European Review for Medical and Pharmacological Sciences 2018; 22: 8805-8813

Long non-coding RNA H19 promotes the osteogenic differentiation of rat ectomesenchymal stem cells via Wnt/β-catenin signaling pathway

Y.-Y. GONG, M.-Y. PENG, D.-Q. YIN, Y.-F. YANG

Department of Prosthodontics, Affiliated Stomatology Hospital of Chongqing Medical University, Chongqing Research Center for Oral Diseases and Biomedical Science, Chongqing, China

Abstract. – OBJECTIVE: To investigate the molecular mechanism of long noncoding RNA (lncRNA) H19 that promotes osteogenic differentiation in rat ectomesenchymal stem cells (EMSCs).

MATERIALS AND METHODS: EMSCs were isolated from rat fetal facial processes by flow cytometry. Osteogenic markers CD29, CD90, CD44, CD57, Nestin and sox10 were detected by fluorescent immunoassay. β-catenin and Wnt pathway target genes were detected by Real-time polymerase chain reaction (RT-PCR) and Western blot after the construction of transient interference H19, a stable expression of H19 EMSCs cell line and the induction of osteogenic differentiation of EMSCs cells. EMSCs of H19 overexpression were treated with Wnt/beta-catenin signaling pathway inhibitor Wnt-C59, and the expressions of beta-catenin and osteogenic markers were detected by RT-PCR and Western blot. Furthermore, the mechanism of H19 regulating Wnt/beta-catenin signaling pathway was explored by transfecting miR-22 and miR-141 mimics and luciferase reporter assays.

RESULTS: EMSCs were successfully isolated and identified, osteogenic markers CD29, CD90, CD44, CD57, Nestin and sox10 were significantly overexpressed. Osteogenesis-induced solution significantly increased the expression of H19 and osteogenic markers ALP, Runx2, BMP and OCN in EMSCs ($p<0.05$). Interference with H19 significantly inhibited the expressions of osteogenic markers, beta-catenin and target genes of Wnt/beta-catenin signaling pathway ($p<0.05$), while upregulation of H19 significantly promoted the expressions of these markers and genes in EMSCs ($p<0.05$). Wnt-C59 inhibitors treatment inhibited the Wnt/beta-catenin signaling pathway and osteogenic differentiation in EMSCs with H19 overexpression ($p<0.05$). Furthermore, H19 could block the inhibitory effect of miR-22 and miR-141 on β-catenin and activate the Wnt/beta-catenin signaling pathway after transfecting miR-22 mimics and miR-141 mimics in EMSCs ($p<0.05$).

CONCLUSIONS: LncRNA H19 can promote the osteogenic differentiation of rat EMSCs by activating Wnt/beta-catenin signal, providing a theoretical basis for the application of EMSCs in tooth tissue engineering regeneration and repair.

Key Words:
LncRNA H19, Osteogenic differentiation, EMSCs, Wnt/β-catenin signaling.

Introduction

Ectomesenchymal stem cells (EMSCs) are a class of mesenchymal stem cells that are differentiated from early cranial neural crest cells along the zygomatic arch to the upper and lower jaws. EMSCs can interact with the epithelium and subsequently differentiate into a variety of mesenchymal stem cells, including neurogenic, myogenic, cartilage, osteoblasts and odontogenic cells. Finally, they will develop into oral and maxillary tissue. It has been showed that[1] EMSCs can differentiate into osteoblasts *in vitro*. Wen et al[2] showed that EMSCs with osteogenic differentiation potential can be used as a cell model for studying tooth development. Recently, Grimm et al[3] have successfully constructed a rat model of alveolar bone regeneration by using EMSCs *in vivo*. Exploring the osteogenic differentiation mechanism of EMSCs may improve our understanding of tooth development and the application prospects for tissue engineering methods to repair periodontal tissues. Wnt/β-catenin signaling pathway plays a key role in embryonic development and participates in cell proliferation, migration and differentiation[4-6]. Numerous researches have found that Wnt/β-catenin signaling pathway is closely related to osteogenic differentiation during tooth development. For example, Han et al[7] reported that the acti-

Corresponding Author: Yongfan Yang, MD; e-mail: mryongfanyang@gmail.com

vation of Wnt/β-catenin signaling could significantly increase the mineralization of periodontal ligament cells and the expression of cementum markers. Yang et al[8] found that osteogenic differentiation of dental follicles could be enhanced through activating Wnt3a in mesenchymal stem cells. Liu et al[9] observed that acetylsalicylic acid can enhance the telomere reverse transcriptase/Wnt/β-catenin signaling cascade and enhance bone regeneration mediated by human dental pulp stem cells. Furthermore, Deng et al[10] observed EMSCs that derived from neural crest stem cells had the characteristics of pluripotent stem cells and the lineage of these stem cells might be determined by certain specific molecules. However, the mechanisms of Wnt/β-catenin signaling pathway and the related upstream molecules that regulate the osteogenic differentiation are rarely reported in EMSCs. Long non-coding RNAs (lncRNAs) are a class of RNAs, about 200-300 nucleotides (nt), which can inhibit target gene expressions of microRNAs (miRNAs) through competitively binding to miRNAs. Researchers[11-13] have found that lncRNAs H19, which is located in chromosome 11, participated in hair follicle development, osteoporosis, and bladder cancer metastasis via activating Wnt/β-catenin signaling pathway. Liang et al[14] found that H19 induced human bone marrow mesenchymal stem cells (hMSCs) to differentiate into osteoblasts through activating Wnt/β-catenin signaling. These above studies have suggested that H19 regulates osteogenic differentiation of MSCs by activating Wnt/β-catenin signaling, but whether H19 participate in osteogenic differentiation of EMSCs by regulating Wnt/β-catenin signaling is unclear. In this study, EMSCs were isolated from rat fetal facial processes by flow cytometry. The cell models of EMSCs with H19 transient interference and H19 stable overexpression were established and then EMSCs were cultured in complete medium with osteogenic inducing drugs for 7 days. Our findings showed that H19 participated in the osteogenic differentiation of EMSCs through Wnt/β-catenin signaling. Furthermore, it was confirmed by transfection of miRNA mimics and luciferase reporter vector that H19 blocked the inhibition function of β-catenin by binding to miR-22 and miR-141. The result confirmed that H19 and Wnt/β-catenin signaling participated in the osteogenic differentiation process in EMSCs cells, providing a theoretical basis for the regeneration and repair of dental tissue engineering

Materials and Methods

Isolation and Identification of EMSCs

10 pregnant Sprague-Dawley (SD) rats were purchased from Saiye Company (Guangzhou Saiye Company, Guangzhou, China). In the embryonic facial processes, rats were cut into 1 mm×1 mm×1 mm at 12.5 d, and these tissues were digested with 1% trypsin containing 1 mM EDTA for 10 min at 37°C. After digestion, the tissue pieces were removed by 75 μm screen Sieve and digested EMSCs were isolated after centrifuging at 800 rpms for 5 minutes. Next, these isolated cells were transferred to the Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS). After being cultured for 3 generations, 1% trypsin was used to digest cells. Antibodies of cell surface marker with fluorescent labels, such as CD29, CD90, CD44, CD57, Nestin and sox10, were purchased from Abcam (Cambridge, MA, USA) and added into cells. Next, they were incubated at 4°C for 1 hour and sorted in flow cytometry.

Construction of EMSCs with H19 Interference and H19 Overexpression

The small RNA interference sequence si-H19 and the control si-NC were purchased from GenePharma (Shanghai GenePharma Co., Ltd., Shanghai, China) and were transfected into the rat EMSCs. The transfection reagent Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), serum-free DMEM, and interference sequence were mixed and incubated for 20 minutes before transfection. Lentiviral vector (Shanghai GenePharma Co., Ltd., Shanghai, China) with H19 overexpression was added to EMSCs cells for 24 hours, then the transfection efficiency was observed under a fluorescent inverted microscope. A stable cell line of EMSCs with H19 overexpression was constructed after screening with puromycin for 1 to 2 weeks.

Cell Culture and Transfection

EMSCs were cultured in Dulbecco's Modified Eagle Medium (DMEM; HyClone, South Logan, UT, USA) with 10% fetal bovine serum (FBS; HyClone, South Logan, UT, USA) at 37°C and 5% CO_2 culture incubator. When the density of EMSCs was about 30%, osteogenic differentiation inducers were added for 7 days, such as 50 mg/ml ascorbic acid, 10 mmol/L 2-phosphoglycerate and 100 nM dexamethasone, which were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). The medium was changed every three

days. Wnt/β-catenin signaling inhibitor Wnt-C59 was added to the stable cell line of EMSCs with H19 overexpression for 24 hours. Cells were planted for 12-24 h until reached to 60-70% confluence, then miR-141 mimics, miR-22 mimics, and negative control were mixed with Lipofectamine 2000 and serum-free Dulbecco's Modified Eagle Medium (DMEM). After incubation for 20 minutes at room temperature, the mixtures were added into EMSCs with H19 overexpression. After transfection for 24 h, the medium was changed for further experiments.

RNA Extraction and Quantitative Real-Time PCR

The total RNA of EMSCs was extracted by TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed by PrimeScript™ RT reagent Kit (TaKaRa, Dalian, China). PCR primers were synthesized by Gene Pharma (Shanghai, China) and sequences were listed in Table I. mRNA expressions were detected by SYBR Premix Ex Taq II (TaKaRa, Dalian, China). The mRNA expressions were normalized to GAPDH and $2^{-\Delta\Delta CT}$ method was used to calculate the relative gene expressions.

Protein Extraction and Western Blot

RIPA protein lysis buffer (Biyuntian, Shanghai, China) was added to EMSCs, after centrifuging at 12000 rpm for 10 minutes at 4°C, and the supernatant was added to 4 volumes of SDS loading buffer. After that, the mixture was placed in a 100°C water bath for 5 minutes. 40 mg proteins were added to 8% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then the separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were blocked at room temperature for 2 h with 10% nonfatty milk powder. After that, these membranes were incubated with primary antibody (ALP, Runx2, BMP, OCN, β-catenin, c-myc and CD44) overnight at 4°C and subsequently incubated with secondary antibodies for 1 h. Proteins were detected by Pierce ECL Western blot substrate (Thermo Fisher Scientific, Waltham, MA, USA) with ECL detection system (Thermo Fisher Scientific, Waltham, MA, USA).

Luciferase Assays

Total RNA was extracted from rat EMSCs, which was reverse transcribed into cDNA. The cDNA was used as a template to amplify the 3'UTR and CDS regions of β-catenin. Primers were designed as follow: β-catenin 3›UTR upstream primers: 5›-TCTGAGGACAAGCCACAAGATTACA-3›, β-catenin 3›UTR downstream primer: 5›-TGGGCACCAATATCAAGTCCAA-3›; β-catenin CDS upstream primer: 5'-ATGGGTAGGGCAAATCAGTAAGAGGT-3', β-catenin CDS downstream primer: 5'-AAGCATCGTATCACAGCAGGTTAC-3'. After successfully construction, the vectors were extracted and named as GLO-miR-141-wt and GLO-miR-22-wt, which were sent to Shanghai Shenggong Co., Ltd. (Shanghai, China) to synthesize deletion mutant pla-

Table I. Sequences of primers used for qRT-PCR.

Genes	Primer sequences
H19	Forward: 5'-CGTTCCTTTAGTCTCCTGAC-3' Reverse: 5'-AGTCCGTGTTCCAAGTCC-3'
ALP	Forward: 5'-GGCTCTGCCGTTGTTTCTCT-3' Reverse: 5'-AAGGTGCTTTGGGAATCTGC-3'
Runx2	Forward: 5'-AGCGGACGAGGCAAGAGTTT-3' Reverse: 5'-CTGTCTGTGCCTTCTTGGTTCC-3'
BMP	Forward: 5'-AGCATGTAGACTGCTGGGGCAA-3' Reverse: 5'-CCTGCAGTAGGTTTCTGCTGCCTTG-3'
OCN	Forward: 5'-CATGTTTTCTGACGGCAACTT-3' Reverse: 5'-CCAGATCACGCCATTTCAC-3'
β-catenin	Forward: 5'-TGATAAAGGCAACTGTTGGATTGA-3' Reverse: 5'-CCGCTGGGTGTCCTGATGT-3'
C-myc	Forward: 5'-AGCGACACAAGAAGCTTCTG-3' Reverse: 5'-CTGAAGCAGCTCCGCCAAAC-3'
CD44	Forward: 5'-CGGACAGGATTGACAGATTGATAGC-3' Reverse: 5'-TGCCAGAGTCTCGTTCGTTATCG-3'
GAPDH	Forward: 5'-CGGACAGGATTGACAGATTGATAGC-3' Reverse: 5'-TGCCAGAGTCTCGTTCGTTATCG-3'

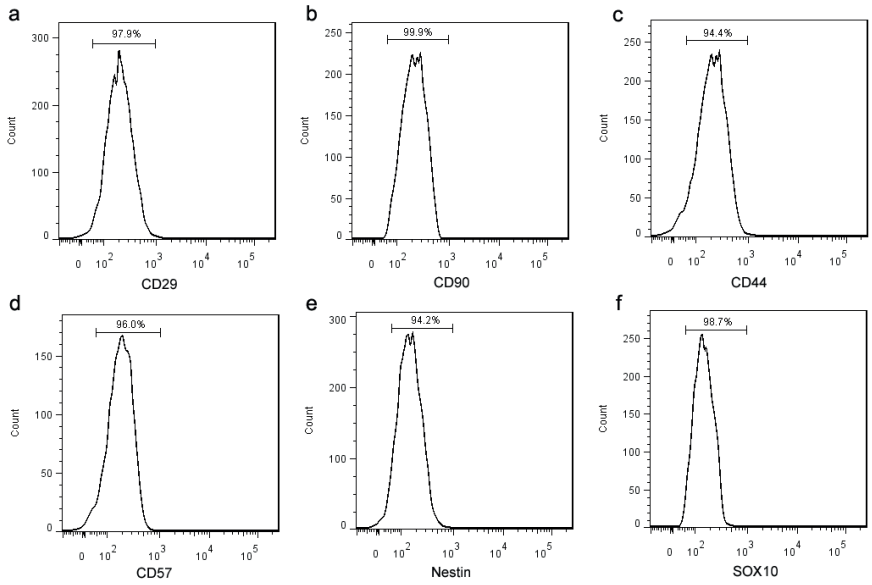

Figure 1. EMSCs were successfully isolated and identified from rat embryonic facial process. ***(a-f)*** Special surface markers of CD29, CD90, CD44, CD57, Nestin and sox10 were significantly overexpressed, which suggested that EMSCs were successfully isolated.

smids named GLO-miR-141-mut and GLO-miR-22-mut. 200 ng of the plasmids were mixed with Lipofectamine 2000 and Dulbecco's Modified Eagle Medium (DMEM) medium. The mixtures were added into EMSCs after placing at room temperature for 20 min. After transfection for 24 h, the cells were lysed and the activity of firefly luciferase and Renilla luciferase was measured using a Promega Luciferase Assay (Madison, WI, USA). Their ratio revealed the relative activity of luciferase.

Statistical Analysis

All data were analyzed by SPSS version 21.0 (SPSS Inc., Armonk, NY, USA). Data were presented by means± SD. The methods of one-way ANOVA and Student's *t*-test were used to analyze these data, and multiple comparisons between groups were performed by S-N-K method. If $p<0.05$, the difference was statistically significant.

Results

EMSCs Were Successfully Isolated and Identified From Rat Embryonic Facial Process

It was reported that EMSCs derived from cranial neural crest would move to embryonic facial process at 11.5 days of embryonic development, and the bud stage is the initial stage of tooth development at 12.5 days[2]. So EMSCs were isolated from rat embryonic facial process at 12.5 days of embryonic development and were identified by special surface markers through flow cytometry. Special surface markers of CD29, CD90, CD44, CD57, Nestin and sox10 were significantly overexpressed, suggesting that EMSCs were successfully isolated (Figure 1a-1f).

Down-Regulation of H19 Repressed Expressions of ALP, Runx2, BMP and OCN, While Up-Regulation of H19 Increased These Osteogenic Markers in EMSCs

To examine the functions of H19 in osteogenic differentiation of EMSCs, si-H19 was transfected into EMSCs and dexamethasone, ascorbic acid and 2-phosphoglyceride were added into the medium for induction of osteogenic differentiation. After culturing for 7 days, osteogenic genes expressions were detected by qRT-PCR. After osteogenic differentiation induction, the H19 expression of si-H19 was significantly repressed, compared with the si-Ctr ($p<0.05$) (Figure 1a). The expressions of osteogenic markers were also significantly repressed, such as ALP, Runx2, BMP and OCN, compared with the si-Ctr ($p<0.05$) (Figure 1b-e). Furthermore, lv-H19 was transfected into EMSCs and expressions of H19, ALP, Runx2, BMP and OCN were significantly increased, compared with the lv-Ctr ($p<0.05$) (Figure 1f). These results suggested that

H19 was a key regulator in inducing osteogenic differentiation of EMSCs.

Down-Regulation of H19 Repressed Expressions of β-Catenin, c-myc and CD44, While Up-Regulation of H19 Increased These Factors in EMSCs

To demonstrate that H19 expression promoted the transduction of Wnt/β-catenin signaling in EMSCs, EMSCs with H19 transient interference and H19 stable overexpression were constructed and extracted. The expressions of Wnt/β-catenin signaling factors were detected by qRT-PCR and Western blot, such as β-catenin, c-myc and CD44 (Figure 2). After osteogenic induction for 7 days in EMSCs with H19 transient interference, the expressions of β-catenin, c-myc and CD44 in the control group were significantly higher than those in 0 day of control group (p<0.01), and the expressions of β-catenin, c-myc and CD44 in si-H19 group were significantly lower than those in the control group at the time 0 day and 7 day (p<0.01) (Figure 3a-3b). The expressions of β-catenin, c-myc and CD44 were much higher than those in control group in EMSCs with H19 stable overexpression (p<0.01) (Figure 3c-3d). The results indicated that Wnt/β-catenin signaling was activated in the process of osteogenic differentiation in EMSCs and H19 was an upstream regulator of Wnt/β-catenin signaling, which suggested that H19 may be involved in the regulation of osteogenic differentiation of EMSCs through Wnt/β-catenin signaling pathway.

H19 Induced Osteogenic Differentiation of EMSCs Through Activating Wnt Signaling

To confirm that Wnt signaling mediated the process of osteogenic differentiation regulated by H19 in EMSCs, Wnt-C59 was used to inhibit the Wnt signaling in EMSCs with H19 overexpression. Results showed that overexpression of H19 (lv-H19) resulted in a significant upregulation of β-catenin and osteogenic markers ALP, Runx2, BMP and OCN (p<0.01), while these expression levels were significantly inhibited after Wnt-C59 treatment in lv-H19 EMSCs (p<0.01) (Figure 4a-4b). These results suggested that H19 was an upstream regulator of the Wnt signaling, which could induce osteogenic differentiation through activating Wnt signaling in EMSCs.

H19 Activated Wnt/β-Catenin Signaling by Inhibiting the Effects of miR-141 and miR-22

Researches[14,15] showed that miR-22 and miR-141 could inhibit β-catenin and block osteogenic differentiation in human mesenchymal stem cells through binding with the 3'-UTR and CDS regions of β-catenin. H19 could bind to the miRNAs ribonucleoprotein complex and then block the binding of the miRNAs to the downstream

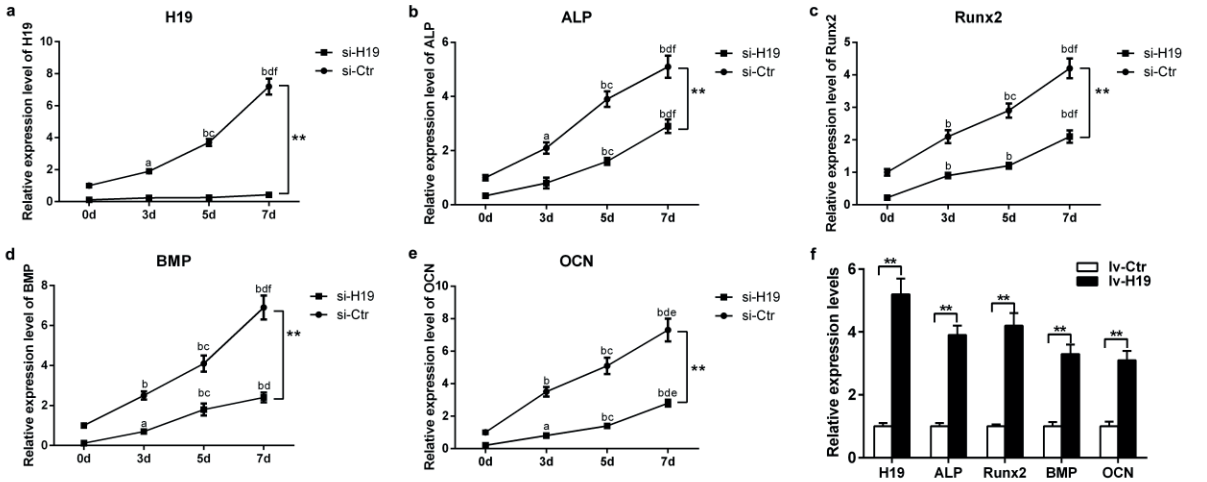

Figure 2. Down-regulation of H19 repressed expressions of ALP, Runx2, BMP and OCN, while up-regulation of H19 increased these osteogenic markers in EMSCs. ***(a)*** The expression of H19 was detected by RT-PCR after transfected with si-H19 and si-Ctr in rat EMSCs for 7 days. ***(b-e)*** The expressions of ALP, Runx2, BMP and OCN were detected by RT-PCR. ***(f)*** The expressions of H19, ALP, Runx2, BMP and OCN were detected by RT-PCR after transfected with lv-H19 and lv-Ctr in rat EMSCs. Data are shown as mean ± SD based on at least three independent experiments, *p < 0.05, ** p <0.01.

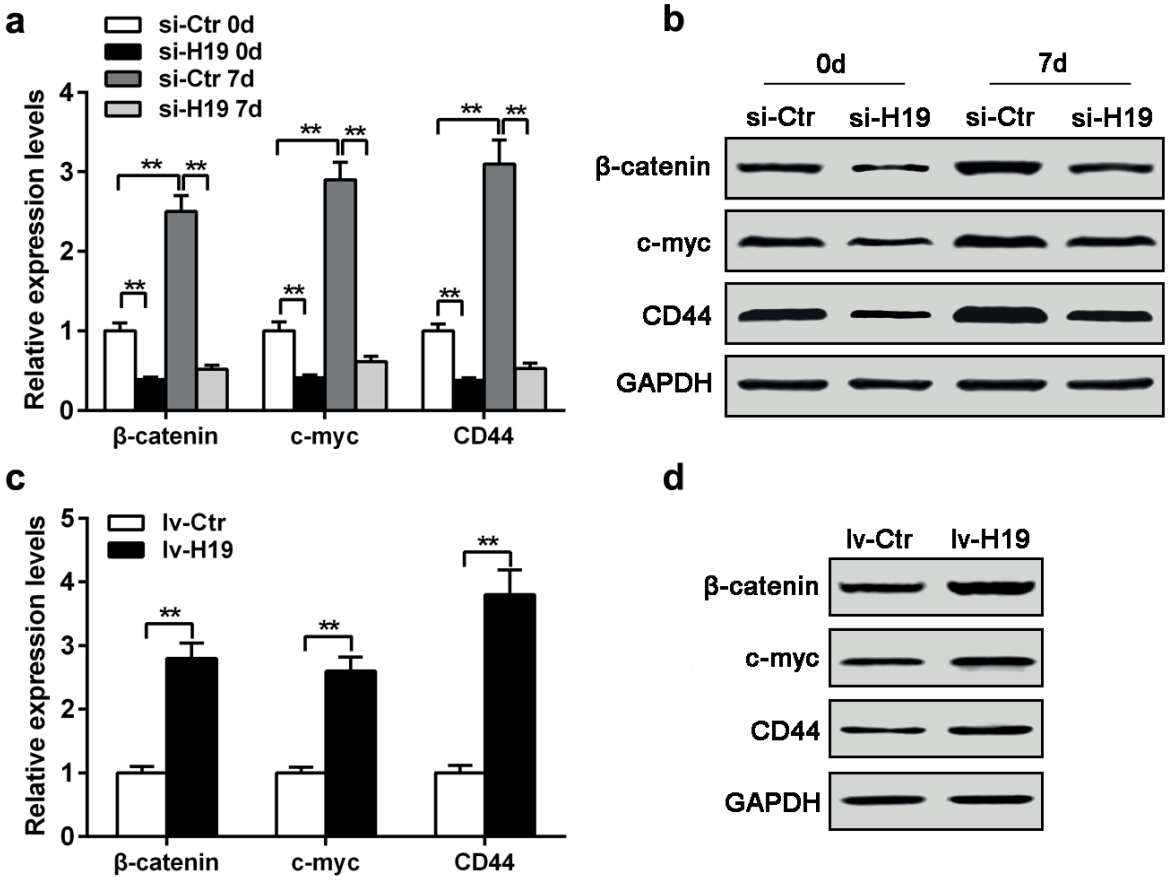

Figure 3. Down-regulation of H19 repressed expressions of β-catenin, c-myc and CD44, while up-regulation of H19 increased these factors in EMSCs. ***(a-b)*** After osteogenic induction for 7 days in EMSCs with H19 transient interference, the expressions of β-catenin, c-myc and CD44 were detected by qRT-PCR and Western blot. ***(c-d)*** Stable cell line of EMSCs with H19 overexpression was constructed and the expressions of β-catenin, c-myc and CD44 were detected by qRT-PCR and Western blot. Data are shown as mean ± SD based on at least three independent experiments, $^{**}p < 0.01$.

target gene, thereby inhibiting the repression of the target gene by the miRNAs[16-18]. In this study, 3'-UTR and CDS regions of β-catenin were cloned into the pmiR-GLO vector, named GLO-miR-141-wt and GLO-miR-22-wt, respectively, and co-transfected with miR-141 mimic and miR-22 mimic in EMSCs with H19 overexpression. Dual luciferase assays were carried out to confirm the blocking effect of H19 on miR-141 and miR-22. The expression of β-catenin in lv-Ctr EMSCs was significantly decreased ($p<0.01$), while the level of β-catenin was not statistically significant in lv-H19 EMSCs ($p>0.05$), after miR-141 mimic and miR-22 mimic transfection (Figure 5a-b). Furthermore, after transfecting with miR-141 mimic and miR-22 mimic in lv-Ctr EMSCs, the luciferase activities of GLO-miR-141-wt and GLO-miR-22-wt were significantly inhibited ($p<0.01$). However, the luciferase activities were not reduced after transfecting with miR-141 mimic and miR-22 mimic in lv-H19 EMSCs ($p>0.05$), thus suggesting that H19 could block the function of miR-141 mimic and miR-22 mimic. In addition, GLO-miR-141-mut and GLO-miR-22-mut were synthesized and transfected into EMSCs; no significant differences had been found in these group ($p>0.05$) (Figure 5c-d). The data suggested that osteogenic differentiation of EMSCs, through activating Wnt/β-catenin signaling, could inhibit the functions of miR-141 and miR-22.

Discussion

Tissue engineering technology to repair periodontal tissue is based on periodontal ligament cells, and the injury of periodontal tissue leads to limited source of periodontal ligament cells. As

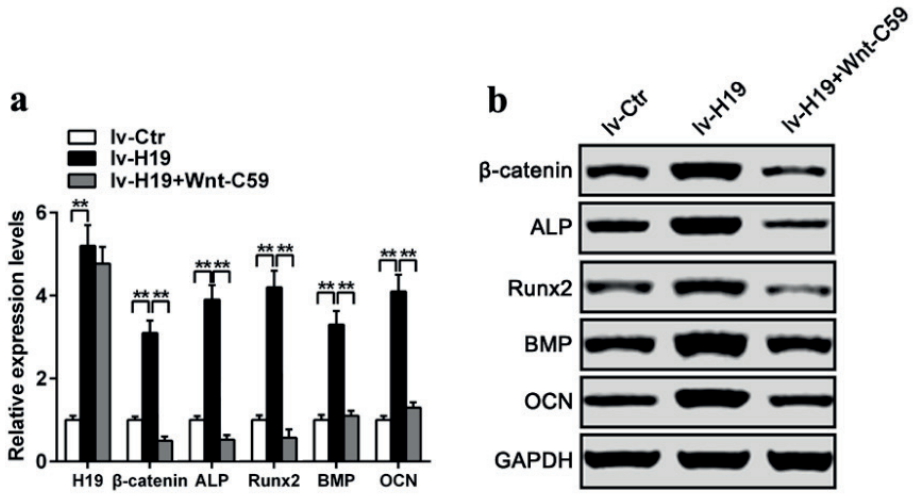

Figure 4. H19 induced osteogenic differentiation of EMSCs through activating Wnt signaling. ***(a-b)*** Wnt-C59 was used to inhibit the Wnt signaling in EMSCs with H19 overexpression, the expression of H19 was detected by qRT-PCR and the expressions of ALP, Runx2, BMP and OCN were detected by qRT-PCR and Western blot. Data are shown as mean ± SD based on at least three independent experiments, ** p <0.01.

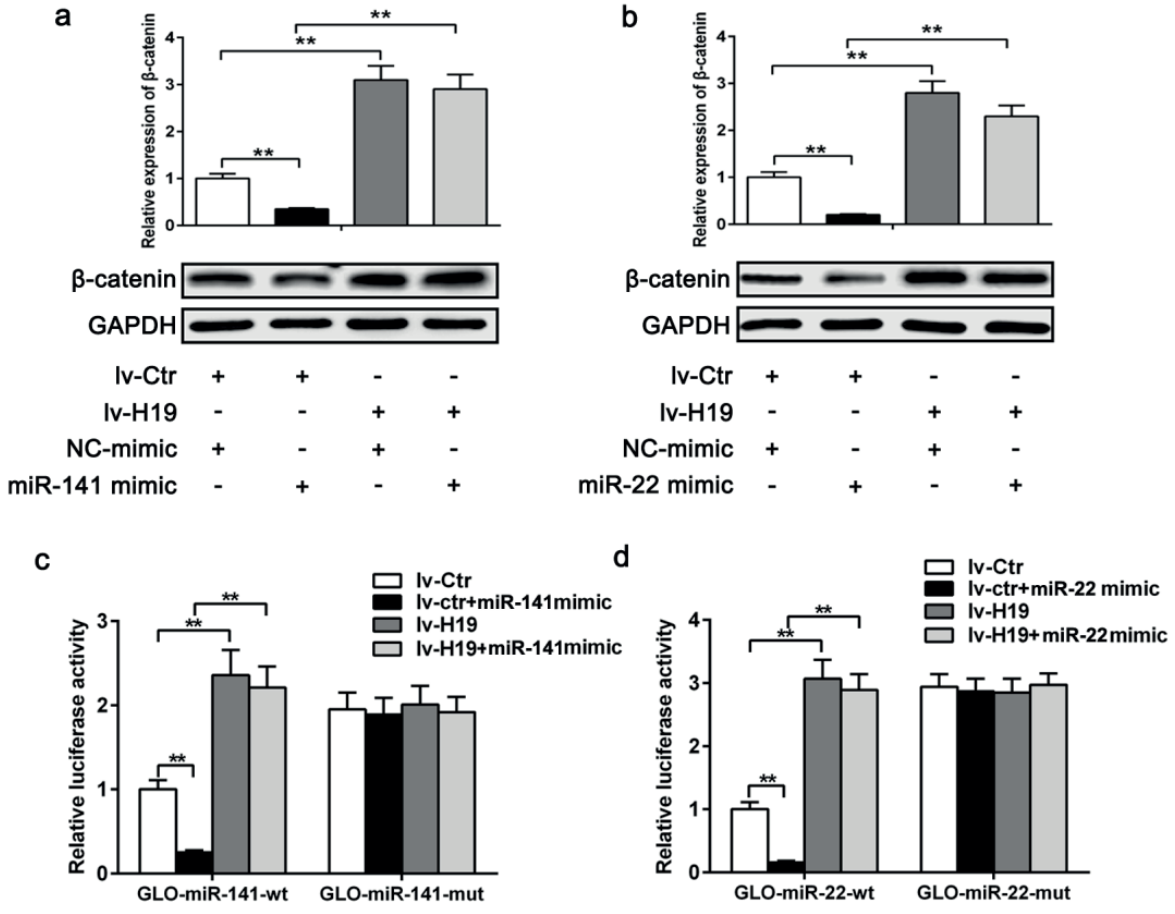

Figure 5. H19 activated Wnt/β-catenin signaling by inhibiting the effects of miR-141 and miR-22. ***(a-b)*** MiR-141 mimic and miR-22 mimic were separately transfected into lv-Ctr and lv-H19 EMSCs, the relative expression of β-catenin was detected by Western blot. ***(c-d)*** GLO-miR-141-wt, GLO-miR-22-wt, GLO-miR-141-mut and GLO-miR-22-mut were separately transfected into lv-Ctr and lv-H19 EMSCs for 24 h, and then miR-141 mimic and miR-22 mimic were separately transfected into corresponding EMSCs for 24 h. After that, Luciferase activity was detected. Data are shown as mean ± SD based on at least three independent experiments, **p<0.01.

a result, to find a kind of seed cells with differentiation potential, as mesenchymal stem cells, embryonic stem cells, etc. is the key point and induce them differentiate into osteoblasts[19-22]. Guzmán-Uribe et al[23] constructed a three-dimensional tissue model of human dental germ-derived EMSCs and successfully transplanted them into the rat's mouth to replace the biological tooth, which suggested that EMSCs might be a kind of new cells for periodontal tissue engineering repairmen. This study first isolated EMSCs from rat fetal facial processes by flow cytometry and the expressions of osteogenic markers, such as ALP, Runx2, BMP and OCN continued to increase under the condition of osteogenic inducers, indicating that EMSCs had the capacity of osteogenic differentiation. Deng et al[10] observed that osteogenic differentiation could be regulated by some upstream specific molecules, such as lncRNAs. Researchers[14,24] had found that LncRNA H19 could promote osteogenic differentiation in different ways, e.g. binding with miRNAs to regulate related signaling pathways, or acting as a competitive endogenous RNA. In this work, we found that osteogenic inducer could induce osteogenic differentiation in EMSCs, and at the same time, it can also induce the expression of H19, suggesting that the osteogenesis of EMSCs was closely related with the expression of H19. Furthermore, the transient interference of H19 leads to a significant decrease in the osteogenic differentiation of EMSCs and the expressions of ALP, Runx2, OCN and BMP were significantly decreased. In addition, EMSCs with H19 overexpression significantly enhanced the osteogenic differentiation capacity, intimating that H19 might be an upstream regulator in osteogenic differentiation of EMSCs; however, the detail mechanism was unknown. Scholars[25-27] have shown that Wnt/β-catenin signaling pathway mediates osteogenic differentiation of mesenchymal stem cells, and the activation of Wnt/β-catenin signaling is critical for this regulatory function. Li et al[28] found that the osteogenic differentiation of EMSCs could be promoted through Wnt/β-catenin signaling, which was activated by LNGFR. We found that interference with H19 leads to inhibit the expression of β-catenin, c-myc and CD44 in EMSCs, while upregulation of H19 leads to increase these gene expressions, suggesting that H19 was an upstream regulator of Wnt/β-catenin signaling. To further confirm that H19 could regulate Wnt/β-catenin signaling during osteogenic differentiation, Wnt-C59, a potent inhibitor of Wnt/β-catenin signaling pathway, was used to treat EMSCs with H19 overexpression. Results showed that the expressions of β-catenin, ALP, Runx2, OCN and BMP, were significantly increased in EMSCs with H19 overexpression, while these genes were decreased after Wnt-C59 treatment, indicating that Wnt-C59 almost completely blocked the activation of Wnt/β-catenin signaling by H19 and inhibited osteogenic differentiation of EMSCs. Researchers[14,15] showed that miR-22 and miR-141 could inhibit β-catenin and block osteogenic differentiation in human mesenchymal stem cells through binding with the 3'-UTR and CDS regions of β-catenin. Results showed that the luciferase activities of GLO-miR-141-wt and GLO-miR-22-wt were not inhibited by miR-22 mimic and miR-141 mimic in EMSCs with H19 overexpression, the same as GLO-miR-141-mut and GLO-miR-22-mut, indicating that H19 could retrieve the expression levels of β-catenin inhibited by miR-22 mimic and miR-141 mimic. Taken together, we found that H19 could activate Wnt/β-catenin signaling pathway by binding to miR-22 and miR-141, as a result, blocking the inhibition of Wnt/β-catenin signaling and promoting osteogenic differentiation of rat EMSCs.

Conclusions

We demonstrated that H19 could promote the osteogenic differentiation of rat EMSCs by activating Wnt/beta-catenin signal, which provides a theoretical basis for the application of EMSCs in tooth tissue engineering regeneration and repair.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Delorme B, Nivet E, Gaillard J, Häupl T, Ringe J, Devèze A, Magnan J, Sohier J, Khrestchatisky M, Roman FS, Charbord P, Sensebé L, Layrolle P, Féron F. The human nose harbors a niche of olfactory ectomesenchymal stem cells displaying neurogenic and osteogenic properties. Stem Cells Dev 2010; 19: 853-866.

2) Wen X, Liu L, Deng M, Zhang L, Liu R, Xing Y, Zhou X, Nie X. Characterization of p75(+) ectomesenchymal stem cells from rat embryonic facial process tissue. Biochem Biophys Res Commun 2012; 427: 5-10.

3) Grimm WD, Dannan A, Giesenhagen B, Schau I, Varga G, Vukovic MA, Sirak SV. Translational research: palatal-derived ecto-mesenchymal stem cells from human palate: a new hope for alveolar bone and cranio-facial bone reconstruction. Int J Stem Cells 2014; 7: 23-29.

4) Bilir B, Kucuk O, Moreno CS. Wnt signaling blockage inhibits cell proliferation and migration, and induces apoptosis in triple-negative breast cancer cells. J Transl Med 2013; 11: 280.

5) Inestrosa NC, Varela-Nallar L. Wnt signalling in neuronal differentiation and development. Cell Tissue Res 2015; 359: 215-223.

6) Zhang Y, Tu C, Zhang D, Zheng Y, Peng Z, Feng Y, Xiao S, Li Z. Wnt/beta-catenin and Wnt5a/Ca pathways regulate proliferation and apoptosis of keratinocytes in psoriasis lesions. Cell Physiol Biochem 2015; 36: 1890-1902.

7) Han P, Wu C, Chang J, Xiao Y. The cementogenic differentiation of periodontal ligament cells via the activation of Wnt/beta-catenin signalling pathway by Li+ ions released from bioactive scaffolds. Biomaterials 2012; 33: 6370-6379.

8) Yang Y, Ge Y, Chen G, Yan Z, Yu M, Feng L, Jiang Z, Guo W, Tian W. Hertwig's epithelial root sheath cells regulate osteogenic differentiation of dental follicle cells through the Wnt pathway. Bone 2014; 63: 158-165.

9) Liu Y, Chen C, Liu S, Liu D, Xu X, Chen X, Shi S. Acetylsalicylic acid treatment improves differentiation and immunomodulation of SHED. J Dent Res 2015; 94: 209-218.

10) Deng MJ, Jin Y, Shi JN, Lu HB, Liu Y, He DW, Nie X, Smith AJ. Multilineage differentiation of ectomesenchymal cells isolated from the first branchial arch. Tissue Eng 2004; 10: 1597-1606.

11) Lin CM, Liu Y, Huang K, Chen XC, Cai BZ, Li HH, Yuan YP, Zhang H, Li Y. Long noncoding RNA expression in dermal papilla cells contributes to hairy gene regulation. Biochem Biophys Res Commun 2014; 453: 508-514.

12) Li B, Zhao J, Ma JX, Li GM, Zhang Y, Xing GS, Liu J, Ma XL. Overexpression of DNMT1 leads to hypermethylation of H19 promoter and inhibition of Erk signaling pathway in disuse osteoporosis. Bone 2018; 111: 82-91.

13) Luo M, Li Z, Wang W, Zeng Y, Liu Z, Qiu J. Long non-coding RNA H19 increases bladder cancer metastasis by associating with EZH2 and inhibiting E-cadherin expression. Cancer Lett 2013; 333: 213-221.

14) Liang WC, Fu WM, Wang YB, Sun YX, Xu LL, Wong CW, Chan KM, Li G, Waye MM, Zhang JF. H19 activates Wnt signaling and promotes osteoblast differentiation by functioning as a competing endogenous RNA. Sci Rep 2016; 6: 20121.

15) Westendorf JJ, Kahler RA, Schroeder TM. Wnt signaling in osteoblasts and bone diseases. Gene 2004; 341: 19-39.

16) Liang WC, Fu WM, Wong CW, Wang Y, Wang WM, Hu GX, Zhang L, Xiao LJ, Wan DC, Zhang JF, Waye MM. The lncRNA H19 promotes epithelial to mesenchymal transition by functioning as miRNA sponges in colorectal cancer. Oncotarget 2015; 6: 22513-22525.

17) Dey BK, Pfeifer K, Dutta A. The H19 long noncoding RNA gives rise to microRNAs miR-675-3p and miR-675-5p to promote skeletal muscle differentiation and regeneration. Genes Dev 2014; 28: 491-501.

18) Zhou X, Ye F, Yin C, Zhuang Y, Yue G, Zhang G. The interaction between MiR-141 and lncRNA-H19 in regulating cell proliferation and migration in gastric cancer. Cell Physiol Biochem 2015; 36: 1440-1452.

19) Liu J, Yu F, Sun Y, Jiang B, Zhang W, Yang J, Xu GT, Liang A, Liu S. Concise reviews: characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. Stem Cells 2015; 33: 627-638.

20) Liu Y, Hu J, Wang S. Mesenchymal stem cell-mediated treatment of oral diseases. Histol Histopathol 2014; 29: 1007-1015.

21) Yeasmin S, Ceccarelli J, Vigen M, Carrion B, Putnam AJ, Tarle SA, Kaigler D. Stem cells derived from tooth periodontal ligament enhance functional angiogenesis by endothelial cells. Tissue Eng Part A 2014; 20: 1188-1196.

22) Wan M, Du W, Zhou X, Xu X, Zheng L. Stem cell-based tooth engineering and their potential in dental medicine. Curr Stem Cell Res Ther 2015; 10: 443-449.

23) Guzmán-Uribe D, Estrada KN, Guillén Ade J, Pérez SM, Ibáñez RR. Development of a three-dimensional tissue construct from dental human ectomesenchymal stem cells: in vitro and in vivo study. Open Dent J 2012; 6: 226-234.

24) Huang Y, Zheng Y, Jia L, Li W. Long noncoding RNA H19 promotes osteoblast differentiation via TGF-beta1/Smad3/HDAC signaling pathway by deriving miR-675. Stem Cells 2015; 33: 3481-3492.

25) Yuan Z, Li Q, Luo S, Liu Z, Luo D, Zhang B, Zhang D, Rao P, Xiao J. PPAR gamma and Wnt signaling in adipogenic and osteogenic differentiation of mesenchymal stem cells. Curr Stem Cell Res Ther 2016; 11: 216-225.

26) He X, Wang H, Jin T, Xu Y, Mei L, Yang J. TLR4 activation promotes bone marrow MSC proliferation and osteogenic differentiation via Wnt3a and Wnt5a signaling. PLoS One 2016; 11: e0149876.

27) Bermeo S, Vidal C, Zhou H, Duque G. Lamin A/C acts as an essential factor in mesenchymal stem cell differentiation through the regulation of the dynamics of the Wnt/beta-catenin pathway. J Cell Biochem 2015; 116: 2344-2353.

28) Li G, Liu J, Wang Y, Yang K, Zhao M, Xiao Y, Wen X, Liu L. LNGFR targets the Wnt/beta-catenin pathway and promotes the osteogenic differentiation in rat ectomesenchymal stem cells. Sci Rep 2017; 7: 11021.