRNAs have been identified to be aberrantly expressed in various diseases. Importantly, many of them have been demonstrated to have crucial biological functions and participate in the occurrence and development of various diseases [166]. In this review, we provide up-to-date knowledge regarding the roles of lncRNAs in osteoporosis, which suggests that lncRNAs play important roles in the pathogenesis of osteoporosis and have great potential as biomarkers and therapeutic targets for osteoporosis. As demonstrated in this review, many lncRNAs can regulate the process of osteogenic differentiation and osteoclastogenesis by

modulating gene expression at different transcriptional levels. At present, the most reported mechanism is that lncRNAs, such as H19, MALAT1, LOC103691336, and lncRNA ODSM, modulate gene expression by acting as endogenous miRNA sponges. In addition, some lncRNAs, such as lnc-ob1, H19, HOTAIR and PGC1β-OT1, can also directly or indirectly mediate epigenetic changes. However, it should be noted that research on lncRNAs in the osteoporosis field is still in its infancy. There are many issues that need to be investigated in future studies. First, although the molecular regulation by lncRNAs in osteoblasts and osteoclasts is broadly discussed in this review, the exact mechanisms of most lncRNAs are not fully investigated. For instance, based on this review, we know that some lncRNAs, such as H19, MALAT, ANCR, and MEG3, can regulate osteoblastic differentiation via multiple molecular mechanisms. This phenomenon may be present in other lncRNAs and in the process of osteoblastic differentiation. Therefore, further studies are needed to investigate the detailed regulatory mechanisms of lncRNAs in osteogenesis and osteoclastogenesis. Second, at present, studies regarding the regulatory roles of lncRNAs in osteoclastic differentiation are far fewer than those regarding osteoblastic differentiation. Therefore, researchers should focus more attention on the regulatory roles of lncRNAs in osteoclastogenesis. Third, exosomes are a class of 40-150 nm extracellular vehicles involved in intercellular communication that can be released by all cell types [167]. Accumulating evidence has demonstrated that exosomes contain abundant proteins, lipids, and ncRNAs, such as lncRNAs, miRNAs, and mRNAs. In recent years, exosomes have become a research hotspot, and an increasing number of studies are focusing on the association between exosomes and various diseases. It has been reported that exosomes can transfer biologically active molecules to osteoclasts and osteoblasts, thus modulating the processes of osteoclastic and osteoblastic differentiation [168-170]. Moreover, exosomal lncRNAs have been verified to participate in tumorigenesis, tumor angiogenesis, and tumor chemoresistance [171]. However, at present, only two studies have investigated the functions of exosomal MALAT1 in osteoclasts and osteoblasts [91, 152]. Therefore, it is necessary to investigate whether other exosomal lncRNAs could regulate osteogenesis and osteoclastogenesis in future studies. Finally, only a few studies with small sample sizes have assessed the diagnostic and prognostic value of lncRNAs in osteoporosis; therefore, more large-sample studies are also needed to evaluate this issue.

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Disclosure of conflict of interest

None.

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