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 (Inc)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 IncRNA, 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.

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

HONG SUN1*, GUOXUAN PENG2*, HONGBIN WU1, MIAO LIU1, GUPING MAO3, XU NING1, HUA YANG1 and JIN DENG2

Departments of 1Orthopaedics and 2Emergency Medicine, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou 550004; 3Department 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

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
2. Roles of IncRNA MEG3 in osteogenic differentiation
3. Roles of IncRNA MEG3 in bone diseases
4. Conclusion

1. Introduction

Long non-coding (Inc)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 IncRNA MEG3 in osteogenic differentiation

Multiple expression profiles showing dysregulated expression of several IncRNAs in patients with osteogenesis have been identified by microarray analyses (4,16). Aberrantly expressed IncRNAs 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 Runx-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 overexpression 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 IncRNAs 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 inhibit 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 IncRNAs 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 IncRNA MEG3 in bone diseases

Numerous studies have confirmed that IncRNAs 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 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 IncRNAs are aberrantly expressed in OS tissues and dysregulated expression is associated with clinical outcomes and disease status (36,37). Studies have shown that IncRNAs 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 IncRNA 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
It has been previously reported that MEG3 is downregulated in bone marrow samples obtained from patients with MM (48). Promoter hypermethylation of the MEG3 gene has been observed in bone marrow samples. 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.

Table I. Roles of MEG3 in osteogenic differentiation.

| Author, year | Study model | Change in expression | Target(s) (increased/decreased) | 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.

BIOMEDICAL REPORTS 13: 15-21, 2020
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.

### 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.

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 IncRNA MEG3 primarily modulates osteogenic differentiation at the transcriptional and post-transcriptional levels. ceRNA mechanisms serve critical roles in MEG3-regulated osteogenic differentiation. Moreover, IncRNA 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.

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