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LncRNA LINC00707 promotes osteogenic differentiation of hBMSCs through the Wnt/β-catenin pathway activated by LINC00707/miR-145/LRP5 axis

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Abstract. – OBJECTIVE: Human bone marrow mesenchymal stem cells (hBMSCs) have a strong self-renewal potential and osteogenic differentiation ability, thus providing a new method for bone defect repair research. LncRNA LINC00707 participates in the regulation of osteogenic differentiation of hBMSCs and our aim was to explore the potential regulatory mechanism.

MATERIALS AND METHODS: Firstly, quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expression levels of LINC00707, miR-145, the low-density lipoprotein receptor-related protein 5 (LRP5) and osteogenesis-related genes. Next, alkaline phosphatase (ALP) activity assay was used to measure the relative activity of ALP in hBMSCs. The protein levels of LRP5 and osteogenesis-related genes were detected by Western blot. Finally, the relationship among LINC00707, miR-145 and LRP5 were predicted by online software and verified by Dual-Luciferase reporter assay, RNA pull-down and RNA immunoprecipitation (RIP).

RESULTS: LINC00707 and osteogenesis-related genes were gradually upregulated during osteogenesis of hBMSCs. Meanwhile, overexpression of LINC00707 promoted osteogenic differentiation of hBMSCs. Interestingly, we found that LINC00707 negatively regulated the miR-145 expression and osteogenic differentiation functions by directly interacting with miR-145, and LINC00707 affected the functions of LRP5 by sponging miR-145 in hBMSCs. Moreover, LINC00707 promoted the Wnt/β-catenin pathway through the LINC00707/miR-145/LRP5 axis.

CONCLUSIONS: LncRNA LINC00707 promoted osteogenic differentiation of hBMSCs by targeting LRP5 mediated by miR-145 through the activation of the Wnt/β-catenin pathway.

Key Words:
hBMSCs, LncRNA LINC00707, MiR-145, LRP5, Wnt/β-catenin pathway.

Introduction

Human bone marrow mesenchymal stem cells (hBMSCs) were found in the bone marrow stroma and had various differentiation potentials, such as bone, cartilage, fat, tendon, myoblasts and tendon[1]. Previous studies reported the application of hematopoietic stem cell transplantation in clinical practice[2]. Osteogenic differentiation plays a vital role in bone formation and bone remodeling of hBMSCs. It was associated with bone diseases, such as femoral head necrosis and osteoporosis. Researchers[3-5] indicated different signaling pathways involved in bone diseases, such as MAPK signaling pathway, Wnt/β-catenin signaling pathway, and PI3K/AKT signaling pathway. However, the molecular regulation mechanism of osteogenic differentiation of hBMSCs needs further improvement.

Long noncoding RNAs (lncRNAs) are >200 nucleotides RNA molecules that play a crucial role in regulating epigenetics in disease and a novel regulator in gene expression[6]. Wang et al[7] showed that lncRNA MEG3 inhibited osteogenic differentiation of hBMSCs. Moreover, TiO2 nanotubes (TNTs) enhanced osteogenic differentiation of mesenchymal stem cells (MSCs) by regulating the expression of lncRNA CCL3[8]. Microarray and bioinformatics showed the expression profile of lncRNA was closely related to abnormal adipogenesis and osteogenic differentiation of hBMSCs[9]. Therefore, exploring the function of lncRNA in hBMSCs has an essential significance in designing new RNA therapies.

Noncoding small molecule RNAs (miRNAs) not only affect the disease by regulating the stability of target mRNA, but also bind to lncRNA

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fully to produce ceRNA[10,11]. MiRNA-27a-3p was reported to promote the differentiation of hMSCs and alleviate osteoporosis by directly regulating ATF3 expression[12]. LncRNA MALAT1 regulated Osterix (OSX) expression by sponging miRNA-143 to promote osteogenic differentiation of hBMSCs[13]. Moreover, miR-145 suppressed osteogenic differentiation by directly regulating osteogenic associated transcription factor OSX[14]. Likewise, lncRNA linc-ROR accelerated osteogenic differentiation as a ceRNA for miR-138 and miR-145[15]. Therefore, miR-145 is a critical regulator in osteogenic differentiation of hBMSCs and our study was committed to researching the functions and the involved signaling pathways.

The low-density lipoprotein receptor-related protein 5 (LRP5) acted as a Wnt/β-catenin pathway receptor and affected bone mineral density by altering the anabolic response of bone to mechanical stress[16]. Li et al[17] proposed that microRNA-23a suppressed the osteogenic differentiation of hBMSCs by regulating LRP5. LRP5/β-catenin/Runx2 pathway participated in improving osteogenic differentiation of light-controlled BMSC sheet-implant complexes[18]. Some authors[19,20] have documented the Wnt/β-catenin signaling pathway promoted osteogenic differentiation in various cells. However, LRP5 as a key receptor of the Wnt/β-catenin pathway, and the function of osteogenesis in hBMSCs, need further studies. In this research, we aimed to investigate the function of LncRNA LINC00707 in osteogenic differentiation of hBMSCs. The LINC00707/miR-145/LRP5 axis was firstly documented and LINC00707 promoted the Wnt/β-catenin pathway through LINC00707/miR-145/LRP5 axis, providing an essential reference for exploring the new RNA therapy.

Materials and Methods

hBMSCs Isolation and Cell Culture

The human bone marrow tissues were obtained from three different donors in the Second Xiangya Hospital of Central South University. Isolation of hBMSCs was according to the description already reported[21]. This research was approved by the Medical Ethics Committee of the Second Xiangya Hospital of Central South University. The hBMSCs were cultured in α-MEM (ScienCell, Carlsbad, CA, USA) added with 10% fetal bovine serum (FBS; ScienCell), 100 U/mL penicillin (ScienCell), and 100 μg/mL streptomycin (ScienCell) at incubator with 5% CO_2 and 37°C.

Osteogenic Differentiation of hBMSCs

In order to induce the osteogenic differentiation of hBMSCs, 10 mM β-glycerophosphate (Yanjin, Shanghai, China), 200 μM ascorbic acid (PureOne, Shanghai, China) and 100 nM dexamethasone (PureOne) were added to the medium and cultured in the incubator with 5% CO_2 and 37°C for 7 d. The medium should be changed every 3 d.

Cell Transfection

The sh-LINC00707, pLINC00707, sh-LRP5 and their controls were obtained from Genepharma (Shanghai, China). The final concentration of synthetic materials was 200 nM and transfected to hBMSCs cultured in 6-well plates by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

RNA Isolation and Quantitative Real-Time PCR

The total RNA was extracted by TRIzol (Invitrogen, Carlsbad, CA, USA) and the RevertAidTM First Strand cDNA Synthesis Kit (Fermentas, Ottawa, Canada) was used for reverse transcription. Then the PCR was completed by an ABI SYBR Green Master Mix (Invitrogen). The RT primers were listed as follows: Forward 5'-CCAACAGGGTATCAGAATTCTC-3' and reverse 5'-TGCTGACAATAGCCATTAGG-3' for LINC00707. Forward 5'-AGTCCACTGGCGTCTTCACC-3' and reverse 5'-CCAGGGGTGCTAAGCAGTTG-3' for Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Forward 5'-TGGCCCGAAACCTCTACTG-3' and reverse 5'-GCACACTCGATTTTAGGGTTCT-3' for LRP5. Forward 5'-AGAATCTGGTGCAGGAATGG-3' and reverse 5'-TCGTATTTCATGTCTCCAGGC-3' for Alkaline phosphatase (ALP). Forward 5'- TGCAGCCTTTGTGTCCAAG-3' and reverse 5'-CCCAGCCATTGATACAGGTAG-3' for Osterix and Osteocalcin (OCN). Forward 5'-TGTCATGGCGGGTAACGAT-3' and reverse 5'-AAGACGGTTATGGTCAAGGTGAA-3' for Runt-related transcription factor 2 (Runx2). Forward 5'-CTCCTTTCACCTGCAGGCAG-3' and reverse 5'-CAGACAGTCAGAAGAGCTGT-3' for OSX. Forward 5'-AGCCGGTCCAGTTTTCCCAGGA-3' and reverse 5'-GTGCAGGGTCCGAGGT-3' for miR-145. Forward 5'-CTCGCTTCGGCAGCACA-3' and reverse 5'-AACGCTTCACGAATTTGCGT-3' for U6[17, 22-24].

ALP Activity Assay

After the osteogenic differentiation of hBMSCs induced for 7 d, the density of the 24-well plates achieved to around 1×10^5 cells per well. Then the ALP activity kit (Anaspec, Fremont, CA, USA) was taken to detect the ALP activity of hBMSCs after induction.

Western Blot Analysis

The total protein was lysed and collected in RIPA lysis buffer (Beyotime, Shanghai, China). After that, the protein samples were separated using 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane (Thermo Fisher Scientific, Waltham, MA, USA). Next, they were incubated by skimmed milk and then primary antibodies were added and incubated with membrane overnight at 4°C. The primary antibodies in our study were as follows: anti-LRP5 antibody (1:1000, Thermo Fisher Scientific), anti-Runx2 antibody (1:1000, Invitrogen), anti-ALP antibody (1:1000, Abcam, Cambridge, MA, USA), anti-OCN antibody (1:1000, Abcam), anti-OSX antibody (1:1000, Abcam), anti-GAPDH antibody (1:1000, Abcam), anti-Axin2 antibody (1:1000, Abcam), anti-β-catenin antibody (1:1000, Abcam). Finally, they were incubated with the secondary antibody (Thermo Fisher Scientific) at room temperature for 1 h. The results were observed on the chemiluminescent image by Kodak film developer (Fujifilm, Minato, Tokyo, Japan).

Dual Luciferase Reporter Assay

The wild type LINC00707 sequences (WT-LINC00707), mutant LINC00707 sequences (MUT-LINC00707), wild type LRP 3'UTR sequences (LRP 3'UTR-WT) and mutant LRP 3'UTR sequences (LRP 3'UTR-MUT) were cloned into the pGL-3 luciferase reporter vector (Promega, Madison, WI, USA). The reporter vectors and miR-145 or miR-NC were transfected into cells together by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Then Dual Luciferase Reporter Assay System (Promega) detected the luciferase activity after transfection 48 h.

RNA Pull-Down and RNA Immunoprecipitation (RIP)

RNA pull-down assay was used to verify the relationship between LINC00707 and miR-145. Firstly, the biotin label joins miR-145 to form the Bio-miR-145 (Bio-NC as the control). Next, the RIPA lysis buffer (Beyotime, Shanghai, China) and RNase inhibitor (Invitrogen, Carlsbad, CA, USA) were used for cell lysing, and the Bio-miR-145 or Bio-NC was added to the supernatant. Next, streptavidin beads were incubated (Invitrogen) and proteinase K was used to isolating RNA. Finally, LINC00707 enrichment was detected by qRT-PCR.

Meanwhile, the Magna RIP RNA-binding Protein Immunoprecipitation Kit (Biomars, Beijing, China) was used to identify the interaction of LINC00707 and miR-145. In brief, the magnetic beads and anti-Ago2 antibody (Abcam) were incubated in cells for 24 h. To purify RNA, we added the proteinase K and the phenol-chloroform-isoamyl alcohol reagent. Finally, qRT-PCR was used to detect LINC00707 enrichment.

Statistical Analysis

The data was displayed as mean ± standard deviation (SD) and statistical analyses were carried out using by SPSS 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA). Student's *t*-test or one-way ANOVA followed by Tukey's test, were utilized to investigate the significant difference. All experiments were repeated three times. $p<0.05$ was considered as statistically significant.

Results

LINC00707 and Osteogenesis-Related Genes were Upregulated During Osteogenesis of hBMSCs

Firstly, we isolated the human hBMSCs and cultured them by osteogenic medium (OM) for 7 d. Then, the expression level of LINC00707 was detected by qRT-PCR and the results indicated that LINC00707 was upregulated during osteogenesis of hBMSCs (Figure 1A). Moreover, ALP activity and the expression of osteogenesis-related genes were used to determine the efficiency of osteogenic differentiation of hBMSCs after induction for 7 d. The results showed that the ALP activity was upregulated (Figure 1B). Meanwhile, qRT-PCR and Western blot revealed that the osteogenesis-related gene ALP, OCN, Runx2 and OSX were also upregulated in hBMSCs after 7 days osteogenic induction (Figure 1C and D), demonstrating that LINC00707 played a crucial role in osteogenic differentiation of hBMSCs.

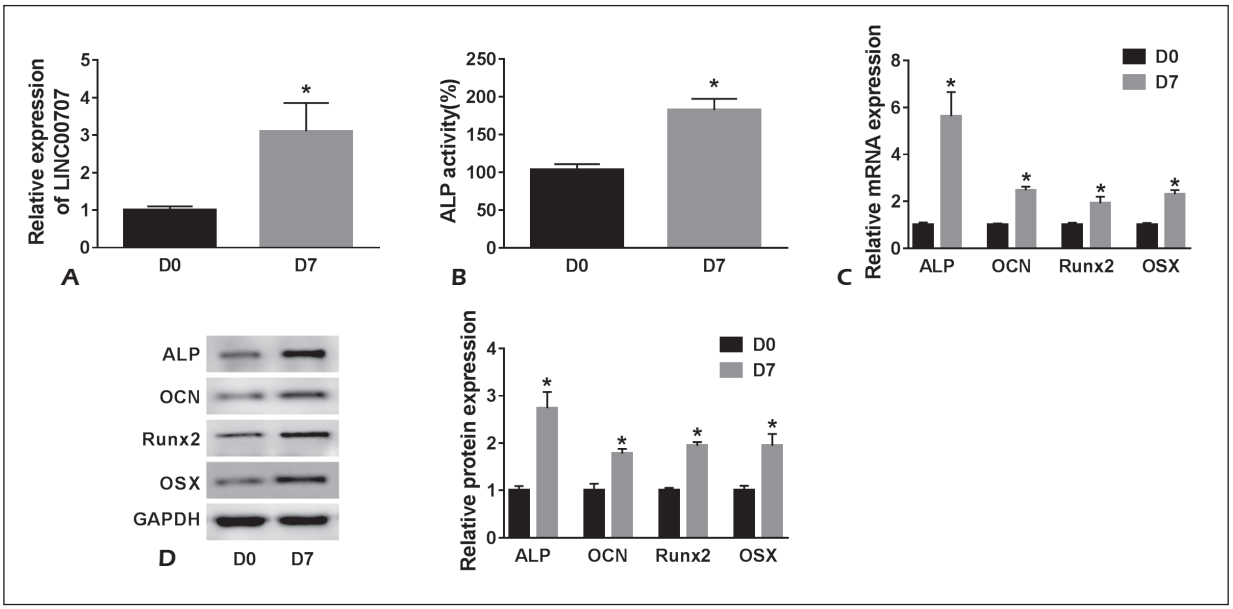

Figure 1. LncRNA LINC00707 and osteogenesis-related genes were upregulated during osteogenesis of hBMSCs. **A**, The expression level of LINC00707 was detected by qRT-PCR in hBMSCs after 7 days osteogenic induction. **B**, The relative activity of ALP in hBMSCs after 7 days osteogenic induction was measured by ALP activity assay. (**C** and **D**) The mRNA and protein levels of osteogenesis-related genes (ALP, OCN, Runx2 and OSX) were detected by qRT-PCR and Western blot in hBMSCs after 7 days osteogenic induction. *$p<0.05$.

LINC00707 Promoted Osteogenic Differentiation of hBMSCs

Furthermore, we aimed to explore the function of LINC00707 in osteogenic differentiation of hBMSCs. After osteogenic induction for 7 d, hBMSCs were transfected with sh-LINC00707 and pLINC00707. Firstly, we detected the transfection efficiency and results showed the expression level of LINC00707 in hBMSCs transfected with sh-LINC00707 was downregulated, and pLINC00707 was upregulated in hBMSCs (Figure 2A). After that, the ALP activity was downregulated in hBMSCs transfected with sh-LINC00707, while in those transfected with pLINC00707 in hBMSCs was significant upregulated (Figure 2B). In addition, qRT-PCR and Western blot detected the expression of ALP, OCN, and Runx2; OSX was downregulated when the expression of LINC00707 was inhibited. In contrast, overexpression of LINC00707 induced the expression of ALP, OCN, Runx2 and OSX (Figure 2C-K), suggesting that LINC00707 promoted osteogenic differentiation of hBMSCs.

LINC00707 Negatively Regulated miR-145 by Directly Targeting miR-145

The online software starBase v2.0 was used to detect the potential binding sites between LINC00707 and miR-145 (Figure 3A). To confirm the relationship of LINC00707 and miR-145, the WT-LINC00707 and MUT-LINC00707 reporter vectors were constructed. Luciferase reporter assay showed the luciferase activity of WT-LINC00707 reporter vector was significantly downregulated than MUT-LINC00707 reporter vector (Figure 3B). Moreover, RNA pull-down and RIP assays further confirmed the relationship between LINC00707 and miR-145 (Figure 3C-E). Then the expression level of miR-145 was detected in hBMSCs transfected with sh-LINC00707 and pLINC00707. Interestingly, the miR-145 level was upregulated in hBMSCs transfected with sh-LINC00707, while the overexpression of pLINC00707 inhibited the expression of miR-145 (Figure 3F). And qRT-PCR showed that miR-145 expression was downregulated in hBMSCs after 7 days osteogenic induction (Figure 3G). To investigate whether pLINC00707 inhibiting the function of miR-145 in hBMSCs, we transfected miR-145 or miR-145 + pLINC00707 into hBMSCs. The expression of miR-145 was significantly inhibited in hBMSCs transfected with miR-145 + pLINC00707, consistently with previous experiment (Figure 4A). Notably, overexpression of miR-145 inhibited the ALP activity,

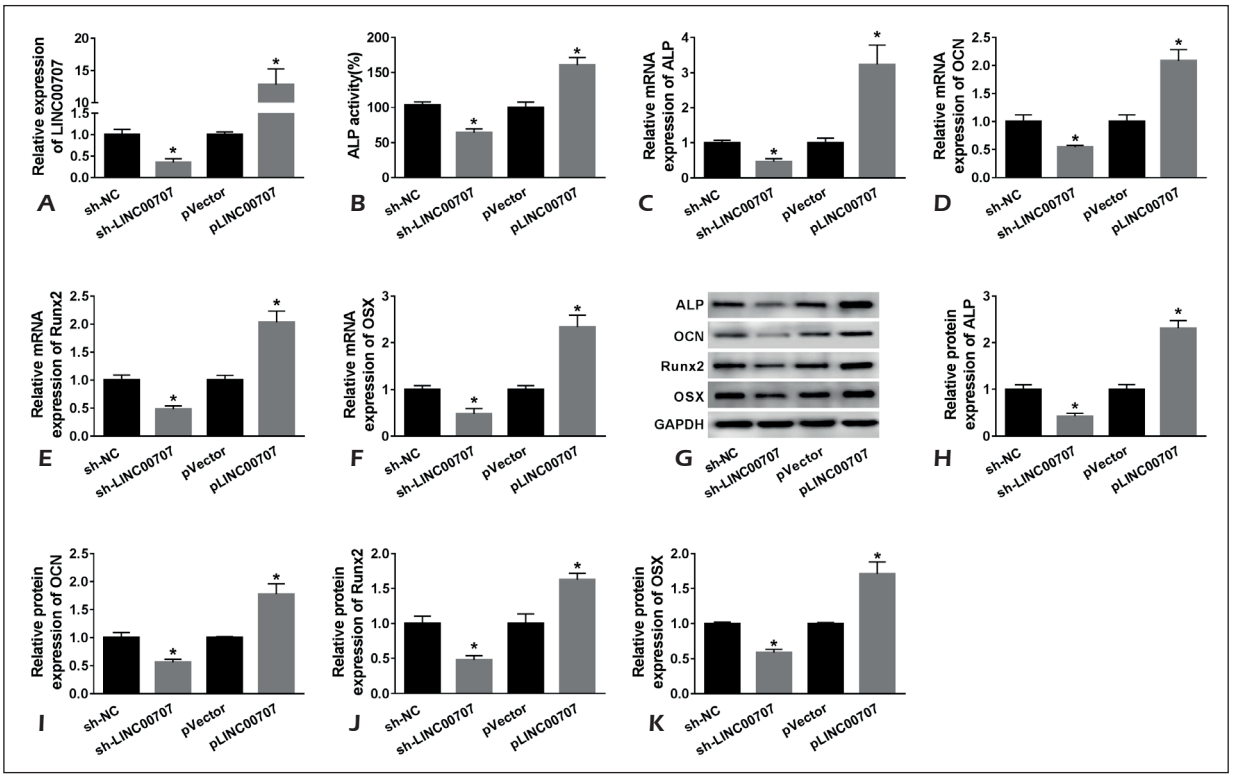

Figure 2. LINC00707 promoted osteogenic differentiation of hBMSCs. **A**, Transfection efficiency of sh-LINC00707 and pLINC00707 in hBMSCs was detected by qRT-PCR. **B**, The relative activity of ALP in hBMSCs transfected with sh-LINC00707 and pLINC00707 was monitored. **C-F**, The mRNA expression levels of osteogenesis-related genes (ALP, OCN, Runx2 and OSX) in hBMSCs transfected with sh-LINC00707 and pLINC00707 were detected via qRT-PCR. **G-K**, The protein levels of ALP, OCN, Runx2 and OSX in hBMSCs transfected with sh-LINC00707 and pLINC00707 were examined. *p<0.05.

but overexpression of LINC00707 reversed the effect (Figure 4B). Finally, the mRNA and protein levels of ALP, OCN, Runx2 and OSX were detected by qRT-PCR and Western blot. The results showed that overexpression of miR-145 inhibited the expression of ALP, OCN, Runx2 and OSX, whereas overexpression of LINC00707 abolished these effects (Figure 4C-K). All data indicated that LINC00707 directly and negatively regulated miR-145.

LINC00707 Regulated LRP5 by Sponging miR-145 in hBMSCs

Likewise, the LRP5 3'UTR contained the binding site of miR-145 by TargetScan (Figure 5A). The luciferase activity of the reporter vector transfected with LRP5 3'UTR-WT was decreased, confirming the interaction of LRP5 and miR-145 (Figure 5B). Interestingly, the expression level of LRP5 was downregulated in hBMSCs transfected with miR-145, whereas when the miR-145 was inhibited, the expression level of LRP5 was upregulated in hBMSCs (Figure 5C and D). Meanwhile, qRT-PCR and Western blot indicated that the expression of LRP5 was upregulated in hBMSCs after seven days osteogenic induction (Figure 5E and F). To further explore the function of LRP5, the sh-LRP5 or sh-LRP5 + pLINC00707 were constructed and transfected into hBMSCs. The results indicated that the downregulated LRP5 expression inhibited the ALP activity of hBMSCs, while overexpressed LINC00707 reversed the effect of LRP5 downregulation on ALP activity of hBMSCs (Figure 6A). Moreover, qRT-PCR and Western blot showed that the expression levels of ALP, OCN, Runx2 and OSX were suppressed in hBMSCs transfected with sh-LRP5, whereas LINC00707 overexpression reversed the inhibitory effects of sh-LRP5 (Figure 6B-J). The data demonstrated that LINC00707 regulated the functions of LRP5 by sponging miR-145 in hBMSCs.

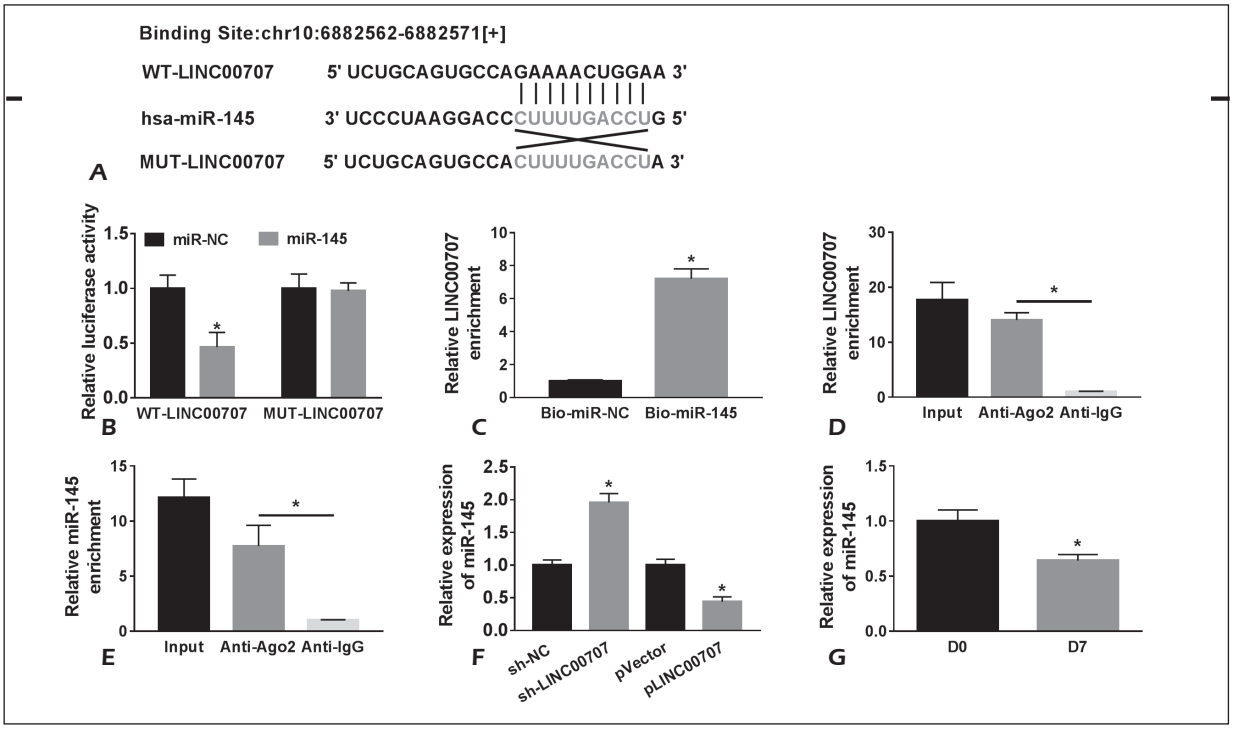

Figure 3. LINC00707 negatively regulated the expression of miR-145 by directly targeting to miR-145. **A**, The binding site between LINC00707 and miR-145 was predicted by online software starBase v2.0. **B**, Luciferase reporter assay was applied to measure the luciferase activity of reporter vectors transfected with WT-LINC00707 and MUT-LINC00707. **C**, RNA pull-down was used to confirm the directly interact relationship of LINC00707 and miR-145. (**D**-**E**) RIP assay was conducted to verify the interaction between LINC00707 and miR-145 (**F**) The expression level of miR-145 in hBMSCs transfected with sh-LINC00707 and pLINC00707 was detected by qRT-PCR. (**G**) The expression level of miR-145 in hBMSCs after 7 days osteogenic induction. *p<0.05.

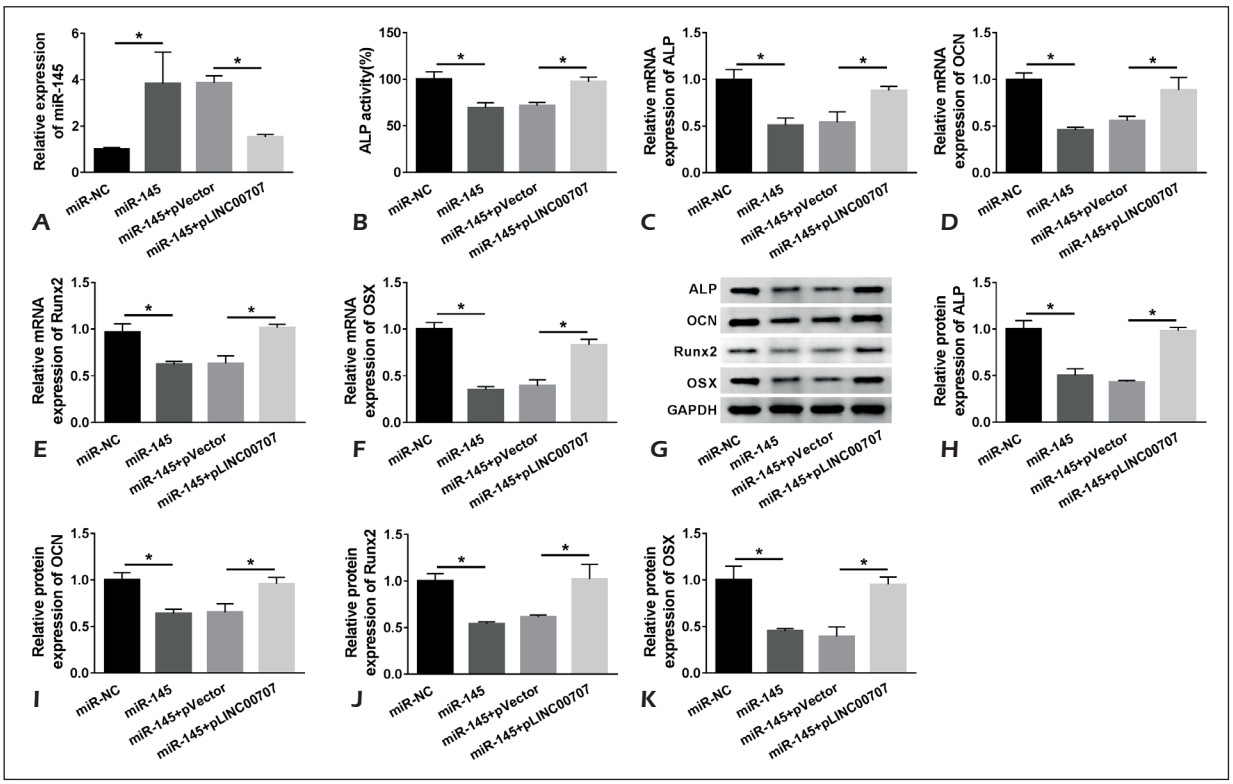

Figure 4. LINC00707 inhibited the effects of miR-145 on osteogenic differentiation of hBMSCs. **A**, The expression level of miR-145 in hBMSCs transfected with miR-145 or miR-145 + pLINC00707 was detected by qRT-PCR. **B**, The ALP activity in hBMSCs transfected with miR-145 or miR-145 + pLINC00707 was detected by ALP activity assay. **C**-**F**, The mRNA expression levels of osteogenesis-related genes (ALP, OCN, Runx2 and OSX) in hBMSCs transfected with miR-145 or miR-145 + pLINC00707 were detected by qRT-PCR. **G**-**K**, Western blot was performed to detect the protein levels of osteogenesis-related genes (ALP, OCN, Runx2 and OSX) in hBMSCs transfected with miR-145 or miR-145 + pLINC00707. *p<0.05.

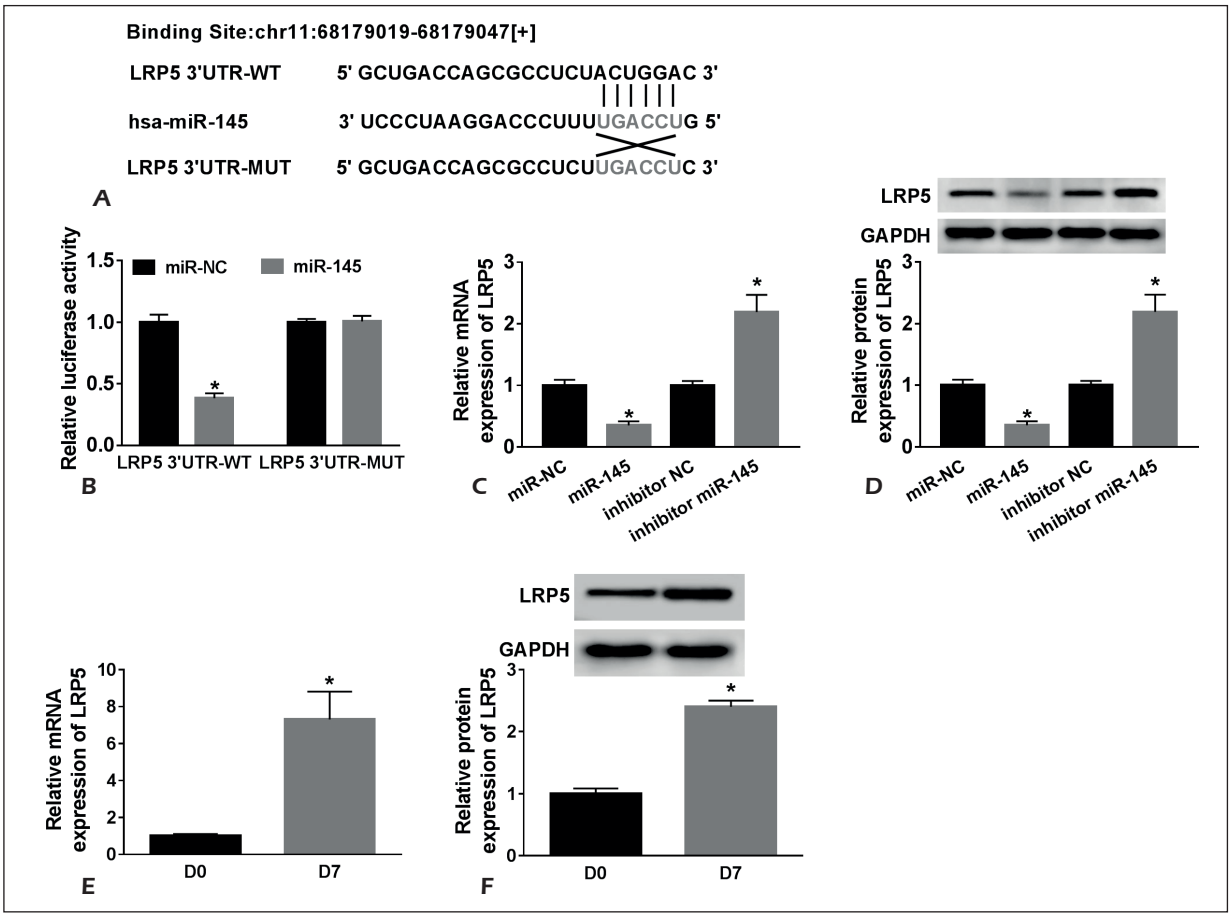

Figure 5. MiR-145 targeted LRP5 and inhibited the expression of LRP5 in hBMSCs. **A**, The binding site between miR-145 and LRP5 was predicted through TargetScan. **B**, The luciferase activity of reporter vectors transfected with LRP5 3'UTR-WT and LRP5 3'UTR-MUT was detected by Luciferase reporter assay. **C-D**, qRT-PCR and Western blot were conducted to test the expression level of LRP5 in hBMSCs transfected with miR-145 and inhibitor miR-145. **E-F**, The mRNA and protein levels of LRP5 were detected by qRT-PCR and Western blot in hBMSCs after 7 days osteogenic induction. *p<0.05.

LINC00707 Promoted the Wnt/β-Catenin Pathway via LINC00707/miR-145/LRP5 Axis

We also aimed to further explore the regulatory mechanism of LINC00707 in osteogenic differentiation of hBMSCs. Western blot was employed to evaluate the expression levels of LRP5 and the Wnt/β-catenin pathway related-proteins β-catenin and Axin2 in hBMSCs transfected with pLINC00707, pLINC00707 + miR-145 or pLINC00707 + sh-LRP5. The results showed that the expression levels of LRP5, β-catenin and Axin2 were upregulated in hBMSCs transfected with pLINC00707, while the effects of LINC00707 overexpression were abolished when hBMSCs were transfected with pLINC00707 + miR-145 or pLINC00707 + sh-LRP5 (Figure 7A-D). These data indicated that LINC00707 promoted the Wnt/β-catenin pathway by LINC00707/miR-145/LRP5 pathway.

Discussion

Osteogenic differentiation of hBMSCs is critical in the treatment of bone diseases, such as femoral head necrosis. Recently, many studies focused on obtaining better treatment options for bone diseases. In the clinical, stem cell homing had great potential for the treatment of many inflammatory and degenerative diseases. Studies have reported that MAPK, PI3K-Akt and Jak/Stat signaling pathways were involved in the activation of hBMSCs homing[25]. The osteogenic differentiation of hBMSCs on the novel Chitosan-PBS (Ch-PBS) biodegradable scaffold provides valid evidence for the development of bone tissue therapy[26].

LncRNA has been reported in the treatment of femoral head necrosis and its abnormal expression is associated with fat increased and decreased osteogenic differentiation[9].

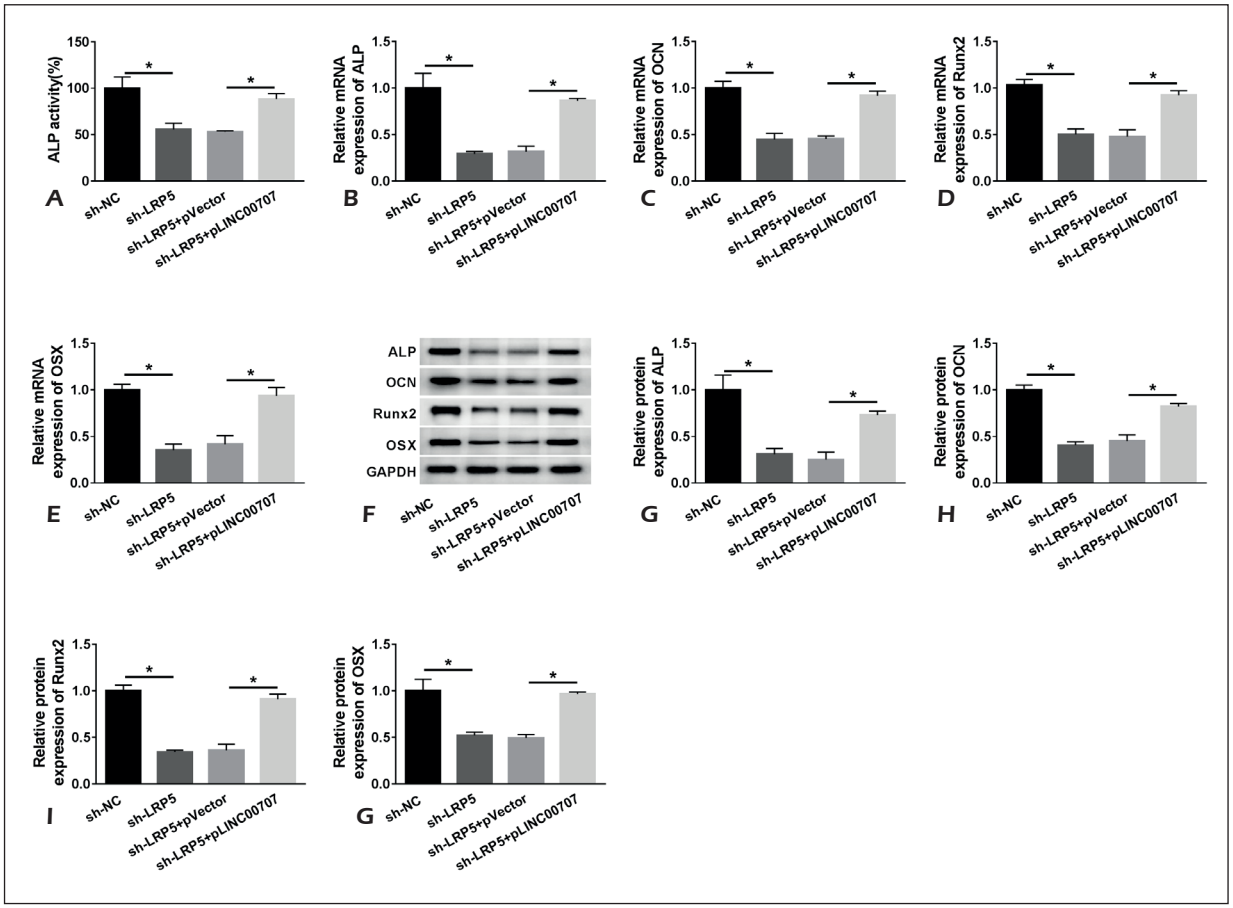

Figure 6. Overexpression of LINC00707 reversed the effects of LRP5 knockdown on osteogenic differentiation of hBMSCs. **A**, ALP activity assay was used to detect the ALP activity of hBMSCs transfected with sh-LRP5 or sh-LRP5 + pLINC00707. **B**-**E**, The mRNA expression levels of osteogenesis-related genes (ALP, OCN, Runx2 and OSX) in hBMSCs transfected with sh-LRP5 or sh-LRP5 + pLINC00707 were detected by qRT-PCR. **F**-**J**, Western blot was used to detect the protein levels of osteogenesis-related genes (ALP, OCN, Runx2 and OSX) in hBMSCs transfected with sh-LRP5 or sh-LRP5 + pLINC00707. *$p<0.05$.

In our study, the abnormal expression of lncRNA LINC00707 during osteogenic differentiation of hBMSCs was detected. A previous study showed that the expression of ALP and OCN could be used to evaluate the proliferation of osteoblasts and the differentiation of early/late osteoblasts[27] Runx2 and OSX were often considered as markers of early osteogenic differentiation[28]. In our study, LINC00707 overexpression increased the expression levels of ALP, OCN, Runx2 and OSX, which suggested that the LINC00707 promoted osteogenic differentiation. Long noncoding RNAs regulating osteogenic differentiation of hBMSCs had been reported by previous studies, such as LncRNA NEAT1[29] and LncRNA KCNQ1OT1[30]. LINC00707 was also reported to be downregulated and inhibit the ability of hBMSCs osteogenic differentiation by targeting miR-370-3p[31]. In the present report, we found that miR-145 was downregulated during osteogenesis of hBMSCs, and miR-145 directly interacted with LINC00707, whereas LINC00707 negatively regulated the functions of miR-145. Liu et al[32] reported miR-145-5p inhibited the osteogenesis of adipose-derived stem cells (hADSCs). miR-145-5p was documented to suppress the osteogenesis of hADSCs by regulating the Toll-like receptor 4 (TLR4) pathway[33]. Besides, miR-145 was reported to derepress the Wnt/β-catenin pathway activator ZEB2 and then suppress the osteogenic differentiation of mesenchymal stem cells (MSCs)[15]. The Wnt/β-catenin pathway associated with the osteogenic differentiation of hBMSCs has also been reported[34,35]. In our study, how LINC00707 regulated LRP5 by

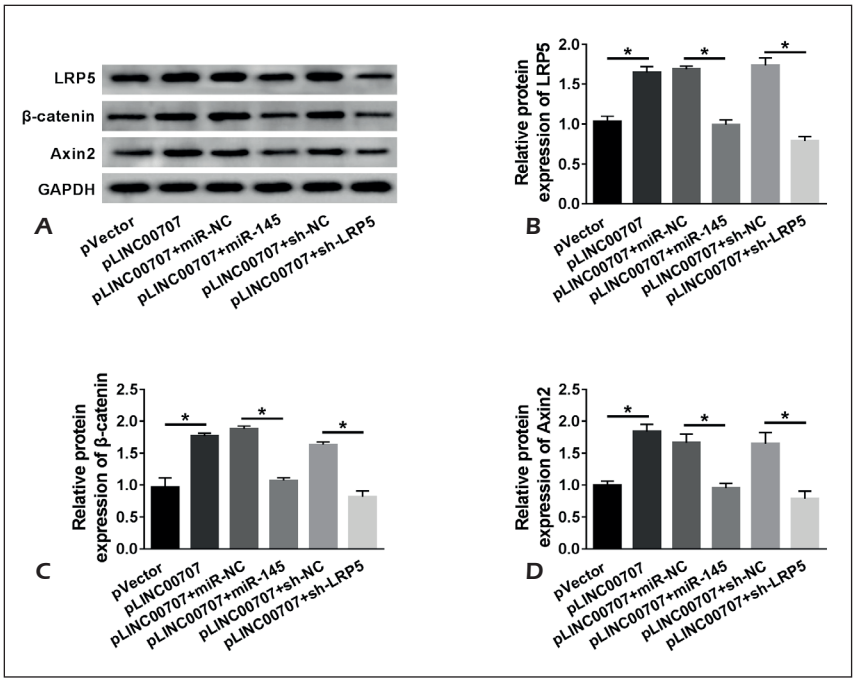

Figure 7. LINC00707 regulated the Wnt/β-catenin pathway by affecting LRP5. **A-D**, The protein expression levels of LRP5, β-catenin and Axin2 in hBMSCs transfected with pLINC00707, pLINC00707 + miR-145 or pLINC00707 + sh-LRP5 were detected by Western blot. *$p<0.05$.

sponging miR-145 was first researched. It has been shown that the gene missense mutations of LRP5 altered the anabolic response of bone to mechanical load, thereby affecting bone density and leading to osteoporosis[36]. LRP5 was a receptor of the Wnt/β-catenin pathway and LRP5 silencing inhibited the osteogenic differentiation through the Wnt/β-catenin pathway[37]. Two previous researches[38,39] suggested that Axin2 regulated ubiquitination of β-catenin and was the target of Wnt/β-catenin signaling. Interestingly, our results demonstrated that LINC00707 affected the expression of the β-catenin and the Axin2 through regulating LRP5.

Conclusions

We demonstrate that LINC00707 could regulate LRP5 expression by acting as the sponge of miR-145. Importantly, LINC00707 promoted the osteogenic differentiation of hBMSCs by activating the LRP5 and Wnt/β-catenin pathway, providing evidence of LINC00707 as the new potential RNA therapeutic target for bone disease.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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