The Roles of Long Non-coding RNAs in Osteogenic Differentiation and Bone Diseases

Sen Qin, Da Liu*, Zimo Zhou and Senxiang Chen

Abstract
Bone marrow mesenchymal stem cells play an important role in osteogenic differentiation, and they complete this important biological process through the coordination of various transcription factors and signal pathways. In recent years, studies have clearly confirmed that long non-coding RNAs (lncRNAs) are involved in osteogenic differentiation, which plays an important biological role in the occurrence and development of osteogenesis-related bone disease. This article reviews the roles and related mechanisms of lncRNAs in osteogenic differentiation, as well as their potential effects on a variety of bone diseases. This understanding may help researchers identify potential therapeutic targets and biological markers in the future.

Keywords
Long non-coding RNA, osteogenesis differentiation, osteosarcoma, osteoarthritis, osteoporosis.

Introduction
Bone marrow mesenchymal stem cells (BMSCs) have a variety of differentiation routes, including adipogenic differentiation, myogenic differentiation, and osteogenic differentiation [1]. Osteoblasts are produced primarily by BMSCs, which have a crucial biological function in maintaining the balance between bone production and resorption. In recent years, abundant evidence has indicated that a variety of molecules can regulate osteogenic differentiation and serve as markers of osteogenic differentiation, including short stature-related transcription factors (RUNX2), alkaline phosphatase (ALP), and osteopontin (OPN) [2]. Although several studies have revealed the factors and pathways regulating the osteogenic differentiation of BMSCs, the specific molecular mechanisms remain unknown.

Long non-coding RNAs (lncRNAs) are usually defined as a type of transcript with a length of more than 200 nucleotides, which have almost no protein coding ability. These lncRNAs are derived mainly from overlapping protein coding regions and intergenic protein coding regions of genes, and transcribed by polymerase II [3]. LncRNAs are named according to their neighboring protein-coding genes. They can be divided into five types according to the correspondence of their spatial position with related protein coding genes: long intergenic, intron, bidirectional, antisense, and sense [4]. LncRNAs have long been considered to be a by-product of RNA polymerase II transcription and to have no biological functions. However, through splicing, lncRNAs obtain the characteristic structure of a 5′ cap and 3′ polyadenylic acid, which activate the transcription process. We briefly summarize several transcription patterns of lncRNAs in Figure 1.

Although the exact roles of most lncRNAs are still being studied, current studies have confirmed that lncRNAs play important roles in the biological processes of cell proliferation, differentiation, and migration [5]. LncRNAs have the ability to mediate alternative splicing, chromatin modification, RNA metabolism, and gene expression at the epigenetic, transcriptional, and post-transcriptional levels, thereby regulating these biological processes [6]. Therefore, lncRNAs are involved in the occurrence and development of many diseases, including bone diseases.

To the best of our knowledge, no literature review has summarized and discussed the osteogenic differentiation...
roles of lncRNAs and their specific roles in bone diseases. In this paper, we conducted a systematic search of the relevant literature, and review the modes of action and mechanisms of lncRNAs in osteogenic differentiation and bone diseases.

**Modes of action and mechanisms of lncRNAs**

lncRNAs perform their biological functions in different ways, and we describe their modes of action (Figure 2) as follows: signal, decoy, guide, and support [7]. lncRNAs play biological roles through one or more of four prototypical mechanistic networks. They perform their functions by participating in each stage of regulating gene expression, including epigenetic modification of chromatin, regulation of transcription, and post-transcriptional regulation.

lncRNAs can be used as scaffolds providing sites for regulation at the level of epigenetic modification of chromatin. They participate in the regulation of related target gene expression by recruiting chromatin remodeling complexes to specific regions or recruiting modifiers to chromatin regulatory regions. *HOTAIR* shows high affinity for target genes and certain proteins. It is capable of attracting polycomb inhibitory complex 2 (PRC2) and histone demethylase 1A (LSD1) [8]. Subsequently, targeting of histone H3K4 demethylation and H3K27 trimethylation affect chromosomal condensation and, ultimately, gene silencing [8].

The regulation of transcription levels is relatively complex and involves multiple mechanisms. First, lncRNAs control the expression of target genes by acting directly on transcription factors. *NRON* inhibits downstream target gene expression by blocking nucleocytoplasmic trafficking of the transcription factor NFAT [9]. Second, lncRNAs compete with transcription factors or function as bait to prevent transcription factors from binding to homologous DNA sites, thereby controlling target gene expression. Glucocorticoid is a negative regulator of bone formation. Its receptor gene binding domain is bound by *Gas5*, which acts as a bait, or is competitively bound, thus resulting in a decline in receptor function and finally encouraging osteogenic differentiation [10]. Furthermore, certain promoter-related lncRNAs can act on RNA-binding proteins and regulate genes by influencing the promoter. Cyclin D1 (CCND1) is transcribed in the 5' upstream region, and the promoter-related non-coding RNA-D (pncRNA-D) specifically binds to the RNA-binding protein TLS, thus inhibiting its activity and exerting a transcriptional inhibitory effect [11]. Finally, lncRNAs recruit transcription factors and act as transcription regulators that exert gene regulation. *Evf2* recruits the transcription factor Dlx2, forms a complex, and then induces the expression of related genes [12].

When stable complementary sequences exist between lncRNAs and miRNAs, the two combine to form a double-stranded complex that stabilizes the miRNA structure.
According to the competitive endogenous RNA (ceRNA) theory, RNA sequences with the same miRNA response element (MRE) might compete for miRNA, serving as an RNA sponge that prevents miRNA from binding to its target [13].

HOTAIR affects the expression of its target gene Smad7 by competitively binding miR-17-5p, thereby participating in the regulation of osteogenic differentiation [14].

Roles of lncRNAs in osteogenic differentiation

In the process of osteogenic differentiation, lncRNAs expressed to different degrees may have varying influence on the normal proliferation, differentiation, and apoptosis of BMSCs. The regulation of osteogenic differentiation by lncRNAs can take the following three forms: as a precursor of miRNA, lncRNAs participate in the regulation of osteogenic differentiation; in the regulation of osteogenic differentiation by regulating various signal pathways; and in the regulation of osteogenic differentiation as ceRNAs or “miRNA sponges.” In the following and in Figure 3, we briefly summarize some lncRNAs and their specific forms of regulation.

LncRNAs that promote osteogenic differentiation

**H19**

H19 is generated from the transcription product of the H19/insulin growth factor 2 (IGF2) gene cluster on human chromosome 11p5.5 [15]. It may be utilized as a precursor of miR-675, which can then be sheared to create two stable miRNAs, miR-675-5p and miR-675-3p, and influence the downstream Wnt signaling pathway to govern stem cell osteogenesis [16, 17]. Second, H19 controls osteogenesis via the Notch molecules’ cellular signaling pathway [18]. The Notch signaling system is crucial in controlling osteogenic differentiation. H19 expression suppression and constitutive expression both result in upregulation of the expression of miRNAs that influence Notch ligands and receptors, encouraging the osteogenic differentiation of MSCs [18]. Similarly to the Notch signaling pathway, the Wnt signaling pathway, as a downstream target of H19, plays an important role in the regulation of osteogenic differentiation. H19’s involvement in inducing osteogenic differentiation via this route has also been well established [19]. Finally, H19 acts as a ceRNA regulating osteogenic differentiation. A binding site exists between H19 and miR-138, which can be utilized as the miRNA’s ceRNA. Both work together to suppress miR-138 and
diminish the inhibitory effects of protein tyrosine kinase (PTK2), the gene encoding focal adhesion kinase (FAK), thus promoting FAK expression and, in turn, osteogenic differentiation [20].

MEG3

Maternally expressed gene 3 (MEG3), as a tumor suppressor, participates in the regulation of osteogenic differentiation of BMSCs and is closely associated with the expression of osteogenic markers. Knockout of MEG3 gene expression decreases RUNX2, OSX, and OCN at the transcriptional level and hinders the formation of mineralized nodules, thereby inhibiting osteogenic differentiation [21]. MEG3 can separate the negative regulator SOX2 from the promoter region of bone morphogenetic protein 4 (BMP4), thus leading to BMP4 activation [21]. In addition, in human adipose stem cells (HASCs), knockout of MEG3 effectively promotes the differentiation of adipocytes while significantly inhibiting osteogenic differentiation [22]. This effect is mediated by miR-140-5p, an miRNA that inhibits osteogenic differentiation, and their expression is negatively correlated. Therefore, when MEG3 is overexpressed, it has a significant positive regulatory effect on osteogenic differentiation.

MALAT1

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is an imprinted gene that is expressed in a range of tissues, and is linked to a number of illnesses and biological processes. In hFoB1.19 osteoblasts, although the relationship between MALAT1 and osteogenic differentiation is not yet clear, MALAT1 has been confirmed to regulate the expression of OPG [23]. Later, Xiao et al. [24] investigated the effect of MALAT1 in calcified aortic valve disease and discovered that it promotes osteogenic differentiation substantially. MALAT1 can be utilized to allow miR-204 to control Smad4 via the ceRNA method, thus causing Smad4 to be upregulated, promoting the production of osteoblast-specific markers and eventually leading to the development of mineralized nodules and bone regeneration [24]. MALAT1 may also be utilized in miR-143 to enhance BMSC osteogenic differentiation through the same method [25].

Other lncRNAs

Some additional lncRNAs are involved in the regulation of osteogenic differentiation. Downregulation of IncRNA-OG in vitro decreases the expression of RUNX2 and also inhibits the activity of ALP and the formation of mineralized nodules. IncRNA-OG interacts with heterogeneous ribonucleoprotein K (hnRNPK), thus enhancing H3K27 acetylation of the IncRNA-OG promoter and upregulating IncRNA-OG transcriptional activity, thereby controlling BMP signal activation [26]. IncRNA AK028326 is the same as IncRNA AK141205, and their osteogenic effects are closely associated with CXC chemokine ligand-13 (CXCL13). High glucose levels normally prevent BMSCs from undergoing osteogenic differentiation. The high expression of AK028326 upregulates CXCL13 and reverses the negative effect of high glucose levels on osteogenic differentiation [27].
Osteogenic growth peptide (OGP) is an osteogenic differentiation promoter that increases ALP activity and osteogenic marker expression. Overexpression of AKT14205 promotes H4 histone acetylation, and inhibits histone deacetylase 1 (HDAC1) and induces CXCL13 expression, thereby promoting OGP activity [28]. MSC-AS1 is a newly discovered lncRNA involved in the osteogenic differentiation of BMSCs [29]. MSC-AS1 and BMP2 are considerably upregulated during BMSC osteogenic development, but miR-140-5p expression is decreased. Consequently, MSC-AS1 may be utilized as the ceRNA of miR-140-5p to enhance BMSC osteogenic differentiation by increasing BMP2 expression [29]. Similarly, KCNQ1OT1 works as a ceRNA toward miR-214 that promotes osteogenic differentiation of BMSCs by further favorably regulating BMP2 expression [30]. Furthermore, KCNQ1OT1 also promotes osteogenic differentiation by activating the Wnt/β-catenin signaling pathway [31].

LncRNAs that inhibit osteogenic differentiation

DANCR

DANCR is the first lncRNA found to inhibit osteogenic differentiation [32]. DANCR interacts with ZESTE homologous gene enhancer 2 (EZH2), thus increasing the methylation of histone H3 lysine 27 (H3K27me3) in the promoter of the RUNX2 gene and resulting in the inhibition of RUNX2 gene expression [32]. In addition, Zhang et al. [33] and Jia et al. [34] found that DANCR has obvious anti-osteogenesis effects in BMSCs and periodontal ligament stem cells, respectively. DANCR overexpression effectively inhibits the phosphorylation of p38, changing the information transduction of p38/MAPK signaling pathway, and inhibits osteogenic differentiation [33]. Furthermore, low DANCR expression stimulates the Wnt/β-catenin signaling pathway and causes RUNX2 production [34].

HOTAIR

HOX transcribed antisense RNA (HOTAIR), which can remodel chromatin to enhance its own inhibitory effect on HOX genes and other target genes, is another lncRNA that can have a negative effect on osteogenesis [16]. In BMSCs of patients with non-traumatic femoral head necrosis, HOTAIR expression is considerably enhanced, and knocking out HOTAIR decreases the DNA methyltransfer level of the miR-17-5p promoter and increases miR-17-5p expression [14]. The upregulation of HOTAIR decreases Runx2 expression and ALP activity, and this effect is reversed by increasing miR-17-5p [14]. Furthermore, when HOTAIR is overexpressed, the expression of Wnt/β-catenin pathway-related specific proteins (β-catenin, cyclin D, and C-myc) considerably decreases, whereas the expression of the pathway’s negative regulator, Dkk1, is significantly elevated [35]. HOTAIR may inhibit the osteogenic differentiation of BMSCs by inhibiting the Wnt/β-catenin signaling pathway.

Other lncRNAs

Some new lncRNAs have also been reported to have anti-osteogenesis effects, including ORLNC1, SNHG1, Hoxa-AS3, and HCG18 [36–39]. In an ovariectomized animal model (OVX), the expression of ORLNC1 is significantly elevated. It acts as a ceRNA that competitively targets miR-296, causing changes in the level of phosphatase and tensin homolog (PTEN), a downstream target, and ultimately inhibiting the osteogenic differentiation of BMSCs in model mice [36]. SNHG1 effectively blocks the activation of p38 through Nedd4, an E3 ubiquitin ligase, and interferes with the important pathway of osteogenic differentiation (p38/MAPK signaling pathway), thereby inhibiting the osteogenic differentiation of BMSCs [37]. Hoxa-AS3 regulates the balance between adipogenic differentiation and osteogenic differentiation and inhibits osteogenic differentiation by regulating EZH2. Overexpressed Hoxa-AS3 binds EZH2, thus decreasing the binding of the latter to the promoter region of the Runx2 gene and decreasing the expression of Runx2 [38]. HCG18 is a newly discovered lncRNA that is abnormally expressed in a variety of malignant diseases. The research on osteoporosis (OP) clearly indicates that HCG18’s inhibitory effect on the downstream target gene NOTCH1 is decreased through sponging miR-30a-5p [39]. Furthermore, HCG18 indirectly increases the expression of NOTCH1, and overexpression of NOTCH1 produced by the two forms inhibits BMSC osteogenic development [39].

LncRNAs and bone diseases

LncRNAs have been linked to the incidence and progression of a number of bone disorders, including osteosarcoma (OS) [40], osteoarthritis (OA) [41, 42], OP [43, 44], ankylosing spondylitis (AS) [45], and rheumatoid arthritis (RA) [46]. In the following content, we summarize the lncRNAs that are associated with bone diseases, as shown in Table 1.

LncRNAs in OS

OS is one of the world’s most frequent primary malignant bone tumors, affecting primarily children and teenagers. Despite substantial improvement in current treatment approaches and tactics for OS, patients still have poor prognoses and high recurrence rates. Elucidating the pathogenic process of OS is critical for identifying novel treatment targets for the illness.

Some lncRNAs are highly expressed in OS tissues, thus suggesting that lncRNAs are associated with the occurrence and development of OS [40, 47–49]. DANCR competes with miR-335-5p and miR-1972 as “ceRNA” that promotes the expression of Rho-associated coiled-coil-containing protein kinase 1 (ROCK1) in OS tissues, thereby promoting tumor cell proliferation and invasion [40]. SNHG3 is highly expressed in OS tissues and absorbs miR-151a-3p through a competitive action, thus decreasing its negative regulatory effect on RAB22A in the RAS oncogene family,
thereby mediating the progression of OS [48]. Similarly, LncRNA-ROR competitively binds miR-185-3p and upregulates the expression of Yes-associated protein 1 (YAP1), which, in turn, affects the aggressiveness of OS cells [49]. The inhibitory effect of YAP1 gene knockout on OS cell proliferation has been clearly confirmed [50]. In addition to the ceRNA effect, certain lncRNAs have been shown to alter OS via established signaling pathways. H19 gene knockout can lead to inactivation of the PI3K/AKT signaling pathway, which in turn results in ineffectiveness of the NF-kB pathway, thus inhibiting the migration of osteosarcoma cells and enhancing the invasion ability [47]. In contrast, the expression of Gas5 is decreased in OS tissues and cell lines. It acts as a ceRNA that engulfs miR-23a-3p and regulates PTEN, which, through its lipid phosphatase activity and PI3K downstream targeting molecule PIP3, blocks the PI3K/ATK pathway and produces a tumor suppressor effect [51].

### Table 1: LncRNAs Associated with Bone Diseases

| Bone diseases | LncRNA name | Expression | Targets | Reference |
|---------------|-------------|------------|---------|-----------|
| Osteosarcoma  | DANCR       | Upregulation | miR-335-5p/ROCK1 axis, miR-1972/ROCK1 axis | [40] |
|               | SNHG3       | Upregulation | miR-151a-3p/RAB22A axis | [48] |
|               | LncRNA-ROR  | Upregulation | miR-185-3p/YAP1 axis | [49] |
|               | H19         | Upregulation | PI3K/ATK and NF-kB pathway | [47] |
|               | Gas5        | Downregulation | miR-23a-3p/PTEN/PI3K/ATK pathway | [51] |
| Osteoarthritis| MF21-AS1    | Upregulation | miR-130a-3p/TCF4 axis | [41] |
|               | Gas5        | Upregulation | Sponge for miR-21 | [53, 60] |
|               | PVT1        | Upregulation | Sponge for miR-488-3p | [54] |
|               | ZFAS1       | Upregulation | Wnt/β-catenin pathway | [55] |
|               | MALAT1      | Upregulation | Sponge for miR-150-5p | [42] |
|               | MEG3        | Downregulation | miR-93/TGFBR2 axis, VEGF | [57, 61] |
|               | SNHG5       | Downregulation | miR-10a-5p/H3F3B axis, miR-181a-5p/TGFBR3 axis | [56, 58] |
|               | HOTAIR      | Downregulation | miR-138/NF-kB axis | [59] |
|               | H19         | Downregulation | DKK4/Wnt/β-catenin axis, DNMT1/H19/ERK-MAPK axis, miR-532-3p/SIRT1 axis, FOX2/WNT4 axis | [63, 64, 69, 70] |
| Osteoporosis  | MALAT1      | Downregulation | PPM1E/AMPK or PPM1E/Nrf2 axis | [73] |
|               | ANCR        | Upregulation | Binding with EZH2 | [71] |
|               | MEG3        | Upregulation | Sponge for miR-133a-3p | [66] |
|               | XIST        | Upregulation | miR-758-5p/caspase 3 axis | [68] |
|               | LOXL1-AS1   | Upregulation | miR-196a-5p/HmgA2/PPARγ axis | [44] |
|               | EPIC1       | Upregulation | Myc | [72] |
| Rheumatoid arthritis | PICSAR | Upregulation | Sponge for miR-4701-5p | [46] |
|               | NEAT1       | Upregulation | miR-204-5p/NF-kB axis | [74] |
|               | H19         | Upregulation | TAK1/NF-kB or TAK1/JNK/p38/MAPK pathway | [75] |
|               | MEG3        | Downregulation | Not explored | [76] |
|               | LOC645166   | Downregulation | TLR/IKK2/IKB/NF-kB axis | [77] |
| Ankylosing spondylitis | H19 | Upregulation | Sponge for miR-22-5p and miR-675-5p | [45] |

**LncRNAs in OA**

OA is a chronic joint disease characterized by progressive deterioration of the structure and function of articular cartilage. The occurrence and development of OA are closely related to the apoptosis of chondrocytes, the degradation of extracellular matrix, abnormal inflammation, and synovial angiogenesis [52]. We summarized the lncRNAs associated with OA and distinguished them according to the following levels:

1. LncRNAs that regulate cell proliferation and apoptosis. Compared with that in normal bones and joints, the expression of MF21-AS1 in OA is upregulated [41]. Overexpression of MF21-AS1 significantly promotes the inhibition of chondrocyte viability and the formation of apoptosis induced by lipopolysaccharide (LPS). This lncRNA acts as a ceRNA that targets and negatively regulates miR-130a-3p, weakening the inhibitory effect of miR-130a-3p on transcription factor 4 (TCF4) [41]. Gas5 and PVT1 are also highly expressed in OA chondrocytes. They sponge miR-21 and miR-488-3p, respectively, thus stimulating chondrocyte apoptosis. They also express miR-21 and miR-488-3p, respectively, thus stimulating chondrocyte apoptosis.

   - **Expression**: Upregulation
   - **Targets**: miR-130a-3p/TCF4 axis
   - **Reference**: [41]

2. LncRNAs that regulate the degradation of extracellular matrix. MALAT1 is highly expressed in OA tissues and cell lines, and it indirectly promotes the expression of AKT3 through sponging miR-150-5p, thus leading to the synthesis of cartilage degrading enzymes such as matrix metalloproteinase-13 (MMP-13) and a disintegrin...
LncRNAs in OP

In recent years, many lncRNAs have been regarded as new regulators of OP. OP is one of the most prevalent systemic metabolic bone disorders, characterized by bone loss, degradation of bone microstructure, bone fragility, and increased fracture risk [62].

Disuse OP (DOP) is caused by a long-term lack of mechanical load or after the limb is stopped for a long time, thus leading to changes in bone structure and integrity and ultimately causing bone loss in the limb. Dkk4 is H19’s downstream target, and mechanical unloading enhances Dkk4 expression by lowering H19 expression [63]. The upregulation of Dkk4 suppresses the Wnt/β-catenin signaling pathway, thus decreasing osteogenic differentiation and the onset and progression of DOP [63]. Another study has also confirmed the role of H19 in DOP. In DOP bone tissue, the expression of DNA methyltransferase-1 (DNMT1) is over-regulated, thus promoting the methylation of the H19 promoter and inhibiting the downstream target ERK [64]. The ERK-MAPK signaling pathway involved in ERK has been demonstrated to play a key role in bone homeostasis and bone development [65].

Postmenopausal OP (PMOP) is a metabolic bone disease. The basic pathogenesis is abnormal bone metabolism caused by a significant decrease in estrogen levels in the body. In PMOP-derived BMSCs, the levels of MEG3 and its downstream target miR-133a-3p are abnormally increased. When the expression of miR-133a-3p, which is positively regulated by MEG3, is upregulated, it significantly inhibits the formation of osteogenic markers and mineralized nodules [66]. Therefore, decreasing the expression of MEG3 is expected to be a treatment to improve the progression of PMOP. Abnormal accumulation of iron can accelerate bone mineral loss in healthy postmenopausal women [67]. In the iron accumulation model, the expression of XIST and caspase 3 increases, and the expression of miR-785-3p decreases [68]. Therefore, XIST may act as the ceRNA of miR-758-3p, regulate caspase 3, and facilitate the formation of PMOP mediated by iron abnormality. In patients with PMOP, the expression of H19 and SIRT1 is downregulated, whereas miR-532-3p is upregulated. H19 interacts directly with miR-532-3p, upregulating the expression of SIRT1, and induces osteogenic differentiation of BMSCs; estrogen reverses the low expression of H19 in bone tissue [69]. In addition, H19 directly binds to forkhead box C2 (FOXC2), enhances the binding ability of FOXC2 and WNT4 promoter, promotes the osteogenic differentiation of BMSCs through the Wnt/β-catenin signaling pathway, and alleviates the progress of PMOP [70]. Furthermore, some other lncRNAs are also involved in PMOP regulation, including ANCR and LOXL1-ASI. The specific binding of ANCR and EZH2 inhibits the expression of Runx2, thereby inhibiting the osteogenesis of PMOP osteoblasts and the formation of osteoid in vivo [71]. In the serum of patients with PMOP, LOXL1-ASI and Hmga2 levels are elevated, and miR-196a-5p levels are diminished. LOXL1-AS regulates Hmga2 as a sponge of miR-196a-5p, inhibits the osteogenic differentiation of BMSCs by activating C/EBP-β-mediated PPARγ expression, and aggravates the progression of PMOP [44].

Glucocorticoid-induced OP is a type of OP caused by long-term use of glucocorticoids, such as dexamethasone (Dex). Studies have indicated that EPIC1 effectively protects osteoblasts from Dex-induced cytotoxicity [72]. EPIC1 targets Myc, thus supporting cell survival, whereas gene silencing increases the toxicity of Dex in cells [72]. The expression of MALAT1 decreases in the hFOB1.19 osteoblast cell line treated with Dex. This lncRNA activates AMPK signal transduction by inhibiting PPM1E (protein phosphatase, Mg2+/Mn2+-dependent 1E) [73]. Meanwhile, MALAT1 increases the activity of nicotinamide adenine dinucleotide phosphate and the activation of the Nrf2 signal [74].

LncRNAs in other bone diseases

RA is an autoimmune disease characterized by chronic inflammation of joint synovium. Its main pathological feature is that the hyperplastic synovial lining tissue contains a large number of activated fibroblast-like synovial cells (FLSS), which interact with a variety of cells and pro-inflammatory cytokines. In RA synovial fluid and RA-FLSS, PICSAR is significantly higher than that in the normal control group. As the targeting ceRNA of miR-4701-5p, this lncRNA promotes the proliferation, migration, and invasion of RA-FLSS cells and also increases and decreases the secretion of pro-inflammatory cytokines and certain proteases, respectively [46]. NEAT1 promotes the proliferation and apoptosis of RA-FLSS cells induced by TNF-α by targeting miR-204-5p and activates the NF-kB signaling pathway, thus mediating the secretion of pro-inflammatory...
cytokines, thereby promoting the progression of RA [74]. TNF-α is responsible for the high expression of H19 in RA, and the increased expression of H19 promotes the phosphorylation of TGF-β-activated kinase 1 (TAK1), which activates the NF-kB and JNK/p38MAPK signaling pathways and promotes the expression of inflammatory cytokines [75]. Unlike the previous IncRNAs, MEG3 is downregulated in RA tissues and is negatively correlated with NOD-like receptor 5 (NLRC5) [76]. NLRC5 is involved in the hypermethylation of the MEG3 gene promoter, which inhibits MEG3 gene transcription, whereas high MEG3 expression lowers NLRC5 and inflammatory cytokines, preventing the development of RA [76].

AS is a chronic systemic inflammatory disease, mainly manifested by arthritis of the spine and sacroiliitis. One study has found that the expression of lncRNA LOC645166 is downregulated in patients with AS [77]. LOC645166 inhibits TLR-mediated activation of IKK2 by binding to the K63-linked polyubiquitin chain, thus resulting in downregulation of IkB phosphorylation. The stable IkB binds to NF-kB, decreases the transfer of NF-kB/IkB complex to the nucleus, and prevents NF-kB from entering the nucleus and subsequently facilitating the transcription of inflammatory cytokine target genes [77]. In 49 patients with AS, compared with healthy controls (49 cases), H19 was significantly expressed in peripheral blood mononuclear cells (PBMC) [45]. H19 acts as a ceRNA targeting miR-22-5p and miR-675-5p, and it significantly increases the expression of vitamin D receptor, TGF-β, and pro-inflammatory factors, such as IL-17A and IL-23 [45].

Summary and outlook

LncRNA research has yielded promising findings in recent years; however, for lncRNAs, compared with miRNAs, further studies are needed. LncRNAs were initially identified in cancer research, but have now been revealed to play a key role in stem cell research across a wide range of fields, including BMSCs. This article reviews the roles of lncRNAs in the osteogenic differentiation of BMSCs. Many studies have identified various processes that govern osteogenic differentiation, including miRNA precursors, signal pathways, and ceRNA mechanisms, by using in situ hybridization technology, luciferase reporter gene experiments, gene chips, and microarray expression profiling. The discovery of these mechanisms helps understand the regulatory role of lncRNAs in osteogenic differentiation.

We further discussed the role of lncRNAs in the pathogenesis of related bone diseases, including bone tumors, OA, OP, RA, and mandatory spondylitis. LncRNAs have multiple regulatory effects through different molecular mechanisms during the occurrence and development of these diseases. The expression of lncRNAs that positively regulate osteogenic differentiation could be promoted in order to improve osteogenic differentiation, or lncRNAs that inhibit osteogenic differentiation could be knocked out to improve bone regeneration. In addition, bone mineral loss after spinal cord injury can also cause OP, which is usually referred to as “fixed OP,” a special type of OP. Although no reports have indicated which type of effect IncRNAs have on this type of OP, the current single-brake disuse theory cannot fully explain this severe bone loss and decline in bone structure degeneration [78]. Therefore, in future research in this field, lncRNAs may serve as a research focus to provide us with new research directions.

Despite the importance of IncRNAs in the regulation of osteogenic differentiation and bone diseases is considerable, future research in this area must be further advanced in order to guide the treatment of orthopedic clinical diseases.

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Not applicable.

Consent for Publication

Not applicable.

Data Availability

Not applicable.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Authors’ Contributions

S.Q. drafted and wrote the manuscript. S.C. and Z.Z. assisted with writing the manuscript and provided helpful suggestions. D.L. reviewed the final manuscript and provided advice on writing the manuscript. All authors read and approved the final manuscript.
References

[1] Abarrategi A, Mian S, Passaro D, Rouault-Pierre K, Grey W, et al. Modeling the human bone marrow niche in mice: from host bone marrow engraftment to bioengineering approaches. J Exp Med 2021;218:729-43. [PMID: 29453226 DOI: 10.1084/jem.20172139]

[2] Crockett J, Rogers M, Coxon F, Hocking L, Helfrich M. Bone remodelling at a glance. J Cell Sci 2011;124:991-8. [PMID: 21402872 DOI: 10.1242/jcs.063032]

[3] Quinn J, Chang H. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet 2016;17:47-62. [PMID: 26666269 DOI: 10.1038/nrg.2015.10]

[4] Ma L, Bajic V, Zhang Z. On the classification of long non-coding RNAs. RNA Biol 2013;10:925-33. [PMID: 23696037 DOI: 10.4161/rna.24604]

[5] Ponting C, Oliver P, Reik W. Evolution and functions of long non-coding RNAs. Cell 2009;136:629-41. [PMID: 19239885 DOI: 10.1016/j.cell.2009.02.006]

[6] Cui Z, Ren S, Lu J, Wang F, Xu W, et al. The prostate cancer-up-regulated long noncoding RNA PcnlncRNA-1 modulates apoptosis and proliferation through reciprocal regulation of androgen receptor. Urol Oncol 2013;31:1117-23. [PMID: 22264502 DOI: 10.1016/j.urolonc.2011.11.030]

[7] Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell 2011;43:904-14. [PMID: 21925379 DOI: 10.1016/j.molcel.2011.08.018]

[8] Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, et al. Long noncoding RNA as modular scaffold of histone modification complexes. Science 2010;329:689-93. [PMID: 20616235 DOI: 10.1126/science.1192002]

[9] Willingham A, Orth A, Batalov S, Peters E, Wen B, et al. A strategy for probing the function of noncoding RNAs finds a repressor of NFAT. Science 2005;309:1570-3. [PMID: 16141075 DOI: 10.1126/science.1116901]

[10] Kino T, Hurt D, Ichijo T, Nader N, Chrousos G. Noncoding RNA and functions as a Dlx-2 transcriptional coactivator. Genes Dev 2006;20:1470-84. [PMID: 16705037 DOI: 10.1101/gad.1416106]

[11] Kurokawa R. Promoter-associated long noncoding RNAs. Cell 2009;136:629-41. [PMID: 19239885 DOI: 10.1016/j.cell.2009.02.006]

[12] Feng J, Bi C, Clark B, Mady R, Shah P, et al. The Evf-2 noncoding RNA is transcribed from the Dlx-5/6 ultraconserved region in mice. Nature 1995;375:34-9. [PMID: 7536897 DOI: 10.1038/375033a0]

[13] Li Z, Jin C, Chen S, Zheng Y, Huang Y, et al. Long non-coding RNA MEG3 inhibits adipogenesis and promotes osteogenesis of human adipose-derived mesenchymal stem cells via miR-140-5p. Mol Cell Biochem 2017;435:51-60. [PMID: 28382492 DOI: 10.1007/s10118-017-0135-z]

[14] Zhuang W, Wu G, Xi Y, Zhang S, Huang M, Zhuang W, et al. Upregulation of IncRNA MEG3 promotes osteogenic differentiation of mesenchymal stem cells from multiple myeloma patients by targeting BMP4 transcription. Stem cells 2015;33:1985-97. [PMID: 25753650 DOI: 10.1002/stem.1989]

[15] Li Z, Jin C, Chen S, Zheng Y, Huang Y, et al. Long non-coding RNA MEG3 inhibits adipogenesis and promotes osteogenesis of human adipose-derived mesenchymal stem cells via miR-140-5p. Mol Cell Biochem 2017;435:51-60. [PMID: 28382492 DOI: 10.1007/s10118-017-0135-z]

[16] Che W, Dong Y, Quan H. RANKL inhibits cell proliferation by regulating MALAT1 expression in a human osteoblastic cell line hFOB 1.19. Cell Mol Biol (Noisy-le-grand) 2015;61:17-4. [PMID: 25817340]

[17] Gao X, Ge J, Li W, Zhou W, Xu L. LncRNA KCNQ1OT1 promotes osteogenic differentiation to relieve osteolysis via Wnt/β-catenin pathway. Int J Mol Med 2018;41:213-9. [PMID: 29115577 DOI: 10.3892/ijm.2017.3215]

[18] Tang S, Xie Z, Wang P, Li J, Wang S, et al. LncRNA-OG promotes the osteogenic differentiation of bone marrow-derived mesenchymal stem cells through the regulation of hnRNPK. Stem Cells 2019;37:270-83. [PMID: 30372259 DOI: 10.1002/stem.2937]

[19] Cao B, Liu N, Wang W. High glucose prevents osteogenic differentiation of mesenchymal stem cells via IncRNA AK028326/CXCL13 pathway. Biomedi Pharmacoch 2016;84:544-51. [PMID: 27693963 DOI: 10.1016/j.biph.2016.09.058]

[20] Li H, Zhang Z, Chen Z, Zhang D. Osteogenic growth peptide promotes osteogenic differentiation of mesenchymal stem cells mediated by LncRNA AK141205 induced upregulation of CXCL13. Biochem Bioph Res Comm 2015;466:82-8. [PMID: 26321662 DOI: 10.1016/j.bbrc.2015.08.112]

[21] Zhang N, Hu X, He S, Ding W, Wang F, et al. LncRNA MSC-AS1 promotes osteogenic differentiation and alleviates osteoporosis through sponging microRNA-140-5p to upregulate BMP2. Biochem Bioph Res Comm 2015;466:82-8. [PMID: 26321662 DOI: 10.1016/j.bbrc.2015.08.112]

[22] Gao Y, Xiao F, Wang C, Wang C, Cui P, et al. Long non-coding RNA MALAT1 promotes osteogenesis via lincRNA AK028326/CXCL13 pathway. Biomedi Pharmacoch 2016;84:544-51. [PMID: 27693963 DOI: 10.1016/j.biph.2016.09.058]

[23] Liao J, Yu X, Hu X, Fan J, Wang J, et al. IncRNA H19 mediates BMP9-induced osteogenic differentiation of mesenchymal stem cells (MSCs) through Notch signaling. Oncotarget 2017;8:53581-601. [PMID: 28881833 DOI: 10.18632/oncotarget.18655]
Review

Zhang L, Xie H, Li S. LncRNA LOXL1-AS1 controls osteogenic differentiation of periodontal ligament stem cells. Arch Oral Biol 2015;60:234-41. [PMID: 25463901 DOI: 10.1016/j.archoralbio.2014.10.007]

Shen J, Zhang C, Chen Z, Wang Z, Yang D, et al. LncRNA HOTAIR inhibits osteogenic differentiation of BMSCs by regulating Wnt/β-catenin pathway. Eur Rev Med Pharmacol Sci 2019;23:7232-46. [PMID: 31353910 DOI: 10.26355/survey_201909_18826]

Yang L, Li Y, Gong R, Gao M, Feng C, et al. The long non-coding RNA-ORLNC1 regulates bone mass by directing mesenchymal stem cell fate. Mol Ther 2019;27:394-410. [PMID: 30638773 DOI: 10.1016/j.mthera.2018.11.019]

Jiang Y, Wu W, Jiao G, Chen Y, Liu H. LncRNA SNHG1 modulates p38 MAPK pathway through Nedd4 and thus inhibits osteogenic differentiation of bone marrow mesenchymal stem cells. Life Sci 2019;226:208-14. [PMID: 31055087 DOI: 10.1016/j.lfs.2019.05.002]

Zhu X, Yan Y, Chen D, Ai C, Lu X, et al. Long non-coding RNA Hoxa-AS3 interacts with EZH2 to regulate lineage commitment of mesenchymal stem cells. Oncotarget 2016;7:63561-70. [PMID: 27566578 DOI: 10.18632/oncotarget.11538]

Che M, Gong W, Zhao Y, Liu M. Long noncoding RNA HCG18 inhibits the differentiation of human bone marrow-derived mesenchymal stem cells in osteoporosis by targeting miR-30a-5p/NOTCH1 axis. Mol Med 2020;26:106. [PMID: 31376682 DOI: 10.1086/640200-020-00219-6]

Wang Y, Zeng X, Wang N, Zhao W, Zhang X, et al. Long noncoding RNA DANCR, working as a competitive endogenous RNA, promotes ROCK1-mediated proliferation and metastasis via decoying miR-335-5p and miR-192-5p in osteosarcoma. Mol Cancer 2018;17:89. [PMID: 29753317 DOI: 10.1186/s12943-018-0837-6]

Luo X, Wang J, Wei X, Wang S, Wang A. Knockdown of LncRNA MFE2-AS1 inhibits lipopolysaccharide-induced osteogenesis progression by miR-130a-3p/TFC4. Life Sci 2020;240:117019. [PMID: 31678554 DOI: 10.1016/j.lfs.2019.117019]

Zhang Y, Wang F, Chen G, He R, Yang L. LncRNA MALAT1 promotes osteosarcoma cells from postmenopausal osteoporosis by targeting miR-133a-3p. DNA Cell Biol 2019;38:794-805. [PMID: 31388174 DOI: 10.1089/dcn.2017.3305]

Bi X, Guo XH, Mo BY, Wang ML, Lao XQ, et al. LncRNA PCSAR promotes cell proliferation, migration and invasion of fibroblast-like synoviocytes by splicing miRNA-4701-5p in rheumatoid arthritis. EBioMedicine 2019;50:408-20. [DOI: 10.1016/j.ebiom.2019.11.024]

Zhao J, Ma ST. Downregulation of LncRNA H19 inhibits migration and invasion of human osteosarcoma through the NF-kB pathway. Mol Med Rep 2018;17:7388-94. [PMID: 29568924 DOI: 10.3892/mmr.2018.8847]

Zheng S, Jiang F, Ge D, Tang J, Chen H, et al. LncRNA SNHG3/miRNA-15a-3p/RAB22A axis regulates invasion and migration of osteosarcoma. Biomed Pharmacother 2019;112:188695. [PMID: 30971514 DOI: 10.1016/j.biopha.2018.108695]

Wang W, Li Y, Zhi S, Li J, Miao J, et al. LncRNA-ROR/microRNA-185-3p/YAP1 axis exerts function in biological characteristics of osteosarcoma cells. Genomics 2021;113:450-61. [PMID: 32898639 DOI: 10.1016/j.ygeno.2020.09.009]

Zhang Y, Zhang M, Xu K, Liu L, Hou W, et al. Knockdown of YAP1 inhibits the proliferation of osteosarcoma cells in vitro and in vivo. Oncol Rep 2014;32:1265-72. [PMID: 24993351 DOI: 10.3829/ or.2014.3305]

Liu J, Chen M, Ma L, Dang X, Du G. LncRNA GAS5 suppresses the proliferation and invasion of osteosarcoma cells via the miR-23a-3p/PTEN/P53/akt pathway. Cell Transplant 2020;29:963687920935093. [PMID: 33121268 DOI: 10.1177/0963689720935093]

Lamonne F, Lapadula G. The pathophysiology of osteoarthritis. Aging Clin Exp Res 2003;15:364-72. [PMID: 14703002 DOI: 10.1007/s00332-007]

Song J, Ahn C, Chun C, Jin E. A long non-coding RNA, GAS5, plays a critical role in the regulation of miR-21 during osteoarthritis. J Orthop Res 2014;32:1628-35. [PMID: 25196853 DOI: 10.1002/jor.22178]

Li Y, Li S, Luo Y, Liu Y, Yu N. LncRNA PVT1 regulates chondrocyte apoptosis in osteoarthritis by acting as a sponge for miR-488-3p. DNA Cell Biol 2017;36:571-80. [PMID: 28520497 DOI: 10.1089/dna.2017.3678]

Ye D, Jian W, Feng J, Xiao L. Role of long noncoding RNA ZFAS1 in proliferation, apoptosis and migration of chondrocytes in osteoarthritis. Biomed Pharmacother 2018;104:825-31. [PMID: 29703568 DOI: 10.1016/j.biopha.2018.04.124]

Chen L, Zhu Z, Peng X, Wang Y, Wang Y, et al. Hepatic magnetic resonance imaging with T2* mapping of ovariectomized rats: correlation between iron overload and postmenopausal osteoporosis. Eur Radiol 2014;24:1715-24. [PMID: 24770554 DOI: 10.1007/s00330-014-3178-x]
[68] Liu H, Wang YW, Chen WD, Dong HH, Xu YJ. Iron accumulation regulates osteoblast apoptosis through lncRNA XIST/miR-758-3p/caspase 3 axis leading to osteoporosis. IUBMB Life 2021;73:432-43. [PMID: 33336851 DOI: 10.1002/iub.2440]

[69] Li T, Jiang H, Li Y, Zhao X, Ding H. Estrogen promotes lncRNA H19 expression to regulate osteogenic differentiation of BMSCs and reduce osteoporosis via miR-532-3p/SIRT1 axis. Mol Cell Endocrinol 2021;527:111171. [PMID: 33577975 DOI: 10.1016/j.mce.2021.111171]

[70] Zhou P, Li Y, Di R, Yang Y, Meng S, et al. H19 and Foxc2 synergistically promotes osteogenic differentiation of BMSCs via Wnt-beta-catenin pathway. J Cell Physiol 2019;234:13799-806. [PMID: 30633332 DOI: 10.1002/jcp.28060]

[71] Cai N, Li C, Wang F. Silencing of LncRNA-ANCR promotes the osteogenesis of osteoblast cells in postmenopausal osteoporosis via targeting EZH2 and RUNX2. Yonsei Med J 2019;60:751-9. [PMID: 31347330 DOI: 10.3349/ymj.2019.60.8.751]

[72] Zhang X, Shan H, Zhang P, She C, Zhou X. LncRNA EPIC1 protects human osteoblasts from dexamethasone-induced cell death. Biochem Biophys Res Commun 2018;503:2255-62. [PMID: 29959919 DOI: 10.1016/j.bbrc.2018.06.146]

[73] Fan J, Zhang Y, Liu W, Zhu X, Xu D, et al. Long non-coding RNA MALAT1 protects human osteoblasts from dexamethasone-induced injury via activation of PPM1E-AMPK signaling. Cell Physiol Biochem 2018;51:31-45. [PMID: 30439702 DOI: 10.1159/000495159]

[74] Xiao J, Wang R, Zhou W, Cai X, Ye Z. LncRNA NEAT1 regulates the proliferation and production of the inflammatory cytokines in rheumatoid arthritis fibroblast-like synoviocytes by targeting miR-204-5p. Hum Cell 2021;34:372-82. [PMID: 33394549 DOI: 10.1007/s13577-020-00461-4]

[75] Yang J, Li Y, Wang L, Zhang Z, Li Z, et al. LncRNA H19 aggravates TNF-alpha-induced inflammatory injury via TAK1 pathway in MH7A cells. Biofactors 2020;46:813-20. [PMID: 32525617 DOI: 10.1002/biof.1659]

[76] Liu Y, Yang L, Xu Q, Lu X, Ma T, et al. Long noncoding RNA MEG3 regulates rheumatoid arthritis by targeting NLRCS5. J Cell Physiol 2019;234:14270-84. [PMID: 30644097 DOI: 10.1002/jcp.28126]

[77] Yu HC, Huang KY, Lu MC, Huang Tseng HY, Liu SQ, et al. Down-regulation of LOC645166 in T cells of ankylosing spondylitis patients promotes the NF-kB signaling via decreasingly blocking recruitment of the IKK complex to K63-linked polyubiquitin chains. Front Immunol 2021;12:591706. [PMID: 33717069 DOI: 10.3389/fimmu.2021.591706]

[78] Liu D, Zhao C, Li H, Jiang S, Jiang L, et al. Effects of spinal cord injury and hindlimb immobilization on sublesional and supraspinal bones in young growing rats. Bone 2008;43:119-25. [PMID: 18482879 DOI: 10.1016/j.bone.2008.03.015]