Long non-coding RNA in osteoporosis

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ABSTRACT

Osteoporosis (OP) is the most common skeletal disease, caused by a lack of balance between osteoclast and osteoblast activity. This results in erosion overriding the deposition of new bone matrix, consequently leading to low-quality bone and an increased risk of incurring fragility fractures. Dual energy X-ray absorptiometry is the gold standard for the diagnosis of OP, while anti-osteoporotic drugs are the gold standard for its treatment. However, due to limitations to their use, researchers have turned to epigenetics as a substantial source of molecules that could potentially be used as diagnostic, prognostic, and therapeutic biomarkers for OP. In particular, long non-coding RNAs (lncRNAs) possess special biological properties that could open new horizons in the field of personalized medicine. This mini review seeks to offer an overview of the studies carried out in the last year on the different lncRNAs that could be involved in the pathogenesis of OP and that could pave the way for the development of innovative therapeutic strategies for this disease.

KEYWORDS

LncRNA, Osteoporosis, miRNAs, osteogenesis.

Introduction

Osteoporosis (OP) means "porous bone". OP is characterized by progressive loss of bone mass and bone quality, due to a lack of balance between the activity of osteoblasts and osteoclasts, and it results in the risk of experiencing fractures [1]. OP afflicts millions of people worldwide, greatly impacting healthcare costs [2]. Typically, OP can be divided into primary OP, which includes male, postmenopausal (PMOP), senile and juvenile forms, and secondary OP which can be caused by pre-existing diseases or different drug treatments [1]. To date, the diagnosis is based on T-score values, which are measured by dual energy X-ray absorptiometry (DEXA) and represent the state of bone mineral density (BMD). Moreover, DEXA is also often juxtaposed with the Fracture Risk Assessment Tool (FRAX) score, which allows the assessment of fracture risk in a patient over a 10-year time span [3]. In addition, bone turnover markers can be measured in the different biological fluids, in order to estimate bone cell metabolic activity [4].

A diet ensuring considerable calcium (Ca^{++}) and vitamin D intake is a fundamental ally to prevent bone mass loss and OP onset, as are physical activity and other lifestyle factors [5]. Obviously, when the presence of the disease is confirmed, it is necessary to intervene pharmacologically, through the administration of anti-osteoporotic drugs, including anti-catabolic and anabolic molecules [6].

However, it should be considered that prolonged administration of these substances results in non-negligible adverse effects in patients [6], which is the reason why it is necessary to find new therapeutic strategies for OP treatment.

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In recent years, the scientific community has identified epigenetics as an interesting source of biomarkers that can be used as therapeutic targets in different diseases, including OP. Among these molecules, long non-coding RNAs (lncRNAs) have emerged as a very interesting class of non-coding RNA [7]. LncRNAs have a length of 200 nucleotides or more and principally can be divided, according to their position, into intergenic lncRNAs (also called lincRNAs) and intragenic lncRNAs [8].

Moreover, depending on their structure and location within the cell, lncRNAs can play different roles, acting as scaffold, decoy, ncRNA sponge molecules, and guide [8].

In the past decade, several works have highlighted the involvement of lncRNAs in the regulation of proliferation, differentiation, activity, and apoptosis of the two main bone cell types. The increased understanding of these molecules' role in the bone remodeling process could pave the way for the identification of new targets for the development of innovative therapeutic lncRNA-based strategies against OP [9].

The works done in the past year on lncRNAs involved in the pathogenesis of OP are summarized below.

LncRNAs involved in osteoporosis

NEAT1

Zhao *et al*. [10] showed that lncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) was upregulated in OP mouse models compared with healthy controls, and demonstrated that knockdown of this lncRNA inhibited the autophagic process in MC3T3-E1 cells by binding to miR-466f-3p and by regulating *hexokinase 2* (*HK2)* expression. In addition, further studies confirmed that the NEAT1/miR-466f-3p/HK2 axis was also capable of exercising its effects on autophagy in an ovariectomized (OVX) mouse model, and that down-expression of NEAT1 led to OP attenuation.

TRG-AS1

Liu *et al*. [11] found lncRNA T cell receptor gamma locus antisense RNA 1 (TRG-AS1) and calcium-binding protein 39 (CAB39) expression to be downregulated in dexamethasone (Dex) induced-OP rats and rat osteoblasts. On the contrary, miR-802 levels were upregulated in these two models. Subsequent analysis revealed that Dex treatment increased cell apoptosis in osteoblasts while decreasing proliferation and differentiation, by regulating lncRNA TRG-AS1 negatively and miR-802 expression positively. Moreover, they discovered that this lncRNA could activate the CAB39/AMPK/SIRT-1/NF-κB pathway by decreasing miR-802 in a targeted manner and alleviating Dex-induced OP.

PTCSC3

Liu *et al*. [12] showed that lncRNA papillary thyroid carcinoma susceptibility candidate 3 (PTCSC3) expression levels were higher in OP patients' plasma with respect to healthy controls, and that they correlated with disease stage. Moreover, *in vitro* studies showed that lncRNA PTCSC3 overexpression promoted osteoblast apoptosis, while its inhibition led to increased cellular vitality. No regulatory effects were observed on osteoclasts with PTCSC3 overexpression and silencing.

MIAT

Li *et al*. [13] showed lncRNA myocardial infarction-associated transcript (MIAT) to be upregulated in peripheral blood mononuclear cells (PBMCs) obtained from PMOP patients compared with the control group. Then, ROC curve analysis supported the suggestion that lncRNA MIAT could represent a good biomarker for OP. Subsequently, they discovered the existence of crosstalk between lncRNA MIAT, miR-216a, and p38MAPK. In fact, MIAT directly interacts with miR-216a to regulate the AMPK/p38MAPK signaling pathway and promote inflammatory cytokine secretion in PBMCs, contributing to PMOP pathogenesis.

ZFAS1

Wu *et al*. [14] demonstrated that lncRNA zinc finger antisense 1 (ZFAS1) is involved in adipogenic and osteogenic differentiation processes of bone marrow-derived mesenchymal stem cells (BMSCs). After seeing that lncRNA expression increased during adipogenesis and decreased during osteogenesis, they found that suppression of ZFAS1 expression inhibited the differentiation of BMSCs in an adipogenic direction, while promoting their differentiation in an osteogenic direction. Moreover, this downregulation arrested cellular senescence and positively regulated autophagy. Finally, they found that lncRNA ZFAS1 was able to sponge miR-499 to upregulate its target *Ephrin Type-A Receptor 5* (*EPHA5*), allowing BMSCs to switch toward the adipogenic phenotype.

TCONS_00072128

Yang *et al*. [15] discovered that lncRNA TCONS_00072128 had extremely decreased expression in exosomes derived from PMOP patients compared with the control group. Subsequent studies in BMSCs revealed that upregulation of this lncRNA was associated with increased levels of caspase 8 expression and osteogenic differentiation, while the opposite effect occurred when there was silencing of TCONS_00072128. Furthermore, they also observed that continuous *Caspase 8* expression could activate the inflammation process mediated by NOD-like receptor family pyrin domain-containing protein 3 (NLRP3) and NF-κB signaling pathways, as well as receptor interacting serine/threonine kinase 1 (RIPK1) phosphorylation. Thus, exosome-derived lncRNA TCONS_00072128 might be involved in PMOP pathogenesis through regulation of *Caspase 8*, which would appear to play an important role in the balance between cell differentiation and activation of inflammatory process pathways.

AWPPH

The study conducted by Qian *et al*. [16] highlighted downregulated lncRNA associated with poor prognosis of hepatocellular carcinoma (AWPPH) expression in the plasma of OP patients with respect to the healthy group, and its correlation with expression of the bone turnover markers P1NP and TRACP-5b. In addition, the ROC curve study showed a high potential of lncRNA AWPPH to discriminate the healthy from the diseased population. Moreover, they observed that AWPPH knockdown in osteoblasts provoked a decrease in type I collagen α2 expression and an increase in type I collagen α1 expression, with a ratio of 1:2 between these proteins. The opposite situation was observed with lncRNA AWPPH overexpression, confirming its potential role in OP.

RAD51-AS1

Li *et al*. [17] reanalyzed the GSE35956 datasets and mainly focused on studying the lncRNA RAD51 antisense RNA 1 (RAD51-AS1), which was found to be down-expressed in OP patients' BMSC samples compared with those from healthy patients. First, they discovered that RAD51-AS1 was principally localized in the nuclear compartment. Then they saw that its knockdown increased cell apoptosis as well as inhibition of osteogenic differentiation and proliferation processes. In contrast, RAD51-AS1 upregulation was associated with a significant increase in proliferation of BMSCs and their ability to give rise to bone cells. Further experiments demonstrated that RAD51-AS1 interacts with Y-Box Binding Protein 1 (YBX1) in order to negatively regulate SMAD7 and SMURF2 expression and promote transcription of the *PCNA* and *SIVA1* genes. Thus, these results establish that a perturbation in the TGF-β pathway could play a pivotal role in bone formation.

BC083743

The qRT-PCR performed by Lu and Tang [18] showed lncRNA BC083743 down-expression in OP patients' serum compared with that of healthy controls. At the same time upregulation of this lncRNA and of osteogenic differentiation-related genes, including *OPG*, *BMP2*, and *RUNX2*, was observed during the osteogenic differentiation of bone marrow-derived stem cells (BMSCs) obtained from the femur of OP patients. In addition, they found that miR-103-3p expression levels were increased in the serum of OP patients but decreased during osteogenic differentiation of BMSCs. Indeed, it has been confirmed that BC083743 behaves as competing endogenous RNA (ceRNA) for miR-103-3p to positively regulate *SATB2* gene expression with a consequent increase in osteogenic differentiation. Therefore, these data suggest that silencing of lncRNA BC083743 might contribute to OP pathogenesis through regulation of the miR-103-3p/SABT2 axis.

KCNQ1OT1

Wang *et al*. [19] discovered that lncRNA KCNQ1 opposite strand/antisense transcript 1 (Kcnq1ot1) expression was increased in MC3T3-E1 cells during osteogenic differentiation, while opposite expression levels were observed for miR-98-5p. In fact, the dual luciferase assay revealed that Kcnq1ot1 may sponge miR-98-5p to enhance the expression of its gene target *T-box Transcription Factor 5* (*TBX5*).

Further investigations also showed that miR-98-5p overexpression and *TBX5* downregulation led to decreased osteogenic differentiation and mineralization in MC3T3-E1 cells. The opposite effect was obtained with lncRNA Kcnq1ot1 overexpression. This led the authors to conclude that Kcnq1ot1 might play an important role in bone formation and OP onset and progression.

TUG

Ouyang *et al*. [20] identified, in BMSCs, a correlation between the expression levels of lncRNA TUG, miR-204, and Sirtuin 1 (SIRT 1), finding that TUG and SIRT 1 increased their expression during the process of osteogenesis, while miR-204 showed the opposite trend. Subsequently, binding between TUG and miR-204 was predicted by bioinformatic analysis and verified by using the Dual-luciferase reporter assay. In fact, they observed that TUG was able to negatively regulate the expression of miR-204, and consequently increase the latter's target, SIRT1. Furthermore, they showed that TUG and SIRT1 promote osteogenesis, while miR-204 inhibits it in BMSCs. Thus, the TUG/miR-204/SIRT1 axis could be an interesting target for the treatment of OP.

Lnc-DIF

Yin *et al*. [21] showed that long non-coding RNA differentiation inhibiting factor (Lnc-DIF) was upregulated in BMSCs isolated by OVX mice. Moreover, they also found a negative relationship between osteogenic marker genes (i.e., *COL IA1*, *ALP*, *RUNX2*, and *OCN*) and Lnc-DIF expression in mouse femur tissue. Further experiments revealed that Lnc-DIF overexpression in MC3T3-E1 cells led to a dramatic decrease in *Col Iα1* and *Runx2* mRNA expression.

On the contrary, the opposite effect was obtained with Lnc-DIF siRNA transfection in the cell line, thus indicating the capability of Lnc-DIF to inhibit osteogenic differentiation. In addition, the same results were obtained in mice transfected with overexpressing and silencing Lnc-DIF plasmid. Furthermore, they revealed that Lnc-DIF could sponge miR-489-3p by regulating its downstream target *SMAD2*, a negative regulator of osteoblastogenesis. Therefore, the authors identified Lnc-DIF as an inhibitor of osteogenesis, which acts via the miR-489-3p/SMAD2 axis.

MALAT1

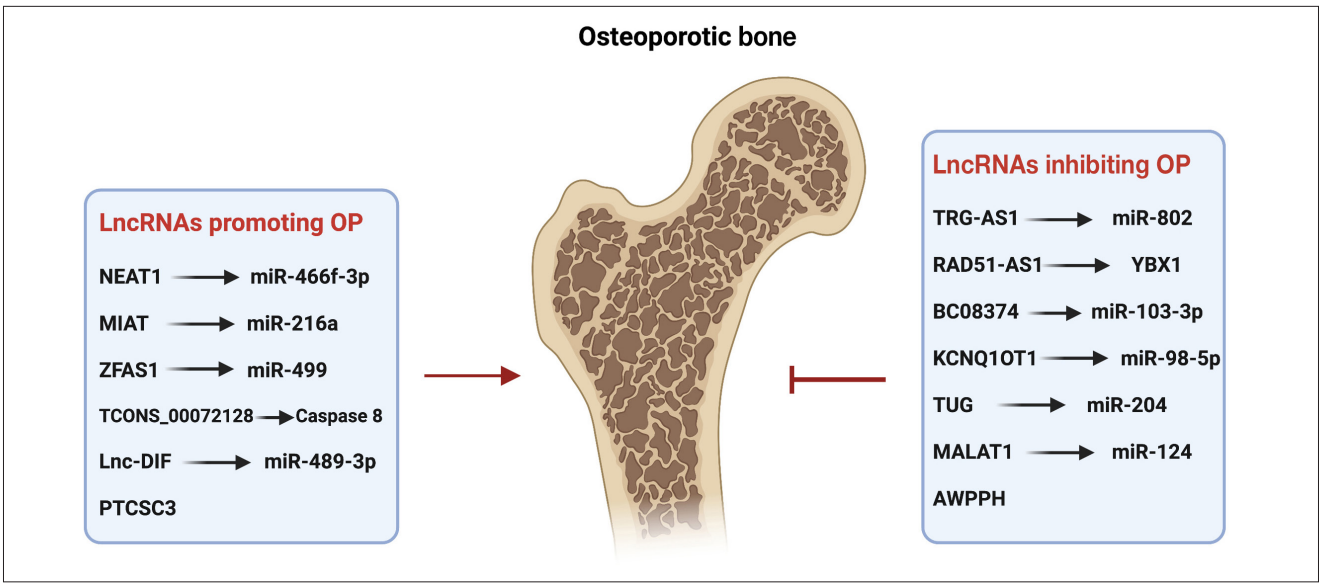
After detecting low MALAT1 expression in the peripheral blood of OP patients compared with controls, Li [22] isolated BMSCs and macrophages (Mø) from both groups to study the possible involvement of this lncRNA in OP. First, he showed that expression of MALAT1 decreased during osteoclastogenesis in Mø and instead increased during osteoblastogenesis in BMSCs. Moreover, MALAT1 downregulation promoted osteoclast formation and inhibited osteoblast formation. Further investigations demonstrated that MALAT1 negatively regulated miR-124, in order to increase *Insulin Like Growth Factor 2 mRNA Binding Protein 1* (*IGF2BP1*) expression. The latter, in turn, was able to activate the Wnt/β-catenin pathway and promote osteogenic differentiation. An interesting aspect was the finding that pc-MALAT1 injection in OVX mice led to augmented activation of the Wnt/β-catenin pathway and attenuation of bone loss, confirming a central role of MALAT1 in OP.

Qian *et al*. [23] discovered that MALAT1 expression was significantly lower in plasma of PMOP patients with respect to healthy individuals, and positively associated with their femoral neck, total hip, and lumbar (L1-L4) spine BMD. Furthermore, dividing the patients into the group that had manifested deformities and vertebral fractures and the group that had never had fractures, it was possible to show that MALAT1 expression was lower in the plasma of the former group than the latter and correlated negatively with Genant grade. The authors also observed that plasma expression of MALAT1 correlated negatively with the Oswestry Disability Index and Visual Analog Scale indices, suggesting its possible involvement in osteoporotic neuropathic pain.

Conclusion and future perspectives

High-throughput RNA sequencing analysis and lncRNA microarray studies have shown that these molecules are differentially expressed in OP patients compared with the healthy population. This review shows how most of the above-mentioned lncRNAs play a pivotal role in BMSC proliferation and differentiation, principally by acting mainly as ceRNA for several miRNAs (Figure 1) to upregulate the expression of their downstream targets, which are often crucial genes involved in osteogenic differentiation. Therefore, these findings could direct the scientific community to consider investigating more thoroughly the mechanism of action of these molecules in OP pathogenesis, in order to develop new diagnostic, prognostic, and therapeutic strategies for this disease.

Figure 1 LncRNAs involved in osteoporosis (This image was created with Biorender software).



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