Platelet lysate induces chondrogenic differentiation of umbilical cord-derived mesenchymal stem cells by regulating the IncRNA H19/miR-29b-3p/SOX9 axis

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Platelet lysate (PL) has been shown to induce chondrogenic differentiation of human umbilical cord-derived mesenchymal stem cells (hUCMSCs). However, the underlying mechanism is still not clear. The aim of this study was to investigate whether long noncoding RNA H19 is involved in this process. hUCMSCs were isolated, identified and cultured in 5% PL-supplemented chondrogenic differentiation medium. Chondrogenic differentiation was assessed by Alcian blue staining. The expressions of H19, miR-29b-3p, SRY-related high-mobility-group box 9 (SOX9), collagen II and aggrecan were determined by quantitative real-time PCR and western blot. The interaction between miR-29b-3p and H19 or SOX9 was analyzed by luciferase reporter assay. During PL-induced chondrogenic differentiation of hUCMSCs, expressions of H19 and SOX9 were increased, whereas miR-29b-3p expression was decreased. H19 overexpression promoted, whereas H19 silencing attenuated the PL-induced chondrogenic differentiation of hUCMSCs. SOX9 was identified as a direct target of miR-29b-3p, and H19 was observed to act as a sponge of miR-29b-3p to up-regulate SOX9 expression. The chondrogenic differentiation-promoting effect of H19 overexpression was negated when miR-29b-3p expression was up-regulated by Lenti-miR-29b-3p infection. In conclusion, PL induced chondrogenic differentiation of hUCMSCs by regulating the H19/miR-29b-3p/SOX9 axis.

Osteoarthritis (OA) is one of the major joint diseases worldwide mainly characterized by articular cartilage degeneration [1]. Articular cartilage is an important component of the synovial joints and lacks the capacity for self-regeneration [2]. Mesenchymal stem cells (MSCs) have emerged as a relevant cell source for cartilage repair in recent years [3,4]. Zheng et al. [5] observed that human chondrocytes could be successfully induced by coculture of human umbilical cord-derived mesenchymal stem cells (hUCMSCs) with rabbit chondrocytes.

Platelet-rich plasma (PRP), a blood extraction product widely used in clinical practice, can not only enhance tissue healing ability and promote tissue cell proliferation and differentiation but also greatly accelerates the healing process of tissue injuries [6–8]. Platelet lysate (PL), a purified product of PRP after lysis [9], contains high levels of growth factors important for MSC proliferation and differentiation, such as transforming growth factor β (TGF-β), fibroblast growth factor and platelet-derived growth factor [10]. Among these, TGF-β is critically important to induce

Abbreviations
hUCMSC, human umbilical cord-derived mesenchymal stem cell; IncRNA, long noncoding RNA; MSC, mesenchymal stem cell; Mut, mutated; NC, negative control; OA, osteoarthritis; PL, platelet lysate; PRP, platelet-rich plasma; qRT-PCR, quantitative real-time PCR; ROCR, regulator of chondrogenesis RNA; SOX9, SRY-related high-mobility-group box 9; TGF-β, transforming growth factor β; WT, wild-type.
chondrogenic differentiation of MSCs [11]. A previous study demonstrated that PL induced chondrogenic differentiation of hUCMSCs, concomitant with elevated expression of SRY-related high-mobility-group box 9 (SOX9), a cartilage-specific transcription factor that plays crucial roles in chondrocyte differentiation [12]. However, the underlying mechanism is still not clear.

Long noncoding RNAs (lncRNAs) are non-protein-coding transcripts longer than 200 nucleotides and play important regulatory roles in various physiological and disease processes [13]. Recent studies have suggested that lncRNA plays a critical role in chondrogenic differentiation of MSCs. For example, Barter et al. [14] reported that lncRNA regulator of chondrogenesis RNA (ROCR) induced chondrogenic differentiation of MSCs by up-regulating SOX9 expression. H19, a kind of lncRNA, has been shown to promote the osteogenic differentiation of MSCs [15,16] but inhibit the differentiation MSCs into adipocytes [17]. Existing evidence has demonstrated that H19 expression can be induced by TGF-β [17], suggesting that H19 might be involved in the PL-induced chondrogenic differentiation of hUCMSCs. In recent years, many studies have indicated that H19 is implicated in tenogenic differentiation [11]. A previous study demonstrated that PL induced chondrogenesis RNA (ROCR) induced chondrogenic differentiation of MSCs [14] and pooled. PRP was isolated from peripheral blood and peripheral blood was collected from each healthy volunteer donors as previously described [12]. In brief, 20 mL of peripheral blood was centrifuged at 15 000 g for 20 min at 37 °C to obtain PL. PL was centrifuged at 15 000 g for 20 min at 4 °C, filtered through 0.22-μm filters and stored at −20 °C until use. Written informed consent was obtained from all volunteers. This experiment was approved by the Ethics Committee of First Affiliated Hospital of Harbin Medical University. The study methodologies conformed to the standards set by the Declaration of Helsinki.

Materials and methods

Preparation of human PLs

Human PLs were prepared from PRP obtained from five donors as previously described [12]. In brief, 20 mL of peripheral blood was collected from each healthy volunteer and pooled. PRP was isolated from peripheral blood and then subjected to three cycles of freeze–thaw (frozen at −80 °C and thawed at 37 °C) to obtain PL. PL was centrifuged at 15 000 g for 20 min at 4 °C, filtered through 0.22-μm filters and stored at −20 °C until use. Written informed consent was obtained from all volunteers. This experiment was approved by the Ethics Committee of First Affiliated Hospital of Harbin Medical University. The study methodologies conformed to the standards set by the Declaration of Helsinki.

Isolation of hUCMSCs

hUCMSCs were isolated from umbilical cords of five cesarean-delivered full-term neonates using the explant method [12]. Informed consent was obtained from the parent/legal guardian. In brief, umbilical cords were cut into smaller pieces of ~5 × 5 cm, transferred to a 25-cm² flask and maintained in Dulbecco’s modified Eagle’s medium (Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 5% PL, 100 μg·mL⁻¹ streptomycin and 100 U·mL⁻¹ penicillin at 37 °C in a humidified atmosphere containing 5% CO₂. The medium was replaced for the first time after 7 days of culture and thereafter every 3–4 days. The umbilical cord segments were removed on the 14th day, and adherent cells reaching 75–85% confluence were detached by 0.05% trypsin/EDTA and further cultivated until the third passage. hUCMSCs at passage 2 were used in the following experiments.

Immunophenotypic characterization of hUCMSCs

hUCMSCs at passage 2 were characterized by flow cytometry. In brief, cells were washed twice with PBS, digested by 0.05% trypsin and suspended at a concentration of 5 × 10⁵ cells per tube. Cells were then stained at 4 °C with the following antibodies: CD34-phycocerythrin (Invitrogen, Carlsbad, CA, USA), CD45-FITC (Abexa, Cambridge, UK), CD44-FITC (Miltenyi Biotec, San Diego, CA, USA) and CD105-phycoerythrin (Invitrogen). After 30 min of incubation in the dark, cells were washed with PBS and analyzed using flow cytometry (Beckman Coulter, Brea, CA, USA).

Chondrogenic differentiation

hUCMSCs at passage 2 (1 × 10⁶ cells) were cultured in a chondrogenic differentiation medium supplemented with Dulbecco’s modified Eagle’s medium, 1 x insulin-transferrin-selenium, 50 mg·L⁻¹ ascorbic acid, 100 nm dexamethasone and 5% PL for 21 days. The culture medium was replaced twice a week.

Cell infection and transfection

The miR-29b-3p precursor-expressing lentiviral particles (Lenti-miR-29b-3p) and control lentivirus particles (Lenti-miR-NC), H19-expressing lentivirus (Lenti-H19) and control lentivirus particles (Lenti-NC), and sh-H19-expressing lentivirus (Lenti-sh-H19) and sh-NC-expressing lentivirus (Lenti-shRNA) were purchased from Hanbio (Shanghai, China). The miR-29b-3p mimic, mimic NC, miR-29b-3p inhibitor and inhibitor NC were purchased from GenePharma (Shanghai, China). The hUCMSCs at 70–80% confluence were infected with these lentiviruses or transfected with the mimics and/or inhibitors using Lipofectamine 3000 (Invitrogen) according to the manufacturer’s
instructions. Cells were harvested 48 h later and subjected to further experiments.

**Alcian blue staining**

After infection with the indicated lentiviruses, hUCMSCs were cultured in a chondrogenic differentiation medium for 21 days as described earlier. Then chondrogenic differentiation was assessed by Alcian blue staining. In brief, the pellets were fixed, dehydrated, embedded in paraffin and sectioned at 5 μm thickness. For detection of proteoglycan and mucopolysaccharides, sections were deparaffinized, rehydrated and then stained with 1% Alcian blue (Sigma-Aldrich, St. Louis, MO, USA) for 30 min.

**Luciferase reporter assay**

The fragments of H19 containing the predicted wild-type (WT) or mutated (Mut) miR-29b-3p binding sites were amplified by PCR and inserted into a pGL3-luciferase vector (Promega, Madison, WI, USA), generating pGL3-H19 WT/pGL3-H19 Mut luciferase constructs. Similarly, the fragments of SOX9 3’-UTR containing the predicted WT or Mut miR-29b-3p binding sites were amplified by PCR and inserted into a pGL3-luciferase vector, generating pGL3-SOX9 WT or pGL3-SOX9 Mut constructs. The primers were as follows: H19 WT construct primer forward, 5’-ACTGGAATTCCTATCATCGGGAGTCC-3’; H19 WT construct primer reverse, 5’-ACTGCTCGAGTGCCTGAAGG-3’; SOX9 WT 3’-UTR construct primer forward, 5’-ACTGGAATTCCTACGGCTTGTCCGTAGGTTCCTTTA-3’; SOX9 WT 3’-UTR construct primer reverse, 5’-ACTGCTCGAGGCGGAGGAGGAG-3’; SOX9 Mut 3’-UTR construct primer forward, 5’-ACACTCGAAGGTGTTGATGCTCAGG-3’; SOX9 Mut 3’-UTR construct primer reverse, 5’-ACACTCGAAGGTGTTGATGCTCAGG-3’. The luciferase activities 24 h after transfection were analyzed using a luciferase reporter assay system (Promega).

**Western blot**

The whole-cell lysates were extracted from hUCMSCs using the radioimmunoprecipitation assay lysis buffer (Beyotime, Haimen, China). Then the protein samples were loaded and separated with 10% SDS/PAGE gels and transferred to poly(vinylidene difluoride) membrane. After being blocked with 5% nonfat milk, the membranes were incubated with primary antibodies (1 : 1000) overnight, followed by the horseradish peroxidase-conjugated secondary antibodies (1 : 2000; Abcam, Cambridge, MA, USA). Blots were examined by an ECL Detection kit (Pierce Biotechnology, Rockford, IL, USA). The primary antibodies used were as follows: anti-collagen II (#ab239007), anti-aggrecan (#ab36861), anti-SOX9 (#ab185230) and anti-a-tubulin (#ab7291) (1 : 1000 dilution; all from Abcam).

**Statistical analysis**

All statistical analyses were performed using ssps 22.0 (SPSS, Inc., Chicago, IL, USA). Statistical significance was analyzed using the Student’s t-test between two groups and one-way ANOVA among three or more groups. A P value <0.05 was considered statistically significant.

**Results**

**H19 expression was increased during PL-induced chondrogenic differentiation of hUCMSCs**

hUCMSCs were isolated from umbilical cords of cesarean-delivered full-term neonates and identified by flow cytometry. Data revealed that the obtained hUCMSCs were positive for CD45 and CD34, but negative for CD45 and CD34 (Fig. 1A), confirming that the cells were MSCs. hUCMSCs were then cultured in 5% PL-supplemented chondrogenic differentiation medium for 0, 7, 14 and 21 days. The results from qRT-PCR analysis demonstrated significant up-regulation of H19 expression during PL-induced chondrogenic differentiation of hUCMSCs (Fig. 1B).
H19 promoted PL-induced chondrogenic differentiation of hUCMSCs

To determine the role of H19 in PL-induced chondrogenic differentiation of hUCMSCs, we overexpressed and silenced H19 in hUCMSCs, and then cultured them in 5% PL-supplemented chondrogenic differentiation medium for 21 days. The overexpression and silencing efficiencies of H19 were confirmed by qRT-PCR analysis (Figs 2A and S1A). Importantly, Alcian blue staining demonstrated that H19 overexpression led to increased production of proteoglycan in the differentiated cells, whereas H19 silencing yielded the opposite results (Fig. 2B). Furthermore, the protein levels of chondrogenic markers (collagen II and aggrecan) in the differentiated cells were significantly up-
regulated by H19 overexpression but down-regulated by H19 silencing (Fig. 2C). These findings indicated that H19 promoted PL-induced chondrogenic differentiation of hUCMSCs.

**H19 acted as a sponge of miR-29b-3p to up-regulate SOX9 expression**

Next, we explored the molecular mechanism by which H19 promotes PL-induced chondrogenic differentiation of hUCMSCs. The results from qRT-PCR analysis revealed that miR-29b-3p expression was notably down-regulated, whereas SOX9 mRNA and protein levels were significantly up-regulated during PL-induced chondrogenic differentiation of hUCMSCs (Fig. 3A–C). Furthermore, H19 overexpression led to a marked increase in SOX9 mRNA and protein levels. In contrast, H19 silencing significantly down-regulated SOX9 expression (Fig. 3D–E). In addition, the luciferase activity was significantly decreased in cells cotransfected with H19 WT reporter and miR-29b-3p mimic (Fig. 3F), indicating that H19 directly interacted with miR-29b-3p. We also observed decreased luciferase activity in cells cotransfected with SOX9 WT reporter and miR-29b-3p mimic (Fig. 3G), suggesting that 3'-UTR of SOX9 was directly targeted by miR-29b-3p. Moreover, transfection with miR-29b-3p mimic resulted in a significant decrease in mRNA and protein levels of SOX9.
On the contrary, inhibition of miR-29b-3p noticeably increased SOX9 expression (Fig. 4A, B). Together, these results manifested that H19 might act as a sponge of miR-29b-3p to up-regulate SOX9 expression.

H19 promoted PL-induced chondrogenic differentiation of hUCMSCs by regulating the miR-29b-3p/SOX9 axis

Finally, we determined whether H19 promotes the PL-induced chondrogenic differentiation of hUCMSCs by regulating the miR-29b-3p/SOX9 axis. To this purpose, we coinfected hUCMSCs with Lenti-NC/Lenti-H19 and Lenti-miR-NC/Lenti-miR-29b-3p, and then cultured them in 5% PL-supplemented chondrogenic differentiation medium for 21 days. The results of the qRT-PCR analysis showed that H19 expression was increased postinfection with Lenti-H19 but decreased postinfection with Lenti-miR-29b-3p (Fig. S1B). Alcian blue staining revealed that Lenti-miR-29b-3p infection reduced production of proteoglycan in the differentiated cells and attenuated the H19
overexpression-mediated promotion of proteoglycan content (Fig. 4C). Furthermore, the up-regulation of protein levels of SOX9, collagen II and aggrecan in the H19-overexpressing cells was rescued following Lenti-miR-29b-3p infection (Fig. 4D). Collectively, these observations suggested that H19 promoted PL-induced chondrogenic differentiation of hUCMSCs by regulating the miR-29b-3p/SOX9 axis.

**Discussion**

MSCs are multipotent stem cells with the potential to differentiate into chondrocytes, and MSC-derived cartilage tissue engineering is a clinical method used for OA treatment [23]. PRP is a blood extraction product widely used in clinical practice to accelerate the wound healing process of some tissues, such as muscle [6], cartilage [7] and tendon [8]. Prepared from PRP, PL has been shown to reduce immunogenicity [10] and promote MSC chondrogenic differentiation, providing broad application prospects for allogeneic or xenogeneic transplantation in cartilage-related diseases [24,25]. A previous study clearly showed that PL induced the differentiation of hUCMSCs into chondrocytes [12]. In this study, we demonstrated for the first time the molecular mechanism by which PL induces MSC chondrogenic differentiation; that is, PL induced chondrogenic differentiation of hUCMSCs by regulating the H19/miR-29b-3p/SOX9 axis.
A number of recent studies have suggested that lncRNA plays a critical role in chondrogenic differentiation of MSCs. An increasing number of lncRNAs have been identified with a potential role in chondrogenesis, such as ROCR [14], differentiation antagonizing non-protein-coding RNA [26], maternally expressed 3 [23] and ADAMTS9 antisense RNA 2 [27]. For instance, lncRNA urothelial cancer-associated 1 (UCA1) promotes chondrogenic differentiation of human bone marrow-derived MSCs via regulating the miR-145-5p/SMAD5 and miR-124-3p/SMAD4 axis [28]. lncRNA H19 has been shown to promote the osteogenic differentiation of MSCs [15,16] and inhibit the differentiation MSCs into adipocytes [17]. Yang et al. [18] demonstrated that in the bovine mammary alveolar cell–T cell line, TGF-β induced up-regulation of H19, which promoted epithelial-to-mesenchymal transition and contributed to mammary gland fibrosis. PL contains a relatively large number of growth factors, including TGF-β that is critical for chondrogenic differentiation [10]. Thus, we speculated that H19 might be involved in the PL-induced chondrogenic differentiation of hUCMSCs. To our knowledge, our findings in this study provided the first evidence that H19 overexpression promoted, whereas H19 silencing attenuated the chondrogenic differentiation of PL-treated MSCs. Evidence indicates that TGF-β1 up-regulated H19 expression through activating the phosphoinositide-3-kinase/AKT pathway in epithelial cells [29]. Whether the phosphoinositide-3-kinase/Akt pathway or other transcriptional regulation mechanisms are involved in the PL-induced H19 up-regulation remains to be further investigated.

Several studies have established that H19 regulates the expression and biological functions of certain miRNAs by acting as a competing endogenous RNA or a miRNA sponge [30,31]. For example, H19 contributes to oxidative damage repair in the early age-related cataract by sponging miR-29a [32]. miR-29b-3p not only plays an important role in tumor initiation and progression [33] but also participates in DNA damage response [34], tissue fibrosis [35] and chondrogenic differentiation [36]. In this study, we performed bioinformatics analysis and luciferase reporter assay to analyze the interaction between miR-29b-3p and H19 or SOX9. Results showed that H19 can function as a sponge RNA for miR-29b-3p that negatively regulates the expression of target gene SOX9. SOX9 gene is a cartilage-specific transcription factor that controls the expression of numerous chondrocyte genes (e.g. collagen II and aggrecan) and plays essential roles in chondrocyte differentiation and cartilage formation [37,38]. Our results demonstrated that H19 positively regulated SOX9 expression and promoted MSC chondrogenic differentiation, and these effects were rescued by miR-29b-3p mimic.

**Conclusion**

The findings in this study demonstrate that H19 expression is induced in PL-stimulated hUCMSCs and that H19 acts as a sponge of miR-29b-3p to up-regulate the expression of the master chondrocyte transcription factor SOX9, thereby promoting PL-induced chondrogenic differentiation of hUCMSCs. These data provide new experimental evidence for elucidating the mechanism of MSC in cartilage repair and a novel therapeutic option for OA.

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**Conflict of interest**

The authors declare no conflict of interest.

**Data accessibility**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Author contributions**

BC designed the study. BC and XD participated in the experiments. BC and XD contributed to the data analysis. BC drafted the paper. All authors approved the paper.

**References**

1 Hunter DJ and Bierma-Zeinstra S (2019) Osteoarthritis. *Lancet* **393**, 1745–1759.
2 Espanha MM (2010) Articular cartilage: structure and histochemical composition. *Acta Reumatol Port* **35**, 424–433.
3 Shen H, Lin H, Sun AX, Song S, Wang B, Yang Y, Dai J and Tuan RS (2020) Acceleration of chondrogenic differentiation of human mesenchymal stem cells by sustained growth factor release in 3D graphene oxide incorporated hydrogels. *Acta Biomater* **105**, 44–55.
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4 Richardson SM, Kalamegam G, Pushparaj PN, Matta C, Memic A, Khademhosseini A, Mobasher R, Poletti FL, Hoyland JA, Mobasher A et al. (2016) Mesenchymal stem cells in regenerative medicine: focus on articular cartilage and intervertebral disc regeneration. Methods 99, 69–80.

5 Zheng P, Ju L, Jiang B, Chen L, Dong Z, Jiang L, Wang R and Lou Y (2013) Chondrogenic differentiation of human umbilical cord blood-derived mesenchymal stem cells by coculture with rabbit chondrocytes. Mol Med Rep 8, 1169–1182.

6 Borrione P, Fagnani F, Di Gianfrancesco A, Mancini A, Pigozzi F and Pitsiladis Y (2017) The role of platelet-rich plasma in muscle healing. Curr Sports Med Rep 16, 459–463.

7 Xu Z, Yin W, Zhang Y, Qi X, Chen Y, Xie X and Zhang C (2017) Comparative evaluation of leukocyte- and platelet-rich plasma and pure platelet-rich plasma for cartilage regeneration. Sci Rep 7, 43301.

8 Filardo G, Di Matteo B, Kon E, Merli G and Marcarelli M (2018) Platelet-rich plasma in tendon-related disorders: results and indications. Knee Surg Sports Traumatol Arthrosc 26, 1984–1999.

9 Burnouf T, Strunk D, Koh MB and Schallmoser K (2016) Human platelet lysate: replacing fetal bovine serum as a gold standard for human cell propagation? Biomaterials 76, 371–387.

10 Fekete N, Gadelorge M, Fürst D, Maurer C, Dausend J, Fleury-Cappellesso S, Mailänder V, Lotfi R, Ignatius A, Sensebé L et al. (2012) Platelet lysate from whole blood-derived pooled platelet concentrates and apheresis-derived platelet concentrates for the isolation and expansion of human bone marrow mesenchymal stromal cells: production process, content and identification of active components. Cytotherapy 14, 540–554.

11 Asen AK, Goebel L, Rey-Rico A, Sohier J, Zurakowski D, Cucchiarini M and Madry H (2018) Sustained spatiotemporal release of TGF-beta1 confers enhanced very early chondrogenic differentiation during osteochondral repair in specific topographic patterns. Faseb J 32, 5298–5311.

12 Hassan G, Bahjat M, Kasem I, Soukkarieh C and Aljamali M (2018) Platelet lysate induces chondrogenic differentiation of umbilical cord-derived mesenchymal stem cells. Cell Mol Biol Lett 23, 11.

13 Quinn JJ and Chang HY (2016) Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet 17, 47–62.

14 Barter MJ, Gomez R, Hyatt S, Cheung K, Skelton AJ, Xu Y, Clark IM and Young DA (2017) The long non-coding RNA ROCR contributes to SOX9 expression and chondrogenic differentiation of human mesenchymal stem cells. Development 144, 4510–4521.

15 Liao J, Yu X, Hu X, Fan J, Wang J, Zhang Z, Zhao C, Zeng Z, Shu Y, Zhang R et al. (2017) IncRNA H19 mediates BMP9-induced osteogenic differentiation of mesenchymal stem cells (MSCs) through Notch signaling. Oncotarget 8, 53581–53601.

16 Huang Y, Zheng Y, Jia L and Li W (2015) Long noncoding RNA H19 promotes osteoblast differentiation Via TGF-beta1/Smad3/HDAC signaling pathway by deriving miR-675. Stem Cells 33, 3481–3492.

17 Huang Y, Zheng Y, Jin C, Li X, Jia L and Li W (2016) Long Non-coding RNA H19 Inhibits adipocyte differentiation and promotes tendon healing through targeting miR-29b-3p and activating TGF-beta1 signaling. Sci Rep 6, 28897.

18 Yang W, Li X, Qi S, Li X, Zhou K, Qing S, Zhang Y and Gao MQ (2017) IncRNA H19 is involved in TGF-beta1-induced epithelial to mesenchymal transition in bovine epithelial cells through PI3K/AKT signaling pathway. PeerJ 5, e3950.

19 Lu YF, Liu Y, Fu WM, Xu J, Wang B, Sun YX, Wu TY, Xu LL, Chan KM, Zhang JF et al. (2017) Long noncoding RNA H19 accelerates tenogenic differentiation and promotes tendon healing through targeting miR-29b-3p and activating TGF-beta1 signaling. Faseb J 31, 954–964.

20 Ding D, Li C, Zhao T, Li D, Yang L and Zhang B (2018) LncRNA H19/miR-29b-3p/PGRN axis promoted epithelial-mesenchymal transition of colorectal cancer cells by acting on Wnt signaling. Mol Cells 41, 423–435.

21 Lv M, Zhong Z, Huang M, Tian Q, Jiang R and Chen J (2017) IncRNA H19 regulates epithelial-mesenchymal transition and metastasis of bladder cancer by miR-29b-3p as competing endogenous RNA. Biochim Biophys Acta Mol Cell Res 1864, 1887–1899.

22 Zhang X, Cheng L, Xu L, Zhang Y, Yang Y, Fu Q, Mi W and Li H (2019) The IncRNA, H19 mediates the protective effect of hypoxia postconditioning against hypoxia-reoxygenation injury to senescent cardiomyocytes by targeting microRNA-29b-3p. Shock 52, 249–256.

23 You D, Yang C, Huang J, Gong H, Yan M and Ni J (2019) Long non-coding RNA MEG3 inhibits chondrogenic differentiation of synovium-derived mesenchymal stem cells by epigenetically inhibiting TRIB2 via methyltransferase EZH2. Cell Signal 63, 109379.

24 Galeano-Garces C, Camilleri ET, Riester SM, Dudakovic A, Larson DR, Qu W, Smith J, Dietz AB, Im H-J, Krych AJ et al. (2017) Molecular validation of chondrogenic differentiation and hypoxia responsiveness of platelet-lysate expanded adipose tissue-derived human mesenchymal stromal cells. Cartilage 8, 283–299.
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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Expression of H19 in hUCMCS postinfection with the indicated lentiviruses. (A) qRT-PCR analysis of H19 expression in hUCMCS infected with Lenti-NC, Lenti-H19, Lenti-shRNA and Lenti-sh-H19 after culture in 5% PL-supplemented chondrogenic differentiation medium for 21 days. **P < 0.01, vs. Lenti-NC; ##P < 0.01, vs. Lenti-shRNA (Student’s t-test). (B) qRT-PCR analysis of H19 expression in hUCMCS coinfected with Lenti-NC/Lenti-H19 and Lenti-miR-NC/Lenti-miR-29b-3p after culture in 5% PL-supplemented chondrogenic differentiation medium for 21 days. **P < 0.01, vs. Lenti-miR-NC + Lenti-NC; ##P < 0.05, vs. Lenti-miR-NC + Lenti-H19 (ANOVA). The quantitative statistics were presented as the mean ± standard deviation (n = 3).