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Long non-coding RNA HULC promotes proliferation and osteogenic differentiation of bone mesenchymal stem cells via down-regulation of miR-195

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Abstract. – OBJECTIVE: LncRNAs HULC has been reported to be important regulators in the development of various human diseases. However, the role of HULC in bone mesenchymal stem cells (BMSCs) remains unclear. The present study aimed to explore the regulatory effect of HULC on proliferation and osteogenic differentiation of BMSCs and the underlying mechanism.

MATERIALS AND METHODS: The expression of HULC and miR-195 in BMSCs were altered by transfection and measured by qRT-PCR. Cell viability was measured by the CCK-8 assay. Osteogenic differentiation of BMSCs was determined by evaluation of osteogenic markers (Ocn, ALP, Runx2, and Col-1) expression levels using Western blot and qRT-PCR. Furthermore, Western blot was performed to assess the expression of proliferation-related factors, Wnt/β-catenin and p38MAPK pathway-related factors.

RESULTS: HULC overexpression significantly increased cell viability, down-regulated p21 expression but up-regulated CyclinD1 expression, and promoted the levels of osteogenic markers. However, the complete opposite effect was observed in HULC knockdown. Notably, miR-195 expression was negatively regulated by HULC and miR-195 exerted a reversed effect of HULC on BMSCs. Moreover, miR-195 mediated the regulatory effect of HULC on BMSCs proliferation and osteogenic differentiation, as miR-195 mimic abolished the effect of HULC overexpression on BMSCs. We also found that HULC overexpression enhanced the activation of Wnt/β-catenin and p38MAPK pathway through down-regulating miR-195.

CONCLUSIONS: We revealed that HULC promoted proliferation and osteogenic differentiation of BMSCs. The potential mechanism might be involved in its negative regulation on miR-195 and enhanced activation of Wnt/β-catenin and p38MAPK pathway.

Key Words:
HULC, Proliferation, Osteogenic differentiation, miR-195, BMSCs.

Introduction

Bone mesenchymal stem cells (BMSCs), derived from the early development of mesoderm and ectoderm, are important members of the stem cell family[1]. BMSCs are the source of osteoblasts in bone tissue, and their number and physiological status directly affect the number and function of osteoblasts[2]. Meanwhile, BMSCs possess the ability of self-renewal and multipotent differentiation and play a critical role in bone homeostasis and regeneration possess[3]. BMSCs can also differentiate into other various cells including chondrocytes and adipogenic cells and it can be widely used in the therapy of various diseases[4-6].

Long non-coding RNAs (lncRNAs) are non-protein-coding RNAs with longer than 200 nucleotides in length, which have been identified as key regulators of various biological processes including cell survival, proliferation, differentiation, apoptosis, and transcriptional regulation[7-9]. Scholars[10-12] suggested that various diseases re-

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sulting from aberrant cellular regulation, such as neurological, autoimmune, cardiovascular conditions, and cancer are frequently associated with ectopic expression levels of lncRNAs. It has been reported[13,14] that lncRNAs play significant roles in the proliferation and differentiation of BMSCs. For example, lncRNA low expression in tumor (LET) has been demonstrated to be down-regulated in rapidly proliferated BMSCs and suppression of LET potentiated BMSCs proliferation by up-regulation of TGF-β1[15]. Meanwhile, lncRNA hypoxia-inducible factor 1α-anti-sense 1 (HIF1α-AS1) which was induced by transforming growth factor-β-mediated targeting of sirtuin 1, promoted osteoblastic differentiation of BMSCs[16]. Hepatocellular carcinoma up-regulated long non-coding RNA (HULC) was first identified from a hepatocellular carcinoma-specific gene expression profiling[17]. HULC has been reported[18-20] to play a significant role in cell proliferation, apoptosis, and metastasis in human cancers, such as gastric cancer, hepatocellular carcinoma, and pancreatic cancer. However, the role of HULC in BMSCs has not been fully reported yet.

The present study aimed to explore the effect of HULC on proliferation and osteogenic differentiation of BMSCs and the underlying mechanism. We found that HULC promoted the proliferation and osteogenic differentiation of BMSCs. Moreover, we also found that microRNA-195 (miR-195) expression was negatively regulated by HULC and miR-195 showed an opposite effect of HULC on BMSCs proliferation and osteogenic differentiation. We demonstrated that HULC exerted its role in BMSCs through down-regulation of miR-195s, which might provide new clues for the treatment of bone injuries-related diseases.

Methods and Materials

Animals and Ethics Statements

Sprague-Dawley (SD) rats (80-120 g) were purchased from the Animal Experiment Center of Yangzhou University (Yangzhou, China). Animals were housed in a 12 h light/dark cycle, with free access to food and water. All experimental procedures and protocols were followed Chinese Council on Animal Care Guidelines, and approved by the Animal Ethical Committee of Yangzhou University. Efforts were made to minimize numbers and suffering of animals used.

Generation of Rat Bone Marrow-Derived MSCs

Briefly, rats were sacrificed and the hindlimbs were aseptically dissected out and bones were dissected free of soft tissues. Then, the femurs and tibias of the rats were removed and both ends of the bones were cut off. The marrow cavities of femur and tibia were flushed with culture medium using a 25-gauge needle. The culture medium consisted of Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12; Gibco, Grand Island, NY, USA), 10% fetal bovine serum (FBS; Gibco), 1% penicillin and streptomycin (Gibco). The cells obtained were suspended in culture medium and seeded into 10 cm^2 culture flasks, and incubated in a humidified atmosphere of 5% CO_2 at 37°C. After incubating for 3 days, non-adherent cells were removed by a frequent medium change. The remaining adherent cells (primary BMSCs) were passaged after digestion using 0.25% trypsin (Beyotime Biotechnology, Shanghai, China). Cells at passage 3 were used in our experiments.

For induction of osteogenic differentiation of BMSCs, cells were cultured in osteogenic medium (OS medium), which consisted of normal culture medium supplemented with dexamethasone (100 nM) and β-glycerophosphate (2 mM) (both purchased from Sigma-Aldrich, St Louis, MO, USA), until they reached the 70-80% influence for 7 and 14 days. The medium was changed every 3 days.

Cell Transfection

The full length-HULC sequences and shRNA directed against HULC were respectively constructed in pcDNA3.1 and U6/GFP/Neo plasmids (GenePharma, Shanghai, China), which were referred as to pc-HULC and sh-HULC. The plasmids carrying non-targeting sequences were used as the negative control (NC) of pc-HULC and sh-NC, which were referred as to pcDNA3.1 and sh-NC. Cell transfections were conducted using lipofectamine 3000 reagent (Life Technologies Corporation, Carlsbad, CA, USA) following the manufacturer's protocol. The stably transfected cells were selected by the culture medium containing 0.5 mg/ml G418 (Sigma-Aldrich, St Louis, MO, USA). After approximately 4 weeks, G418-resistant cell clones were established.

For miR-195 transfection, miR-195 mimic, miR-195 inhibitor and NC were synthesized (Life Technologies Corporation) and transfected into BMSCs using lipofectamine 3000 reagent (Life Technologies Corporation) on the basis of manu-

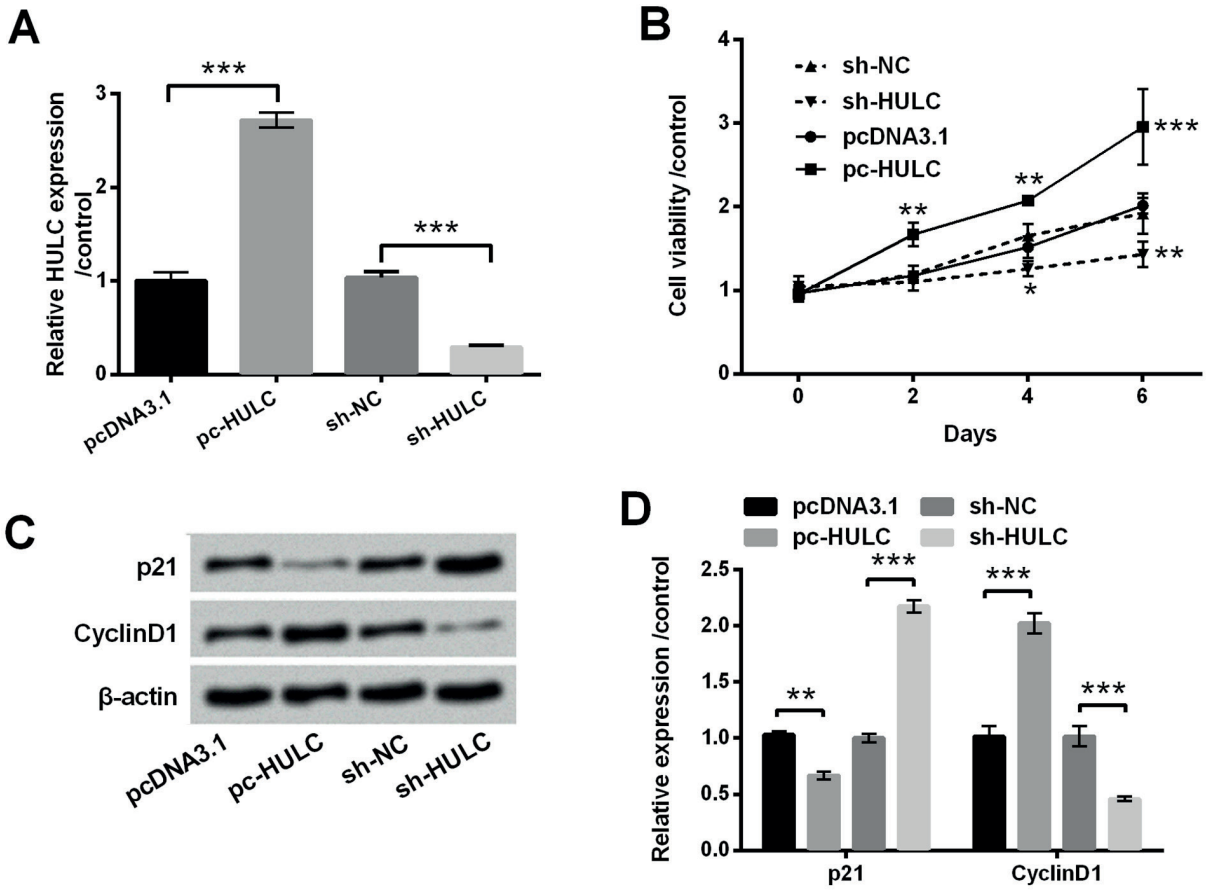

Figure 1. HULC promoted the proliferation of BMSCs. BMSCs were transfected with pc-HULC, sh-HULC or their corresponding controls, *i.e.*, pcDNA3.1 and sh-NC. ***A,*** The transfected efficiency was verified by qRT-PCR. ***B,*** Relative cell viability was measured by CCK-8 assay. ***C,*** The protein immunoblots of proliferation-related factors by Western blot assay. ***D,*** Relative expression levels of p21 and CyclinD1. The β-actin acted as an internal control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

facture's manner. After transfection for 48 h, cells were collected for further investigation.

Cell Viability Assay

Cell viability was assessed by Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Gaithersburg, MD, USA) assay. Briefly, BMSCs were seeded onto 96-well plates at a density of 1×10^4 cells/well and were incubated for 24 h. At the end of each culture period, 10 μl CCK-8 solution was added to each well and the cultures were incubated for another 1 h at 37°C in humidified 95% air and 5% CO_2. The absorbance was measured at a wavelength of 450 nm using a Microplate Reader (Bio-Rad, Hercules, CA, USA).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA of cells was extracted by using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The One Step SYBR®PrimeScript® PLUS RT-RNA PCR Kit (TaKaRa, Dalian, China) was used for the qRT-PCR analysis to evaluate the expression level of HULC. For the analysis of mRNAs expression levels, reverse transcription was performed using RNA PCR Kit (AMV) Ver.3.0 (TaKaRa), and qPCR analysis was performed using SYBR®Premix Ex TaqTM II (TaKaRa). For the test of miR-195, reverse transcription was performed using TaqMan MicroRNA Reverse Transcription Kit and the followed RT-PCR was performed with Taqman Universal Master Mix II according to the manufacturer's protocols (both from Applied Biosystems, Foster City, CA, USA). Relative expressions of HULC, mRNAs, and miR-195 were calculated using the $2^{-\cdots CT}$ method[21]. The GAPGH was used for normalization of HULC and mRNAs, and the U6 was used for miR-195.

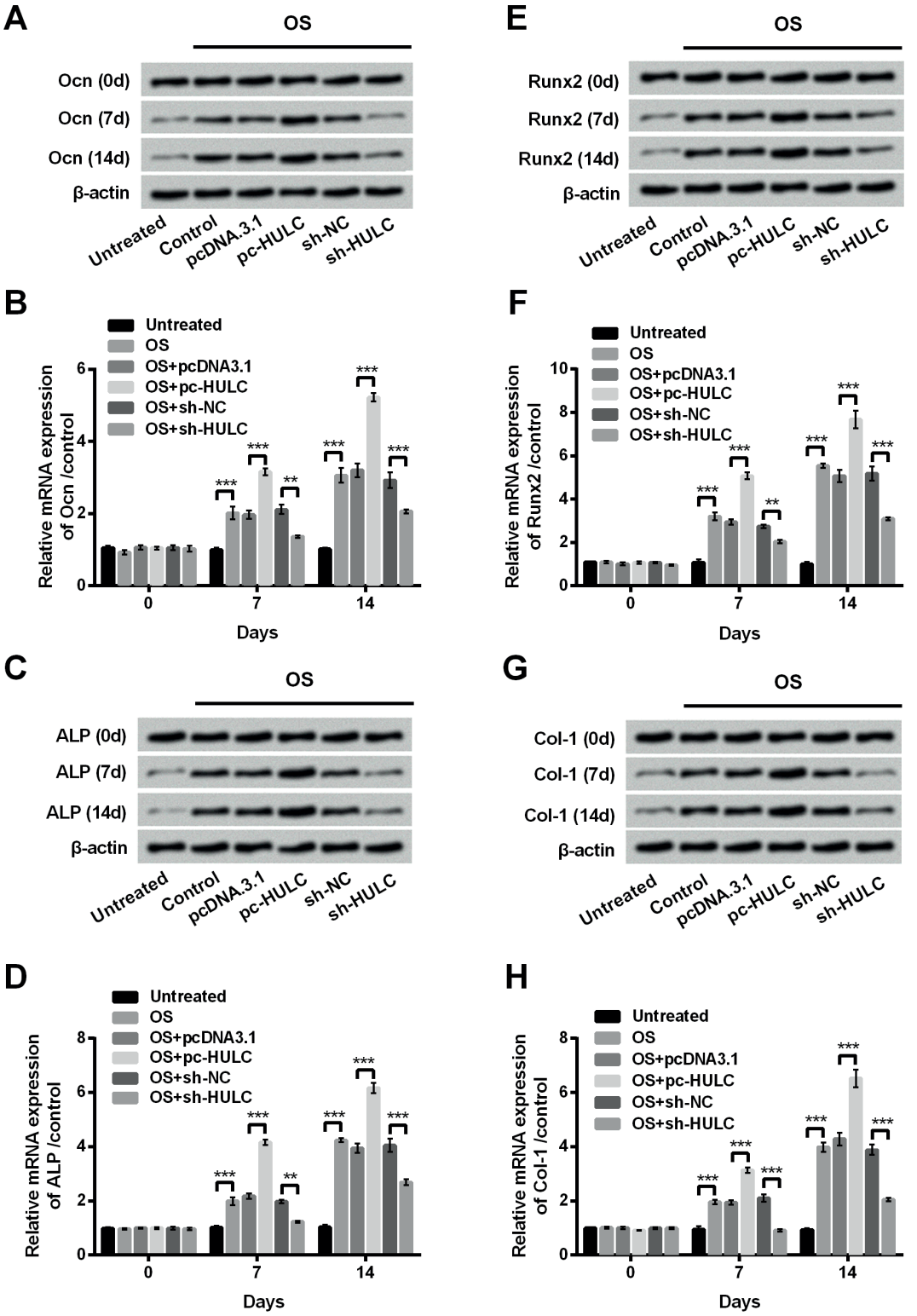

Figure 2. HULC promoted the osteogenic differentiation of BMSCs. BMSCs were transfected with pc-HULC, sh-HULC or their corresponding controls, *i.e.*, pcDNA3.1 and sh-NC. The transfected and non-transfected cells were cultured in OS medium for 7 and 14 days. Then, the ***(A)*** protein and (**B**) mRNA expression of Ocn was determined by Western blot and qRT-PCR. The ***(C)*** protein and ***(D)*** mRNA expression of ALP was determined by Western blot and qRT-PCR. The ***(E)*** protein and (**F**) mRNA expression of Runx2 was determined by Western blot and qRT-PCR. The ***(G)*** protein and ***(H)*** mRNA expression of Col-1 was determined by Western blot and qRT-PCR. $^{**}p < 0.01$, $^{***}p < 0.001$.

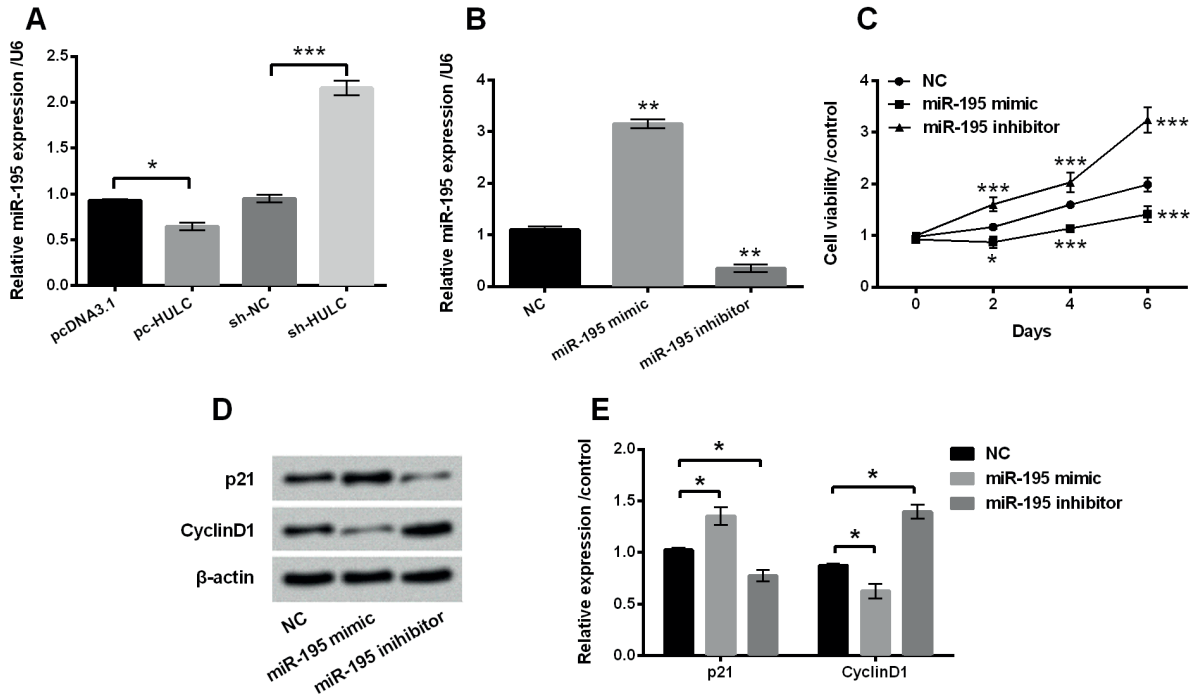

Figure 3. miR-195 expression was negatively regulated by HULC and miR-195 inhibited the proliferation of BMSCs. BMSCs were transfected with pc-HULC, sh-HULC or their corresponding controls, *i.e.*, pcDNA3.1 and sh-NC. ***A,*** The expression of miR-195 was measured by qRT-PCR. BMSCs were transfected with miR-195 mimic, miR-195 inhibitor and NC. ***B,*** Then, the transfected efficiency was verified by qRT-PCR. ***C,*** Relative cell viability was measured by CCK-8 assay. ***D,*** The protein immunoblots of proliferation-related factors by Western blot assay. ***E,*** Relative expression levels of p21 and CyclinD1. The β-actin acted as an internal control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Western Blot Analysis

The proteins of BMSCs were extracted by using lysis buffer (Beyotime Biotechnology, Shanghai, China) and supplemented with (Roche, Basel, Switzerland). BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). They were used for quantification of protein samples. An equal amount of samples (30 μg) were separated by sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore Corporation, Billerica, MA, USA). Next, the PVDF membranes were blocked with 5% bovine serum albumin (BSA; Roche) for 1 h at room temperature. Then, the blocked membranes were incubated with corresponding primary antibodies, which were prepared in 5% BSA at a dilution of 1:1000, at 4°C overnight, followed by the incubation with secondary antibody marked by horseradish peroxidase (HRP) for 1 h at room temperature. The signals were captured and analyzed using Image Lab™ software (Bio-Rad). The primary antibodies used in the study were as follows: p21 (ab109199), CyclinD1 (ab134175), osteocalcin (Ocn; ab13418), alkaline phosphatase (ALP; ab16695), Runt-related transcription factor (Runx2; ab23981), collagen type-1 (Col-1; ab34710), Wnt-5a (ab72583) Wnt-3a (ab28472), β-catenin (ab32572); they were purchased from Abcam (Cambridge, MA, USA); β-actin (#4970), p-p38MAPK (#9211) and t-p38MAPK (#9212) were purchased from Cell Signaling Technology (Danvers, MA, USA).

Statistical Analysis

All experiments were repeated three times. The results of multiple experiments are presented as the mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). The *p*-values were calculated using a one-way analysis of variance (ANOVA). *p*-value of <0.05 was considered to indicate a statistically significant result.

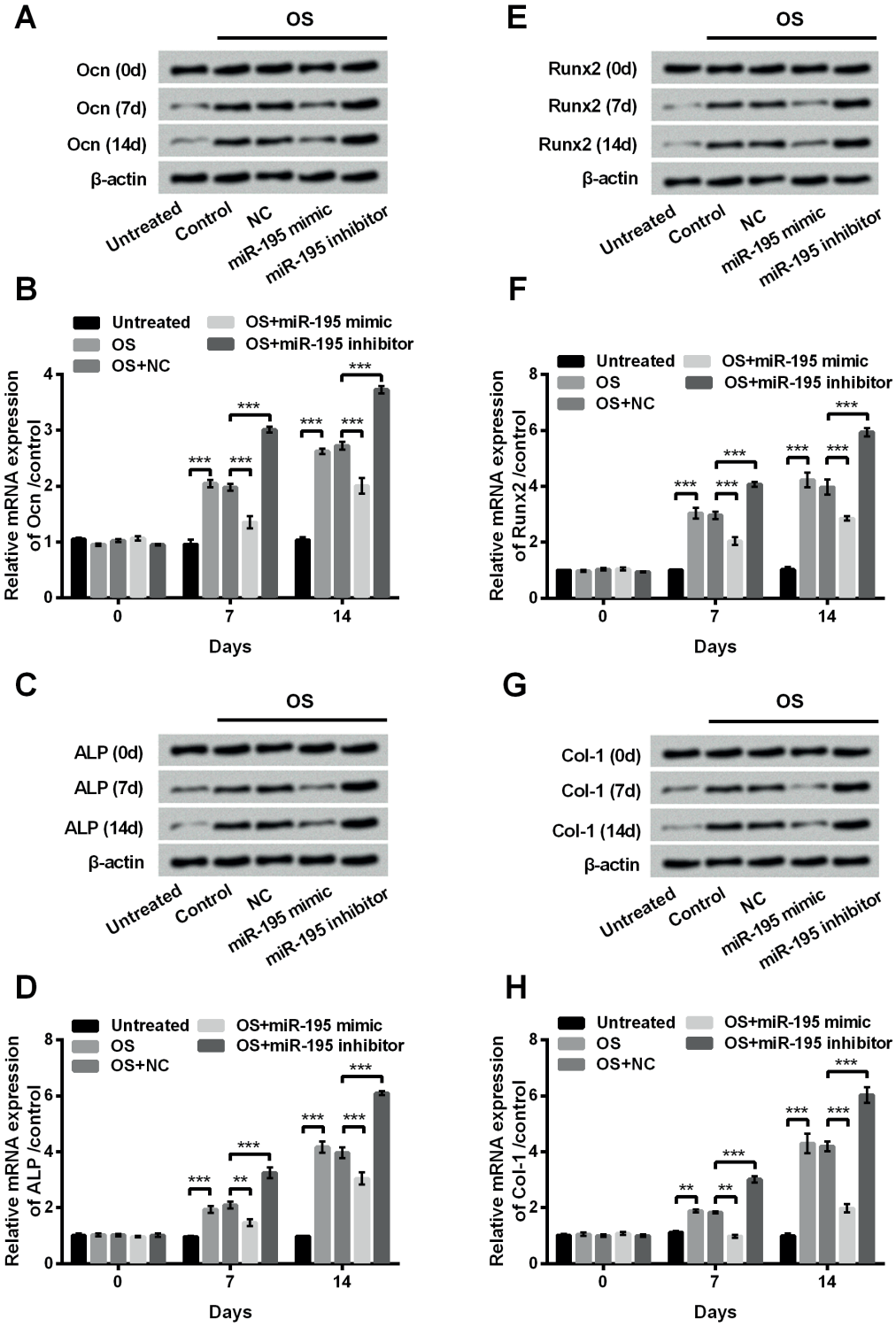

Figure 4. miR-195 inhibited the osteogenic differentiation of BMSCs. BMSCs were transfected with miR-195 mimic, miR-195 inhibitor and NC. The transfected and non-transfected cells were cultured in OS medium for 7 and 14 days. Then, the ***(A)*** protein and ***(B)*** mRNA expression of Ocn was determined by Western blot and qRT-PCR. The ***(C)*** protein and ***(D)*** mRNA expression of ALP was determined by Western blot and qRT-PCR. The ***(E)*** protein and ***(F)*** mRNA expression of Runx2 was determined by Western blot and qRT-PCR. The ***(G)*** protein and ***(H)*** mRNA expression of Col-1 was determined by Western blot and qRT-PCR. $^{**}p < 0.01$, $^{***}p < 0.001$.

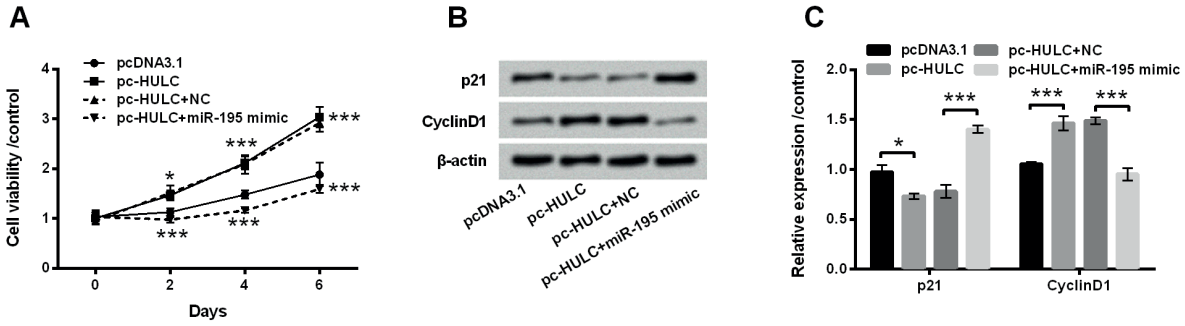

Figure 5. miR-195 mimic reversed the effect of HULC overexpression on the proliferation of BMSCs. BMSCs were transfected with pc-HULC, or co-transfected with miR-195 mimic and pc-HULC. ***A,*** Relative cell viability was measured by CCK-8 assay. ***B,*** The protein immunoblots of proliferation-related factors by Western blot assay. ***C,*** Relative expression levels of p21 and CyclinD1. The β-actin acted as an internal control. *$p < 0.05$, ***$p < 0.001$.

Results

HULC Enhanced the Proliferation and Osteogenic Differentiation of BMSCs

To investigate the function of HULC in cell proliferation and osteogenic differentiation of BMSCs, BMSCs were transfected with pc-HULC or sh-HULC to overexpress or knockdown the expression of HULC. The efficiency of transfection was verified by qRT-PCR. As expected, HULC expression was significantly up-regulated by pc-HULC transfection but was down-regulated by sh-HULC transfection (Figure 1A; $p<0.001$). Results in Figure 1B showed that HULC overexpression significantly increased cell viability while HULC knockdown reduced cell viability of BMSCs ($p<0.05$, $p<0.01$, or $p<0.001$). The expression levels of proliferation-related core factors were also assessed by Western blot. As shown in Figure 1C and 1D, HULC overexpression markedly inhibited the expression of p21 but enhanced the expression of CyclinD1 ($p<0.01$, or $p<0.001$). On the contrary, knockdown of HULC increased the expression of p21 and reduced CyclinD1 expression ($p<0.001$). These results suggested that HULC enhanced the proliferation of BMSCs.

We further assessed the effect of HULC on osteogenic differentiation of BMSCs. BMSCs were cultured in an OS medium containing β-glycerophosphate and dexamethasone, which were widely used in *in vitro* osteogenesis model[22]. After osteogenic induction, BMSCs highly expressed osteogenic markers including Ocn, ALP, Runx2, and Col-1 at 7 days and 14 days (Figure 2A-2F; $p<0.001$). Of note, overexpression of HULC further accelerated the protein and mRNA expression of those four osteogenic markers while inhibition of HULC suppressed the up-regulated expressions of those four factors induced by osteogenic induction ($p<0.01$, or $p<0.001$), suggesting that HULC promoted osteogenic differentiation of BMSCs. Above, these results indicated that HULC enhanced the proliferation and osteogenic differentiation of BMSCs.

HULC Negatively Regulated the Expression of miR-195 in BMSCs

As shown in Figure 3A, we found that the expression of miR-195 was negatively regulated by HULC in BMSCs, as there was a significant decrease of miR-195 expression observed in HULC-overexpressing cells and an increase of miR-195 expression observed in HULC-silencing cells ($p<0.05$, or $p<0.001$).

miR-195 Suppressed Cell Proliferation and Osteogenic Differentiation of BMSCs

Next, BMSCs were transfected with miR-195 inhibitor, or miR-195 mimic to investigate the role of miR-195 in cell proliferation and osteogenic differentiation. The transfected efficiency was identified by qRT-PCR and the results indicated that the expression of miR-195 was significantly enhanced by miR-195 mimic transfection while suppressed by a miR-195 inhibitor (Figure 3B; $p<0.01$). Results in Figure 3C showed that over-

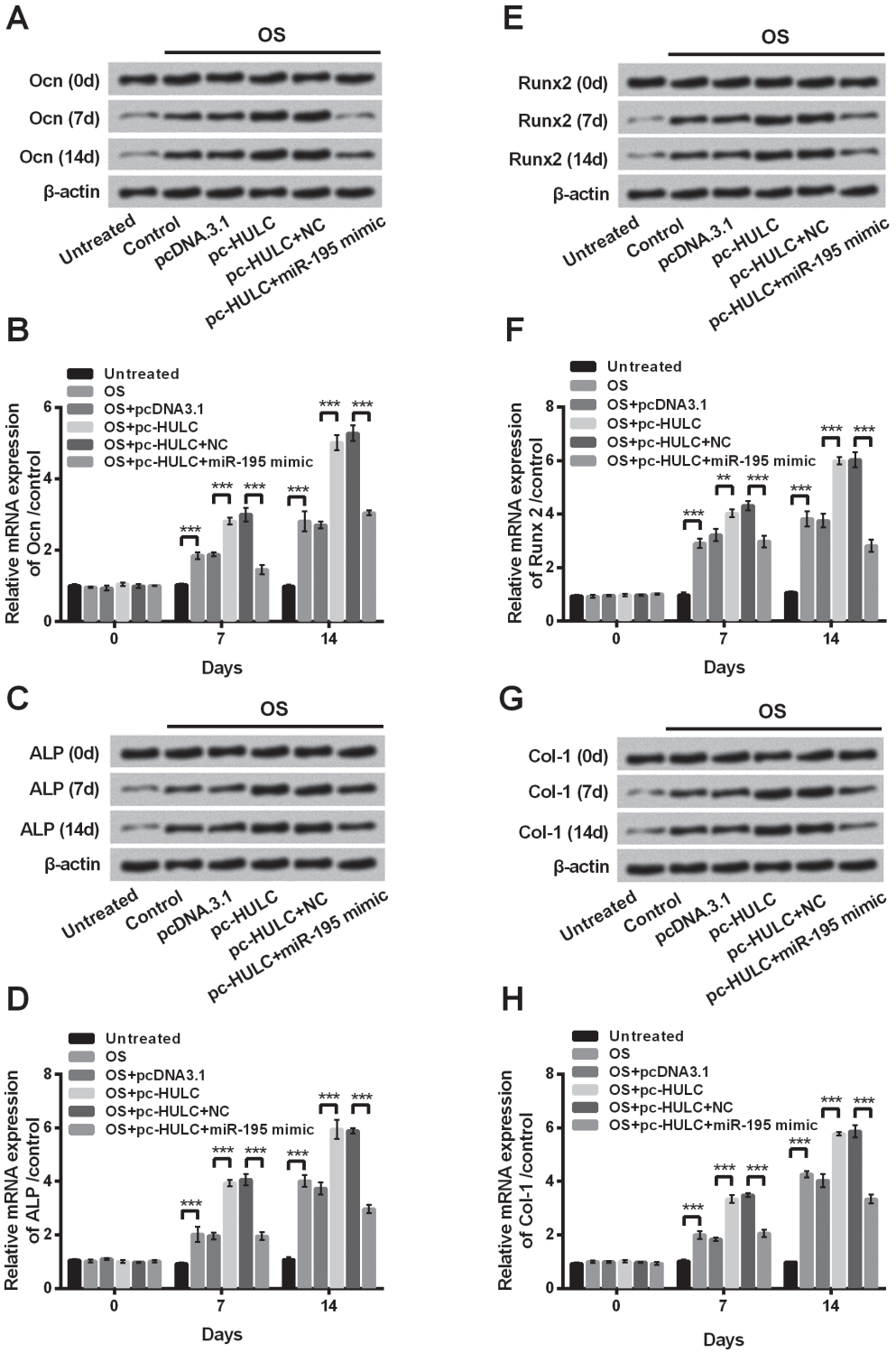

Figure 6. miR-195 mimic reversed the effect of HULC overexpression on the osteogenic differentiation of BMSCs. BMSCs were transfected with pc-HULC, or co-transfected with miR-195 mimic and pc-HULC. The transfected and non-transfected cells were cultured in OS medium for 7 and 14 days. Then, the (**A**) protein and (**B**) mRNA expression of Ocn was determined by Western blot and qRT-PCR. The (**C**) protein and (**D**) mRNA expression of ALP was determined by Western blot and qRT-PCR. The (**E**) protein and (**F**) mRNA expression of Runx2 was determined by Western blot and qRT-PCR. The (**G**) protein and (H) mRNA expression of Col-1 was determined by Western blot and qRT-PCR. ***$p < 0.001$.

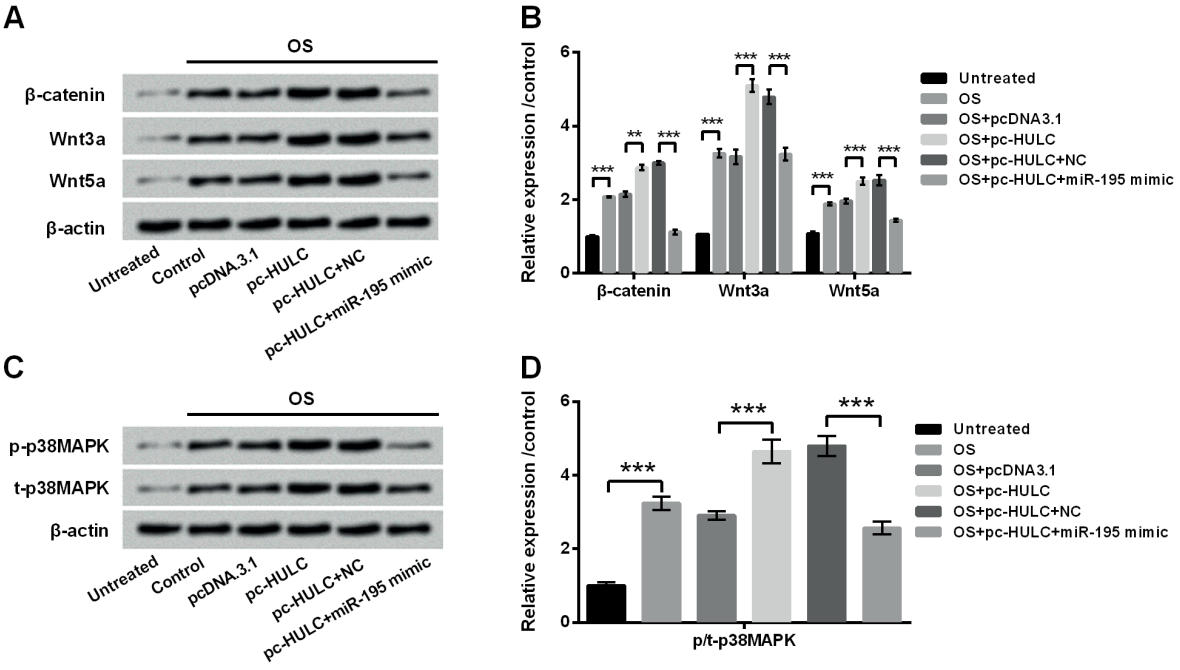

Figure 7. HULC overexpression enhanced the activation of Wnt/β-catenin and p38MAPK signaling pathway through down-regulation of miR-195. BMSCs were transfected with pc-HULC, or co-transfected with miR-195 mimic and pc-HULC. The transfected and non-transfected cells were cultured in OS medium for 14 days. ***A,*** The protein immunoblots of Wnt/β-catenin pathway-related factors by Western blot assay. ***B,*** Relative expression levels of β-catenin, Wnt3a and Wnt5a. The β-actin acted as an internal control. ***C,*** The protein immunoblots of p38MAPK pathway-related factors by Western blot assay. ***D,*** Relative expression levels of p-p38MAPK and t-p38MAPK. The β-actin acted as an internal control. $^{**}p < 0.01$, $^{***}p < 0.001$.

expression of miR-195 significantly reduced the viability of BMSCs and knockdown of miR-195 increased cell viability ($p<0.05$ or $p<0.001$). Similar results were observed in the protein expression levels of proliferation-related factors, which showed that miR-195 overexpression markedly up-regulated p21 expression and down-regulated CyclinD1 levels, while miR-195 inhibitor exerted an opposite effect (Figure 3D and 3E; $p<0.05$).

We also assessed the effect of miR-195 on expression levels of osteogenic markers by qRT-PCR and Western blot. Results in Figure 4A and 4B showed that both protein and mRNA expression of Ocn was down-regulated by miR-195 mimic while was further up-regulated by miR-195 inhibitor in OS-treated BMSCs at 7 days and 14 days ($p<0.001$). Similar data were found in the expression levels of another three osteogenic markers, which showed that miR-195 mimic significantly inhibited the protein and mRNA levels of ALP, Runx2 and Col-1, while miR-195 inhibitor enhanced the expression levels of these three factors (Figure 4C-4H; $p<0.01$ or $p<0.001$). Generally, these findings suggested that miR-195 might possess a reversed role of HULC in cell proliferation and osteogenic differentiation of BMSCs.

HULC Accelerated Cell Proliferation and Osteogenic Differentiation of BMSCs Through Inhibition of miR-195

According to the above results, we hypothesized that miR-195 was associated with the regulatory effect of HULC on proliferation and osteogenic differentiation of BMSCs. BMSCs were transfected with pc-HULC or co-transfected with pc-HULC and miR-195 mimic, and then, cell viability, proliferation-related factors expressions and osteogenic markers expressions were assessed. The CCK-8 assay analysis showed that the increased viability of BMSCs induced by HULC overexpression was abolished by miR-195 mimic, as a significant decrease of viability was observed in the pc-HULC + miR-195 mimic group compared with those in the pc-HULC + NC group (Figure 5A; $p<0.05$ or $p<0.001$). As shown in Figure 5B and 5C, miR-195 mimic significantly enhanced the protein expression of p21 but suppressed the expression of CyclinD1 in

HULC-overexpressing cells ($p<0.05$ or $p<0.001$).

We also found that miR-195 mimic reversed the effect of HULC overexpression on osteogenic markers levels, for the fact that miR-195 mimic markedly reduced the protein and mRNA expression of Ocn (Figure 6A and 6B, $p<0.001$), ALP (Figure 6C and 6D, $p<0.001$), Runx2 (Figure 6E and 6F, $p<0.01$ or $p<0.001$) and Col-1 (Figure 6G and 6H, $p<0.001$) in OS-treated HULC-overexpressing cells. Taken together, these results demonstrated that HULC promoted cell proliferation and osteogenic differentiation of BMSCs through inhibition of miR-195 levels.

HULC Enhanced the Activation of Wnt/β-catenin and p38MAPK Signaling Pathway Through Down-Regulation of miR-195

As shown in Figure 7A and 7B, OS treatment induced the expression of Wnt3a, Wnt5a, and β-catenin of BMSCs ($p<0.001$). HULC overexpression further enhanced the expression levels of these three proteins ($p<0.01$ or $p<0.001$). However, miR-195 overexpression inhibited the up-regulated expression of these three proteins induced by HULC overexpression ($p<0.001$), suggesting that HULC promoted the activation of Wnt/β-catenin pathway through down-regulation of miR-195. Moreover, we also found that miR-195 mimic suppressed the phosphorylation of p38MAPK, which was induced by OS treatment and HULC overexpression in BMSCs (Figure 7C and 7D; $p<0.001$). These results suggested that HULC might activate the Wnt/β-catenin and p38MAPK signaling pathway through regulating miR-195 in BMSCs.

Discussion

BMSCs, as mesenchymal stromal cells, contribute to the generation of mesenchymal tissues and are essential in supporting the growth and differentiation of primitive hemopoietic cells within bone marrow microenvironment[23,24]. The promising therapeutic advantage of BMSCs in tissue engineering has been attracted more and more attention in recent years[15]. Our present work demonstrated that lncRNA HULC overexpression increased cell viability, regulated the protein expression levels of proliferation-related factors, as well as promoted osteogenic markers levels in BMSCs. On the contrary, HULC knockdown inhibited cell proliferation and osteogenic differentiation of BMSCs.

HULC has been reported[25-27] as a novel biomarker for various carcinomas as its expression level was associated with worse survival and high risk of cancer metastasis. Zhaol[18] revealed that overexpression of HULC promoted proliferation and invasion but inhibited cell apoptosis in SGC7901 cells while HULC inhibition showed an opposite effect. Moreover, Kong and Wang et al[28] indicated that knockdown of HULC suppressed proliferation, migration and invasion, and enhanced apoptosis through inhibiting miR-122 expression levels in osteosarcoma. However, the role of HULC in BMSCs has not been elucidated yet. Our results firstly demonstrated that HULC promoted proliferation and osteogenic differentiation of BMSCs, which was partially similar to previous studies of HULC role in tumor cells.

It has been reported that several miRNAs were associated with the regulatory effect of HULC on the cellular process including miR-200a[29], miR-372[26], miR-122[28], miR-9[30]. In the present investigation, we found that miR-195 was negatively regulated by HULC in BMSCs and was associated with the effect of HULC on BMSCs. Previous researches[31-33] have demonstrated the aberrant expression of miR-195 and its suppressive role in various kinds of human cancers. Overexpression of miR-195 could significantly suppress the tumor cell growth and enhanced chemosensitivity to sorafenib in renal cell carcinoma cells[33]. The regulatory role of miR-195 in BMSCs proliferation and osteogenic differentiation has been rarely reported. In our results, overexpression of miR-195 showed a reversed function of HULC overexpression and abrogated the modulatory effect of HULC overexpression on BMSCs, suggesting that HULC promoted proliferation and osteogenic differentiation of BMSCs through inhibition of miR-195.

Ling et al[34] showed that Wnt/β-catenin signaling pathway plays a critical role in BMSCs and that the activation of Wnt/β-catenin pathway could promote osteogenic differentiation and proliferation of BMSCs. Moreover, miR-195 has been demonstrated to suppress tumor cell growth through inhibiting the activation of Wnt/β-catenin pathway in renal cell carcinoma cells and colorectal cancer cells[29,35]. Thus, we suggested that HULC overexpression might activate Wnt/β-catenin pathway through miR-195 in BMSCs, thus promoting osteogenic differentiation. Our data showed that HULC overexpression enhanced the activation of Wnt/β-catenin pathway, which was abolished by a miR-195 mimic. We also investi-

gate the p38MAPK pathway, which has widely recognized important roles in the osteogenic differentiation of BMSCs[36]. We found that HULC overexpression could promote the activation of the p38MAPK pathway through down-regulating miR-195.

Conclusions

We demonstrated that HULC could enhance the proliferation and osteogenic differentiation of BMSCs via down-regulation of miR-195. What's more, HULC could promote the activation of Wnt/β-catenin and p38MAPK signaling pathways through miR-195, which might be involved in the regulatory effect of HULC on proliferation and osteogenic differentiation of BMSCs. Our results might provide a new potential target for the therapies of bone injuries-related diseases.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interest.

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