Long non-coding RNA MEG3 is involved in osteogenic differentiation and bone diseases (Review)

HONG SUN[1*], GUOXUAN PENG[2*], HONGBIN WU[1], MIAO LIU[1], GUPING MAO[3], XU NING[1], HUA YANG[1] and JIN DENG[2]

Departments of [1]Orthopaedics and [2]Emergency Medicine, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou 550004; [3]Department of Joint Surgery, Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong 510080, P.R. China

Received October 2, 2019; Accepted March 13, 2020

DOI: 10.3892/br.2020.1305

Abstract. Osteogenic differentiation originating from mesenchymal stem cells (MSCs) requires tight co-ordination of transcriptional factors, signaling pathways and biomechanical cues. Dysregulation of such reciprocal networks may influence the proliferation and apoptosis of MSCs and osteoblasts, thereby impairing bone metabolism and homeostasis. An increasing number of studies have shown that long non-coding (lnc)RNAs are involved in osteogenic differentiation and thus serve an important role in the initiation, development, and progression of bone diseases such as tumors, osteoarthritis and osteoporosis. It has been reported that the lncRNA, maternally expressed gene 3 (MEG3), regulates osteogenic differentiation of multiple MSCs and also acts as a critical mediator in the development of bone formation and associated diseases. In the present review, the proposed mechanisms underlying the roles of MEG3 in osteogenic differentiation and its potential effects on bone diseases are discussed. These discussions may help elucidate the roles of MEG3 in osteogenic differentiation and highlight potential biomarkers and therapeutic targets for the treatment of bone diseases.

Correspondence to: Professor Hua Yang, Department of Orthopaedics, Affiliated Hospital of Guizhou Medical University, 28 Guiyi Road, Guiyang, Guizhou 550004, P.R. China
E-mail: yanghua0203@gmc.edu.cn

Professor Jin Deng, Department of Emergency Medicine, Affiliated Hospital of Guizhou Medical University, 28 Guiyi Road, Guiyang, Guizhou 550004, P.R. China
E-mail: 1049218742@qq.com

*Contributed equally

Key words: long non-coding RNAs, maternally expressed gene 3, osteogenic differentiation, bone diseases

Contents

1. Introduction

Long non-coding (lnc)RNAs are RNA transcripts >200 nucleotides in length, which have low or no protein-coding potential (1). LncRNAs serve an important role in numerous biological processes including cell proliferation, differentiation and migration (2). LncRNAs regulate these processes through histone modifications, chromatin methylation, binding of transcription factors and other post-transcriptional mechanisms (3). Therefore, lncRNAs are involved in the development of multiple diseases, including bone diseases.

LncRNA maternally expressed gene 3 (MEG3), encoded by the MEG3 gene, is ~1,600 nucleotides in length (4). MEG3 gene belongs to the imprinted DLK-MEG3 locus that is located at chromosome 14q32.3 in humans. Multiple factors are involved in the regulation of MEG3 gene expression including the DNA methyltransferase family (5), cyclic adenosine monophosphate (6) and nuclear factor-κB (NF-κB) (7). MEG3 expression is downregulated in several primary human tumors and tumor cell lines, and it may function as a novel tumor suppressor (4,8,9). An increasing number of studies have shown that MEG3 serves an important role in the development of osteogenic differentiation and thus regulates bone formation, metabolism, and regeneration (10,11). Additionally, dysregulation of MEG3 is associated with various bone diseases including bone tumors, joint degeneration, osteoporosis and rheumatoid arthritis (RA) (12-15). To the best of our knowledge, there are no literature reviews summarizing the specific roles of MEG3 in the development of osteogenic differentiation and bone diseases. In this review, the relevant literature was systematically searched, and the mechanisms underlying the roles of MEG3 in osteogenic differentiation and its potential effects in bone diseases are discussed.

2. Roles of lncRNA MEG3 in osteogenic differentiation

Multiple expression profiles showing dysregulated expression of several lncRNAs in patients with osteogenesis have been identified by microarray analyses (4,16). Aberrantly expressed lncRNAs may exhibit varying effects on proliferation and apoptosis of mesenchymal stem cells (MSCs) and osteoblasts. MEG3 expression is associated with the expression of key osteogenic markers such as Runt-related transcription factor 2 (RUNX2), Osterix (Osx) and osteocalcin (OCN) (10). MEG3 expression is dysregulated and acts as a crucial regulator of several processes through transcriptional and post-transcriptional regulatory mechanisms, as shown in Table I.

Transcriptional regulation. LncRNAs interact with the promoters of multiple genes and regulate the binding of transcription factors to the promoter region, thereby regulating the expression of specific genes (17). Bone morphogenic protein 4 (BMP4) is a crucial regulator that promotes osteogenic differentiation (18). MEG3, which is located near the BMP4 gene, can dissociate the transcription factor SOX2 from the BMP4 promoter (17). In 2015, Zhuang *et al* (10) showed that MEG3 upregulation activates transcriptional activity of BMP4 by directly inhibiting SOX2 activity, thereby stimulating the osteogenic differentiation of MSCs.

In addition, MEG3 mediated histone methylation is also involved in transcriptional regulation of osteogenic differentiation. It has been reported that MEG3 endogenously binds to enhancer of zeste homologue 2 (EZH2), which is responsible for the activation of H3K27 trimethylation. Moreover, EZH2 can directly inhibit the expression of Wnt genes such as β-catenin and Wnt ligand expression through H3K27 trimethylation. Elevated levels of MEG3 increases EZH2 expression, and attenuates osteogenic differentiation by inhibiting the Wnt/β-catenin signaling pathway in human dental follicle stem cells (19).

Post-transcriptional regulation. Numerous studies have suggested that lncRNAs can modulate gene expression at multiple levels due to the diversity of their regulatory mechanisms (20,21). MEG3 has been reported to regulate osteogenic gene expression during post-transcriptional mRNA modification by acting as competing endogenous RNA (ceRNA) (13,14).

BMP2 is an important molecule responsible for regulation of osteogenesis (18). Modifications of BMP2 mRNA are associated with heterogeneous nuclear ribonucleoprotein I (hnRNPI), and the downregulation of hnRNPI can in turn decrease BMP2 protein expression levels (11). It has been shown that MEG3 can compete with BMP2 mRNA for interaction with hnRNPI. Therefore, MEG3 overexpression suppresses BMP2 levels and attenuates osteogenic differentiation in periodontal ligament cells (11).

MEG3 can regulate osteogenic differentiation through ceRNA activity. miRNAs are negative regulators of gene expression which can hybridize to target mRNAs and thereby regulate the translation or stability of those mRNAs (22). Previous studies have increasingly focused on the crosstalk between miRNAs and lncRNAs in numerous biological processes (23,24). LncRNAs may regulate gene expression by sponging miRNAs, reducing the levels of available miRNAs within the cell, thereby acting as a negative regulator of miRNA function (25). MEG3 expression is upregulated during the osteogenesis of human adipose-derived stem cells (hASCs), and further functional study suggested that MEG3 is negatively correlated with miR-140-5p; thus, stimulating hASCs osteogenesis (26).

3. Roles of lncRNA MEG3 in bone diseases

Numerous studies have confirmed that lncRNAs participate in the initiation and progression of several types of bone diseases including bone tumors (27), osteoarthritis (OA) (28,29), osteoporosis (30,31), RA (32) and ankylosing spondylitis (AS) (33). MEG3 has been shown to be involved in the development of the all these bone diseases, as shown in Table II.

MEG3 and bone tumors. Osteosarcoma (OS) is the most common malignant bone tumor with an incidence rate of 4.4 cases per million individuals, occurring primarily in children and adolescents (34). Despite advances in the available treatment strategies, OS still leads to unfavorable clinical outcomes and high rates of disability in patients (35). Therefore, understanding the mechanisms underlying OS and exploring novel therapeutic targets is of great clinical significance. It has been shown that lncRNAs are aberrantly expressed in OS tissues and dysregulated expression is associated with clinical outcomes and disease status (36,37). Studies have shown that lncRNAs which exhibit dysregulated expression may serve as prognostic biomarkers and therapeutic targets for patients with OS (12,27).

MEG3 is considered a vital regulator of a range of biological processes and a tumor suppressor in several types of cancer, particularly in OS (4,38). Various studies have suggested that MEG3 expression is significantly reduced in OS cells and tissues (39-41). MEG3 upregulation significantly reduces proliferation and invasion, as well as increasing apoptosis of OS cells (39,40). Furthermore, the Notch and transforming growth factor (TGF)-β signaling pathways participate in MEG3-induced inhibition of OS cell proliferation and metastasis (40). Decreased expression of MEG3 induced by lncRNA EWSAT1 facilitates OS cell growth and metastasis (42). Downregulated expression of MEG3 is associated with advanced clinical stage cancer, distant metastasis and poor overall survival (41).

An important mechanism underlying the pathogenesis of OS is that MEG3 acts as a ceRNA by sponging miRNAs (12). Sahin *et al* (43) showed that MEG3 attenuates proliferation and migration of OS cells through directly interacting with miR-664a. In addition, MEG3 can directly bind to miR-361-5p and increase its expression, downregulating the expression of FoxM1 (44). In this case, MEG3 abrogates development of OS via a miR-361-5p/FoxM1 axis instead of acting as a ceRNA. Conversely, Wang and Kong (45) demonstrated that MEG3 interacts with miR-127 and forms a network to regulate ZEB1. MEG3 overexpression increased growth and metastasis of OS cells, and prevented their apoptosis by upregulating ZEB1-induced activation of the JNK and Wnt signaling pathways (45). In general, the majority of studies demonstrated that that MEG3 serves as a tumor suppressor in the pathogenesis of OS.

Multiple myeloma (MM) is also one of the most frequent tumors to involve bones; it is characterized by the aberrant proliferation and accumulation of plasma cells in the bone

Table I. Roles of MEG3 in osteogenic differentiation.

Author, year	Study model	Change in expression	Target(s)	Cellular process affected (increased/decreased)	(Refs.)
Zhuang *et al*, 2015	hBMSCs	Downregulated	SOX2/BMP4	Osteogenic differentiation (increased)	(10)
Liu *et al*, 2019	hPDLCs	Downregulated	hnRNPI/BMP2	Cell viability (decreased)/Apoptosis (increased)	(11)
Wang *et al*, 2017	hBMSCs	Downregulated	miR-133a-3p/ SLC39A1	Osteogenic differentiation (decreased)	(14)
Deng *et al*, 2018	hDFSCs	Downregulated	EZH2/H3K27me	Osteogenic differentiation (decreased)	(19)
Li *et al*, 2017	hASCs	Upregulated	miR-140-5p	Adipogenesis (decreased)/Osteogenesis (increased)	(26)

MEG3, maternally expressed gene; hBMSC, bone marrow mesenchymal stem cell; hPDLC, human periodontal ligament cell, human; hDFSC, human dental follicle stem cell; hASC, human adipose-derived stem cell; BMP, bone morphogenic protein; hnRNPI, heterogeneous nuclear ribonucleoprotein I; miR, microRNA; EZH2, enhancer of zeste homologue 2.

marrow (46). Bone lesions caused by a severe imbalance in bone remodeling are an important feature of MM (47). Promoter hypermethylation of the MEG3 gene has been observed in bone marrow samples obtained from patients with MM (48). MEG3 expression is significantly downregulated in both human MM cells and MSCs from patients with MM (10,49). Shen *et al* (49) demonstrated that MEG3 functions as a ceRNA and directly binds to miR-181a, preventing the pathogenesis of MM by attenuating the inhibition of HOXA11 through miR-181a. Furthermore, elevated MEG3 expression facilitated osteogenic differentiation in bone marrow MSCs from patients with MM by increasing transcription of BMP4 (10). These findings highlight the potential of MEG3 as a promising therapeutic target for MM.

MEG3 and OA. OA is characterized by degradation of articular cartilage (50). Results from several expression profiling studies have suggested an association between several lncRNAs and degraded articular cartilage (28,51,52). MEG3 is downregulated in human OA cartilage compared with normal articular cartilage (53). OA is closely associated with angiogenesis, and inhibition of angiogenesis assists in alleviating inflammation and pain in OA (54). Su *et al* (53) reported that angiogenesis is increased in OA, but inversely associated with MEG3 levels, suggesting that MEG3 may prevent development of OA through the regulation of angiogenesis. MEG3 expression is also downregulated in IL-1β induced chondrocytes (13). Chen *et al* (13) showed that MEG3 interacts with miR-93 and forms a ceRNA network to regulate TGF-β receptor 2 (TGFBR2). Overexpression of MEG3 decreased the expression of miR-93, and thus decreased apoptosis and degradation of chondrocytes by increasing TGFBR2 induced activation of the TGF-β signaling pathway (13). Furthermore, MEG3 serves as a protective regulator by directly binding to miR-203, antagonizing the inhibition of sirt1, and attenuating LPS-induced inflammatory responses by activating the PI3K/AKT and NF-κB signaling pathways in ADTC-5 chondrocytes (55).

It has been previously reported that MEG3 is downregulated and its target, miR-16, is upregulated in rat OA cartilage tissues (56). MEG3 increases the expression of SMAD7, by sponging miR-16. MEG3 also reduced proliferation and increased apoptosis of inflammatory chondrocytes, thereby inhibiting the progression of OA (56). Methylene blue is an anti-oxidative and anti-inflammatory agent which may regulate inflammatory response and relieve clinical pain syndromes in patients with OA (57). Li *et al* (58) investigated the molecular mechanism of methylene blue in pain and inflammation relief in an OA rabbit model. Results showed that MEG3 is significantly increased in methylene blue treated knee joints of rabbits and alleviates OA-associated pain and inflammation by suppressing P2X3 expression (58). Hence, these findings from *in vivo* and *in vitro* studies suggest MEG3 is an inhibitor of the progression of OA.

MEG3 and osteoporosis. Recently, lncRNAs have emerged as novel regulatory markers of osteoporosis (30,31). Osteoporosis is a systemic skeletal disorder characterized by low bone mass, and is associated with an increase in the risk of bone fracture (59). Postmenopausal osteoporosis (PMOP) is a type of metabolic bone disease caused by decreased levels of estrogen in the body (60). The major mechanism underlying PMOP involves an imbalance between bone resorption by osteoclasts and bone formation by osteoblasts (61). RNA sequencing results have shown there are 51 PMOP-associated differentially expressed lncRNAs in the peripheral whole blood of PMOP patients compared with healthy individuals, which may serve as novel biomarkers of PMOP (62).

Bone marrow mesenchymal stem cells (BMSCs) are responsible for bone remodeling, and in particular, in maintaining the balance between bone formation and resorption. The expression levels of MEG3 and miR-133a-3p are increased in BMSCs extracted from patients with PMOP (14). In contrast, the expression levels of MEG3 and miR-133a-3p are significantly decreased when BMSCs differentiate into osteoblasts (14). MEG3 overexpression significantly increases miR-133a-3p expression by direct binding, and thus decreases mineralized nodule formation, ALP activity and the expression of osteogenic markers, including SLC39A1, RUNX2, OCN and OPN (14). Several

Table II. Changes in expression and the target genes of MEG3 in bone diseases.

A, Osteosarcoma

Author, year	Change in MEG3 expression	Target	(Refs.)
Sahin *et al*, 2017	Downregulated	miR-664a	(43)
Shen *et al*, 2019	Upregulated	miR-361-5p	(44)
Wang and Kong, 2018	Downregulated	miR-127	(45)

B, Osteoarthritis

Author, year	Change in MEG3 expression	Target	(Refs.)
Xu and Xu, 2017	Downregulated	miR-16	(56)
Chen *et al*, 2019	Downregulated	miR-93	(13)
Wang *et al*, 2018	Downregulated	miR-203	(55)
Li *et al*, 2018	Downregulated	P2X3	(58)

C, Multiple myeloma

Author, year	Change in MEG3 expression	Target	(Refs.)
Shen *et al*, 2018	Downregulated	miR-181a	(49)
Zhuang *et al*, 2015	Downregulated	BMP4	(10)

D, Osteoporosis

Author, year	Change in MEG3 expression	Target	(Refs.)
Wang *et al*, 2017	Upregulated	miR-133a-3p	(14)
Chen *et al*, 2018	Downregulated	BMP4	(63)

E, Rheumatoid arthritis

Author, year	Change in MEG3 expression	Target	(Refs.)
Liu *et al*, 2019	Downregulated	NLRC5	(15)
Lu and Qian, 2019	Downregulated	STAT3	(68)

F, Ankylosing spondylitis

Author, year	Change in MEG3 expression	Target	(Refs.)
Liu *et al*, 2019	Downregulated	-	(66)

miR, microRNA; MEG3, maternally expressed gene.

studies have confirmed that MEG3-induced activation of BMP4 signaling pathway is associated with osteogenic differentiation of BMSCs obtained from patients with MM or osteoporosis (10,63). Chen *et al* (63) showed that DEP domain containing DEPTOR results in the progression of osteoporosis. Further analysis showed that DEPTOR upregulation impaired osteogenic differentiation by inhibiting MEG3-mediated activation of the BMP4 signaling pathway (14). Thus, MEG3 may increase osteogenic differentiation of BMSCs in osteoporosis through activation of the BMP4 signaling pathway. These two studies have revealed the contradictory roles of MEG3 in osteogenic differentiation of BMSCs in osteoporosis (14,63). The exact functions of MEG3 in osteoporosis should be further evaluated.

MEG3 and other bone diseases. AS and RA are classified as autoimmune-type arthritis and chronic inflammatory

diseases (64,65). Both are characterized by long-term inflammation of connective tissues. The serum levels of MEG3 are significantly decreased in patients with AS compared with healthy subjects (66). Furthermore, patients with upregulated expression levels of MEG3 had shorter hospitalization times and a lower re-admission rate within 2 years after discharge. NOD-like receptors 5 (NLRC5) is closely associated with inflammatory and autoimmune diseases (67). NLRC5 expression is upregulated and MEG3 expression is downregulated in the synovial tissues of Complete Freund's Adjuvant-induced RA rats. Further analysis demonstrated that NLRC5 is responsible for hypermethylation of the MEG3 gene promoter and prevents MEG3 gene transcription. Increased expression of MEG3 reduced the levels of NLRC5 and inflammatory cytokines, thereby inhibiting the development of RA (15). Lu and Qian (68) showed that MEG3 expression is downregulated in the fibroblast-like synoviocytes of patients with RA. Further analysis demonstrated that MEG3 can reduce proliferation and invasion, and promote apoptosis of fibroblast-like synoviocytes via the STAT3 signaling pathway (68). These findings suggest that MEG3 may act as a negative regulator in progression of AS and RA.

In addition, MEG3 is crucial to tibia-fraction healing (69). It has been found that MEG3 expression is increased in non-union fracture bone samples compared with healing fracture samples. Knockdown of MEG3 expression increases the levels of Col2a1, RUNX2, Osx and OCN, and callus growth. Furthermore, silencing MEG3 expression accelerates fracture construction, faster fracture healing and functional recovery by activating the Wnt/β-catenin signaling pathway (69).

4. Conclusion

Numerous studies have shown that lncRNAs are novel regulators involved in the expression of multiple genes. In the present review, the role of MEG3 in the osteogenic differentiation of MSCs was discussed. It is reported that lncRNA MEG3 primarily modulates osteogenic differentiation at the transcriptional and post-transcriptional levels. ceRNA mechanisms serve critical roles in MEG3-regulated osteogenic differentiation. Moreover, lncRNA MEG3 is also closely associated with several types of bone diseases including bone tumors, OA, osteoporosis, RA and AS. MEG3 shows negative regulatory effects in the development of these bone diseases. These findings highlight a novel target for diagnosis or treatment of such bone diseases. However, only relatively few studies have investigated the role of MEG3 in bone disease. The underlying mechanisms of MEG3 in bone disease should thus be further investigated.

Acknowledgements

We would like to thank Professor Chuan Ye and Professor Xianwen Shang at the Affiliated Hospital of Guizhou Medical University (Guiyang, Guizhou, China) for their assistance in writing the manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (grant nos. 81772403 and 81660317).

Availability of data and materials

Not applicable.

Authors' contributions

The subject of this review was conceived by HS and HY. HS and GP prepared the original draft. The literature search was performed by HW and ML. Data curation and methodology were accomplished by GM and XN. JD finished the formal analysis and provided the funding. JD and HY reviewed and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kopp F and Mendell JT: Functional classification and experimental dissection of long noncoding RNAs. Cell 172: 393-407, 2018.
2. Ponting CP, Oliver PL and Reik W: Evolution and functions of long noncoding RNAs. Cell 136: 629-641, 2009.
3. Schmitz SU, Grote P and Herrmann BG: Mechanisms of long noncoding RNA function in development and disease. Cell Mol Life Sci 73: 2491-2509, 2016.
4. Zhou Y, Zhang X and Klibanski A: MEG3 noncoding RNA: A tumor suppressor. J Mol Endocrinol 48: R45-R53, 2012.
5. Yan J, Guo X, Xia J, Shan T, Gu C, Liang Z, Zhao W and Jin S: MiR-148a regulates MEG3 in gastric cancer by targeting DNA methyltransferase 1. Med Oncol 31: 879, 2014.
6. Zhao J, Zhang X, Zhou Y, Ansell PJ and Klibanski A: Cyclic AMP stimulates MEG3 gene expression in cells through a cAMP-response element (CRE) in the MEG3 proximal promoter region. Int J Biochem Cell Biol 38: 1808-1820, 2006.
7. He C, Yang W, Yang J, Ding J, Li S, Wu H, Zhou F, Jiang Y, Teng L and Yang J: Long noncoding RNA MEG3 negatively regulates proliferation and angiogenesis in vascular endothelial cells. DNA Cell Biol 36: 475-481, 2017.
8. Chunharojrith P, Nakayama Y, Jiang X, Kery RE, Ma J, De La Hoz Ulloa CS, Zhang X, Zhou Y and Klibanski A: Tumor suppression by MEG3 lncRNA in a human pituitary tumor derived cell line. Mol Cell Endocrinol 416: 27-35, 2015.
9. Zhang X, Zhou Y, Mehta KR, Danila DC, Scolavino S, Johnson SR and Klibanski A: A pituitary-derived MEG3 isoform functions as a growth suppressor in tumor cells. J Clin Endocrinol Metab 88: 5119-5126, 2003.
10. Zhuang W, Ge X, Yang S, Huang M, Zhuang W, Chen P, Zhang X, Fu J, Qu J and Li B: Upregulation of lncRNA MEG3 promotes osteogenic differentiation of mesenchymal stem cells from multiple myeloma patients by targeting BMP4 transcription. Stem Cells 33: 1985-1997, 2015.
11. Liu Y, Zeng X, Miao J, Liu C, Wei F, Liu D, Zheng Z, Ting K, Wang C and Guo J: Upregulation of long noncoding RNA MEG3 inhibits the osteogenic differentiation of periodontal ligament cells. J Cell Physiol 234: 4617-4626, 2019.
12. Jiang M, Wang YR, Xu N, Zhou L and An Q: Long noncoding RNA MEG3 play an important role in osteosarcoma development through sponging microRNAs. J Cell Biochem 120: 5151-5159, 2019.

13. Chen K, Zhu H, Zheng MQ and Dong QR: LncRNA MEG3 inhibits the degradation of the extracellular matrix of chondrocytes in osteoarthritis via targeting miR-93/TGFBR2 axis. Cartilage: Jun 28, 2019 (Epub ahead of print).
14. Wang Q, Li Y, Zhang Y, Ma L, Lin L, Meng J, Jiang L, Wang L, Zhou P and Zhang Y: LncRNA MEG3 inhibited osteogenic differentiation of bone marrow mesenchymal stem cells from postmenopausal osteoporosis by targeting miR-133a-3p. Biomed Pharmacother 89: 1178-1186, 2017.
15. Liu YR, Yang L, Xu QQ, Lu XY, Ma TT, Huang C and Li J: Long noncoding RNA MEG3 regulates rheumatoid arthritis by targeting NLRC5. J Cell Physiol 234: 14270-14284, 2019.
16. Huang G, Kang Y, Huang Z, Zhang Z, Meng F, Chen W, Fu M, Liao W and Zhang Z: Identification and characterization of long non-coding RNAs in osteogenic differentiation of human adipose-derived stem cells. Cell Physiol Biochem 42: 1037-1050, 2017.
17. Long Y, Wang X, Youmans DT and Cech TR: How do lncRNAs regulate transcription? Sci Adv 3: eaao2110, 2017.
18. Zhang X, Guo J, Zhou Y and Wu G: The roles of bone morphogenetic proteins and their signaling in the osteogenesis of adipose-derived stem cells. Tissue Eng Part B Rev 20: 84-92, 2014.
19. Deng L, Hong H, Zhang X, Chen D, Chen Z, Ling J and Wu L: Down-regulated lncRNA MEG3 promotes osteogenic differentiation of human dental follicle stem cells by epigenetically regulating Wnt pathway. Biochem Biophys Res Commun 503: 2061-2067, 2018.
20. Stephens KE, Miaskowski CA, Levine JD, Pullinger CR and Aouizerat BE: Epigenetic regulation and measurement of epigenetic changes. Biol Res Nurs 15: 373-381, 2013.
21. Dykes IM and Emanueli C: Transcriptional and post-transcriptional gene regulation by long non-coding RNA. Genomics Proteomics Bioinformatics 15: 177-186, 2017.
22. Filipowicz W, Bhattacharyya SN and Sonenberg N: Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? Nat Rev Genet 9: 102-114, 2008.
23. Xue M, Zhuo Y and Shan B: MicroRNAs, long noncoding RNAs, and their functions in human disease. Methods Mol Biol 1617: 1-25, 2017.
24. Yoon JH, Abdelmohsen K and Gorospe M: Functional interactions among microRNAs and long noncoding RNAs. Semin Cell Dev Biol 34: 9-14, 2014.
25. Paraskevopoulou MD and Hatzigeorgiou AG: Analyzing miRNA-lncRNA interactions. Methods Mol Biol 1402: 271-286, 2016.
26. Li Z, Jin C, Chen S, Zheng Y, Huang Y, Jia L, Ge W and Zhou Y: Long non-coding RNA MEG3 inhibits adipogenesis and promotes osteogenesis of human adipose-derived mesenchymal stem cells via miR-140-5p. Mol Cell Biochem 433: 51-60, 2017.
27. Yang Z, Li X, Yang Y, He Z, Qu X and Zhang Y: Long noncoding RNAs in the progression, metastasis, and prognosis of osteosarcoma. Cell Death Dis 7: e2389, 2016.
28. Fu M, Huang G, Zhang Z, Liu J, Zhang Z, Huang Z, Yu B and Meng F: Expression profile of long noncoding RNAs in cartilage from knee osteoarthritis patients. Osteoarthritis Cartilage 23: 423-432, 2015.
29. Sun H, Peng G, Ning X, Wang J, Yang H and Deng J: Emerging roles of long noncoding RNA in chondrogenesis, osteogenesis, and osteoarthritis. Am J Transl Res 11: 16-30, 2019.
30. Silva AM, Moura SR, Teixeira JH, Barbosa MA, Santos SG and Almeida MI: Long noncoding RNAs: A missing link in osteoporosis. Bone Res 7: 10, 2019.
31. Wu QY, Li X, Miao ZN, Ye JX, Wang B, Zhang F, Xu RS, Jiang DL, Zhao MD and Yuan FL: Long non-coding RNAs: A new regulatory code for osteoporosis. Front Endocrinol (Lausanne) 9: 587, 2018.
32. Li Z, Li X, Jiang C, Qian W, Tse G, Chan MTV and Wu WKK: Long non-coding RNAs in rheumatoid arthritis. Cell Prolif 51, 2018.
33. Xu Z, Zhou X, Li H, Chen Q and Chen G: Identification of the key genes and long non-coding RNAs in ankylosing spondylitis using RNA sequencing. Int J Mol Med 43: 1179-1192, 2019.
34. Geller DS and Gorlick R: Osteosarcoma: A review of diagnosis, management, and treatment strategies. Clin Adv Hematol Oncol 8: 705-718, 2010.
35. Mirabello L, Troisi RJ and Savage SA: Osteosarcoma incidence and survival rates from 1973 to 2004: Data from the surveillance, epidemiology, and end results program. Cancer 115: 1531-1543, 2009.
36. Ren K, Ni Y, Li X, Wang C, Chang Q, Li Y, Gao Z, Wu S, Shi X, Song J, *et al*: Expression profiling of long noncoding RNAs associated with vasculogenic mimicry in osteosarcoma. J Cell Biochem 120: 12473-12488, 2019.
37. Zhang S, Ding L, Li X and Fan H: Identification of biomarkers associated with the recurrence of osteosarcoma using ceRNA regulatory network analysis. Int J Mol Med 43: 1723-1733, 2019.
38. Al-Rugeebah A, Alanazi M and Parine NR: MEG3: An oncogenic long non-coding RNA in different cancers. Pathol Oncol Res 25: 859-874, 2019.
39. Shi Y, Lv C, Shi L and Tu G: MEG3 inhibits proliferation and invasion and promotes apoptosis of human osteosarcoma cells. Oncol Lett 15: 1917-1923, 2018.
40. Zhang SZ, Cai L and Li B: MEG3 long non-coding RNA prevents cell growth and metastasis of osteosarcoma. Bratisl Lek Listy 118: 632-636, 2017.
41. Tian ZZ, Guo XJ, Zhao YM and Fang Y: Decreased expression of long non-coding RNA MEG3 acts as a potential predictor biomarker in progression and poor prognosis of osteosarcoma. Int J Clin Exp Pathol 8: 15138-15142, 2015.
42. Sun L, Yang C, Xu J, Feng Y, Wang L and Cui T: Long noncoding RNA EWSAT1 promotes osteosarcoma cell growth and metastasis through suppression of MEG3 Expression. DNA Cell Biol 35: 812-818, 2016.
43. Sahin Y, Altan Z, Arman K, Bozgeyik E, Koruk OM and Arslan A: Inhibition of miR-664a interferes with the migration of osteosarcoma cells via modulation of MEG3. Biochem Biophys Res Commun 490: 1100-1105, 2017.
44. Shen B, Zhou N, Hu T, Zhao W, Wu D and Wang S: LncRNA MEG3 negatively modified osteosarcoma development through regulation of miR-361-5p and FoxM1. J Cell Physiol 234: 13464-13480, 2019.
45. Wang Y and Kong D: Knockdown of lncRNA MEG3 inhibits viability, migration, and invasion and promotes apoptosis by sponging miR-127 in osteosarcoma cell. J Cell Biochem 119: 669-679, 2018.
46. Marino S and Roodman GD: Multiple myeloma and bone: The fatal interaction. Cold Spring Harb Perspect Med 8: pii: a031286, 2018.
47. Terpos E, Politou M and Rahemtulla A: The role of markers of bone remodeling in multiple myeloma. Blood Rev 19: 125-142, 2005.
48. Benetatos L, Dasoula A, Hatzimichael E, Georgiou I, Syrrou M and Bourantas KL: Promoter hypermethylation of the MEG3 (DLK1/MEG3) imprinted gene in multiple myeloma. Clin Lymphoma Myeloma 8: 171-175, 2008.
49. Shen X, Bai H, Zhu H, Yan Q, Yang Y, Yu W, Shi Q, Wang J, Li J and Chen L: Long non-coding RNA MEG3 functions as a competing endogenous RNA to regulate HOXA11 expression by sponging miR-181a in multiple myeloma. Cell Physiol Biochem 49: 87-100, 2018.
50. Wang M, Shen J, Jin H, Im HJ, Sandy J and Chen D: Recent progress in understanding molecular mechanisms of cartilage degeneration during osteoarthritis. Ann N Y Acad Sci 1240: 61-69, 2011.
51. Pearson MJ, Philp AM, Heward JA, Roux BT, Walsh DA, Davis ET, Lindsay MA and Jones SW: Long intergenic noncoding RNAs mediate the human chondrocyte inflammatory response and are differentially expressed in osteoarthritis cartilage. Arthritis Rheumatol 68: 845-856, 2016.
52. Xing D, Liang JQ, Li Y, Lu J, Jia HB, Xu LY and Ma XL: Identification of long noncoding RNA associated with osteoarthritis in humans. Orthop Surg 6: 288-293, 2014.
53. Su W, Xie W, Shang Q and Su B: The long noncoding RNA MEG3 is downregulated and inversely associated with VEGF levels in osteoarthritis. Biomed Res Int 2015: 356893, 2015.
54. Ashraf S and Walsh DA: Angiogenesis in osteoarthritis. Curr Opin Rheumatol 20: 573-580, 2008.
55. Wang Z, Chi X, Liu L, Wang Y, Mei X, Yang Y and Jia T: Long noncoding RNA maternally expressed gene 3 knockdown alleviates lipopolysaccharide-induced inflammatory injury by up-regulation of miR-203 in ATDC5 cells. Biomed Pharmacother 100: 240-249, 2018.
56. Xu J and Xu Y: The lncRNA MEG3 downregulation leads to osteoarthritis progression via miR-16/SMAD7 axis. Cell Biosci 7: 69, 2017.
57. Zheng J and Li Q: Methylene blue regulates inflammatory response in osteoarthritis by noncoding long chain RNA CILinc02. J Cell Biochem 120: 3331-3338, 2019.

58. Li X, Tang C, Wang J, Guo P, Wang C, Wang Y, Zhang Z and Wu H: Methylene blue relieves the development of osteoarthritis by upregulating lncRNA MEG3. Exp Ther Med 15: 3856-3864, 2018.
59. Tu KN, Lie JD, Wan CKV, Cameron M, Austel AG, Nguyen JK, Van K and Hyun D: Osteoporosis: A review of treatment options. P T 43: 92-104, 2018.
60. Riggs BL: The mechanisms of estrogen regulation of bone resorption. J Clin Invest 106: 1203-1204, 2000.
61. Raisz LG: Pathogenesis of osteoporosis: Concepts, conflicts, and prospects. J Clin Invest 115: 3318-3325, 2005.
62. Fei Q, Bai X, Lin J, Meng H, Yang Y and Guo A: Identification of aberrantly expressed long non-coding RNAs in postmenopausal osteoporosis. Int J Mol Med 41: 3537-3550, 2018.
63. Chen S, Jia L, Zhang S, Zheng Y and Zhou Y: DEPTOR regulates osteogenic differentiation via inhibiting MEG3-mediated activation of BMP4 signaling and is involved in osteoporosis. Stem Cell Res Ther 9: 185, 2018.
64. Garcia-Montoya L, Gul H and Emery P: Recent advances in ankylosing spondylitis: Understanding the disease and management. F1000Res 7 (F1000 Faculty Rev): 1512, 2018.
65. Aletaha D and Smolen JS: Diagnosis and management of rheumatoid arthritis: A Review. JAMA 320: 1360-1372, 2018.
66. Liu W, Huang L, Zhang C and Liu Z: lncRNA MEG3 is downregulated in ankylosing spondylitis and associated with disease activity, hospitalization time and disease duration. Exp Ther Med 17: 291-297, 2019.
67. Liu YR, Yan X and Yu HX: NLRC5 promotes cell proliferation via regulating the NF-κB signaling pathway in Rheumatoid arthritis. Mol Immunol 91: 24-34, 2017.
68. Lu X and Qian J: Downregulated MEG3 participates in rheumatoid arthritis via promoting proliferation of fibroblast-like synoviocytes. Exp Ther Med 17: 1637-1642, 2019.
69. Liu YB, Lin LP, Zou R, Zhao QH and Lin FQ: Silencing long non-coding RNA MEG3 accelerates tibia fraction healing by regulating the Wnt/β-catenin signalling pathway. J Cell Mol Med 23: 3855-3866, 2019.