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Short title: Tensile strain regulates BMSC differentiation through IncRNA-MEG3

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Abstract

Background: With the aging of the population, osteoporosis is becoming more and more common. This progressive bone disease increases the risk of fractures and pain and causes serious harm to people’s health and quality of life. Several studies, including our previous studies, confirmed that tensile strain can promote bone marrow mesenchymal stem cell (BMSC) osteogenic differentiation in vitro. In this study, we further explored the mechanism by which tensile strain regulates BMSC differentiation.

Methods: A device designed by our group was used to apply tensile strain to BMSCs to study the effects of tensile strain on their differentiation. LncRNA-MEG3 overexpression and silencing models of BMSCs were constructed by lentivirus transfection to study the involvement of LncRNA-MEG3. We assessed osteogenic differentiation of BMSCs by alkaline phosphatase (ALP) staining and the expression of Runx2 mRNA and BMP2 mRNA, while adipogenic differentiation was evaluated by oil red staining and the expression of PPARγ mRNA and C/EBPα mRNA.

Results: We demonstrated that proper tensile strain can promote osteogenic differentiation of BMSCs while inhibiting differentiation into adipocytes, and simultaneously promote the expression of LncRNA-MEG3. The overexpression of LncRNA-MEG3 further promotes osteogenic differentiation of stressed BMSCs and inhibits expression of miR-140-5p; the knockdown of LncRNA-MEG3 induces the opposite effects.

Conclusion: Appropriate mechanical stimulation can inhibit the expression of miR-140-5p by promoting LncRNA-MEG3 expression, thereby promoting the osteogenic differentiation of BMSCs. Our results provide a theoretical basis for physical exercise to improve the prevention and treatment of osteoporosis.

Keywords: Stretch strain; LncRNA-MEG3; miRNA-140-5p; bone marrow mesenchymal stem cells; osteogenic differentiation; adipogenic differentiation
Introduction

With the aging of the population, osteoporosis is becoming more and more common. This progressive bone disease increases the risk of fracture and continuous pain, which causes serious harm to people’s health and quality of life and imposes high medical costs. Therefore, it is urgent to improve the prevention and treatment strategies for osteoporosis. It is believed that proper physical exercise can effectively increase bone density, prevent and treat osteoporosis, and reduce visceral fat; it is considered the cornerstone of preventing osteoporosis and obesity (Howe et al., 2011; Keating et al., 2015). From the point of view of cellular mechanics, the effects of physical exercise are mediated by the tensile strain of the cells. Tensile strain is a mechanical stimulation, and bone marrow mesenchymal stem cells (BMSCs) are mechanical stimulation-sensitive cells. When BMSCs are exposed to tensile strain, their various biological activities change, including their differentiation activity. Tensile strain can regulate the differentiation of BMSCs. For example, tensile strain can effectively promote the myocardial differentiation of BMSCs in vitro (Gu et al., 2017), and appropriate cyclic stress can promote the differentiation of fibrochondroid cells from BMSCs (Connelly et al., 2010). In our previous studies, we have shown that the exposure of BMSCs to strain can promote osteogenic differentiation, which may be related to the TGFβ1/Smad2 pathway (Li et al., 2013; Li et al., 2015a, 2015b). It is of great significance for the prevention and treatment of osteoporosis to further explore the mechanism and signaling pathway of distraction strain as a promoter of osteogenic differentiation.

Long noncoding RNA (lncRNA) is a type of noncoding RNA with a length greater than 200 nucleotides; it plays critical regulatory roles in various biological processes, such as cell cycle regulation, embryonic development, and cell differentiation (Fatica and Bozzoni, 2014; Kopp and Mendell, 2018). Among lncRNAs, lncRNA-MEG3 has been confirmed in multiple studies to participate in the regulation of differentiation activities of various types of cells. For example, Sun et al. (2018) examined diabetes-related erectile dysfunction and showed that the endothelial differentiation of BMSCs was promoted when lncRNA-MEG3 was downregulated. Moreover, lncRNA-MEG3 has also been linked to the differentiation of osteoblasts from stem cells (Zhuang et al., 2015; Xia et al., 2019). Some studies have pointed
out that lncRNA-MEG3 can participate in the regulation of mesenchymal stem cell (MSC) differentiation and metabolism through interaction with microRNA (miRNA), e.g., adipogenic and osteogenic differentiation of human adipose stem cells (ADSC) through miR-140-5p, and osteogenic differentiation of PDLSC in periodontitis through regulating the miR-27a-3p/IGF1 axis (Li et al., 2017; Liu et al., 2019).

Although there have been several studies on the role of lncRNA-MEG3 in BMSCs differentiation, no studies have been performed to clarify whether lncRNA-MEG3 is involved in the regulation of BMSCs differentiation by tensile strain. The purpose of this study was to examine the effects of tensile strain on osteogenic and adipogenic differentiation of BMSCs, and to explore whether lncRNA-MEG3 plays a role in this process.

Materials and methods

Isolation and culture of rat BMSCs

All of the animal experiments were conducted in accordance with relevant national and international guidelines and were approved by the Animal Care and Use Committee of Southern Medical University. BMSCs were separated from the bone marrow cavities of the femur and tibia of Sprague–Dawley rats (80–100 g) and cultured in DMEM low-glucose medium containing 10% FBS (GIBCO), 100 U/mL penicillin, and 100 µg/mL streptomycin in a cell incubator at 37°C with a humid 5% CO₂. After 24 hours, nonadherent cells were removed by washing with PBS, fresh medium was added for further growth, and the medium was refreshed every 2–3 days. The cells were separated with 0.25% trypsin when cell confluence reached about 85%. Then, 1 × 10⁴ cells/cm² were subcultured and collected at the second or third generation. The expression levels of CD29, CD34, CD44, and CD45 were detected by flow cytometry (BD).

Osteogenic or adipogenic differentiation induction with/without tensile strain

BMSCs at logarithmic growth stage (1 × 10⁵ cells/mL) were inoculated to a Bioflex 6-well plate and incubated at 37°C with 5% CO₂. BMSCs differentiation was induced by culture conditions. In the osteogenic differentiation group, the cells were cultured in a medium
containing 10% FBS, 1% penicillin/streptomycin, 100 nmol/L dexamethasone, 50 µmol/L ascorbic acid 2-phosphate, and 10 mmol/L β-glycerophosphate. In the adipogenic differentiation group, the cells were cultured in DMEM containing 10% FBS, 1% penicillin/streptomycin, 1 µmol/L dexamethasone, 0.5 mmol/L IBMX, 10 mg/L insulin, and 200 µmol/L indomethacin. In the control group, the cells were cultured normally, with medium changed every 48 hours. Tensile strain was set at 5% deformation rate, 0.5 Hz, and 6 hours/day (Zhu et al., 2019).

**Alkaline phosphatase and oil red staining**

Osteogenic differentiation was determined using an ALP Staining Kit (Tianjian Biotechnology Co., Ltd.) in accordance with the manufacturer’s instructions. In brief, the cells were fixed with 4% paraformaldehyde for 12 minutes, then stained with 2 mL ALP solution per well at room temperature for 30 minutes. ALP-positive cells were observed under a microscope. For adipogenic differentiation determination, the cells were fixed with 10% neutral buffered formalin for 1 hour at room temperature, then incubated with 60% isopropanol for 1 minute, and finally stained with oil red O for 15 minutes. Positive lipid droplets were observed.

**RT-PCR analysis**

Briefly, BMSCs were harvested, and RNA was extracted using TRIzol reagent. Total RNA was reverse-transcribed into cDNA using a K1622 kit (Thermo Scientific). PCR analysis was prograemed applying an All-in-One™ qPCR kit (Genecopoeia) with specific primers. The expression levels of the tested genes were standardized using GAPDH as control with the $2^{-\Delta\Delta C_{t}}$ method. The primer sequences were as follows:

miR-140-5p-F: 5’-CAGTGGTTTTACCTATGG-3’,
miR-140-5p-R: 5’-GTGCAGGGTCCGAGGT-3’;
Meg3-F: 5’-CTGCCCATCTACACCTCACG-3’,
Meg3-R: 5’-CTCTCCGCCGTCTGCGCTAGGGGCT-3’;
Runx2-F: 5’-GGACCGACACAGCCATATAAA-3’,
Runx2-R: 5’-GCCTCATTCCCTAACCTGAAA-3’;
BMP2-F: 5’-CAGTGGGAGAGCTTTGATGT-3’,
BMP2-R: 5’-ACCTGGCTTCTCCTCTAAGT-3’;
PPARγ-F: 5’-GACCTGAAGCTCCAAGAATACC-3’,
PPARγ-R: 5’-TTCATGTGGCCTGTTGTAGAG-3’;
C/EBP-F: 5’-CCTCTGGGATGGATCGATTATG-3’,
C/EBP-R: 5’-GGGACCTTAGTTTCTGGTCTTG-3’.

Overexpression and knockdown of lncRNA-MEG3
In order to evaluate the effect of lncRNA-MEG3 on differentiation, we constructed the lncRNA-MEG3 overexpression and silencing models of BMSC cells transiently transfected with recombinant lentiviruses LV-shMEG3-GFP and LV-MEG3-GFP (GenePharma) at a MOI of 50 and cultured in a fresh medium with polybrene (5 µg/mL). After 16 hours, the cells were collected and grown in BMSC differentiation-inducing conditions as described above.

Detection of the interaction between lncRNA-MEG3 and miR-140-5p using dual-luciferase reporter assay
293T cells were seeded and cultured on a 24-well plate until the cell confluence reached 60%. A vector encoding wild-type or mutant lncRNA-MEG3 3’-UTR was transfected into 293T cells using X-treme GENE™ HP DNA transfection reagent. After incubation for 48 hours, the cells were dissolved in 1× PLB (Passive Lysis Buffer) until the cells were completely lysed. Luciferase activity was measured lightless by a Dual-Luciferase® Reporter Assay System (Promega) in line with the manufacturer’s instructions.

Statistical analysis
All of the data are expressed as mean ± standard deviation (SD) of at least three independent experiments. GraphPad Prism version 8.0 software was utilized to analyze intergroup differences by Student’s t test. P ≤ 0.05 was considered to be statistically significant.
Results

BMSC morphology and identification

The primary MSCs adhered to the wall 2–4 days after inoculation, and the morphology of MSCs was spindle-shaped. On days 3–4, the cells proliferated rapidly in clusters after the first medium replacement; hematopoietic cells were gradually eliminated after repeated medium replacement and PBS washing. Cell confluence reached 80% after 12–14 days. After the first passage, MSC growth rate increased and full confluence was obtained in 7–10 days. The cells were slender, spindle, spiral, and confluent. Consistent with the known phenotype of MSCs, the cells were positive for CD29 (98.2%), CD34 (1.1%), CD44 (98.7%), and CD45 (1.3%), which is consistent with the previous results (Li et al., 2015a, 2015b).

Expression changes of lncRNA-MEG3 during osteogenic and adipogenic differentiation of BMSCs with or without tensile strain

To evaluate the molecular mechanism of the differentiation of BMSCs under tensile strain, we examined the expression changes of lncRNA-MEG3 during the induced differentiation. As shown in Figure 1A, during the 2-week osteogenic differentiation of BMSCs, the expression level of lncRNA-MEG3 gradually increased in a time-dependent manner, and the increase rate gradually decreased. The expression level of lncRNA-MEG3 peaked on day 14. In the osteogenic differentiation group with simultaneous loading of tensile strain, the expression level of lncRNA-MEG3 also gradually increased with time. Notably, the expression level of lncRNA-MEG3 in BMSCs with simultaneous osteogenic differentiation and exertion of tensile strain was significantly higher than that in the osteogenic differentiation group. In contrast, as shown in Figure 1B, during adipogenic differentiation of BMSCs, the expression level of lncRNA-MEG3 gradually decreased in a time-dependent manner. In the adipogenic differentiation + tensile strain group, the expression level of lncRNA-MEG3 also gradually decreased with time, and the downward trend was similar to that in the simple osteogenic differentiation group. At each time point, the expression level of lncRNA-MEG3 in the adipogenic differentiation group loaded with tensile strain was higher than that in the adipogenic differentiation group.
Effects of lncRNA-MEG3 overexpression on osteogenic and adipogenic differentiation of BMSCs under tensile strain

In order to study the effects of lncRNA-MEG3 on the differentiation of BMSCs under tensile strain, we used recombinant lentivirus LV-MEG3-GFP to transfect BMSCs to construct a lncRNA-MEG3 overexpression cell model. As shown in Figure 2A, the fluorescence imaging was performed 48 hours after the completion of infection, and the obviously enhanced fluorescence intensity indicated that the transfection was successful and effective. We used qPCR to detect lncRNA-MEG3 expression on the fifth-day post-transfection and confirmed the significantly upregulated expression of lncRNA-MEG3 (Figure 2B). As shown in Figure 2C, the expression levels of biomarkers of osteogenic differentiation, including Runx2 mRNA and BMP2 mRNA, in BMSCs with overexpressed lncRNA-MEG3 were significantly higher than those in the control group. The expression levels of adipogenic differentiation markers when lncRNA-MEG3 was overexpressed were significantly lower than those in the control group (Figure 2D). In addition, western blot analysis and ALP and oil red staining revealed that the expression of osteogenic differentiation indexes was promoted, while the index of adipogenic differentiation was suppressed (Figure 2C–2E). Collectively, these results demonstrate that lncRNA-MEG3 overexpression simultaneously promotes osteogenic differentiation of stressed BMSCs and inhibits adipogenic differentiation.

Effects of lncRNA-MEG3 silencing on osteogenic and adipogenic differentiation of BMSCs under tensile strain

To further study the effects of lncRNA-MEG3 on the differentiation of BMSCs under tensile strain, we used recombinant lentivirus LV-shMEG3-GFP to construct a cell model with lncRNA-MEG3 silencing. Fluorescence imaging was performed 48 hours after cell infection. As shown in Figure 3A, the green fluorescent signal inside the cells confirmed the successful transfection. In contrast, the detection of lncRNA-MEG3 by qPCR confirmed that its expression level was significantly downregulated in shMEG3 group (Figure 3B). Subsequently, follow-up results showed that the expression of markers of BMSCs osteogenic
differentiation, including Runx2 mRNA and BMP2 mRNA, was significantly downregulated in the lncRNA-MEG3 silencing group (Figure 3C). At the same time, the expression level of adipogenic markers when lncRNA-MEG3 was silenced was significantly higher than that in the control group (Figure 3D). Similarly, the results of western blot, ALP, and oil red staining further showed that osteogenic differentiation was suppressed in the lncRNA-MEG3 silencing group, while adipogenic differentiation was promoted (Figure 2C-2E). It can be concluded that lncRNA-MEG3 knockdown promotes the differentiation of BMSCs induced by tensile strain and inhibits their osteogenic differentiation.

**Effects of lncRNA-MEG3 on the expression of miR-140-5p in BMSCs under tensile strain**

miR-140-5p has previously been reported to be capable of suppressing osteogenesis in undifferentiated human MSCs (Guo et al., 2019; Hwang et al., 2014). Herein, we wondered whether lncRNA-MEG3 promotes osteogenic differentiation of BMSCs under tensile strain via regulating miR-140-5p. The results of the luciferase reporter gene assay showed that the overexpression of miR-140-5p significantly reduced the expression of luciferase in the 3’UTR region of the lncRNA-MEG3 group compared with the miR-NC group (Figure 4A); however, after mutating the 3’UTR of lncRNA-MEG3, this phenomenon was eliminated (Figure 4A). These results showed that miR-140-5p and lncRNA-MEG3 could interact directly. Furthermore, we assessed the expression changes of miR-140-5p in the lncRNA-MEG3 overexpression and silencing cell models. As shown in Figure 4B, in BMSCs under tensile strain stimulation, lncRNA-MEG3 overexpression resulted in a significant reduction in miR-140-5p expression levels, while lncRNA-MEG3 silencing induced the opposite effects. These results further confirmed the interaction between lncRNA-MEG3 and miR-140-5p.

**Discussion**

Osteoporosis is characterized by decreased bone density and bone mass, and increased bone loss and increased bone fragility. Regular exercise is considered an effective method to stimulate bone formation in osteoporotic patients to increase bone mass, which can reduce
osteoporosis-induced risk of fracture (Benedetti et al., 2018). Previous research has suggested that exercise transmits force through bones, thereby generating mechanical stimuli; these stimuli can cause a series of cellular biochemical reactions, including the regulation of gene expression or protein activity, thereby promoting the formation of bone tissue. Tensile strain is a type of mechanical stimulation. Several studies, including our previous research, have confirmed that proper tensile strain can promote osteogenic differentiation and inhibit adipogenic differentiation (Li et al., 2013; Li et al., 2015a, 2015b). In this study, we showed that the expression of lncRNA-MEG3 was gradually upregulated with the progress of osteogenic differentiation and was further upregulated after the stimulation by tensile strain. In contrast, there was an opposite trend in adipogenic differentiation. These results demonstrate that tensile strain can regulate the expression of lncRNA-MEG3, further confirming that the mechanical stimulation induces gene regulation in BMSCs.

To further demonstrate the role of lncRNA-MEG3 in the regulation of BMSCs differentiation by tensile strain, we used lentiviral transfection technology to construct lncRNA-MEG3 overexpression and silencing models of BMSCs. The results showed that after overexpression of lncRNA-MEG3, osteogenic differentiation of BMSCs was significantly promoted, while adipogenic differentiation was restrained. On the contrary, when lncRNA-MEG3 was silenced, the opposite trend was observed. These data fully illustrate that the expression of lncRNA-MEG3 is a factor that promotes osteogenic differentiation and inhibits adipogenic differentiation. Combined with the upregulated expression of lncRNA-MEG3 upon tensile loading, we found that strain loading may promote osteogenic differentiation and inhibit adipogenic differentiation through upregulating lncRNA-MEG3. Actually, similar regulatory activities have been reported in multiple studies. For example, after the intervention of circulatory mechanical stretching on human aortic smooth muscle cells, 30586 IncRNAs were screened and 580 differentially expressed IncRNAs were identified (Mantella et al., 2017). These differentially expressed IncRNAs have been linked to cell differentiation, stress response, and response to external stimuli. Also, in Huang’s study (Huang et al., 2020), 155 differentially expressed IncRNAs were detected after circulating mechanical stretching of endothelial cells; IncRNA-NEAT1 was shown to be an important factor for endothelial
dysfunction, and tensile strain was able to regulate the expression of lncRNA so as to achieve the regulation of cell activity.

LncRNAs can execute their regulatory functions through a variety of pathways (Zhuang et al., 2015; Li et al., 2017; Sun et al., 2018; Xia et al., 2019). Among them, the main mechanism by which lncRNAs regulate life activities is based on miRNA “sponging” to change the expression of miRNA, so as to modify the translation of miRNA’s target mRNA (Salmena et al., 2011). Gao et al. have demonstrated that lncRNA-MALAT1 promotes OSX expression and osteogenic differentiation of hMSCs through regulating miR-143 (Gao et al., 2018). Therefore, we also attempted to explore miRNAs that interact with lncRNA-MEG3. Li et al. found, through intervention experiments, that lncRNA-MEG3 can regulate osteogenic differentiation of human adipose stem cells (hASCs) by regulating miR-140-5p (Li et al., 2017). In our experiments, we performed luciferase report gene assay on lncRNA-MEG3 and miR-140-5p; we determined that lncRNA-MEG3 possessed targeting sites for miR-140-5p. Furthermore, after interfering with the expression of lncRNA-MEG3, we found that the enhanced expression of lncRNA-MEG3 in BMSCs under tensile strain significantly inhibited the expression level of miR-140-5p and promoted osteogenic differentiation, whereas silencing of lncRNA-MEG3 induced the opposite effects. All these results indicate that lncRNA-MEG3 can regulate the activity of miR-140-5p in BMSCs upon tensile strain.

In conclusion, our study demonstrated that tensile strain promotes osteogenic differentiation of BMSCs by upregulating lncRNA-MEG3. We also verified the relationship between lncRNA-MEG3 and miR-140-5p in osteogenic differentiation of BMSCs induced by tensile strain; we found that lncRNA-MEG3 can regulate the activity of miR-140-5p. These results can provide more research directions for the prevention and treatment of osteoporosis.
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Author contributions
R.L. designed this program. G.Z., C.Z. and Y.Q. operated the cell and animal experiments. G.Z., S.Y., Z.Y. and S.Z. conducted the data collection and analysis. G.Z., C.Z. and Y.Q. produced the manuscript which was checked by R.L. All the authors have confirmed the submission of this manuscript.

Conflict of interest
The authors declare no conflict of interest.

Data Availability Statement
The data supporting the results of the questionnaires are available from the corresponding author upon reasonable request.

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None.
References:


liver fat and visceral adiposity. J. Hepatol. 63, 174-182.


ganglion cell dysplasia in congenital intestinal atresia through directly regulating miR-211-5p/GDNF axis. Biomed. Pharmacother 111, 436-442.


Figure legends

Figure 1. The expression changes of lncRNA-MEG3 in BMSCs during osteogenic or adipogenic differentiation. (A) Changes in the expression level of lncRNA-MEG3 at different stages of osteogenic differentiation of BMSCs with or without tensile strain. (B) Changes in the expression level of lncRNA-MEG3 at different stages of adipogenic differentiation of BMSCs with or without tensile strain. Data are presented as mean and SD. * P < 0.05

Figure 2. LncRNA-MEG3 overexpression promotes tensile strain-induced osteogenic differentiation and inhibits adipogenic differentiation of BMSCs. (A) Fluorescence imaging was performed 48 hours post-transfection by LV-MEG3-GFP. (B) qPCR was performed to detect expression of lncRNA-MEG3 in different groups of BMSCs induced to osteogenic differentiation or adipogenic differentiation. (C) The expression of osteogenic differentiation markers Runx2 mRNA and BMP2 mRNA in BMSCs with or without lncRNA-MEG3 overexpression was detected by qPCR. (D) The expression of adipogenic differentiation markers PPARγ mRNA and C/EBPα mRNA in BMSCs with or without lncRNA-MEG3 overexpression was detected by qPCR. (E) The staining of BMSCs with or without lncRNA-MEG3 overexpression after induced osteogenic or adipogenic differentiation. Left: ALP staining after osteogenic differentiation; Right: oil red staining after adipogenic differentiation. Data are presented as mean and SD. * P < 0.05

Figure 3. LncRNA-MEG3 knockdown inhibits tensile strain-induced osteogenic differentiation and promotes adipogenic differentiation of BMSCs. (A) Fluorescence imaging was performed 48 hours post-transfection by LV-shMEG3-GFP. (B) qPCR was performed to detect expression of lncRNA-MEG3 in different groups of BMSCs induced to osteogenic differentiation or adipogenic differentiation. (C) The expression of osteogenic differentiation markers Runx2 mRNA and BMP2 mRNA in BMSCs with or without lncRNA-MEG3 knockdown was detected by qPCR. (D) The expression of adipogenic differentiation markers PPARγ mRNA and C/EBPα mRNA in BMSCs with or without
LncRNA-MEG3 knockdown was detected by qPCR. (E) The staining of BMSCs with or without LncRNA-MEG3 knockdown after induced osteogenic or adipogenic differentiation. Left: ALP staining after osteogenic differentiation; Right: oil red staining after adipogenic differentiation. Data are presented as mean and SD. * P < 0.05

**Figure 4. LncRNA-MEG3 regulates differentiation of BMSCs through regulating miR-140-5p.** (A) The luciferase reporter gene assay was performed in 293T cells to confirm the interaction between LncRNA-MEG3 and miR-140-5p. (B) The expression of miR-140-5p in BMSCs with or without LncRNA-MEG3 overexpression was detected after osteogenic differentiation or adipogenic differentiation. (C) The expression of miR-140-5p in BMSCs with or without LncRNA-MEG3 knockdown was detected after osteogenic differentiation or adipogenic differentiation. Data are presented as mean and SD. * P < 0.05