Supplemental data

LncRNA SNHG16 promotes human placenta-derived mesenchymal stem cell proliferation capacity through the PI3K/AKT pathway under hypoxia

Xudong Feng[1], Jiahang Zhou[1], Junyao Chen[1], Bing Feng[1], Ruitian Hu[3], Jian Wu[1,2], Qiaoling Pan[1], Jinfeng Yang[1], Jiong Yu[1], Hongcui Cao*[1, 4]

1 State Key Laboratory for the Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, 79 Qingchun Rd., Hangzhou City 310003, China

2 Jinan Microecological Biomedicine Shandong Laboratory, Jinan, Shandong 250117, , China

3 Department of Chemistry, Duke University, 124 Science Drive, Durham, NC 27708, United States

4 Key Laboratory of Diagnosis and Treatment of Aging and Physic-chemical Injury Diseases of Zhejiang Province, 79 Qingchun Rd, Hangzhou City 310003, China.

***Corresponding author:**

Hongcui Cao, M.D.

State Key Laboratory for the Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, 79 Qingchun Rd., Hangzhou City 310003, China. Tel: 86-571-87236451; Fax: 86-571-87236459

E-mail: hccao@zju.edu.cn

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Methods

Cell culture

hP - MSCs were cultured in specialized medium (MesenCult® Human Basal Medium plus MesenCult® Human Supplement; STEMCELL Technologies Inc., Vancouver, Canada) in 25 cm2 cell culture flasks (Nunc™ EasYFlask™; Thermo Fisher Scientific Inc., Waltham, MA). During the continuous culture period, the culture medium was replaced every 3 days, and the cells were trypsinized and passaged upon reaching 70–80% confluence. The cells were stabilized in a standard humidified incubator (HERAcell150, Thermo Fisher Scientific Inc., Waltham, MA, United States) with a 21% O2 and 5% CO2 atmosphere. The hypoxic groups were placed in a humidified, water-jacketed CO2 incubator with oxygen control (Forma™ Series II, Thermo Fisher Scientific Inc.) in an atmosphere containing 2.5% O2 and 5% CO2. The normoxia group continued to be incubated in the standard humidified incubator.

Colony-forming unit-fibroblast (CFU-F) assay

For the CFU-F assay, 1000 hP-MSCs were plated on six-well plates in triplicate and cultured in complete medium for 14 days under normoxic or hypoxic conditions with medium changes every 3 days. The culture dishes were rinsed with PBS, fixed with 4% paraformaldehyde and then stained with crystal violet. After rinsing with PBS, colonies consist of more than 50 stained cells were counted. Total colony numbers was determined.

Flow cytometry analysis of cell cycle

The cells were collected with trypsin and fixed with cold 70% ethanol for at least 2 h. After fixation, cells were transferred from 4 °C to the bench top and equilibrated to room temperature. The cells were washed with cold PBS and incubated in the dark with 400 μL staining buffer containing RNase A and propidium iodide at 37 °C for 30 min. A flow cytometer (BeamCyte-1026) was

used to analyze the cell cycle distribution with ModFit LT ver. 5.0 (Verity Software House).

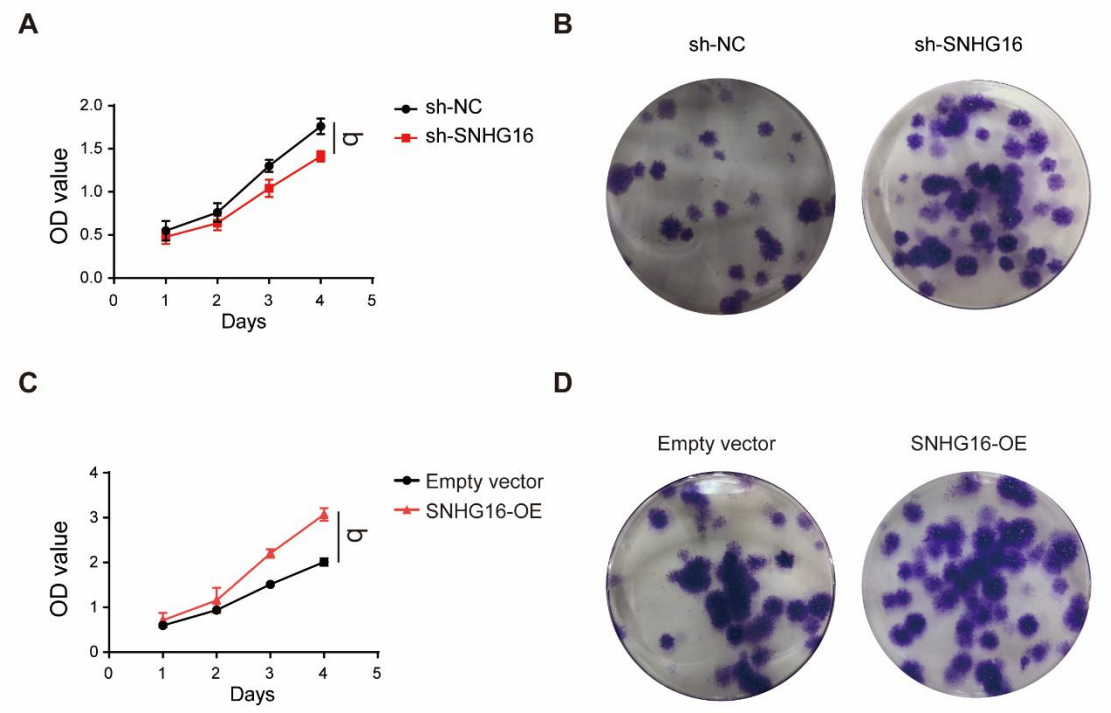
Western blot assay

The cellular protein was harvested by solubilizing MSCs in RIPA lysis buffer (Beyotime) mixed with protease inhibitor cocktail and phosphatase inhibitor cocktail. The protein concentration was determined using a BCA kit (Beyotime). A total of 20 μg of proteins were run on 4–15% Mini‐PROTEAN® TGX™ gels (Bio‐Rad Laboratories, Inc., Hercules, CA) and transferred onto a polyvinylidene difluoride
membrane (Merck KGaA, Darmstadt, Germany). Then, the membranes were incubated in QuickBlock™ Blocking Buffer (Beyotime Biotech Co., Ltd.) at room temperature. After blocking, the membranes were immunoblotted with specific primary antibodies overnight at 4°C. After washing, the membranes were incubated with horseradish peroxidase‐conjugated goat anti‐rabbit IgG (1:4,000; Abcam, Cambridge, UK) for 1 hr at room temperature. After rinsing with Tris‐buffered saline with 0.1% Tween, the membranes were incubated with Pierce™ ECL Western blot analysis Substrate (Thermo Fisher Scientific Inc.) for 1 min and detected with the ChemiScope Western Blot Imaging System (Clinx Science Instruments Co., Ltd., Shanghai, China). The primary antibodies were anti-β-actin (Abcam, Cambridge, UK), anti-GAPDH (Abcam), anti-HIF1α (Cell Signaling Technology, Danvers, MA, USA), anti-c-MYC (Abcam), anti-PCNA (Abcam), anti-CDK2 (Abcam), anti-CDK4 (Abcam), anti-CDK6 (Abcam), anti-CyclinD1 (Abcam), anti-CyclinE1 (Abcam), anti-AKT (Abcam), and anti-phospho-AKT (Abcam).

Cell transfection

Once the cells reached 50% confluence, the hPMSCs were distributed into four groups for different treatments to acquire distinct levels of SNHG16 expression. Recombinant lentiviruses for SNHG16 over-expression or the

corresponding empty vectors (Genomeditech, Shanghai, China) were separately added to the medium to infect the respective groups of hPMSCs with an MOI of 50:1, with the aim of acquiring hPMSC that over-expressed SNHG16 and a corresponding negative control, empty vector. Similarly, two other groups of hPMSCs were individually infected with an SNHG16 shRNA for gene silencing or a scrambled shRNA for the negative control. The four groups of processed hPMSCs were cultured as usual for 48 hours, and the culture medium was replaced. Puromycin (Sigma-Aldrich Co. LLC, St. Louis, MO, USA) was mixed into each medium and filtered. After 2–3 days of extended culture, the medium was replaced and the filtering was repeated. Then, successful infection was confirmed by qRT-PCR.



Supplementary Figure 1 SNHG16 is a potential promoter of the hP-MSC proliferative rate under hypoxia. A: The cell proliferation ability of SNHG16-knockdown hP-MSCs under hypoxic culture was assessed by CCK-8 assay; B: Colony formation of SNHG16-knockdown hP-MSCs under hypoxic culture; C: The cell proliferation ability of SNHG16-overexpression hP-MSCs under hypoxic culture; D: Colony formation of SNHG16-overexpression hP-MSCs under hypoxic culture. Data are presented as means ± SD. $^{b}P < 0.01$.