Involvement of the long noncoding RNA H19 in osteogenic differentiation and bone regeneration

Zimo Zhou, Mohammad Showkat Hossain and Da Liu *

Abstract

Osteogenic differentiation and bone regeneration are complex processes involving multiple genes and multiple steps. In this review, we summarize the effects of the long noncoding RNA (lncRNA) H19 on osteogenic differentiation.

Osteogenic differentiation includes matrix secretion and calcium mineralization as hallmarks of osteoblast differentiation and the absorption of calcium and phosphorus as hallmarks of osteoclast differentiation. Mesenchymal stem cells (MSCs) form osteoprogenitor cells, pre-osteoblasts, mature osteoblasts, and osteocytes through induction and differentiation. IncRNAs regulate the expression of coding genes and play essential roles in osteogenic differentiation and bone regeneration. The IncRNA H19 is known to have vital roles in osteogenic induction.

This review highlights the role of H19 as a novel target for osteogenic differentiation and the promotion of bone regeneration.

Keywords: Long noncoding RNA, H19, Osteogenic differentiation, Bone regeneration

Introduction

Bone regeneration is a complex process involving the synergistic effects of mesenchymal stem cell (MSC)-derived osteoblasts and hematopoietic stem cell-derived osteoclasts. After fracture or the onset of osteoporosis and other diseases, the damaged bone releases cytokines. These cytokines induce osteoblastic matrix secretion and calcium mineralization. MSCs gradually differentiate into bone progenitor cells, pre-osteoblasts, and osteoblasts. Then, osteoblasts begin to synthesize and secrete matrix, repair the tissue microenvironment, and induce bone regeneration. Moreover, with the differentiation of osteoclasts, the organic and inorganic compounds released by the damaged bone are absorbed. Ca^{2+}, (PO_4)^{3-}, and other degradation products enter the blood circulation. These processes work effectively through complex multigene processes, with multistep regulation.

MSCs are stem cells with multipotent differentiation capacity. Many studies have demonstrated that MSCs play crucial roles in maintaining and repairing various connective tissues, including cartilage, muscle tissue, bone, and adipose tissues [1]. As an essential process in bone regeneration and cell repair, the osteogenic differentiation potential of MSCs is induced by the extracellular microenvironment. Indeed, mechanical and molecular signals regulate osteogenic differentiation at the transcriptional and post-transcriptional levels [2] (Fig. 1).

In prior studies, the roles of protein-coding genes and noncoding microRNAs in osteogenic differentiation have been extensively studied. However, long noncoding RNAs (IncRNAs), which account for a large proportion of the genome sequence, have not been sufficiently...
studied. With the recent development of high-throughput RNA sequencing (RNA-seq) and other technologies, lncRNAs, previously regarded as transcriptional noise, have been shown to have positive roles in regulating nuclear chromatin structure and gene expression. Zuo et al. [3] first reported the relationship between lncRNAs and bone generation in 2013. In response to bone morphogenetic protein-2 (BMP-2), lncRNA expression profiles are significantly altered in C3H10T1/2 cells, demonstrating a correlation between lncRNAs and osteoblast differentiation. Moreover, researchers have identified 116 differentially expressed lncRNAs, facilitating further studies of these sequences in osteogenesis.

In this review, we summarize the effects of the lncRNA \( H19 \) on osteogenic differentiation. We also discuss the roles of other lncRNAs associated with this process and highlight the potential applications of this information regarding the understanding and management of bone-related diseases.

**Structure and function of lncRNAs**

lncRNAs, as by-products of RNA polymerase II transcription, belong to a family of noncoding RNAs (ncRNAs) with lengths of 200–100,000 nt. These molecules have little or no protein-coding potential [4, 5]. Functionally, lncRNAs act as regulatory ncRNAs and include microRNAs (miRNAs), small interfering RNAs (siRNAs), and Piwi-interacting RNAs [6]. Compared with miRNAs, lncRNAs show lower expression levels and exhibit relatively low homology among species. However, promoters and exons are conservative to some extent, indicating that the functions of lncRNAs are relatively conserved [7]. Many lncRNAs contain conserved secondary structures and exhibit alternative splicing and subcellular localization. In addition, many lncRNAs show specific expression during various stages of tissue development.

lncRNAs can be divided into five types: sense, antisense, bidirectional, intronic, and intergenic; the functions of these lncRNAs differ to some extent [2, 8]. In general, lncRNAs have no coding potential; however, Matsumoto et al. [9] showed that a small polypeptide encoded by the lncRNA \( LINC00961 \) could inhibit the amino acid-induced activation of skeletal muscle mammalian target of rapamycin complex 1 in SPAR-polypeptide-specific-knockout mice, demonstrating that lncRNAs could encode short peptides under exceptional circumstances.

**Regulatory mechanisms of lncRNAs in osteogenic differentiation**

The regulatory mechanisms of lncRNAs are highly complex. The mechanisms of action of lncRNAs can be summarized into four levels: epigenetic, transcriptional,
post-transcriptional, and other regulatory mechanisms. In osteogenic differentiation, lncRNAs show three general functional roles, as follows: (1) they mediate epigenetic modification to regulate osteogenic differentiation; (2) they regulate osteogenic differentiation through the modulation of signaling pathways; and (3) they regulate osteogenic differentiation by serving as miRNA sponges or precursor structures.

Roles of lncRNAs in mediating epigenetic modifications to regulate osteogenic differentiation

Epigenetics refers to heritable genetic phenotypes and gene expression changes through DNA methylation, histone modification, and chromatin remodeling without changes in the DNA sequences. DNA methylation can directly regulate the expression of Runx-related transcription factor 2 (Runx2) and osterix (Osx), which affect bone formation [10]. Kino et al. [11] showed that during osteogenic differentiation, the lncRNA Gas5 could bind to the glucocorticoid receptor gene binding domain as bait and inhibit receptor function. As negative regulators of bone formation, glucocorticoids cannot bind to glucocorticoid receptors.

Roles of lncRNAs in regulating osteogenic differentiation through modulation of signaling pathways

A series of regulatory factors and cells are involved in osteogenesis and osteogenic differentiation. These regulators play important roles by activating or inhibiting relevant signaling pathways. The Wnt/β-catenin, mitogen-activated protein kinase (MAPK), and BMP/Smad pathways have been extensively studied [12–14]. The core transcription factor of osteogenic differentiation, Runx2, can be modulated by BMPs, Wnt protein, estrogen, and glucocorticoids, resulting in alterations in the phosphorylation or expression of downstream elements, such as β-catenin and Smads [15, 16]. siRNAs have inhibitory effects on the activity of the lncRNA AK045490, which can promote osteoblastic differentiation in the context of osteoporosis. Moreover, experimental results have shown that AK045490 downregulates T cell factor 1 (TCF1), lymphoid enhancer-binding factor 1 (LEF1), and Runx2 by inhibiting the nuclear translocation of β-catenin, blocking the β-catenin/TCF1/Runx2 signaling pathway, and ultimately suppressing the differentiation and bone formation of osteoblasts [15]. Additionally, HOX transcript antisense RNA (HOTAIR) can directly reduce Wnt inhibitory factor 1 (WIF-1) expression by promoting histone H3K27 methylation in the promoter region, thereby regulating the Wnt/β-catenin signaling pathway and activating matrix metalloproteinase-13 (MMP-13) expression in chondrocytes to block cartilage damage [17–19]. Inflammatory signals play essential roles in inducing osteogenic differentiation through the matrix microenvironment. In the osteogenic differentiation of human MSCs, the lncRNA differentiation antagonizing non-protein-coding RNA (DANCR) induces the expression of interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) in mononuclear cells, thereby enhancing the osteoclastic activity of bone resorption [20]. IL-1β can also activate osteogenic differentiation via upregulation of the extracellular signal-regulated kinase (ERK) 1/2 signaling pathway. However, IL-1β eventually inhibits osteoblast differentiation via the strong activation of p38 signaling. Matrix stiffness can also regulate osteogenic differentiation by modulating the MAPK pathway [21, 22]. However, no reports have described the modulation of osteogenic differentiation through lncRNA-dependent inflammatory signals.

Roles of lncRNAs as miRNA sponges or precursors to regulate osteogenic differentiation

miRNAs, which cause translational repression or degradation of target mRNAs, regulate the expression of genes involved in the osteogenic differentiation of MSCs. For example, miR-138 inhibits osteoblast differentiation in bone marrow mesenchymal stem cells (BMSCs) and phosphorylation of focal adhesion kinase (FAK), ERK1/2, and Runx2. Moreover, miR-138-dependent downregulation of Runx2 is also essential for the platelet-derived growth factor (PDGF)-mediated inhibition of BMSC osteogenic differentiation [23], and miR-705, miR-124, miR-204, miR-30a, and miR-705 regulate the balance between lipid formation and osteogenic differentiation in BMSCs by modulating Runx2 and Osx expression [24–28]. Studies have shown that lncRNAs can competitively associate to limited miRNA-specific sites and regulate miRNA levels. The lncRNA KCNQ1OT1 interacts directly with miR-214 to form an miRNA sponge during the regulation of BMSC osteogenic differentiation, and miR-214 can bind to the 3′-untranslated region (UTR) of BMP-2 to inhibit the expression of this protein [29, 30]. Notably, some miRNAs can be transcribed from genomic regions of lncRNA gene sequences. As miRNA precursors, these molecules regulate downstream targets after being cleaved [31]. Additionally, lncRNAs can also facilitate the cleavage of pri-miRNAs, modulate the production of mature miRNAs, and play important regulatory role [32]. In one study using RNA-seq to elucidate the involvement of lncRNAs in the osteogenic differentiation of immortalized mesenchymal stem cells (iMSC#3), 32 new lncRNAs were screened out as miRNA precursors (including miR-689, miR-640, miR-601, and miR-544) [33]. Thus, further studies are expected to identify more functions of lncRNAs as miRNA sponges or precursors.
Types and mechanisms of lncRNAs in osteogenic differentiation

Many lncRNAs have been shown to be involved in tumor growth, immune system diseases, and other diseases. For example, Luan et al. [34] knocked down the lncRNA NPPA-AS1 in human normal cervical epithelial cells (H8 cells) and human cervical cancer cells (C33A, CaSki, HeLa, and SiHa cells) and showed that this lncRNA impaired cell proliferation and migration. Moreover, lncRNAs are known to participate in the progression of lung cancer, breast cancer, and cervical cancer [34–37]. Additionally, various lncRNAs can affect disease occurrence and outcomes through multiple molecular pathways. The various molecular mechanisms through which lncRNAs regulate osteogenic differentiation in disease are summarized in Table 1.

DANCR

The lncRNA DANCR was the first lncRNA shown to regulate progenitor differentiation [38]. The function of DANCR in chondrogenic differentiation of human synovium-derived MSCs and osteogenic differentiation of periodontal ligament stem cells (PDLSCs) has been reported [39, 40]. Additionally, Lin et al. [41] evaluated the expression of lncRNAs in hFOB1.19 human fetal osteoblastic cells and found that DANCR targets EZH2 and regulates the expression of Runx2 in osteogenic differentiation. During osteogenic differentiation, the canonical Wnt signaling pathway can be activated via ANCR-RNAi in PDLSCs during proliferation and osteogenic induction [42]. Moreover, DANCR has been shown to regulate the proliferation and osteogenic differentiation of human bone marrow-derived MSCs (PTA-1058 cells) via the p38/MAPK pathway [43].

HOTAIR

HOTAIR is an lncRNA formed by HOXC gene transcription. HOTAIR can inhibit the activity of HOX and other target genes by chromatin remodeling [44]. In the osteogenic differentiation of BMSCs, HOTAIR mediates the Wnt/β-catenin pathway, and downregulation of HOTAIR results in the increased expression of Wnt/β-catenin pathway-related proteins [45]. Furthermore, during osteogenic differentiation and proliferation in nontraumatic osteonecrosis of the femoral head (ONFH), HOTAIR regulates osteogenic differentiation and proliferation by modulating the activity of the miR-17-5p and its target gene Smad7 [46].

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1)

MALAT1 is a highly abundant and conserved imprinted gene. By investigating the function of MALAT1 in calcific aortic valve disease, Xiao et al. [27] demonstrated that MALAT1 could promote osteogenic differentiation. Additionally, Smad4 can be regulated by the MALAT1/miR-204 sponge, promoting the osteogenic differentiation of calcific valves after osteogenic induction in human aortic valve interstitial cells. In another study, researchers found that MALAT1 could regulate Osx expression by sponging miR-143 to promote the osteogenic differentiation of human bone marrow-derived MSCs [47]. MALAT1 can also promote osteogenic differentiation by sponging miR-30, miR-214, miR-124, and miR-34c [48–51].

Maternally expressed gene 3 (MEG3)

The lncRNA MEG3 is associated with various bone diseases, such as bone tumors, osteoporosis, and rheumatoid arthritis [52]. Zhao et al. [53] showed that MEG3

Table 1 The different types and roles of lncRNAs in osteogenic differentiation

| Approved symbol | Gene locus | Change in expression | Target(s) | Stem cell types | Refs. |
|-----------------|------------|----------------------|-----------|-----------------|-------|
| DANCR           | 4q12       | Downregulated        | miR-1305-Smad 4 axis EZH2, Runx2, OCN p38/MAPK pathway | PDLSCs hBMSCs | [36–41] |
| HOTAIR          | 12q13.13   | Downregulated        | Wnt/β-catenin pathway miR-17-5p Histone modification | BMSCs | [42–44] |
| MALAT1          | 11q13.1    | Upregulated          | Sponging for miR-30 Sponging for miR-214 Sponging for miR-124 Sponging for miR-34c Sponging for miR-204 | hBMSCs VICs | [27, 45–49] |
| MEG3            | 14q32.2    | Upregulated          | miRNA-543/SMURF1/RUNX2 axis miR-27a-3p/IGF1 axis BMP4 signaling pathway | hDPSCs PDLSCs | [50–53] |
| GAS5            | 1q21       | Upregulated          | miRNA-498/ RUNX2 axis miR-26-5p/PTEN axis miR-135a-3p/FOXO1 pathway GDF5 and p38/INK signaling pathway | MSCs HASMCs | [1, 54–56] |
could inhibit the osteogenic differentiation of human dental pulp stem cells via the miR-543/Smad ubiquitin regulatory factor 1/Runx2 axis. Similarly, downregulation of MEG3 suppresses the osteogenic differentiation of PDLS Cs through the miR-27a-3p/insulin-like growth factor (IGF) 1 axis in periodontitis [54]. In one study, researchers showed that the upregulation of MEG3 suppresses osteogenic differentiation by downregulating BMP-2 expression in PDLSCs [55]. Furthermore, Chen et al. suggested that MEG3-mediated activation of BMP-4 signaling may promote the osteogenic differentiation of BMSCs. This process is regulated by the DEP domain-containing mammalian target of rapamycin-interacting protein.

GAS5
In recent studies, many diseases have been shown to be associated with GAS5 [1]. However, few studies have described the roles of GAS5 in bone diseases. Feng et al. [1] showed that GAS5 overexpression prevents the development of osteoporosis by promoting the osteogenic differentiation of MSCs via targeting miR-498 to regulate Runx2. As a direct target of phosphatase and tensin homolog, miR-26-5p was shown to bind to GAS5 [56]. GAS5 can also promote osteogenic differentiation via the miR-135a-5p/FOXO1, growth differentiation factor 5, and p38/c-Jun N-terminal kinase signaling pathways [57, 58].

Roles of the lncRNA H19 in osteogenic differentiation
The lncRNA H19 is transcribed from the H19/IGF2 gene located on human chromosome 11p15.5 and has a molecular weight of 2.3 kilobase [59, 60]. Several studies have shown that H19 is related to the development of cancer [61–64], and the H19 locus can show tumor-suppressive effects in some cancers [65]. However, in oral squamous cell carcinoma, hepatocellular carcinoma, breast cancer, and bladder cancer, H19 is aberrantly upregulated and can act as a biomarker [63].

H19 is upregulated during the osteogenic induction of primitive stem cells and plays important functional roles in regulating osteogenic differentiation. The expression of H19 varies during different stages of osteogenic differentiation. In some in vitro studies, the osteogenic differentiation of human adipogenic stem cells (hASCs) is induced by the inhibition of H19 expression, resulting in the upregulation of the expression of pro-osteogenic genes. Additionally, overexpression of H19 downregulates the expression of pro-osteogenic genes [66]. Liao et al. [67] firstly reported a method for the generation of functional H19 using the AdEasy system and identified the biphasic effects of H19 on MSC osteogenic differentiation in immortalized mouse adipose-derived progenitors.

Functionally, H19 can participate in the regulation of osteogenic differentiation as an miRNA precursor. Moreover, H19 can act as a competitive endogenous RNA by adsorbing and inhibiting the expression of miRNAs. Inhibition of miR-22 and miR-141 by H19 results in the upregulation of Wnt/β-catenin/Runx2, thereby promoting the osteogenic differentiation of MSCs. The miR-138 sponge, through competitive binding with H19, reduces the inhibition of PTK2 gene expression to promote FAK expression and induce the osteogenic differentiation of MSCs [68]. Similarly, H19 mediates ligand-dependent nuclear receptor corepressor to affect the osteogenic and adipogenic differentiation of BMSCs through sponging miR-188 [69]. Additionally, H19 also regulates osteogenic differentiation through various other signaling pathways. The TP53 gene blocks cell cycle progression and inhibits cell proliferation by enhancing the transcription of different genes. During the osteogenic differentiation of MSCs, H19 binds directly to the p53 protein, inhibits the activity of p53, and promotes the proliferation of osteoblasts from MSCs [70, 71]. In a C57/BL6 mouse strain and A2lox-miR-675 cells, Keniry et al. [60] showed that H19 downregulates transforming growth factor (TGF)-1 expression through miR-675/TGF-1, inhibits the phosphorylation of Smad3, and downregulates histone deacetylase (HDAC) 4/5, enabling HDACs to target the promoter of Runx2. Other studies have also shown that H19 can act as a precursor of miR-675 and produce two mature miRNAs (miR-675-5p and miR-675-3p) by shearing, thereby regulating osteogenic differentiation through the Wnt/β-catenin signaling pathway [2, 72] (Fig. 2).

Conclusions
Compared with coding RNAs and miRNAs, many lncRNAs have still not been extensively studied, and the mechanisms and functions of these lncRNAs have not been clarified. Importantly, various lncRNAs have been shown to play roles in bone regeneration and osteogenic differentiation. Additionally, advancements in technology have facilitated the study of lncRNAs in different fields. For example, RNA-binding protein immunoprecipitation (RIP) has been widely used to explore the interactions between proteins and lncRNAs in vivo. Then, after confirming the target protein, quantitative reverse transcription polymerase chain reaction can be used to isolate and quantify the lncRNA [73]. Wang et al. [74] used RIP to identify the association between the lncRNA MIAT and miR-200a in the differentiation of bone marrow-derived MSCs into endothelial cells in a rat model of erectile dysfunction [75]. Although the interactions of RNA-binding proteins with different RNAs are critical for RNA regulation, these interactions are difficult to detect. Crosslinking immunoprecipitation (CLIP) can also be used to solve this problem of identifying RNA/protein interactions in vivo [76, 77]. In CLIP, cells are irradiated
with ultraviolet light to generate covalent bonds between the target RNA and protein when RNA/protein complexes come in close contact. After this step, RNA-binding proteins can be purified [75]. Moreover, RNA-pulldown assays and chromatin isolation by RNA purification can also be used to evaluate, identify, and test lncRNAs. However, the differential expression of many lncRNAs in various disease states and cell types has still not been clarified. Accordingly, bioinformatics studies, such as microarray analyses, are expected to have important applications in functional studies of lncRNAs. For example, Wang et al. [78] explored the potential roles of lncRNAs in ONFH via microarray and bioinformatics analyses of the lncRNA expression profiles of BMSCs isolated from patients with steroid-induced ONFH.

Overall, in this review, we summarized the functions and mechanisms of H19, which plays important roles in osteogenic differentiation. Many studies of H19 regulation have been reported, including the regulatory effects of H19 on gene expression, signaling pathways, lncRNA/miRNA sponging, and miRNA precursors. These mechanisms and potential biomarkers are expected to guide diagnoses, clinical treatments, and prognostic judgments in the future. However, the regulatory mechanisms of H19 have not been fully elucidated. For example, there is still a lack of information regarding the microarray expression profiles of H19-overexpressing or H19-knockdown cells during osteogenic differentiation; thus, the effects of H19 on the expression of downstream factors has not been determined. Such studies may improve our understanding of this important lncRNA. Studies of H19 are still in the primary research stage, and the potential clinical applications of this lncRNA are unclear. However, the biological potential of lncRNAs is obvious, and further studies of the clinical importance of lncRNAs, including H19, in bone diseases, such as osteoporosis, fracture, and other diseases, may lead to improvements in therapeutic strategies for, and outcomes of, bone-associated diseases.

Abbreviations
MSC: Mesenchymal stem cell; lncRNA: Long noncoding RNA; RNA seq: RNA sequencing; ncRNA: Noncoding RNA; siRNA: Small interfering RNA; miRNA: MicroRNA; CC: Cervical cancer; DIANOR: Differentiation antagonizing non-protein-coding RNA; PDLC: Periodontal ligament stem cell; Runx2: Runx-related transcription factor 2; BMP-2: Bone morphogenetic protein 2; HOTAIR: HOX transcript antisense RNA; ONFH: Nontraumatic osteonecrosis of the femoral head; MALAT1: Metastasis-associated lung adenocarcinoma transcript 1; Oste: Osterix; MEG3: Maternally expressed gene 3; CLIP: Crosslinking immunoprecipitation; RIP: RNA-binding protein immunoprecipitation
Acknowledgements
This work was supported by grants from the National Natural Science Foundation of China [grant no. 81972156] and the Natural Science Foundation of Liaoning Province [grant no. 2019-ZD-0781].

Authors’ contributions
ZM Zhou drafted and wrote the manuscript. Mohammad Showkat Hossein assisted with writing the manuscript and provided helpful suggestions. Dr. Da Liu reviewed the final manuscript and provided advice on writing the manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by grants from the National Natural Science Foundation of China [grant no. 81972156] and the Natural Science Foundation of Liaoning Province [grant no. 2019-ZD-0781].

Availability of data and materials
Not applicable.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 5 October 2020 Accepted: 6 January 2021

Published online: 21 January 2021

References
1. Feng J, Wang XJ, Li CH. LncRNA GAS5 overexpression alleviates the development of osteoporosis through promoting osteogenic differentiation of MSCs via targeting microRNA-488 to regulate RUNX2. Eur Rev Med Pharmac. 2019;23(18):7757–65.
2. Peng SP, Cao LH, He SW, Zhong YC, Ma HT, Zhang YR, Shuai CJ. An overview of long noncoding RNAs involved in bone regeneration from mesenchymal stem cells. Stem Cells Int. 2018;2018. https://doi.org/10.1155/2018/8727548.
3. Zuo CQ, Wang ZG, Lu HY, Dai Z, Liu YG, Cui L. Expression profiling of IncRNAs in C3H10T1/2 mesenchymal stem cells undergoing early osteoblast differentiation. Mol Rep. 2013;8(2):463–7.
4. Jathar S, Kumar V, Srivastava J, Tripathi V. Technological developments in IncRNA biology. Adv Exp Med Biol. 2017;1008:283–323.
5. Zhang WY, Dong R, Diao S, Du J, Fan ZP, Wang F. Differential long non-coding RNA/mRNA expression profiling and functional network analysis during osteogenic differentiation of human bone marrow mesenchymal stem cells. Stem Cell Res Ther. 2017;8. https://doi.org/10.1186/s13287-017-0485-6.
6. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell. 2009;136(4):629–41.
7. Yang L, Froberg JE, Lee JT. Long non-coding RNAs: fresh perspectives into the RNA world. Trends Biochem Sci. 2014;39(1):35–43.
8. Zhu L, Zhu J, Liu YF, Chen YJ, Li YL, Huang LR, Chen SS, Li T, Dang YH, Chen T. Methamphetamine induces alterations in the long non-coding RNAs expression profile in the nucleus accumbens of the mouse. BMC Neurosci. 2015;16. https://doi.org/10.1186/s12866-015-0157-3.
9. Matsumoto A, Parasut A, Matsumoto M, Yamashita R, Fung J, Monteleone E, Saghatelian A, Nakayama K, Clohessy JG, Pandolfi PP. mTORC1 and muscle regeneration are regulated by the LINC00961-encoded SPAR polypeptide. Nature. 2017;541(7636):228–32.
10. de Andres MC, Kingham E, Imagawa K, Gonzalez A, Roach H, Wilson DJ, Oreffo RO. Epigenetic regulation during fetal femur development: DNA methylation matters. PloS One. 2013;8(11):e75497.
11. Kino T, Hurt DE, Ichijo T, Nader N, Chrousos GP. Noncoding RNA GAS5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. Sci Signal. 2010, 3(107). https://doi.org/10.1126/scisignal.2000568.
12. Fu X, Zhu X, Qin F, Zhang Y, Lin J, Ding Y, Yang Z, Wang Y, Wang L, Zhang Q, et al. Linc00210 drives Wnt/beta-catenin signaling activation and liver tumor progression through CTNNB1-dependent manner. Mol Cancer. 2018;17(1):73.
13. Chew CL, Conos SA, Uenal T, Tergaonkar V. Noncoding RNAs: master regulators of inflammatory signaling. Trends Mol Med. 2018;24(1):65–84.
14. Tang Z, Wang Z, Qing F, Ni Y, Fan Y, Tian Y, Zhang X. Bone morphogenetic protein Smads signaling in mesenchymal stem cells affected osteoinductive calcium phosphate ceramics. J Biomed Mater Res A. 2015;103(2):1001–10.
15. Li D, Tian Y, Yin C, Huai Y, Zhao Y, Su P, Wang X, Pei J, Zhang K, Yang C, et al. Silencing of IncRNA AK045490 promotes osteoblast differentiation and bone formation via beta-Catenin/TCF71/Runx2 signaling axis. Int J Mol Sci. 2019;20(24). https://doi.org/10.3390/ijms202446239.
16. Yu C, Li L, Xie F, Guo S, Liu F, Dong N, Wang Y. LncRNA TUG1 sponges miR-204-5p to promote osteoblast differentiation through up-regulating Runx2 in aortic valve calcification. Cardiovasc Res. 2018;114(1):168–79.
17. Bouaaziz W, Sigaux J, Modowski D, Devignes CS, Funck-Brentano T, Richette P, Da HK, Provot S, Cohen-Solal M, Hay E. Interaction of HIF alpha/beta-catenin inhibits matrix metalloproteinase 13 expression and prevents cartilage damage in mice. Proc Natl Acad Sci U S A. 2016;113(19):5453–8.
18. Song J, Kim D, Han J, Kim Y, Lee M, Jin EJ. PBMSC and esosomes-derived Hotair is a critical regulator and potent marker for rheumatoid arthritis. Clin Exp Med. 2015;15(1):121–6.
19. Zhou W, He X, Chen Z, Fan D, Wang Y, Feng H, Zhang G, Lu A, Xiao L. LncRNA HOTAIR-mediated Wnt/beta-catenin network modeling to predict and validate therapeutic targets for cartilage damage. BMC Bioinformatics. 2019;20(1):412.
20. Chen L, Song Z, Huang S, Wang R, Qin W, Guo J, Lin Z. IncRNA DANC5 suppresses odontoblast-like differentiation of human dental pulp cells by inhibiting Wnt/beta-catenin pathway. Cell Tissue Res. 2016;364(2):309–18.
21. Kyrtakis JM, Avnuch J. Mammalian MAPK signal transduction pathways activated by stress and inflammation: A 10-year update. Physiol Rev. 2012;92(4):679–737.
22. Wan W, Cheng B, Zhang C, Ma Y, Li A, Xu F, Lin M. Synergistic effect of matrix stiffness and inflammatory factors on osteogenic differentiation of MSCs. Biophys J. 2019;117(1):129–42.
23. Qu B, Xia X, Wu HH, Tu CQ, Pan XM. PDGFR-regulated miRNA-138 inhibits the osteogenic differentiation of mesenchymal stem cells. Biochem Biophys Res Commun. 2014;448(3):241–7.
24. Liao L, Yang X, Su X, Hu C, Zhu X, Yang N, Chen X, Shi, Shi, Shi, Jin Y. Redundant miR-3077-5p/miR-705 mediate the shift of mesenchymal stem cell lineage commitment to adipocyte in osteoporosis bone marrow. Cell Death Dis. 2012;3:e600.
25. Liu HP, Hao DJ, Wang XD, Hu HM, Li YB, Dong XH. miR-30a-3p promotes ovariectomy-induced osteoporosis in rats via targeting SFRP1. Eur Rev Med Pharmacol Sci. 2019;23(22):9754–60.
26. Qadir AS, Um S, Lee J, Baek K, Seo BM, Lee G, Kim GS, Woo KM, Ryoo HM, Baek JH. miR-124 negatively regulates osteogenic differentiation and in vivo bone formation of mesenchymal stem cells. J Cell Biochem. 2015;116(5):730–42.
27. Xiao X, Zhou T, Guo S, Guo C, Zhang Q, Dong N, Wang Y. LncRNA MALAT1 sponges miR-204 to promote osteoblast differentiation of human aortic valve interstitial cells through up-regulating Smad4. Int J Cardiol. 2017;243:404–12.
28. Yang X, Yang K, Liao K, Jin Y. Effect of miR-705 on osteogenic differentiation of mouse embryo osteoblast precursor cells MCT3T3-E1. Zhejiang Da Xue Xue Bao. 2016;45(6):575–80.
29. Wang CG, Liao Z, Xiao H, Liu H, Hu YH, Liao QD, Zhong D. LncRNA KCNQ1/OT1 promotes BMP2 expression to regulate osteogenic differentiation by sponging miRNA-214. Exp Mol Pathol. 2019;107:77–84.
30. Wang CL, Xiao F, Wang CD, Zhu JF, Shen C, Zuo B, Wang H, Li WW, Feng WJ, et al. Gremlin2 suppression increases the BMP-2-induced osteogenesis of human bone marrow-derived mesenchymal stem cells via the BMP-2/Smad/Runx2 signaling pathway. J Cell Biochem. 2017;118(2):286–97.
31. Cai X, Cullen BR. The imprinted H19 non-coding RNA is a primary microRNA precursor. RNA (New York). 2007;13(3):313–6.
32. Jiang L, Shao C, Wu Qi, Chen G, Zhou J, Yang B, Li H, Gou LT, Zhang Y, Wang Y, et al. NEAT1 scaffolds RNA-binding proteins and the microprocessor to globally enhance pri-miRNA processing. Nat Struct Mol Biol. 2017;24(1):80–4.
33. Song WQ, Gu WQ, Qian YB, Ma X, Yao YQ, Liu WJ. Identification of long non-coding RNA involved in osteogenic differentiation from mesenchymal stem cells using RNA-Seq data. Genet Mol Res. 2015;14(4):18268–9.
34. Luan Y, Xie B, Wei W. REST-repressed lncRNA NPPA-AS1 regulates cervical cancer progression by modulating miR-30e/DKX1/Wnt/b-catenin signaling pathway. J Cell Biochem. 2021;122(1):16-28. https://doi.org/10.1002/jcb. 29701.
35. Li YQ, Sun N, Zhang CS, Li N, Wu B, Zhang JL. Inactivation of IncRNA HOTAIR caused by histone methyltransferase RIZ1 accelerated the proliferation and invasion of liver cancer. Eur Rev Med Pharmacol Sci. 2020; 24(17):8767–77.

36. Liu Z, Mi M, Li X, Zheng X, Wu G, Zhang L. A IncRNA prognostic signature associated with immune infiltration and tumour mutation burden in breast cancer. J Cell Mol Med. 2020;24(21):4344–56. https://doi.org/10.1111/1749-6023.15762.

37. Shi YB, Liu SL, Mou XR, Liao J, Che JP, Fei XQ, Wang AR. Long non-coding RNA HOXA-AS2 acts as an oncogene by targeting miR-145-3p in human non-small cell lung cancer. Eur Rev Med Pharmacol Sci. 2020;24(17):8629.

38. Kretz M, Webster DE, Flockhart RJ, Lee CS, Zehnder A, Lopez-Pajares V, Qu K, Zheng GX, Chow J, Kim GE, et al. Suppression of progenitor differentiation requires the long non-coding RNA ANCR. Genes Dev. 2012;26(4):338–43.

39. Zhang L, Sun X, Chen S, Yang C, Shi B, Zhou L, Zhao J. Long non-coding RNA DANCER regulates miR-1305-3md-4 axis to promote chondrogenic differentiation of human synovium-derived mesenchymal stem cells. Biosci Rep. 2017;37(4). https://doi.org/10.1042/BSR201701037.

40. Wang Z, Huang Y, Tan L. Downregulation of IncRNA DANCER promotes osteogenic differentiation of periodontal ligament stem cells. BMC Dev Biol. 2020;20(1):12.

41. Zhu L, Xu PC. Downregulated LncRNA-ANCR promotes osteoblast biodegradation by targeting EZH2 and regulating Runx2 expression. Biochem Biophys Res Co. 2013;432(4):612–7.

42. Jia Q, Jiang W, Ni L. Down-regulated non-coding RNA (IncRNA-ANCR) promotes osteogenic differentiation of periodontal ligament stem cells. Arch Oral Biol. 2015;60(2):234–41.

43. Zhang J, Tao Z, Wang Y. Long non-coding RNA DANCER regulates the proliferation and osteogenic differentiation of human bone-derived marrow mesenchymal stem cells via the p38 MAPK pathway. Int J Mol Med. 2018; 41(1):213–9.

44. Li L, Liu B, Wapinski OL, Tsai MC, Qu K, Zhang J, Carlson JC, Lin M, Fang F, Gupta RA, et al. Targeted disruption of Hotair leads to homoeotic transformation and gene deserepression. Cell Rep. 2013;5(1):3–12.

45. Shen JJ, Zhang CH, Chen ZW, Wang XZ, Yang DC, Zhang FL, Feng KH. LncRNA HOTAIR inhibited osteogenic differentiation of BMSCs by regulating Wnt/beta-catenin pathway. Eur Rev Med Pharmacol Sci. 2019;23(17):7232–46.

46. Wei B, Wei W, Zhao B, Guo X, Liu S. Long non-coding RNA HOTAIR inhibits miR-17-5p to regulate osteogenic differentiation and proliferation in non-traumatic osteonecrosis of the femoral head. Plos One. 2017;12(2):e0169097.

47. Gao Y, Xiao F, Wang C, Wang C, Cui P, Zhang X, Chen X. Long non-coding RNA MALAT1 promotes osteoex expression to regulate osteogenic transformation and gene derepression. Cell Rep. 2013;5(1):3–6.

48. Yang Q, Han Y, Liu P, Huang Y, Li X, Jia L, Zheng Y, Li W. Long noncoding RNA GASS promotes osteogenic differentiation of human periodontal ligament stem cells by regulating GDFs and p38/JNK signaling pathway. Front Pharmacol. 2020;11:701.

49. Li J, Hao D, He T, Huang W. Recombinant adenovirus (AdEasy system) mediated exogenous expression of long non-coding RNA H19 (IncRNA H19) biphasic regulating osteogenic differentiation of mesenchymal stem cells (MSCs). Am J Trans Res. 2020;12(5):1700–13.

50. Wu J, Zhao J, Sun L, Pan Y, Wang H, Zhang WB. Long non-coding RNA H19 mediated mechanical tension-induced osteogenesis of bone marrow mesenchymal stem cells via FAK by sponging miR-138. Bone. 2018;106:27–37.

51. Wang Y, Liu W, Liu Y, Cui J, Zhao Z, Cao H, Fu Z, Liu B. Long non-coding RNA H19 mediates LCoR to impact the osteogenic and adipogenic differentiation of mBMSCs in mice through sponging miR-188. J Cell Physiol. 2018;233(9):7435–46.

52. Watkins AJ, Wilkins A, Cunningham C, Perry VH, Svet M, Ormond C, Eckert JJ, Tomens C, Gagampang RF, Caal J, et al. Low protein diet fed exclusively during mouse oocyte maturation leads to behavioral and cardiovascular abnormalities in offspring. J Physiol. 2008;586(23):2231–44.

53. Zhou QP, Zhang F, Zhang J, Ma D. H19 promotes the proliferation of osteocytes by inhibiting p53 during fracture healing. Eur Rev Med Pharmacol Sci. 2018;22(8):2226–32.

54. Liang WC, Fu WN, Wang YB, Sun YK, Xu LL, Wong CW, Chan KM, Li G, Waye MM, Zhang JF. H19 activates Wnt signaling and promotes osteoblast differentiation by functioning as a competing endogenous RNA. Sci Rep. 2016;6:30121.

55. Zheng X, Mao J, Liu C, Wei F, Liu D, Zheng Z, Ting K, Wang C, Guo J. Upregulation of long non-coding RNA MEG3 inhibits the osteogenic differentiation of periodontal ligament cells. J Cell Physiol. 2019;234(4):4617–26.

56. Chang Z, Yan G, Zheng J, Liu Z. The IncRNA GASS inhibits the osteogenic differentiation and calcification of human vascular smooth muscle cells. Calcif Tissue Int. 2020;107(1):86–95.

57. Wang X, Zhao D, Zhu Y, Dong Y, Liu Y. Long non-coding RNA GASS promotes osteogenic differentiation of bone marrow mesenchymal stem cells by regulating the miR-135a-5p/FOXO1 pathway. Mol Cell Endocrinol. 2019;496:110534.

58. Yang Q, Han Y, Liu P, Huang Y, Li X, Jia L, Zheng Y, Li W. Long noncoding RNA GASS promotes osteogenic differentiation of human periodontal ligament stem cells by regulating GDFs and p38/JNK signaling pathway. Front Pharmacol. 2020;11:701.

59. Kallen AN, Zhou XB, Xu J, Qiao C, Ma J, Yan L, Lu L, Liu C, Yi JS, Zhang H, et al. The imprinted H19 IncRNA antagonizes let-7 microRNAs. Mol Cell. 2013;52(1):101–12.

60. Kendry A, Osley D, Monnier P, Kyba M, Dandolo L, Smits G, Reik W. The H19 IncRNA is a developmental reservoir of miR-675 suppresses growth and Igf1. Nat Cell Biol. 2012;14(7):659–65.

61. Dugimont T, Curgy JJ, Wemert N, Delobbelle A, Raes MB, Joublé A, Stehelin D, Coll J. The H19 gene is expressed within both epithelial and stromal components of human invasive adenocarcinomas. Biol Cell. 1995;85(2):13–21.

62. El-Rini M, Shevelev A, Schulte E, Tykocinsky M, Cooper M, Ariel I, Pode D, Kopf E, de Groot N, Hochberg A. The expression of the imprinted H19 and IGF-2 genes in human bladder carcinoma. FEBS Lett. 1995;374(1):57–61.

63. Ghafouri-Fard S, Esmaeili M, Taheri M. H19 IncRNA: roles in tumorigenesis. Biomed Pharmacother. 2020;123:109774.

64. Verhaegh GW, Verkleij L, Vermeulen SH, Den Heijer M, Witjes JA, Kiemeney LA. Polycombings in the H19 gene and the risk of bladder cancer. Eur Urol. 2008;54(3):1118–26.

65. Yoshimizu T, Miraglio A, Ripoche MA, Gabony A, Vennucci M, Riccio A, Colnot S, Godard C, Terris B, Jammes H, et al. The H19 locus acts in vivo as a tumor suppressor. Proc Natl Acad Sci U S A. 2008;105(34):12417–22.

66. Huang G, Kang Y, Huang Z, Zhang Z, Meng F, Chen W, Fu M, Liao W, Zhang Z. Identification and characterization of long non-coding RNAs in osteogenic differentiation of human adipose-derived stem cells. Cell Physiol Biochem. 2017;42(3):1037–50.

67. Liao J, Xiao H, Dai G, He T, Huang W. Recombinant adenovirus (AdEasy system) mediated exogenous expression of long non-coding RNA H19 (IncRNA H19) biphasic regulating osteogenic differentiation of mesenchymal stem cells (MSCs). Am J Trans Res. 2020;12(5):1700–13.

68. Wu J, Zhao J, Sun L, Pan Y, Wang H, Zhang WB. Long non-coding RNA H19 mediates mechanical tension-induced osteogenesis of bone marrow mesenchymal stem cells via FAK by sponging miR-138. Bone. 2018;106:27–37.

69. Wang Y, Liu W, Liu Y, Cui J, Zhao Z, Cao H, Fu Z, Liu B. Long non-coding RNA H19 mediates LCoR to impact the osteogenic and adipogenic differentiation of mBMSCs in mice through sponging miR-188. J Cell Physiol. 2018;233(9):7435–46.
76. Cottrell KA, Djuranovic S. Urb-RIP - an adaptable and efficient approach for immunoprecipitation of RNAs and associated RNAs/proteins. PLoS One. 2016;11(12)e0167877.
77. Yoon JH, Gorospe M. Cross-linking immunoprecipitation and qPCR (CLIP-qPCR) analysis to map interactions between long noncoding RNAs and RNA-binding proteins. Methods Mol Biol. 2016;1402:11–7.
78. Wang Q, Yang Q, Chen G, Du Z, Ren M, Wang A, Zhao H, Li Z, Zhang G, Song Y. LncRNA expression profiling of BMSCs in osteonecrosis of the femoral head associated with increased adipogenic and decreased osteogenic differentiation. Sci Rep. 2018;8(1):9127.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.