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SYSTEMATIC REVIEW

Long non-coding RNA in osteogenesis

A NEW WORLD TO BE EXPLORED

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Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides with limited coding potential, which have emerged as novel regulators in many biological and pathological processes, including growth, development, and oncogenesis. Accumulating evidence suggests that lncRNAs have a special role in the osteogenic differentiation of various types of cell, including stem cells from different sources such as embryo, bone marrow, adipose tissue and periodontal ligaments, and induced pluripotent stem cells. Involved in complex mechanisms, lncRNAs regulate osteogenic markers and key regulators and pathways in osteogenic differentiation. In this review, we provide insights into the functions and molecular mechanisms of lncRNAs in osteogenesis and highlight their emerging roles and clinical value in regenerative medicine and osteogenesis-related diseases.

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Keywords: lncRNA, Molecular mechanism, Osteogenesis, Osteogenic differentiation

Article focus

- Accumulating evidence highlights the significant roles of lncRNAs in osteogenesis.
- The detailed mechanisms of lncRNAs in osteogenesis remain to be elucidated.
- This systematic review summarizes the roles and molecular mechanisms of osteogenesis-related lncRNAs, identifies limitations of current research, and offers future research directions.

Key messages

- High-throughput technologies have been applied to identify critical osteogenesis-related lncRNAs.
- lncRNAs regulate osteogenic markers or key regulators and pathways in complex mechanisms to participate in osteogenesis.
- Most studies have focused on the cross-talk between lncRNAs and microRNA, providing insights into the mechanism by which non-coding RNA coordinate to regulate the osteogenesis, and few have focused on the subcellular localization of lncRNAs and discussed the possibility of the competing mode of regulation.

Introduction

Long non-coding RNAs (lncRNAs) are a class of transcripts longer than 200 nucleotides with a low coding potential.[1-3] However, a subset of lncRNAs longer than 10 000 nucleotides contain small open reading frames that undergo active translation,[4] redefining lncRNA. Although the precise roles of the most lncRNAs are still under investigation,[5] they have been identified as the essential regulators in many biological and pathological processes, such as growth, development, and oncogenesis.[6-10] lncRNAs participate in these processes by regulating gene expression patterns at the transcriptional and post-transcriptional level.[11-13]

Accumulating evidence has provided insights into the major roles of lncRNAs in osteogenesis. A growing number of lncRNAs, including H19,[14] DANCR,[15] MALAT1,[16] MEG3,[17] and HOTAIR,[18] have been identified as differentially expressed in osteogenesis and further confirmed to regulate osteogenic markers or key regulators and pathways in osteogenic differentiation, such as the Wnt/β-catenin signalling pathway.[19-21] The mechanisms of competing endogenous RNAs (ceRNAs) during osteogenic processes have been expounded, and a connection between lncRNAs and microRNAs (miRNAs) which has been widely observed in osteogenesis,[14,18,22-24] has been identified. Moreover, lncRNAs serve as scaffolds or guides to modulate the function of key regulators, such as EZH2[24] and FOXO1.[15] The present review

Table I. Overview of lncRNAs and their roles in osteogenesis

Name	Cell type	Expression*	Functional role	Related molecules	Related pathways	Reference
H19	hMSC/mMSC/ MC3T3-E1/UMR10	Upregulation	Promotion	miR-675/TGF-β1/Smad3/HDAC; miR-675-5p /miR-141/miR-22; OPN; DKK4; miR-449a/miR-449b/miR-107/miR-106/miR-125a/miR27b/miR-34a/miR-17; miR-141/miR-22; miR-675/NOMO1; miR-138/FAK	Wnt/β-catenin signalling	14,19,22,23,26-28
DANCR	MSC/hBMSC/ hDTSC†/ hFOB1.19	Downregulation	Inhibition	EZH2/Runx2; p-GSK-3β/β-catenin; FOXO1/SKP2; Runx2; p38	Wnt/β-catenin signalling; MAPK signalling	15,25,29-32
MEG3	hMSC/hBMSC/ hASC	ND	Controversy	SOX2/BMP4/OSX/OCN; miR-140-5p; miR-133a-3p/SLC39A1	ND	17,33,34
HOTAIR	hBMSC/hAVIC	Downregulation	Inhibition	β-catenin/BMP1/BMP4/BMP6/BMPR6/ COL1A1; miR-17-5p/Smad7/Runx2/ COL1A1/ALP	Wnt/β-catenin signalling	18,21
MALAT1	hFOB 1.19/hAVIC	Upregulation	Promotion	OPG; miR-204/Smad4	ND	16,35
AK007000	MC3T3-E1/C2C12/ C3H10T1/2	Upregulation	Promotion	BMP2	ND	36
AK089560	C3H10T1/2	Downregulation	ND	Sema3a	ND	37
AK138929	MC3T3-E1	Downregulation	Inhibition	miR-489-3p/PTPN6	ND	38
AK141205	rBMSC	Upregulation	Promotion	OPG/CXCL13/H4 histone	ND	39
AK028326	hBMSC	ND	Promotion	CXCL13	ND	40
MIR31HG	hASC	ND	Inhibition	NF-κB/p65/IκB-α	NF-κB signalling	41
NONHSAT009968	hBMSC	ND	Inhibition	ND	ND	42
POIR	hPDLSC	ND	Promotion	miR-182/FOXO1/TCF-4/β-catenin	Wnt/β-catenin signalling	43
HCG18	NPC	ND	Promotion	miR-146a-5p/TARF6/NFκB	NFκB signalling	44
HOXA-AS3	mMSC	No change	Inhibition	EZH2/Runx2/H3K27me3	ND	45
lncRUNX2-AS1	hBMSC	ND	Inhibition	Runx2	ND	46
MIAT	hASC	Downregulation	Inhibition	ND	ND	8
MODR	MSMSC	Upregulation	Promotion	miR-454	ND	47
HIF1A-AS2	hPDLSC	ND	Inhibition	ND	ND	48
TUG1	hAVIC	ND	Promotion	miR-204-5p/Runx2	ND	24
TCONS_00041960	rBMSC	ND	Promotion	Glucocorticoid/miR-204-5p/miR-125a-3p/ Runx2/GILZ	ND	49

*lncRNA expression during osteogenic differentiation
†hDTSC, human dental tissue-derived stem cell, including human periodontal ligament stem cell (hPDLSC), human dental stem pulp cell (hDPSC), and human stem cell from the apical papilla (hSCAP).
MSC, mesenchymal stem cell; hMSC, human mesenchymal stem cell; mMSC, mouse mesenchymal stem cell; rMSC, rat mesenchymal stem cell; hBMSC, human marrow mesenchymal stem cell; rBMSC, rat marrow mesenchymal stem cell; hASC, human adipose-derived stem cell; hAVIC, human aortic valve interstitial cell; NPC, nucleus pulposus cell; MSMSC, maxillary sinus membrane stem cell; ND, not determined.

summarizes the current evidence of the differential expression and molecular mechanisms of lncRNAs and provides insights into their roles in osteogenesis. An overview of lncRNAs and their roles in osteogenesis is provided in Table I and Fig. 1.[8,14-19,21-24,25-49]

A total of 508 studies were identified by a literature search in Pubmed, Embase, and Web of Science, up to 27 February 2018. The combined search terms were: "osteogenesis" or "osteogenic" or "osteogenic differentiation" or "bone formation" or "osteoblast" or "MC3T3" and "long noncoding RNA" or "long non-coding RNA" or "lncRNA". After excluding 424 irrelevant or duplicated studies by screening, 84 studies were further assessed using the following criteria: (1) research focus on screening for osteogenesis-related lncRNA; (2) research focus on the roles of lncRNA in osteogenesis; (3) not review articles; (4) full text available. Finally, 73 eligible studies involving 21 lncRNAs and eight types of cell, including mesenchymal stem cells (MSC) from various sources and species, dental tissue-derived stem cells and induced pluripotent stem cells, were included in this systematic review (Table I, Fig. 1).

High-throughput technology, such as RNA sequencing (RNA-Seq) and microarray profiling, has been applied to investigate the patterns of expression of lncRNA during osteogenic differentiation, and has successfully characterized various osteogenesis-related lncRNAs. Identification of functional lncRNAs in osteogenesis has mainly focused on types of MSC from various sources, including the embryo and bone marrow. For example, Zuo et al[50] identified 116 differentially expressed lncRNAs in BMP2-treated C3H10T1/2 stem cells, among which 24 pairs of co-expressed lncRNAs and nearby mRNAs such as lincRNA0231-EGFR and MEG3-DLK1 were identified. Cheng et al[51] identified 24 downregulated lncRNAs in BMP2-treated C3H10T1/2 stem cells, among which AK035085 was shown to inhibit osteogenic differentiation. In order to identify potential lncRNAs involved in osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs), Luo et al[52] screened for the lncRNAs near to osteogenesis-related genes Smurf1, MSX1, and BMP1, and identified promising regulators AK096529 and uc003ups, which may positively regulate Smurf-1. Meanwhile, Gordon et al[53] identified 1912 annotated lncRNAs expressed during osteoblast differentiation of mouse MSCs, among which 198 lncRNAs were differentially expressed. The analysis,

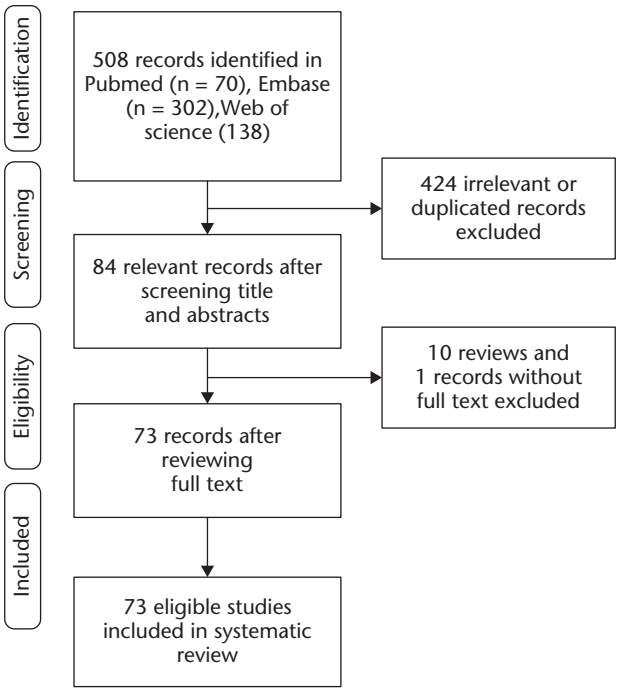

Fig. 1

Flow diagram of the systematic review.

combined with chromatin immunoprecipitation sequencing data, revealed that > 40% of the genomic loci of these lncRNAs were bound with Runx2, suggesting that many are potentially regulated by Runx2. In addition, Harris et al[54] found that 3764 lncRNAs were differentially expressed during the differentiation of α-SMA-positive BMSCs into mineralizing osteoblasts-osteocytes, among which 745 intersected with H3K27ac active enhancers, indicating a set of candidate enhancer RNAs (eRNA) for osteoblast differentiation. Based on public RNA-Seq data, Song et al[55] identified that the expression of 574 lncRNAs significantly altered during the osteogenic differentiation of MSCs. Of these, the lncRNAs TCONS_00046478, TCONS_00027225, and TCONS_00007697 may function in osteogenic differentiation by acting as miRNA precursors (e.g. miR-544, miR-601, miR-640, and miR-689) and regulating the expression of co-expressed genes (COL4A4, COL21A1, and WNT2). In lncRNA microarray analyses of hBMSCs, Wang et al[56] identified 1206 lncRNAs that were differentially expressed during osteogenic differentiation, among which H19 and uc022axw.1 probably have important roles in osteogenesis. Xie et al[57] found that 520 lncRNAs exhibited differential expression in osteogenically differentiated hBMSCs from patients with ankylosing spondylitis (ASMSCs), among which lnc-USP50-2, lnc-FRG2C-3, lnc-LIN54-1, and lnc-ZNF354A-1 may be involved in the mechanism leading to the imbalance between BMP2 and NOG that promotes the abnormal osteogenic differentiation of ASMSCs. Moreover, 339 differentially expressed lncRNAs were identified in hBMSCs co-cultured with human amnion-derived mesenchymal stem cells (hAMSCs), among which DANCR may participate in complex regulatory mechanisms of hAMSC-derived osteogenic differentiation of hBMSCs.[25] Zhang et al[58] revealed six core regulators (NR_024031, XR_111050, FR148647, FR406817, FR401275, and FR374455) among 1408 differentially expressed lncRNAs of hBMSCs during osteogenic differentiation, of which XR_111050 promoted the osteogenic potential of hBMSCs. Finally, Qiu et al[59] identified 433 upregulated and 232 downregulated lncRNAs in the osteogenic differentiation of hBMSCs, among which ENST00000502125.2 may be a promising target to promote osteogenesis.

Some studies have investigated other types of cell, such as human periodontal ligament stem cells (hPDLSCs), human adipose-derived stem cells (hASCs), the mouse osteoblast cell line MC3T3-E1, and human induced pluripotent stem cells (iPSCs). Qu et al found that 994 lncRNAs were upregulated and 1177 were downregulated in the osteogenic differentiation of hPDLSCs, among which 393 lncRNAs were closely related to osteogenesis-related mRNAs (ALP, BMP2, BMP5, BMP6, IL6, COL1A1, and COL1A2). Moreover, RP11-305L7.6, RP4-613B23.1, RP11-45A16.4, XLOC_002932, and AC078851.1 were recognized as key candidate lncRNAs. MEG3 was upregulated after osteogenic induction, indicative of its critical functions in the osteogenesis in hPDLSCs. Furthermore, AC078851.1 was negatively correlated with IL6, while TCONS_00007046 was positively correlated with BMP2 and ALP.[60] Meanwhile, Gu et al[61] confirmed that a total of 960 lncRNAs were differentially expressed during hPDLSC osteogenic differentiation. In particular, TCONS_00212979 and TCONS_00212984 might interact with miR-34a and miR-146a to modulate the osteogenic differentiation of hPDLSCs via the MAPK pathway. Furthermore, Hu et al[62] identified 857 lncRNAs that were significantly altered during MC3T3-E1 osteoblast differentiation under simulated microgravity, as well as 132 pairs of lncRNA and nearby coding genes, among which NONMMUT044983-Ptbp2, NONMMUT023474-Ext1, and NONMMUT018832-Tnpo1 were screened for possible functions in osteoblast differentiation. Huang et al[63] found that 1460 upregulated and 1112 downregulated lncRNAs in the osteogenic differentiation of hASCs, and constructed the lncRNA-mRNA co-expression network that included 12 lncRNAs and 157 mRNAs. Finally, Paik et al[64] analyzed the transcriptome of iPSCs induced by BMP2 using RNA-Seq and identified that the lncRNA SNHG1 was upregulated in response to BMP2 among the 5566 differentially expressed transcripts, of which lncRNAs accounted for 4%.

lncRNAs in osteogenesis. The lncRNA H19 has been found to be one of the most abundant and conserved non-coding transcripts in mammalian development, which has profound effects on proliferation, differentiation, and carcinogenesis.[65] Significant upregulation of H19 in osteogenesis was first proposed by Huang et al,[26] and

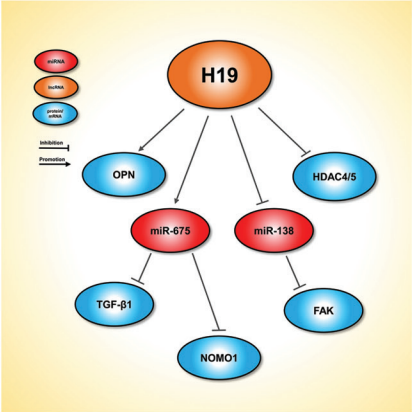

Fig. 2a

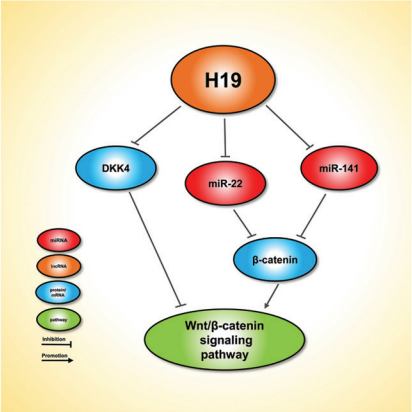

Fig. 2b

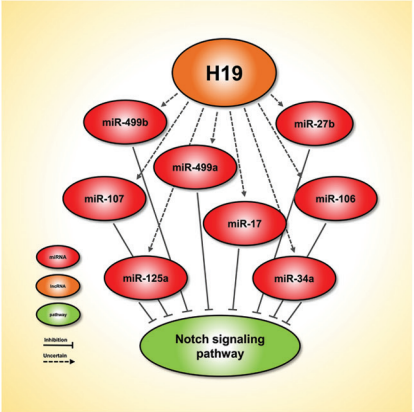

Fig. 2c

The molecular mechanisms of H19 in osteogenesis. (a) H19 regulates osteogenesis-related genes. (b) H19 regulates the Wnt/β-catenin signalling pathway. (c) H19 regulates the MAPK signalling pathway.

subsequently confirmed by other studies in osteoblasts and hBMSCs.[22,27] These findings ignited academic interest in the role of H19 in osteogenesis.

Accumulating evidence has suggested a crucial role of H19 as a ceRNA. Huang et al[26] proposed that H19 could promote the osteogenic differentiation of hBMSCs through the miR-675/TGF-β1/Smad3/HDAC pathway (Fig. 2a) Meanwhile, Liang et al[22] demonstrated that H19 promoted osteogenesis *in vivo* and *in vitro*, by functioning as a ceRNA to sponge miR-22 and miR-141, two negative regulators of osteogenesis targeting β-catenin, ultimately activating the Wnt/β-catenin pathway and enhancing osteogenesis (Fig. 2b). Moreover, the feedback loop between H19 and its encoded miR-675-5p may partially account for the regulation of osteoblast differentiation.[22] Furthermore, Dong et al[27] found that H19 was upregulated in 20(R)-ginsenoside Rh2 (Rh2)-treated MC3T3-E1, which increased the expression of osteopontin by inhibiting acetylation of histones H3 and H4 in its promoter to participate in Rh2-mediated proliferation. Huang et al[26] showed that the silencing of H19 reduced the expression of osteogenesis-related genes in hASCs, including ALPL and Runx2.[27] Li et al[19] applied RNA-Seq to investigate the functions of H19 in disuse osteoporosis. Among 464 differentially expressed lncRNAs, H19 decreased with a fold-change of 2.86 in response to mechanical unloading, with related genes that were mainly involved in the Wnt signalling pathway. Knockdown of H19 upregulated Dkk4, which suppressed Wnt signalling and inhibited osteogenesis in UMR106 cells. These findings partially demonstrate the pivotal roles of H19 in osteoporosis of hindlimb-unloaded rats (Fig. 2b).[19]

Liao et al[66] showed the dynamic changes in H19 during BMP9-induced osteogenesis of mouse MSCs, in which H19 was sharply upregulated during the early stage, followed by a rapid decrease and gradual return to basal levels, accompanied by expression changes of osteogenic markers. However, the well-coordinated biphasic expression of H19 may be critical in osteogenic differentiation, because either overexpression or silencing of H19 impaired osteogenesis by dysregulating Notch signalling-targeting miRNAs (Fig. 2c).[66] Liang et al[22] found that H19 activated the Wnt/β-catenin pathway by sponging miR-141 and miR-22, resulting in the potentiated expression of β-catenin and other osteogenic markers to promote osteogenesis in hBMSCs (Fig. 2b). Zhao et al[23] demonstrated that the mutation of DLX3 interferes with bone formation partially through the H19/miR-675/NOMO1 axis in tricho-dento-osseous (TDO) syndrome. Finally, Wu et al[14] showed that mechanical tension (10%, 0.5 Hz) could enhance osteogenic differentiation by increasing H19 expression in hBMSCs, which sponged miR-138, and increasing its target FAK (Fig. 2a).

Differentiation antagonizing non-protein coding RNA (DANCR), previously called anti-differentiation non-coding RNA (ANCR), is a novel lncRNA downregulated during stem cell differentiation that maintains epidermal stem cells or osteoblast cells in an undifferentiated state.[28] The upregulation of DANCR has been confirmed during osteogenesis in many cell types, including hFOB1.19 bone cells,[29] hPDLSCs,[30] human dental stem pulp cells (hDPSCs),[20] human stem cells from the apical papilla (hSCAP)[31] and, hBMSCs.[32] For example, Zhu et al[29] found that the downregulation of DANCR promoted osteoblast differentiation in hFOB1.19, whereas DANCR overexpression inhibited this process. In particular, DANCR suppressed the expression of Runx2 by physically interacting with EZH2 in Runx2 gene promoters, subsequently blocking osteoblast differentiation. Meanwhile, Jia et al[30] showed that DANCR suppressed proliferation of hPDLSCs, which is promising for the use of dental tissue-derived stem cells (DTSCs) in tissue engineering. Downregulation of DANCR enhanced the osteogenic potential of hPDLSCs by promoting the Wnt signalling pathway and upregulating osteogenic markers. They also investigated the regulatory effects of DANCR on the proliferation and differentiation of

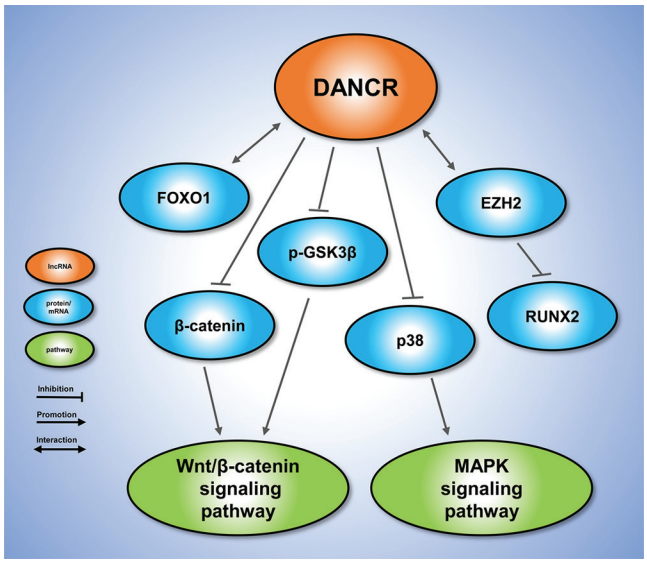

Fig. 3

The molecular mechanisms of MEG3 in osteogenesis.

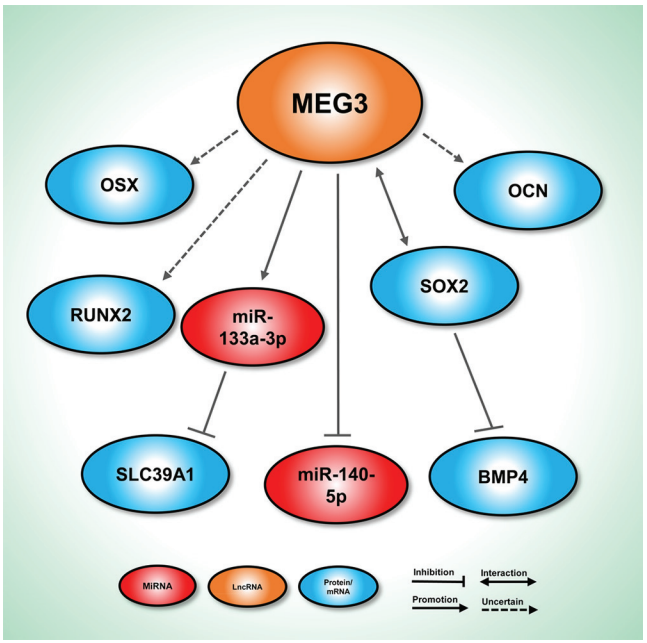

Fig. 4

The molecular mechanisms of DANCR in osteogenesis. DANCR regulates the Wnt/β-catenin signalling and MAPK signalling pathways.

two other types of DTSCs, hDPSCs, and hSCAPs. Although few effects on the proliferation of hDPSC and hSCAP were observed, the downregulation of DANCR promoted the osteogenic, adipogenic, and neurogenic differentiation of DTSCs (including hPDLSCs), indicating that DANCR is an important regulator of stem cell differentiation.[31] In an integrated lncRNA profiling analysis of hDPSCs, a total of 139 lncRNAs were differentially expressed during hDPSC differentiation into odontoblast-like cells, among which DANCR was significantly downregulated in a time-dependent manner. The upregulation of DANCR significantly decreased the expression of p-GSK-3β and β-catenin to block mineralized nodule formation, suggesting that DANCR can suppress the Wnt/β-catenin pathway during the odontoblast-like differentiation of hDPSCs.[20] Wang et al[25] identified that DANCR was significantly decreased in the hBMSCs co-cultured with hAMSCs, and DANCR overexpression inhibited the enhanced osteogenic effect of hAMSCs on hBMSCs by suppressing Runx2 upregulation. In addition, Zhang et al[32] found that DANCR was abnormally decreased in hBMSCs during osteogenic differentiation, and significantly inhibited the proliferation and osteogenic differentiation of hBMSCs by suppressing p38 MAPK activation, rather than ERK1/2 or JNK MAPKs. Tang et al[15] detected the expression of DANCR in clinical samples and MSCs to investigate its functions in osteolysis following total hip arthroplasty. In periprosthetic tissues, DANCR was upregulated while FOXO1 was downregulated. Polymethyl methacrylate (PMMA), a common adhesive agent used in arthroplasty, inhibited MSC osteogenesis via the DANCR/FOXO1 pathway. In this mechanism, PMMA increases the expression of DANCR, which binds to FOXO1 and promotes Skp2-mediated ubiquitination of FOXO1 to decrease its expression. (Fig. 3)

MEG3 is an important regulator involved in human development and various diseases.[67-70] Accumulating evidence for the biological and clinical significance of MEG3 in osteogenesis has been highlighted in recent years. However, the role of MEG3 in osteogenesis remains controversial. Zhuang et al[33] identified lower MEG3 expression in hBMSCs isolated from patients with multiple myeloma than in those from normal donors during osteogenic differentiation. MEG3 exhibited a critical transcriptional regulatory function by dissociating SOX2 from the BMP4 promoter, thereby relieving the suppression effect of SOX2 on BMP4. In addition, MEG3 positively regulates other key osteogenic markers, including Runx2, osterix, and osteocalcin (OCN).[33] Similarly, Li et al[71] found that MEG3, which was downregulated during adipogenesis and upregulated during osteogenesis in hASCs, regulated the balance of adipogenesis and osteogenesis in hASCs by suppressing miR-140-5p. Nevertheless, Wang et al[17] found that both MEG3 and miR-133a-3p were increased in hBMSCs during postmenopausal osteoporosis. By contrast, the expression of MEG3 and miR-133a-3p was markedly decreased in the differentiation of hBMSCs into osteoblasts. MEG3 was confirmed to regulate positively miR-133a-3p which targets SLC39A1, thereby leading to the inhibition of osteogenesis in hBMSCs.[17] Overall, the role of MEG3 in osteogenesis appears to differ substantially depending on the cell type and conditions (Fig. 4).

The functional roles of HOTAIR in human cancers have been largely elucidated[35,72-74] and its biological functions in other diseases and physiological processes have been

partly clarified.[75-78] Carrion et al[21] reported that HOTAIR was mechanoresponsive to cyclic stretching in human aortic valve interstitial cells (hAVICs), and inhibited aortic valve calcification by elevating two osteogenic genes, ALPL and BMP2. Targeting HOTAIR, certain osteogenic genes such as BMP1, BMP4, BMP6, BMPR6, and COL1A1 were upregulated, and the differentially expressed genes were involved in ossification. In addition, Wei et al[18] found higher HOTAIR levels in samples of non-traumatic osteonecrosis of the femoral head compared with osteoarthritis, which was negatively related to miR-17-5p. Meanwhile, decreasing in BMP2-induced osteoblastic differentiation, HOTAIR reduced the expression of osteogenic markers, including Runx2, COL1A1, and ALP by inhibiting miR-17-5p and promoting its target Smad7 in hBMSCs.[18] These findings indicate its critical roles in osteogenic differentiation.

Several studies have examined the roles of MALAT1 in osteogenesis. Che et al[35] first reported that MALAT1 regulated OPG expression in hFOB1.19 bone cells, although the relationship between MALAT1 and osteoblast differentiation was unclear. Subsequently, Xiao et al[16] observed an elevated expression of MALAT1 in calcific valves and hAVICs during osteogenesis, where MALAT1 acted as a ceRNA to elevate Smad4 by sponging miR-204, thereby increasing the expression of osteoblast-specific markers, such as ALP and OCN, and promoting bone matrix formation in hAVICs.

Looking at others, Gao et al[36] screened an upregulated lncRNA, AK007000, from microarray analyses of MC3T3-E1, C2C12, and C3H10T1/2 induced by BMP2, which was subsequently shown to be positively related to osteogenic differentiation markers. Meanwhile, Zuo et al[37] found that AK089560 was decreased in both osteogenic and adipogenic differentiation of C3H10T1/2 cells, which is transcribed from the intron of Sema3a and may regulate Sema3a expression to participate in the multidirectional differentiation of MSCs. In addition, Yin et al[38] found that AK138929, a novel osteogenic regulator, inhibited osteoblast differentiation by targeting miR-489-3p and promoting PTPN6 in MC3T3-E1. Moreover, Li et al[39] identified the lncRNA AK141205, which was upregulated in osteogenic growth peptide-induced osteogenesis, and promoted ALP activity, formation of calcium salt nodules, and osteogenic differentiation markers, suggesting its key regulatory role in osteogenesis. Further experiments indicated that AK141205 positively promoted CXCL13 expression via acetylation of histone H4 in its promoter region. Cao et al[40] found that AK028326 was decreased during high glucose-induced inhibition of osteogenic differentiation in hBMSCs, which was further confirmed to positively regulate CXCL13 expression. In addition, high glucose suppressed the osteogenic differentiation of hBMSCs via the reduction of CXCL13 expression mediated by AK028326.

Jin et al[41] showed that knockdown of MIR31HG significantly promoted osteogenic differentiation, completely antagonizing inflammation-induced osteogenic inhibition. The MIR31HG-NFκB regulatory loop suppressed osteogenic differentiation of hASCs, in which p65 promoted MIR31HG expression by binding to the MIR31HG promoter, and MIR31HG directly interacted with IκB-α, in turn participating in NFκB activation. They also investigated the roles of myocardial infarction-associated transcript (MIAT) in the osteogenic differentiation of hASCs. MIAT, which was downregulated during osteogenesis of hASCs, suppressed osteogenic differentiation both *in vitro* and *in vivo*.[8]

Among 2033 differentially expressed lncRNAs in staphylococcal protein A (SpA)-treated hBMSCs, NON HSAT009968 was upregulated 3.8-fold, and was subsequently confirmed to participate in SpA-induced osteogenic suppression in hBMSCs.[42] Meanwhile, POIR positively regulated osteogenic differentiation of hPDLSCs from patients with periodontitis (pPDLSCs) by acting as a ceRNA for miR-182, leading to de-repression of its target gene, FOXO1, which increased bone formation of pPDLSCs by competing with TCF-4 for β-catenin and inhibiting the canonical Wnt pathway.[43] Xi et al[44] found that HCG18 was upregulated in patients with degeneration of intervertebral discs and functioned as the miR-146a-5p sponge in nucleus pulposus cells, in which osteogenic differentiation was promoted via the miR-146a-5p/TARF6/NFκB axis. Zhu et al[45] found that although HOXA-AS3 remained unchanged during osteogenic induction, knockdown of HOXA-AS3 expression promoted osteogenesis and the expression of osteogenic markers, including COL1A1, Runx2, SP7, BGLAP, SPP1, and IBSP. HOXA-AS3 was confirmed to bind to EZH2 and regulate the expression of Runx2 via H3K27me3. Xu et al[46] reported a highly abundant lncRNA in MSCs from multiple myeloma, namely lncRUNX2-AS1, which could be packaged into exosomes and transferred to hBMSCs, inhibiting osteogenic differentiation by forming an RNA duplex with Runx2 and decreasing its stability. Weng et al[47] reported that a gradually upregulated lncRNA during osteogenic differentiation, namely MODR, acted as a ceRNA to sponge miR-454, resulting in elevated Runx2 expression and promoting osteogenesis of maxillary sinus membrane stem cells. Chen et al[48] revealed that HIF1A-AS2 had a negative effect on the osteogenic differentiation of periodontal ligament cells. Yu et al[24] found that TUG1 positively regulated Runx2 expression by sponging miR-204-5p, subsequently enhancing osteogenic differentiation in calcific aortic valve disease. Finally, TCONS_00041960, identified as a downregulated lncRNA in the microarray analysis of rat glucocorticoid-treated BMSCs, promoted the expression of the osteogenic genes Runx2 and GILZ by sponging miR-204-5p and miR-125a-3p, leading to enhanced osteogenesis.[49]

In conclusion, osteogenesis is related to various biological or pathological processes, leading to growth, development,

and disease. Although many studies have partially revealed the regulatory mechanisms of osteogenesis and explored the treatments of osteogenesis-related disease,[79,80] it is still unsatisfactory. An increasing number of studies have confirmed the differential expression of lncRNAs during osteogenesis and revealed their roles and molecular mechanisms *in vivo* and *in vitro*. Most have focused on the crosstalk between lncRNAs and miRNAs, providing insights into non-coding RNA coordination to regulate osteogenesis. Some studies have proposed other mechanisms, such as physical binding to transcription factors and decaying target mRNA. Nevertheless, the detailed regulatory mechanisms of lncRNAs remain unclear. In particular, the mechanism by which ceRNA sponges miRNA and modulates other osteogenesis-related genes is essential, but incomplete, for lncRNAs. Moreover, few studies have focused on the subcellular localization of lncRNAs, which is indispensable for establishing the ceRNA regulation mode. For example, if a lncRNA was mainly located in the nucleus, the ceRNA would not be in the "critical" regulation mode, as proposed by some researchers. RNA-binding protein, as its name implies, should show promise to demonstrate the mechanisms of lncRNAs in osteogenic differentiation. Given that alternative splicing is remarkable in terms of differentiation and development, future investigations should focus on the different functions of various alternative transcripts in osteogenesis.

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Author contributions

- J. Zhang: Wrote and approved the final manuscript.
- X. Hao: Study conception and literature search.
- M. Yin: Study conception and literature search.
- T. Xu: Wrote and approved the final manuscript.
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