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Original Article

Long noncoding RNA urothelial carcinoma associated 1 protects human placental vascular endothelial cells from hypoxia-induced damage by regulating the miR-197-3p/histone deacetylase-2 axis in patients with pregnancy-induced hypertension

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Abstract: Purpose: Pregnancy-induced hypertension (PIH) is a major cause of mortality among pregnant women, fetuses, and newborns. This study assessed the role of long noncoding RNA (lncRNA) urothelial carcinoma associated 1 (UCA1) in PIH development. Methods: Serum samples were collected from 30 pregnant women with PIH and 30 healthy pregnant women. Serum UCA1, miR-197-3p, and histone deacetylase-2 (HDAC2) mRNA level was evaluated using quantitative polymerase chain reaction. The expression of UCA1, miR-197-3p and HDAC2 in human placental vascular endothelial cells (HPVECs) was regulated by transfection. HPVECs were treated with hypoxia reoxygenation (H/R) to establish the PIH cell model. Methyl thiazolyl tetrazolium (MTT) assay, the terminal transferase uridyl nick end labelling (Tunel) assay and tubule formation assay were performed to assess the viability, apoptosis and angiogenesis of HPVECs. Dual-luciferase reporter gene assay, RNA pull-down assay, and RNA immunoprecipitation assay were performed to identify the binding between two genes. Western blot analysis was used for protein expression detection. Results: In pregnant women with PIH, serum UCA1 and HDAC2 expression was downregulated and serum miR-197-3p expression was upregulated. H/R induction decreased the viability and angiogenesis of HPVECs, and increased the apoptosis of HPVECs. In H/R-induced HPVECs, UCA1 upregulation increased the viability and angiogenesis, and decreased the apoptosis. Downregulation of UCA1 had a contrasting result. UCA1 competitively binds to miR-197-3p to upregulate the expression of HDAC2. HDAC2 knockdown counteracted the effect of UCA1 upregulation on the viability, apoptosis and angiogenesis of HPVECs. Conclusions: LncRNA UCA1 protected HPVECs from hypoxia-induced damage by regulating the miR-197-3p/HDAC2 axis in PIH.

Keywords: PIH, LncRNA UCA1, apoptosis, angiogenesis, miR-197-3p/HDAC2

Introduction

Pregnancy-induced hypertension (PIH) is a common complication that poses a serious threat to the lives of pregnant women, fetuses, and newborns [1]. PIH is defined as a systolic blood pressure of > 140 mmHg and a diastolic blood pressure of > 90 mmHg in pregnant women [2]. Fetal hypoxia caused by abnormal placental function-induced by PIH is the main cause of fetal and neonatal mortality, and PIH-induced heart failure is mainly associated with maternal mortality [3]. Endothelial cell damage in maternal and placental angiogenic disorders is an important factor affecting PIH [2]. However, significant efforts must be exerted to elucidate the molecular mechanisms of PIH pathogenesis, which can then help control and treat the condition.

Long noncoding RNAs (lncRNAs) are a class of transcripts with > 200 nucleotides without protein-coding function [4]. LncRNAs are associated with multiple physiological and pathological processes in human diseases [5]. The expression of some serum lncRNAs is aberrantly high

in pregnant women with PIH and is correlated with the severity of PIH. These lncRNAs can be early biomarkers for the prediction of PIH [6]. The expression of serum LncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is high in pregnant women with PIH. MALAT1 can promote inflammation and oxidative stress, thereby facilitating the development of PIH [7]. Nevertheless, the function of lncRNAs in PIH remains in its infancy. The actual functions of a large number of lncRNAs in PIH development remain unknown.

LncRNA urothelial carcinoma associated 1 (UCA1) is located at the human chromosome 19p13.12 [8]. It is an oncogene in several types of human cancers [9, 10]. However, its function in PIH is not fully elucidated. We conducted a preliminary research, and the results showed that the serum lncRNA UCA1 expression was up-regulated in pregnant women with PIH compared with healthy pregnant women. Therefore, UCA1 may be involved in the regulation of PIH. In this study, a series of experiments were performed to validate this hypothesis. In addition, by predicting ENCORI (http://starbase.sysu.edu.cn), miR-197-3p was found to have a binding site with UCA1 and histone deacetylase-2 (HDAC2) in the 3'UTR region. Previous studies have shown that miR-197-3p is overexpressed in the maternal plasma of patients with pre-eclampsia (PE), and the HDAC2 level was significantly downregulated in the placental tissues of patients with severe PE [11, 12]. PE after 20 weeks of gestation is characterized by hypertension and proteinuria [13]. Moreover, our preliminary research revealed that compared with healthy pregnant women, pregnant women with PIH had over-regulated serum miR-197-3p expression and down-regulated serum HDAC2 expression. Taken together, this study aimed to validate the effect of UCA1 on the development of PIH by targeting the miR-197-3p/HDAC2 axis. The findings might provide an effective molecular target for the treatment of PIH in clinical practice.

Materials and methods

Ethics approval

This study was approved by the ethics committee of The Second Affiliated Hospital of Nantong University (2020KY010) and was performed in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Patients and blood samples

This study enrolled 30 pregnant women with PIH (PIH group) and 30 healthy pregnant women (normal group) in The Second Affiliated Hospital of Nantong University from November 2020 to March 2021. Blood samples were collected at 33-40 weeks of pregnancy. The serum was separated to detect the expression of UCA1, miR-197-3p, and HDAC2 mRNA. Patients with a singleton pregnancy, those diagnosed with PIH, and those without other organ diseases and chronic diseases were included in the study and were classified under the PIH group.

Cell culture and transfection

Human placental vascular endothelial cells (HPVECs, Catalog #7100, ScienCell, Wuhan, China) were cultured in Endothelial Cell Medium (ECM, Solarbio, Beijing, China) at 37°C, 5% CO_2 in a humidified incubator.

PcDNA-UCA1 vectors, empty vectors, UCA1 shRNA and negative control (NC), miR-197-3p mimic, mimic NC, miR-197-3p inhibitor, inhibitor NC, HDAC2 shRNA, and scramble shRNA were purchased from GeneChem (Shanghai, China). HPVECs grown in serum-free ECM were transfected using Lipofectamine™ 2000 Transfection Reagent (Thermo Fisher Scientific, Shanghai, China). The transfection process was implemented in accordance with the manufacturer's instructions.

Hypoxia reoxygenation (H/R) induction to construct the PIH cell model

After transfection, HPVECs were subjected to H/R induction, as described in a previous study [14]. In summary, HPVECs were cultured overnight in ECM at 37°C with 5% CO_2. Thereafter, for hypoxia treatment, HPVECs were placed at 37°C and 2% O_2 for 4 h. Next, HPVECs were cultured under normal conditions (37°C, 5% CO_2) for reoxygenation. HPVECs subjected to neither transfection nor H/R induction were used as control.

Methyl thiazolyl tetrazolium (MTT) assay

HPVECs (1×10^4 cells) were cultured in 100 μL ECM in 96-well plates for 48 h at 37°C and 5%

CO_2 after H/R induction. Subsequently, MTT solution (5 mg/mL, 10 μL) was added into wells to treat HPVECs for 4 h at 37°C. Before treatment with 150 μL of dimethyl sulfoxide (Solarbio, Beijing, China), the waste liquid in each well was removed. After treatment with dimethyl sulfoxide for 15 min, the absorbance value of each well was assessed with a microplate reader (BioTek, Shoreline, WA, the USA) at a wavelength of 450 nm.

The terminal transferase uridyl nick end labelling (Tunel) assay

After hypoxia treatment, HPVECs (1×10^5 cells) were cultured in 6-well plates for 48 h at 37°C and 5% CO_2. Then, the In Situ Apoptosis Detection Kit (Roche, Mannheim, Germany) was used to detect the apoptotic capability of HPVECs according to the manufacturer's instructions. Under a fluorescence microscope (Olympus, Tokyo, Japan), apoptotic HPVECs showed red fluorescence, and the nuclei showed blue fluorescence using 4', 6-diamidino-2-phenylindole (Beyotime, Shanghai, China).

Tubule formation assay

Growth factor reduced Matrigel (200 μL, BD Biosciences, Bedford, MA, the USA) was precoated into 24-well plates. After hypoxia treatment, HPVECs (1×10^5 cells) were inoculated into 24-well plates and cultured for 48 h at 37°C and 5% CO_2. Tube formation was evaluated, and images were obtained using a microscope (Olympus, Tokyo, Japan). The tube length was measured to evaluate the angiogenic capability of HPVECs.

Dual-luciferase reporter gene assay

By predicting ENCORI (http://starbase.sysu.edu.cn), miR-197-3p was found to possess UCA1 and HDAC2 binding sites in the 3'UTR region. Then, 293T cells (Catalog #EY-X0563, ATCC, Shanghai, China) were used to conduct the dual-luciferase reporter gene assay. Dulbecco's modified eagle medium without fetal bovine serum was used to suspend 293T cells at a density of 1×10^6 cells/mL. After inoculation into 6-well plates, 293T cells were transfected with miR-197-3p mimic and negative control (NC) mimic using Lipofectamine™ 2000 Transfection Reagent (Thermo Fisher Scientific, Shanghai, China). In addition, the full length of UCA1 and HDAC2 sequences, including wild-type (WT) and mutant-type (MUT) sequences, were designed, synthesized, and loaded onto luciferase reporters (GeneChem, Shanghai, China). These luciferase reporters were co-transfected into 293T cells. After transfection, 293T cells were cultured with Dulbecco's modified eagle medium containing 10% fetal bovine serum for 48 h. The firefly and *Renilla* luciferase activity was then monitored using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). The relative firefly luciferase activity was normalized to the Renilla luciferase activity.

RNA pull-down assay

HPVECs transfected with pcDNA-UCA1 vectors and empty vectors were harvested after 48 h of transfection. Next, they were lysed and centrifuged, and the supernatant was collected and incubated using the biotin-labeled UCA1 probe for 4 h at room temperature. Then, Dynabeads M-280 Streptavidin (Dynal AS, Oslo, Norway) was added to capture the UCA1-miRNAs complexes. After 12 h of capture, the beads were obtained via centrifugation. RNA immunoprecipitation (RIP) wash buffer with proteinase K (Beyotime, Shanghai, China) was added to the beads to elute the UCA1-miRNAs complexes. The extraction of RNAs in the UCA1-miRNAs complexes was implemented by incubating with TRIzol reagent (Beyotime, Shanghai, China). Quantitative polymerase chain reaction (qPCR) was performed to determine the expression of miR-197-3p.

RIP assay

HPVECs were harvested and lysed using RNA lysis buffer. RIP immunoprecipitation buffer (Beyotime, Shanghai, China) was then used to incubate the lysate. Notably, Protein A/G Sepharose beads (Biolab Technology, Beijing, China) conjugated with Ago-2 antibody (1:100, FNab00214, Fine Biotechnology, Wuhan, China) or IgG antibody (1:100, GWB-T00987, LMAI Bio, Shanghai, China) were contained in the RIP immunoprecipitation buffer. The RNeasy Mini Kit (#74106, Qiagen, Beinuo Bio-medical Technology Co.,Ltd., Shanghai, China) was used to extract the immunoprecipitated RNA. The level of immunoprecipitated UCA1 and miR-197-3p was determined via qPCR.

qPCR

HPVECs were collected after 48 h of H/R induction. TRIzol reagent (Beyotime Biotechnology, Shanghai, China) was used to extract total RNA in HPVECs. Reverse transcription (RT) reaction was performed using the cDNA kit (D7170L, Beyotime, Shanghai, China) and the miRNA RT kit (QP013, GeneCopoeia, Rockville, MD, USA) based on the manufacturer's instructions. PCR was performed using the ABI 7500 qPCR instrument (Applied Biosystems, Foster City, CA, the USA) under the following conditions: 95°C, 20 s; 40 repetitions (95°C, 15 s and 58°C, 60 s). The primers were: UCA1: forward, 5'-CTCTCCATTGGGTTCACCATTC-3', reverse, 5'-GCGGCAGGTCTTAAGAGATGAG-3'; HDAC2: forward, 5'-CGTGTAATGACGGTATCATTCC-3', reverse, 5'-ACCAGATAATGAGTCTGCACC-3'; β-actin: forward, 5'-TCCCTGGAGAAGAGCTACGA-3', reverse, 5'-AGCACTGTGTTGGCGTACAG-3'; miR-197-3p: forward, 5'-GTTCACCACCTTCTCCAC-3', reverse, 5'-GTGCAGGGTCCGAGGT-3'; and U6: forward, 5'-CTCGCTTCGGCAGCACA-3',reverse,5'-AACGCTTCACGAATTTGCGT-3'. β-actin was used as a control for UCA1 and HDAC2 and U6 for miR-197-3p. The expression of genes was determined using the $2^{-\Delta\Delta Ct}$ method and was normalized to the relative control.

Western blot analysis

HPVECs were harvested after 48 h of H/R induction. RIPA lysis buffer (Beyotime, Shanghai, China) was utilized to extract total proteins in HPVECs. The BCA kit (Beyotime, Shanghai, China) was used to identify total proteins. Then, sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed to separate proteins. The proteins were transferred to polyvinyl difluoride membranes and then blocked by 5% skimmed milk. After probing with primary antibodies (dilution ratio of 1:1000) overnight at 4°C, the proteins were treated with secondary antibodies (dilution ratio of 1:2000) for 2 h at room temperature. The following antibodies were purchased from Abcam (Shanghai, China): mouse anti-HDAC2 (ab12169), rabbit anti-Bcl-2 (ab59348), rabbit anti-Bax (ab53154), rabbit anti-cleaved caspase-3 (ab2302), rabbit anti-caspase-3 (ab44976), rabbit anti-β-actin (ab8227), and horseradish peroxidase-labeled goat anti-mouse (1:2000, ab6789) or anti-rabbit (ab6721, 1:2000) secondary antibodies. The visualization and quantification of protein blots were implemented using the enhanced chemiluminescence detection system (Pierce, Rockford, USA) and Image J (NIH, Bethesda, Maryland, USA) sequentially. β-actin was used as a control.

Statistical analysis

Quantitative data were expressed as mean ± standard deviation. The Statistical Package for the Social Sciences software version 19.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The Student's *t*-test (for two groups) and one-way analysis of variance (for more than two groups) were performed for data comparison. A P value of < 0.05 was considered statistically significant.

Results

Downregulation of serum UCA1 and HDAC2 expression and upregulation of serum miR-197-3p expression in pregnant women with PIH

Serum samples were collected from pregnant women with PIH (n = 30, PIH group) and healthy pregnant women (n = 30, normal group). The expression of serum UCA1, miR-197-3p, and HDAC2 mRNA was monitored via qPCR. Compared with healthy pregnant women, pregnant women with PIH had an aberrantly lower serum UCA1 and HDAC2 mRNA expression and a higher serum miR-197-3p expression ($P < 0.01$) (**Figure 1A-C**). Based on Pearson's correlation analysis, in pregnant women with PIH, the serum UCA1 level was negatively correlated with the serum miR-197-3p level ($P = 0.0087$) and positively correlated with the serum HDAC2 mRNA level ($P < 0.0001$). Moreover, the miR-197-3p level was negatively correlated with the HDAC2 mRNA level ($P = 0.0246$) (**Figure 1D-F**). Taken together, pregnant women with PIH had a downregulated UCA1 and HDAC2 and upregulated miR-197-3p in the serum.

Increased viability and decreased apoptotic capability of H/R-induced HPVECs with UCA1 upregulation

The HPVECs of the H/R group had a lower UCA1 expression than those of the control group ($P < 0.01$). Hence, H/R induction significantly decreased the expression of UCA1 in HPVECs. The HPVECs of the H/R + pcDNA-UCA1

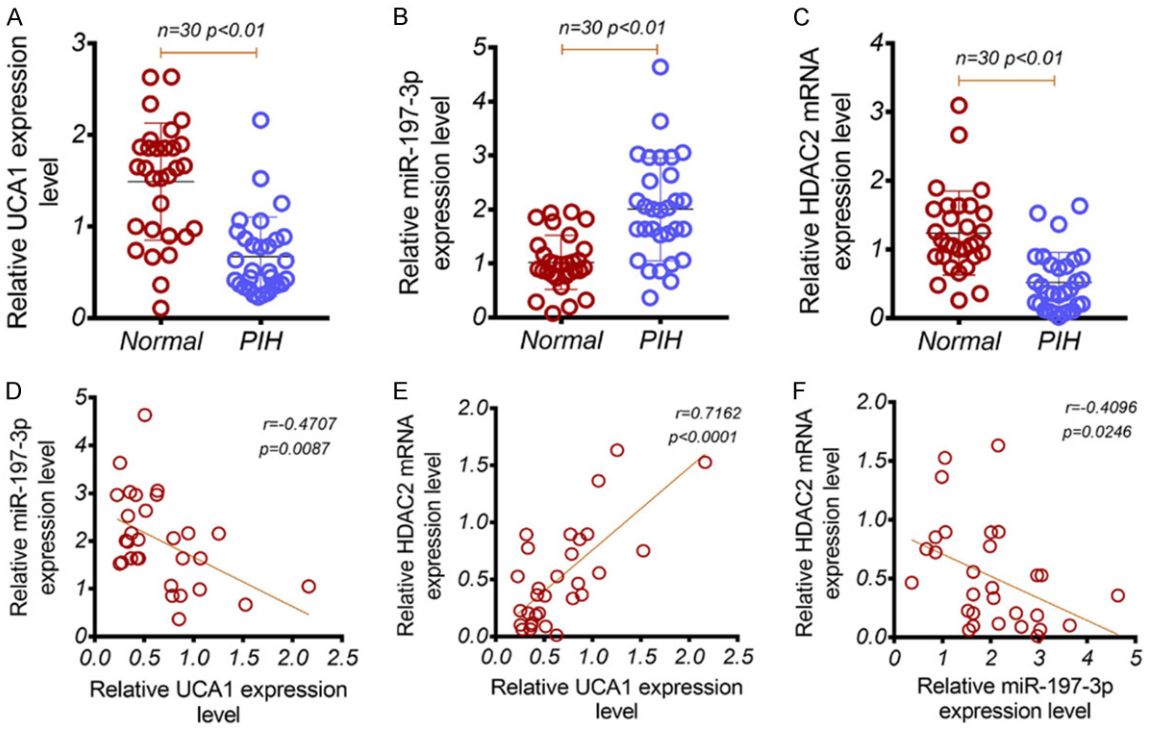

Figure 1. Down-regulated UCA1 and HDAC2 and up-regulated miR-197-3p were found in the serum of pregnant women with PIH. A-C. The expression of UCA1, miR-197-3p and HDAC2 mRNA in the serum of pregnant women with PIH (PIH group) and healthy pregnant women (Normal group) was monitored by qPCR. D-F. Pearson's correlation analysis was implemented to establish a relationship between UCA1, miR-197-3p and HDAC2 mRNA level in the serum of pregnant women with PIH. Abbreviations: UCA1, urothelial carcinoma associated 1; HDAC2, histone deacetylase-2; PIH, pregnancy-induced hypertension; qPCR, quantitative polymerase chain reaction.

group had an aberrantly elevated UCA1 expression, and the HPVECs of the H/R + shUCA1 group had a lower UCA1 expression ($P < 0.01$) (**Figure 2A**). Therefore, HPVECs were effectively induced by H/R and transfected with pcDNA-UCA1 vectors and UCA1 shRNA.

The MTT assay was performed to assess the viability of HPVECs. H/R induction (H/R group) significantly decreased the viability of HPVECs as compared with the control group ($P < 0.01$). Comparatively, the upregulation of UCA1 remarkably increased the viability of H/R-induced HPVECs. Meanwhile, the downregulation of UCA1 had an opposite effect ($P < 0.01$) (**Figure 2B**).

The Tunel assay showed that H/R induction increased the apoptosis of HPVECs ($P < 0.01$). Interestingly, the upregulation of UCA1 decreased the apoptosis of H/R-induced HPVECs. Meanwhile, the downregulation of UCA1 had an opposite effect on the apoptosis of H/R-induced HPVECs ($P < 0.01$) (**Figure 2C**). The expression of apoptosis-related proteins was evaluated via Western blot analysis. Results showed that H/R-induced HPVECs had lower Bcl-2 protein expression and a higher Bax and cleaved caspase-3/caspase-3 protein expression ($P < 0.01$). Intriguingly, the upregulation of UCA1 significantly increased the expression of Bcl-2. However, it reduced the expression of Bax and cleaved caspase-3/caspase-3 protein in H/R-induced HPVECs ($P < 0.05$ or $P < 0.01$). Conversely, the downregulation of UCA1 decreased the expression of Bcl-2. However, it increased the expression of Bax and cleaved caspase-3/caspase-3 proteins in H/R-induced HPVECs ($P < 0.01$) (**Figure 2D**). Therefore, the upregulation of UCA1 increased the viability and decreased the apoptotic capability of H/R-induced HPVECs.

Upregulation of UCA1 increased angiogenesis of H/R-induced HPVECs

The tubule formation assay was performed to evaluate the tube-forming capability of HPVECs.

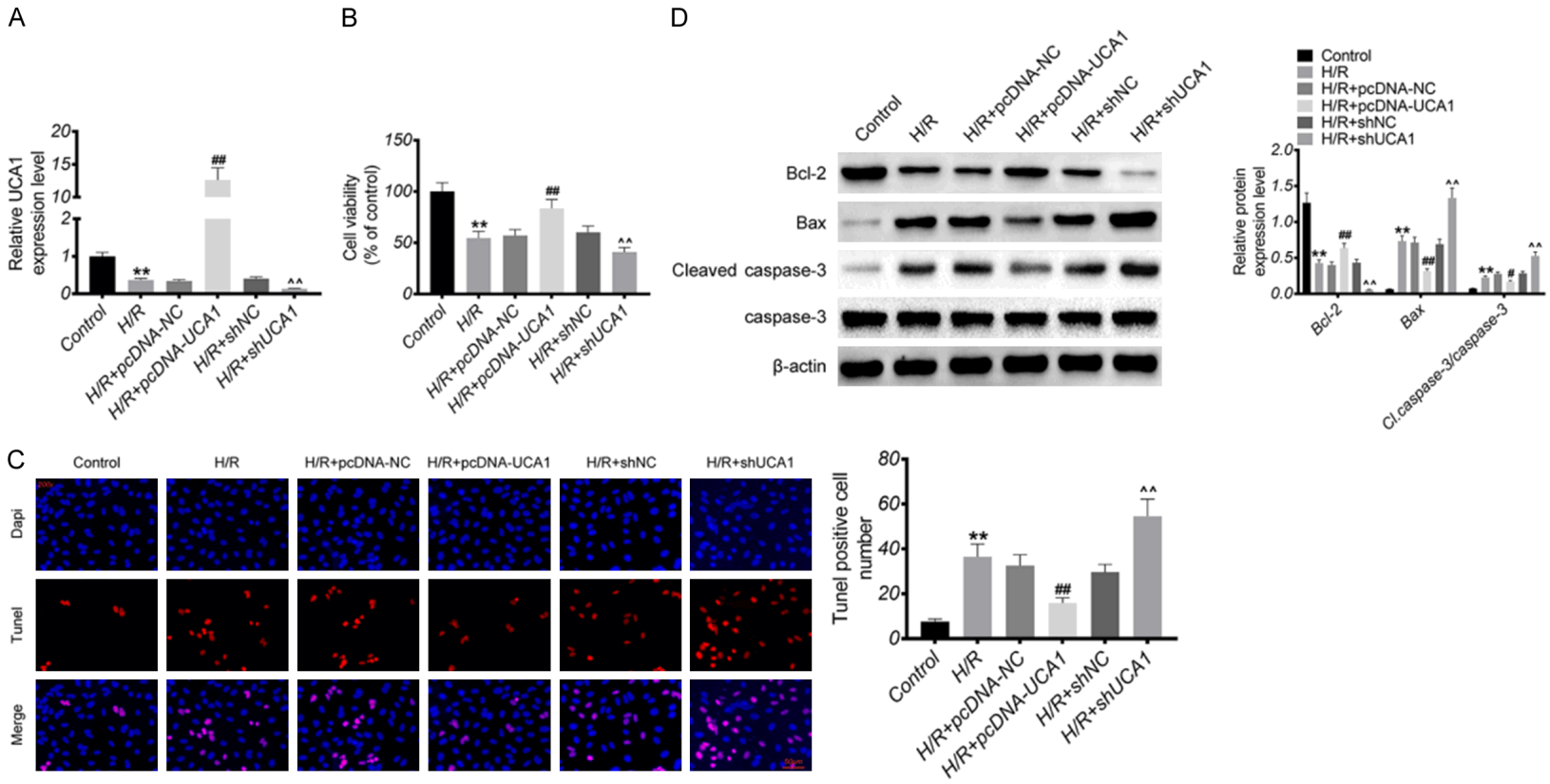

Figure 2. UCA1 up-regulation enhanced viability and attenuated apoptosis of H/R-induced HPVECs. A. UCA1 expression in HPVECs was determined by qPCR. B. MTT assay was implemented to study the viability of HPVECs. C. Tunel assay was executed to exam the apoptosis of HPVECs. Magnification: 200 ×. D. The expression of apoptosis-related proteins in HPVECs was analyzed by Western blot. $^{**}P < 0.01$ vs. Control group. $^{\#}P < 0.05$ or $^{\#\#}P < 0.01$ vs. H/R + pcDNA-NC group. $^{\wedge\wedge}P < 0.01$ vs. H/R + shNC group. Abbreviations: UCA1, urothelial carcinoma associated 1; HPVECs, human placental vascular endothelial cells; H/R, hypoxia reoxygenation; qPCR, quantitative polymerase chain reaction; MTT, methyl thiazolyl tetrazolium; Tunel, the terminal transferase uridyl nick end labelling.

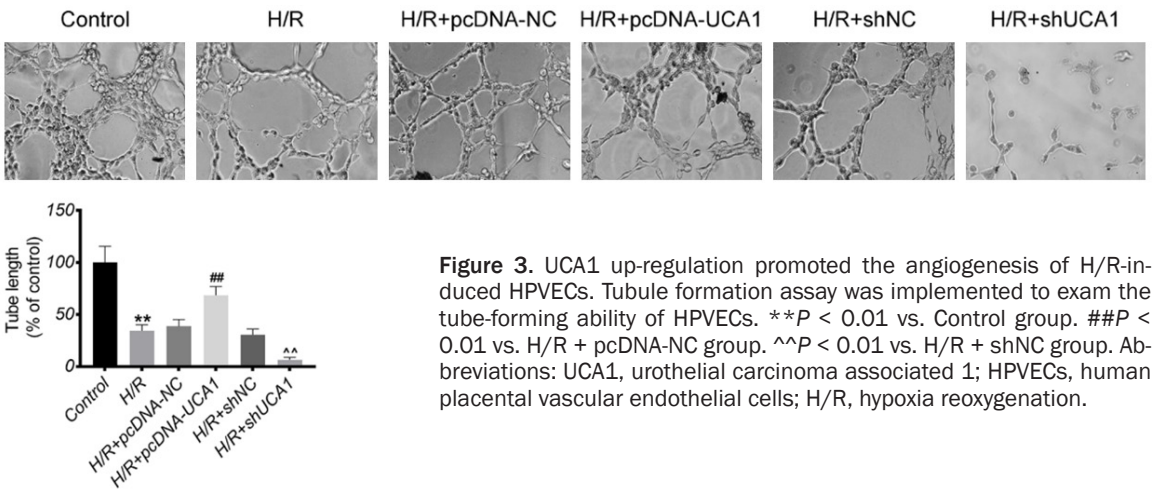

Figure 3. UCA1 up-regulation promoted the angiogenesis of H/R-induced HPVECs. Tubule formation assay was implemented to exam the tube-forming ability of HPVECs. $**P < 0.01$ vs. Control group. $\#\#P < 0.01$ vs. H/R + pcDNA-NC group. $\wedge\wedge P < 0.01$ vs. H/R + shNC group. Abbreviations: UCA1, urothelial carcinoma associated 1; HPVECs, human placental vascular endothelial cells; H/R, hypoxia reoxygenation.

In the control group, H/R induction significantly reduced the tube length formed by HPVECs ($P < 0.01$). Intriguingly, the upregulation of UCA1 increased the tube-forming capability of H/R-induced HPVECs, as evidenced by the longer tube length formed by the HPVECs of the H/R + pcDNA-UCA1 group relative to that of the H/R + pcDNA-NC group ($P < 0.01$). However, the downregulation of UCA1 had an opposite effect on the tube-forming capability of H/R-induced HPVECs ($P < 0.01$) (**Figure 3A**). Therefore, the upregulation of UCA1 increased the angiogenesis of H/R-induced HPVECs.

UCA1 directly bound to miR-197-3p

The dual-luciferase reporter gene assay was conducted with 293T cells to evaluate the bond between UCA1 and miR-197-3p. As shown in **Figure 4A**, the difference in the relative luciferase activity of the UCA1-MUT reporter did not significantly differ between the NC mimic and miR-197-3p mimic groups. However, miR-197-3p mimic remarkably reduced the relative luciferase activity of UCA1-WT reporter than NC mimic ($P < 0.01$). The RNA pull-down assay showed that the overexpression of UCA1 could enrich more miR-197-3p ($P < 0.01$) (**Figure 4B**). The RIP assay revealed that UCA1 and miR-197-3p were more abundant in the anti-Ago2 and input groups than those in the anti-IgG group ($P < 0.01$). Simultaneously, UCA1 and miR-197-3p were more significantly abundant in the anti-Ago2 group than those in the input group ($P < 0.01$) (**Figure 4C**). In H/R-induced HPVECs (H/R group), the expression of miR-197-3p was significantly higher than that in the control group ($P < 0.01$). However, the upregulation of UCA1 reduced the expression of miR-197-3p in H/R-induced HPVECs ($P < 0.01$). Conversely, the downregulation of UCA1 increased the expression of miR-197-3p in H/R-induced HPVECs ($P < 0.01$) (**Figure 4D**). Based on these data, UCA1 could directly suppress the expression of miR-197-3p.

UCA1 competitively binds to miR-197-3p to upregulate the expression of HDAC2

The dual-luciferase reporter gene assay showed that the relative luciferase activity of HDAC2-WT reporter was significantly decreased by miR-197-3p mimic rather than NC mimic ($P < 0.01$). However, compared with the NC mimic, the miR-197-3p mimic did not significantly affect the relative luciferase activity of the HDAC2-MUT reporter (**Figure 5A**). Relative to the control group, H/R induction remarkably increased miR-197-3p expression but reduced HDAC2 expression at the mRNA and protein levels in HPVECs ($P < 0.01$). Compared with the HPVECs of the H/R + NC mimic group, the HPVECs of the H/R + the miR-197-3p mimic group had a significantly higher miR-197-3p expression but a lower HDAC2 mRNA and protein expression ($P < 0.01$). By contrast, compared with the HPVECs of the H/R + NC inhibitor group, the HPVECs of the H/R + miR-197-3p inhibitor group had a lower miR-197-3p expression and a higher HDAC2 mRNA and protein expression ($P < 0.01$) (**Figure 5B**, **5C**). Furthermore, in H/R-induced HPVECs, the upregulation of UCA1 significantly increased the expression of HDAC2 protein. However, the downregu-

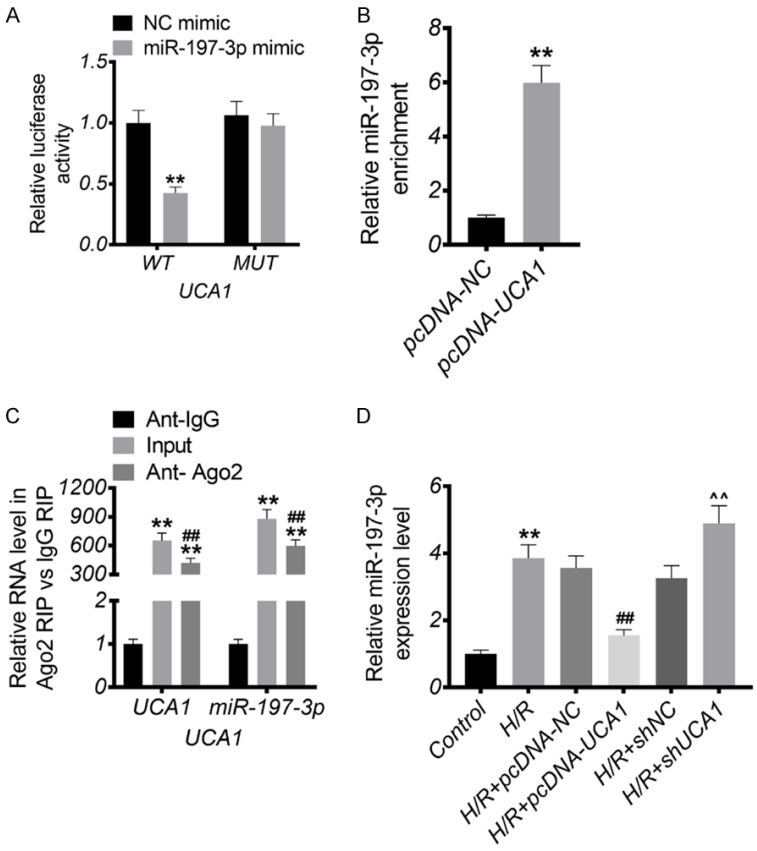

Figure 4. UCA1 directly bound to miR-197-3p. A. Dual-luciferase reporter gene assay was conducted with 293T cells to exam the binding between UCA1 and miR-197-3p. $**P < 0.01$ vs. NC mimic group. B. RNA pull-down assay was performed with HPVECs to exam the binding between UCA1 and miR-197-3p. $**P < 0.01$ vs. pcDNA-NC group. C. RIP assay was implemented with HPVECs to monitor the binding between UCA1 and miR-197-3p. $**P < 0.01$ vs. Anti-IgG group. ## $P < 0.01$ vs. Input group. D. miR-197-3p expression in HPVECs was detected by qPCR. $**P < 0.01$ vs. Control group. $##P < 0.01$ vs. H/R + pcDNA-NC group. $\hat{}\hat{}P < 0.01$ vs. H/R + shNC group. Abbreviations: UCA1, urothelial carcinoma associated 1; HPVECs, human placental vascular endothelial cells; H/R, hypoxia reoxygenation; RIP, RNA immunoprecipitation; qPCR, quantitative polymerase chain reaction.

lation of UCA1 had an opposite effect ($P < 0.01$) (**Figure 5D**). Intriguingly, the HPVECs of the H/R + pcDNA-UCA1 + NC mimic group had a significantly higher HDAC2 protein expression than the H/R + pcDNA-NC + NC mimic group, and the H/R + pcDNA-UCA1 + miR-197-3p mimic group ($P < 0.01$) (**Figure 5E**). Hence, UCA1 competitively bound to miR-197-3p upregulated the expression of HDAC2.

UCA1 decreased H/R-induced damage and increased the angiogenesis of HPVECs by promoting the expression of HDAC2

As presented in **Figure 6A-C**, compared with the HPVECs of the H/R + pcDNA-NC + Scramble group, the HPVECs of the H/R + pcDNA-UCA1 + Scramble group had higher cell viability and tube length and a lower apoptotic capability. Further, the HPVECs of the H/R + pcDNA-NC + shHDAC2 group had lower cell viability and tube length and higher apoptosis ($P < 0.01$). Compared with the HPVECs of the H/R + pcDNA-NC + shHDAC2 group, the HPVECs of the H/R + pcDNA-UCA1 + shHDAC2 group had higher cell viability and tube length and a lower apoptotic capability ($P < 0.01$). Based on the data obtained via Western blot analysis, the HPVECs of the H/R + pcDNA-UCA1 + Scramble group had an aberrantly higher HDAC2 and Bcl-2 protein expression and a lower Bax and cleaved caspase-3/caspase-3 protein expression compared with those of the H/R + pcDNA-NC + Scramble group ($P < 0.01$). However, compared with the HPVECs of the H/R + pcDNA-NC + Scramble group, the HPVECs of the H/R + pcDNA-NC + shHDAC2 group had a lower HDAC2 and Bcl-2 protein expression and a higher Bax and cleaved caspase-3/caspase-3 protein expression ($P < 0.01$). The HPVECs of the H/R + pcDNA-UCA1 + shHDAC2 group had a significantly higher HDAC2 and Bcl-2 protein expression and a remarkably lower Bax and cleaved caspase-3/caspase-3 protein expression than the HPVECs of the H/R + pcDNA-NC + shHDAC2 group ($P < 0.01$) (**Figure 6D**). Thus, UCA1 might protect HPVECs from H/R-induced damage by promoting HDAC2 expression.

Discussion

This research showed that pregnant women with PIH had a downregulated serum lncRNA UCA1 and HDAC2 expression and an upregulated serum miR-197-3p expression. UCA1 increased the viability and apoptotic inhibi-

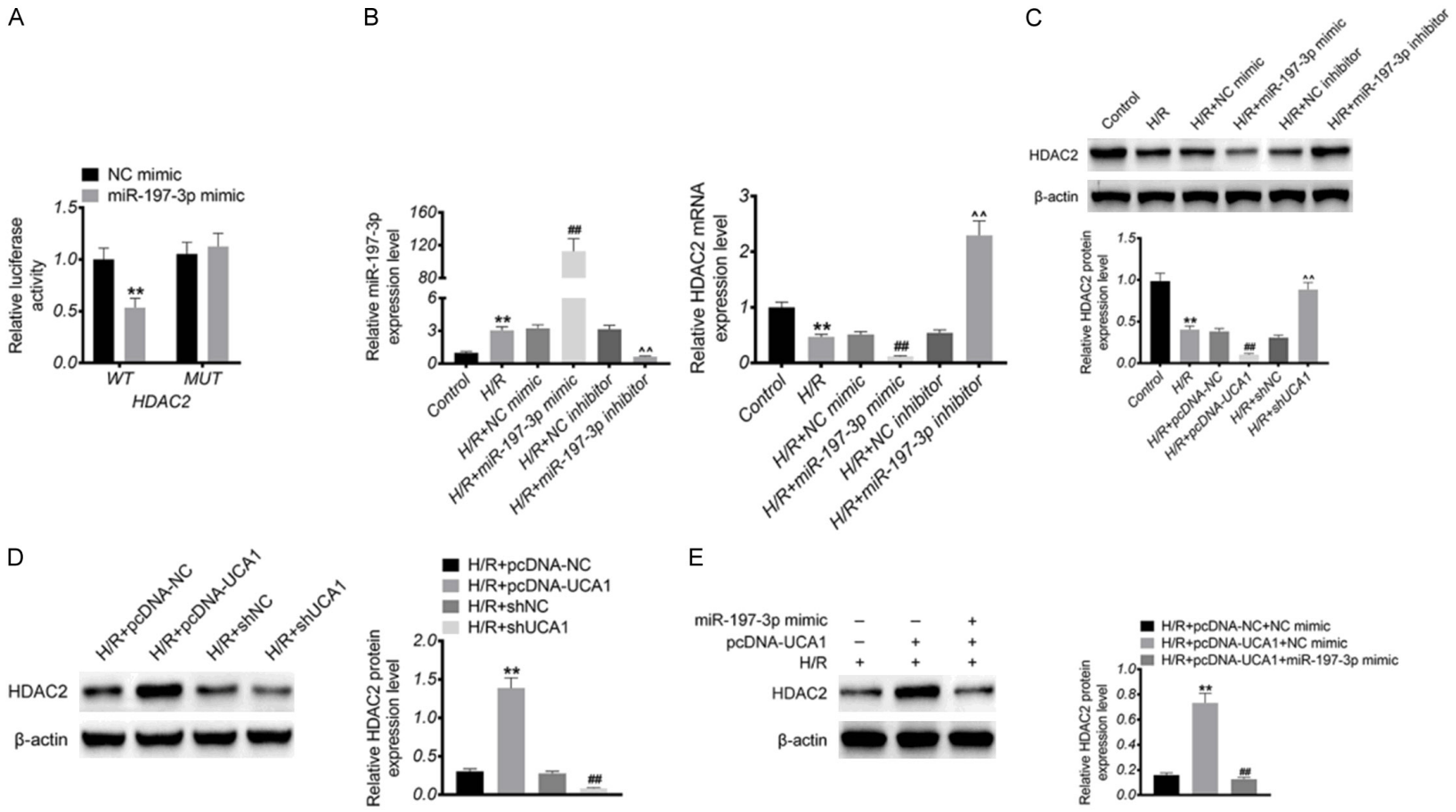

Figure 5. UCA1 competitively bound to miR-197-3p to up-regulate the expression of HDAC2. A. Dual-luciferase reporter gene assay was conducted with 293T cells to exam the binding between miR-197-3p and HDAC2. $^{**}P < 0.01$ vs. NC mimic group. B and C. HDAC2 mRNA and protein expression in HPVECs was assessed by qPCR and Western blot. $^{**}P < 0.01$ vs. Control group. ## $P < 0.01$ vs. H/R + NC mimic group. ^^ $P < 0.01$ vs. H/R + NC inhibitor group. D. HDAC2 protein expression in HPVECs was evaluated by Western blot. $^{**}P < 0.01$ vs. H/R + pcDNA-NC group. ##$P < 0.01$ vs. H/R + shNC group. E. HDAC2 protein expression in HPVECs was monitored by Western blot. $^{**}P < 0.01$ vs. H/R + pcDNA-NC + NC mimic group. ##$P < 0.01$ vs. H/R + pcDNA-UCA1 + NC mimic group. Abbreviations: UCA1, urothelial carcinoma associated 1; HDAC2, histone deacetylase-2; HPVECs, human placental vascular endothelial cells; qPCR, quantitative polymerase chain reaction; H/R, hypoxia reoxygenation.

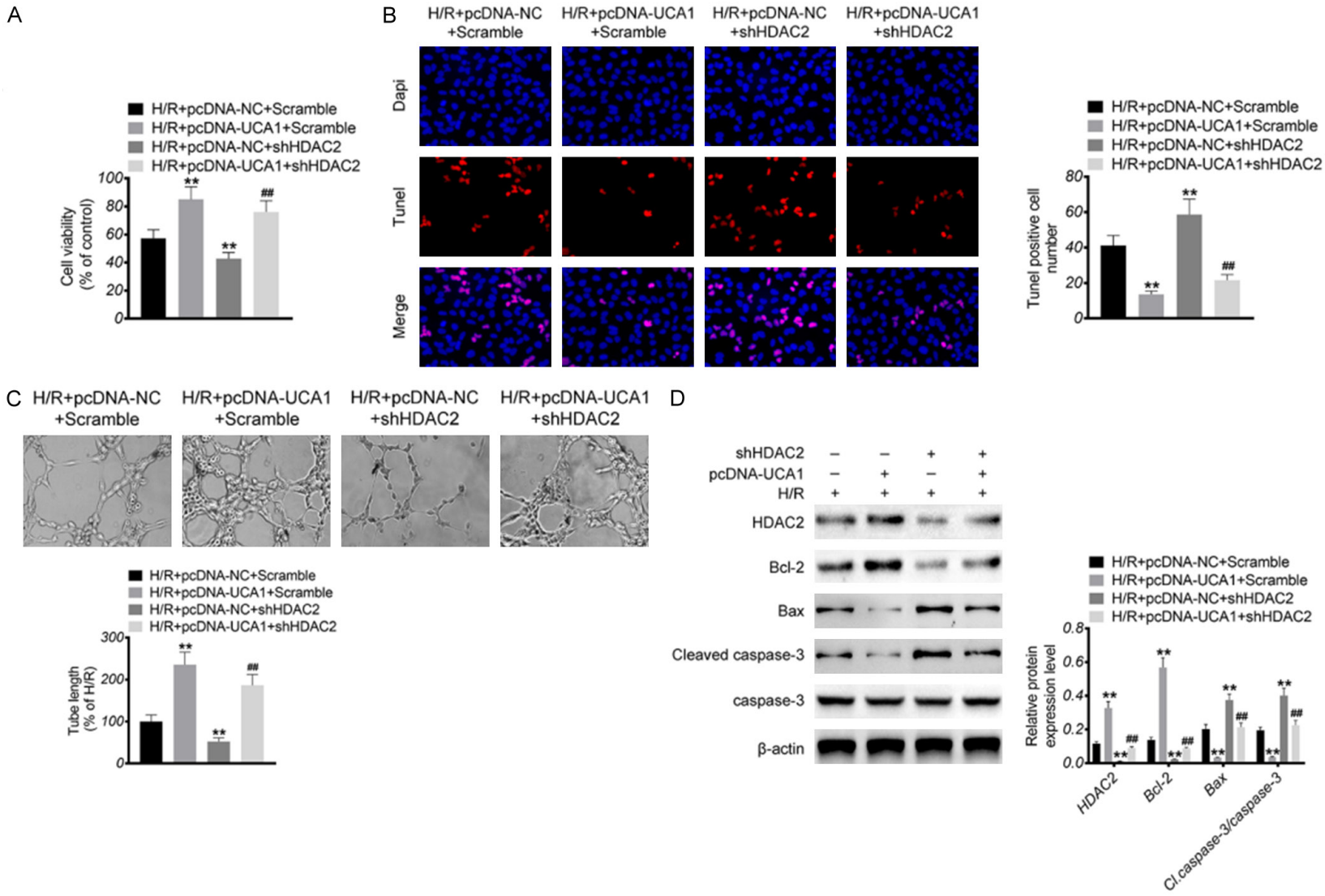

Figure 6. UCA1 alleviated H/R-induced damage and promoted angiogenesis of HPVECs by increasing the expression of HDAC2. A. The viability of HPVECs was evaluated by MTT assay. B. The apoptosis of HPVECs was investigated by Tunel assay. Magnification: 200 ×. C. The tube formation ability of HPVECs was assessed by tubule formation assay. D. Western blot was executed to assess the expression of proteins in HPVECs. $^{**}P < 0.01$ vs. H/R + pcDNA-NC + Scramble group. $^{\#\#}P < 0.01$ vs. H/R + pcDNA-NC + shHDAC2 group. Abbreviations: UCA1, urothelial carcinoma associated 1; H/R, hypoxia Reoxygenation; HPVECs, human Placental vascular endothelial cells; HDAC2, histone deacetylase-2; MTT, methyl thiazolyl tetrazolium; Tunel, the terminal transferase uridyl nick end labelling.

tion and angiogenic capability of H/R-induced HPVECs by competitively binding to miR-197-3p to upregulate the expression of HDAC2.

The clinical symptoms of PIH gradually disappear after the placenta is delivered. Therefore, it is currently believed that the pathogenesis of PIH is correlated with the placenta [15]. Normal intraplacental vascular development is an important factor for transporting sufficient nutrients and oxygen to the fetus [16]. Vascular endothelial cells line all blood vessels and are essential for maintaining normal vascular functions, including blood pressure control [7]. In this study, HPVECs were adopted to construct the PIH cell model via H/R induction. Results showed that H/R induction significantly decreased the viability and angiogenic capability of HPVECs and increased the apoptotic capability of HPVECs. Interestingly, the up-regulation of UCA1 reversed the effects of H/R induction, as evidenced by the increased viability and angiogenic capability of H/R-induced HPVECs and the decreased apoptotic capability of H/R-induced HPVECs via the upregulation of UCA1. UCA1 is involved in the development of PE because metformin can prevent PE by suppressing the expression of UCA1 in trophoblast cells [17]. Moreover, UCA1 is aberrantly expressed in placental diseases. Hence, its downregulation is correlated with the dysregulation of some key trophoblast physiology genes. However, these trophoblast physiology genes are associated with some important physiological processes such as oxidative stress and proliferation [18]. This study showed that the up-regulation of UCA1 increased the viability and angiogenic and apoptotic inhibition capability of the PIH cell model. In addition, the up-regulation of UCA1 increased the expression of Bcl-2 protein but decreased the expression of Bax and cleaved caspase-3/caspase-3 proteins in the PIH cell model. Bcl-2 is a type of anti-apoptotic protein. Meanwhile, Bax and cleaved caspase-3 (cleaved form of caspase-3) are important pro-apoptotic proteins [19-21]. Therefore, the up-regulation of UCA1 might inhibit the apoptosis of H/R-induced HPVECs by regulating the expression of these apoptosis-related genes.

More importantly, the up-regulation of UCA1 might inhibit the development of PIH by competitively binding to miR-197-3p to upregulate the expression of HDAC2. The effect of miR-197-3p has been widely assessed in human cancers, such as prostate cancer, multiple myeloma, and ovarian cancer. MiR-197-3p is considered a tumor suppressor in these human cancers by inhibiting drug resistance, epithelial-mesenchymal transition, and malignant proliferation of tumor cells [22-24]. To date, only one study has shown that miR-197-3p is upregulated in the maternal plasma of patients with PE [11]. However, it did not identify the exact function of miR-197-3p in the development of PE. The current study first identified the overexpression of serum miR-197-3p in pregnant women with PIH. It could be sponged by UCA1 and involved in the regulation of PIH development.

MiRNAs can play a role in multiple cellular events by targeting the amounts of coding genes that maintain homeostasis in several human organs, including the placenta [2]. This article revealed that pregnant women with PIH had a low expression of serum HDAC2 and that miR-197-3p could directly bind to HDAC2 to suppress the expression of the latter. The expression of HDAC2 is downregulated in the placental tissues of patients with severe PE [12]. The pathogenesis of PE is partly attributed to the enhanced death of trophoblast cells. However, HDAC2 is poorly expressed in the placental tissues of patients with PE, and its upregulation can decrease the apoptosis of trophoblast cells [13]. Furthermore, the upregulation of HDAC2 facilitates the proliferation of trophoblast cells. Thus, it was considered a novel molecular target for the treatment of PE [25]. In this research, HDAC2 knockdown counteracted the upregulation of UCA1 on the development of PIH. Taken together, UCA1 might inhibit the development of PIH by regulating the miR-197-3p/HDAC2 axis in PIH.

This work first showed the downregulation of serum UCA1 and HDAC2 expression and the upregulation of serum miR-197-3p expression in pregnant women with PIH. The up-regulation of UCA1 could suppress the development of PIH by promoting the viability and apoptotic inhibition and angiogenic capability of HPVECs. This could be attributed to the fact that UCA1 competitively bound to miR-197-3p to upregulate the expression of HDAC2. UCA1 was initially found to prevent the development of PIH.

Therefore, UCA1 might be a promising target in PIH treatment in clinical practice. Nevertheless, further research must be conducted to provide sufficient evidence.

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Disclosure of conflict of interest

None.

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