Zygote

cambridge.org/zyg

lncRNA SNHG14 involved in trophoblast cell proliferation, migration, invasion and epithelial–mesenchymal transition by targeting miR-330-5p in preeclampsia

Yulei Zhang and Muling Zhang

Department of Obstetrics, Huai'an First People's Hospital/The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, Huai'an, 223300, Jiangsu, China

Research Article

Cite this article: Zhang Y and Zhang M (2021) lncRNA SNHG14 involved in trophoblast cell proliferation, migration, invasion and epithelial–mesenchymal transition by targeting miR-330-5p in preeclampsia. *Zygote*. **29**: 108–117. doi: 10.1017/S0967199420000507

Received: 6 July 2020
Revised: 27 July 2020
Accepted: 27 July 2020
First published online: 9 November 2020

Keywords:
lncRNA SNHG14; miR-330-5p; Preeclampsia; Trophoblast cell

Author for correspondence:
Muling Zhang. Department of Obstetrics, Huai'an First People's Hospital/The Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University, No. 1 Huanghe West Road, Huaiyin District, Huai'an 223300, China.
E-mail: mulingzhangd@163.com

Summary

Preeclampsia (PE), a pregnancy-specific disease, has become one of the leading causes of maternal and neonatal morbidity and mortality. Pathogenesis of PE has still not been fully addressed and there is a great need to develop early diagnosis markers and effective therapy. This study aimed to determine if lncRNA SNHG14 has a protective effect on placental trophoblast and prevents PE. SNHG14 levels in the peripheral blood from patients with PE or from women with healthy pregnancies were detected using RT-qPCR. The relationship between SNHG14 and miR-330-5p was determined using a dual-luciferase reporter assay. In addition, cell proliferation and cell cycle were evaluated by performing CCK8 assays and flow-cytometric analysis, respectively. Wound-healing and transwell assays were performed to assess cell migration and invasion ability. lncRNA SNHG14 was downregulated in PE patients; it was involved in trophoblast proliferation and regulated cell proliferation during G1/S transition. In addition, lncRNA SNHG14 promoted migration, invasion and epithelial–mesenchymal transition (EMT) in HTR-8/SVneo cells. Luciferase reporter assay indicated that lncRNA SNHG14 served as a molecular sponge for miR-330-5p and negatively regulated miR-330-5p expression in PE. Furthermore, the effects of silenced SNHG14 on trophoblast proliferation, migration, invasion and EMT were reversed by addition of miR-330-5p inhibitor, suggesting that in PE lncRNA SNHG14 functions by competitively binding to miR-330-5p. Taken together, the current study demonstrated that in PE lncRNA SNHG14 is a vital regulator by binding to miR-330-5p. SNHG14 might serve as a therapeutic application in PE progression.

Introduction

Preeclampsia (PE), a pregnancy-specific diseases, is characterized by hypertension and proteinuria after 20 weeks (Tang *et al.*, 2019). To date, PE has become one of the leading causes of both maternal and neonatal morbidity and mortality (Mol *et al.*, 2016). Severe PE is associated with preterm birth, fetal distress, stillbirth and neonatal asphyxia (Zhong *et al.*, 2019). Despite extensive research, conventional therapeutics for PE are still not satisfactory and pathogenesis of the disease has still not been fully addressed (Zhong *et al.*, 2010). There is still a lack of adequate and accurate diagnostic approaches or biomarkers for PE (Dong *et al.*, 2019), therefore, it is vital to develop early diagnosis markers and valid therapy for PE patients in clinical settings.

A substantial body of evidence has demonstrated that development of PE is a multifactorial process including abnormal angiogenesis, placental and endothelial dysfunction, increased apoptosis and impaired invasion of trophoblast cells (Xie *et al.*, 2019). Dysfunction of trophoblast cells has emerged as a vital factor in PE pathogenesis (Fan *et al.*, 2019). EMT has been identified as playing a key role in the regulation of cell migration and invasion (Mao *et al.*, 2018). Accumulating evidence supports the idea that inadequate migration and invasion of cytotrophoblasts into the uterine artery can lead to placental ischaemia and reduce utero-placental perfusion (Redman and Sargent, 2005). Therefore, EMT is an important factor involved in underlying mechanisms of PE.

MicroRNAs (miRNAs) are a class of small noncoding RNAs of 20–22 nucleotides in length (Zhang *et al.*, 2017). miRNAs negatively modulate gene expression through targeting the 3′-untranslated region (3′UTR) of their target mRNAs. Increasing evidence has supported the idea that miRNAs are associated with the metabolism and pathogenesis of many diseases (Bartel, 2018). In addition, multiple miRNAs in the placenta can regulate trophoblast cell proliferation, apoptosis, migration and invasion (Xiao *et al.*, 2017). More recently, work by Tong and colleagues suggested that miR-330-5p plays a vital role in human decidualization and the occurrence of PE (Tong *et al.*, 2018). Upregulation of miR-330-5p could inhibit EMT processes and the capability for proliferation, invasion and migration (Liu *et al.*, 2019). As hypothesized,

CAMBRIDGE UNIVERSITY PRESS

miR-330-5p may participate in the pathological process of PE by regulating the proliferation, migration, invasion and EMT of trophoblast cells.

Long noncoding RNAs (lncRNAs), a subset of noncoding RNAs of more than 200 nucleotides in length, are involved in a large array of diseases including PE (Li *et al.*, 2019a,b). A previous study has demonstrated that knockdown of lncRNA SNHG14 could significantly suppress cell proliferation, migration and invasion by regulating miR-206/YWHAZ (Ji *et al.*, 2019). However, lncRNA SNHG14 has not been identified to modulate the associated functions of trophoblast cells and PE development.

Collectively, the purpose of this study was to analyze and elucidate the effects of lncRNA SNHG14 and miR-330-5p in the regulation of trophoblast behaviour. This study will highlight the potential role and underlying mechanisms of SNHG14 and miR-330-5p in the development of PE, introducing novel targets for PE diagnosis and therapy.

Materials and methods

Clinical specimen collection

Patients diagnosed with PE were chosen for this study at Huai'an First People's Hospital from 2018 to 2019. The present study was approved and authorized by the Ethic Committee of Huai'an First People's Hospital. Blood samples were collected from 30 patients with preeclampsia and from 30 women with healthy pregnancies. Blood samples were immediately frozen and stored at −80°C for further study. All patients in the study submitted written informed consent forms and approved the study.

Cell culture and transfection

HTR-8/SVneo cells were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were cultured in RPMI 1640 medium (Gibco, NY, USA) supplemented with 10% fetal bovine serum (Cor., New York, NY, USA) and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin) in an incubator at 37°C in a 5% CO_2 : 95% air atmosphere for regular maintenance. HTR8/SVneo cells were transfected with siRNAs purchased from Shanghai GenePharma, Co. Ltd (Shanghai, China) using Lipofectamine 2000 transfection reagent (Invitrogen, CA, USA) in line with the manufacturer's instructions. Cells were then maintained for 48 h for further analysis.

Cell Counting Kit-8 (CCK-8) assay

Cell proliferation was assessed by CCK8 assay in line with the standard protocol. Here, 100 μl of cell suspension at a density of 5×10^4/ml were incubated in a standard culture condition (37°C, 5% CO_2 and 95% humidity) for 24 h, 48 h and 72 h. CCK-8 reagent (10 μl; Beyotime, Shanghai, China) was added into each well and applied to detect cell proliferation index. Absorbance at 450 nm was measured and read on a microplate reader (Hercules, CA, USA).

Cell-cycle assay

HTR-8/SVneo cells transfected with plasmids were collected and fixed with 75% ice-cold ethanol at −20°C overnight. Following washing with phosphate-buffered saline (PBS), the fixed cells were treated with propidium iodide (PI; Beyotime, Shanghai, China) at room temperature for 30 min. Then, cell samples were analyzed using a flow cytometer (BD Biosciences, CA, USA).

Wound-healing assay

Cell migratory ability was evaluated by wound-healing assay. For the migration assay, 1×10^5 HTR-8/SVneo cells were seeded in six-well plates and cultured in a humidified incubator under 5% CO_2 in air at 37°C. Briefly, confluent monolayers of HTR-8/SVneo cells were mechanically wounded by dragging a 10-μl sterile plastic tip at the centre of the well. Cells were allowed to migrate for 24 h and the wounded images were photographed using a Leica microscope (Leica, Wetzlar, Germany) in the same position at 0 and 24 h after wounding.

Transwell invasion assay

Transwell assay was conducted using transwell chambers. The invasive ability of HTR-8/SVneo cells was evaluated based on the ability to cross the pores of migration chambers. Cells were cultured with fetal bovine serum (FBS)-free medium in the upper transwell chambers and the medium containing 10% FBS was added to the lower chamber as a chemoattractant. After incubation for 24 h, non-invaded cells in the upper chamber were removed using a cotton swab. Invaded cells in the bottom chamber were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet. Cell invasive capacity was assessed by counting the number of invaded cells in five randomly selected microscopic fields under an inverted microscope.

RNA isolation and real-time quantitative PCR

Total RNA was isolated from HTR-8/SVneo cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and then reverse-transcribed using a PrimeScript™ reverse transcription reagent kit (TaKaRa Bio, Tokyo, Japan) in line with the manufacturer's instructions. RT-qPCR was carried out using the ABI PRISM® 7300 real-time PCR system (Applied Biosystems, Foster City, CA, USA) in accordance with the standard instructions. The relative amount of lncRNA SNHG14 was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and the relative amount of miR-330-5p was normalized to U6 as internal controls. Gene expression data were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Primer sequences were as follows: SNHG14-Forward: 5′-CAGGCTGAACTGAGGCAGGCAT-3′; SNHG14-Reverse: 5′-ACATCTCATTCTATAGTCAATGT-3′; miR-330-5p-Forward: 5′-TCTCTGGGCCTGTGTCTTAGGC-3′; miR-330-5p-Reverse: 5′-TTAATGGGGTGATTGGTGGT-3′; U6-Forward: 5′-CTCGCTTCGGCAGCACA-3′; U6-Reverse: 5′-AACGCTTCACGAATTTGCGT-3′; GAPDH-Forward: 5′-AAATCCCATCACCATCTTCCAG-3′; GAPDH-Reverse: 5′-TGATGACCCTTTTGGCTCCC-3′.

Western blot assay

After indicated treatments, HTR-8/SVneo cells were washed with ice-cold PBS and lysed in RIPA lysis buffer (Beyotime, Shanghai, China) supplemented with proteinase inhibitor. Total cellular proteins in the supernatants were determined by BCA assay, in accordance with the manufacturer's instructions (Beyotime, Shanghai, China). Equal amounts of protein were loaded and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membranes. Membranes were blocked with 5% fat-free milk followed by an incubation at 4°C overnight with the following primary antibodies: CDK2 (Abcam, ab32147, 1:2000), P21 (Abcam, ab188224, 1:1000), cyclinD1 (Abcam, ab134175, 1:10,000), MMP2 (Abcam, ab181286,

https://doi.org/10.1017/S0967199420000507 Published online by Cambridge University Press

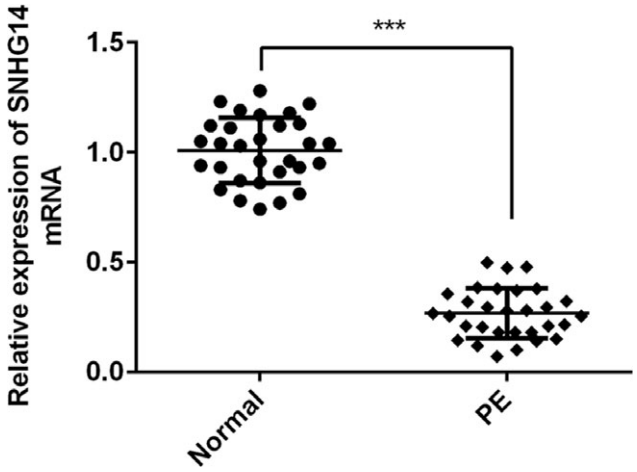

Figure 1. lncRNA SNHG14 was downregulated in PE tissues. lncRNA SNHG14 expression determined by RT-qPCR. ***$P < 0.001$ versus normal pregnancies.

1:1000), MMP9 (Abcam, ab228402, 1:1000), E-cadherin (Abcam, ab212059, 1:1000), vimentin (Abcam, ab137321, 1:3000), β-catenin (Abcam, ab6302, 1:4000) and GAPDH (Abcam, ab8245, 1:10,000). Membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies (Abcam, ab205718, 1:20,000) at room temperature for 1 h. β-Catenin and GAPDH served as internal loading controls. Immunoreactive bands on PVDF membranes were visualized using a ECL detection kit (Beyotime, Shanghai, China) and protein intensity was measured with BandScan software.

Immunofluorescence staining

Transfected HTR-8/SVneo cells were rinsed with PBS and then fixed in 4% paraformaldehyde for 15 min at room temperature. Next, cells were treated for 30 min with PBS containing 0.25% Triton X-100 and 1% BSA and then blocked with 5% BSA for 1 h. Subsequently, cells were incubated with anti-E-cadherin (Abcam, ab231303, 1:1000) or anti-vimentin (Abcam, ab8978, 1:200) antibody overnight at 4°C. After washing three times in PBS, cells were incubated with fluorescence-conjugated secondary antibody (Abcam, ab150113, 1:1000) for 2 h in the dark. Finally, cells were stained with DAPI and analyzed under a fluorescence microscope.

Dual-luciferase reporter assay

The 3′UTR sequence of SNHG14 mRNA containing miR-330-5p binding sites and the corresponding mutated sequence were cloned into the pGL3 promoter vector (Realgene, Shanghai, China). HTR-8/SVneo cells were seeded in 24-well plates and cultured until 70% confluency. Afterwards, cells were co-transfected with miR-330-5p mimics and wild-type/mutant reporter plasmid vectors using Lipofectamine 2000 (Invitrogen, CA, USA). Following 48 h transfection, firefly luciferase activity was measured using a dual-luciferase reporter gene assay system (Promega, WI, USA) and normalized to the Renilla value.

Statistical analysis

All figures were performed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). All reactions were performed in triplicate and data were presented as mean ± standard deviation (SD). The statistical differences among groups were assessed by *t*-test or one-way analysis of variance (ANOVA) followed by Tukey's post hoc test in SPSS 22.0 software. A *P*-value < 0.05 was considered to indicate statistically significant difference.

Results

lncRNA SNHG14 was downregulated in PE

To investigate the potential role of lncRNA SNHG14 in PE, expression of lncRNA SNHG14 in blood samples was determined by RT-qPCR. Results showed that the SNHG14 level in PE patients was visibly lower compared with that in blood from women with normal pregnancies (Fig. 1A).

lncRNA SNHG14 was involved in sufficient trophoblast proliferation

To investigate whether lncRNA SNHG14 was involved in the regulation of trophoblast proliferation, HTR-8/SVneo cells were transfected with pcDNA SNHG14 and transfection efficiency was verified. lncRNA SNHG14 expression was greatly upregulated following transfection (Fig. 2A). Subsequent CCK-8 assay demonstrated that upregulation of SNHG14 markedly promoted cell proliferation in HTR-8/SVneo cells (Fig. 2B).

lncRNA SNHG14 regulated HTR-8/SVneo cell proliferation by G1/S transition

To determine whether lncRNA SNHG14 enhancement of the growth of HTR-8/SVneo cells was accompanied by an influence on the cell cycle, we assessed cycle distribution using flow cytometry. Compared with the control, the proportion of cells in S and G2 phases markedly increased and the proportion of cells in the G0/G1 phase dramatically reduced following SNHG14 overexpression (Fig. 3A, B). In addition, we explored the mechanism by which SNHG14 regulated cell proliferation by performing western blot analysis. SNHG14 overexpression caused a decrease in p21 level and an increase in cyclin D1 and CDK2 levels (Fig. 3C, D).

lncRNA SNHG14 promoted HTR-8/SVneo cell migration and invasion

To evaluate the biological functions of lncRNA SNHG14 in cell migration and invasion, the migration activities and invasion capacities of HTR-8/SVneo cells were detected by wound-healing assay and transwell assay, respectively. Compared with the control, upregulation of SNHG14 could significantly enhance migration activities (Fig. 4A, B) and invasion capacities (Fig. 4C, D) of HTR-8/SVneo cells. Protein levels of MMP2 and MMP9 were also examined to further investigate the role of SNHG14 in cell migration and invasion. As expected, MMP2 and MMP9 expression levels were elevated drastically following SNHG14 overexpression in HTR-8/SVneo cells (Fig. 4E).

lncRNA SNHG14 regulated EMT in HTR-8/SVneo cells

To investigate whether lncRNA SNHG14 could regulate EMT, levels of EMT-related proteins including E-cadherin, β-catenin and vimentin in HTR-8/SVneo cells were examined by western blot analysis. SNHG14 overexpression clearly enhanced vimentin expression, but reduced E-cadherin and β-catenin expression (Fig. 5A). Furthermore, immunofluorescence results indicated that E-cadherin expression was strikingly decreased (Fig. 5B) and

https://doi.org/10.1017/S0967199420000507 Published online by Cambridge University Press

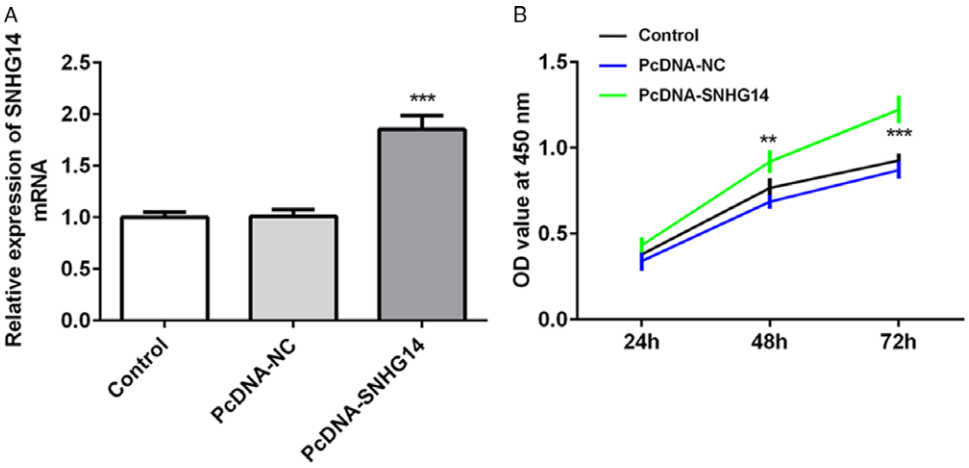

Figure 2. lncRNA SNHG14 was involved in sufficient trophoblast proliferation. (*A*) HTR-8/SVneo cells were transfected with pcDNA SNHG14 and RT-qPCR was applied to determine SNHG14 mRNA expression. (*B*) Cell proliferation detected by CCK-8 assay. $^{**}P < 0.01$, $^{***}P < 0.001$ versus Control.

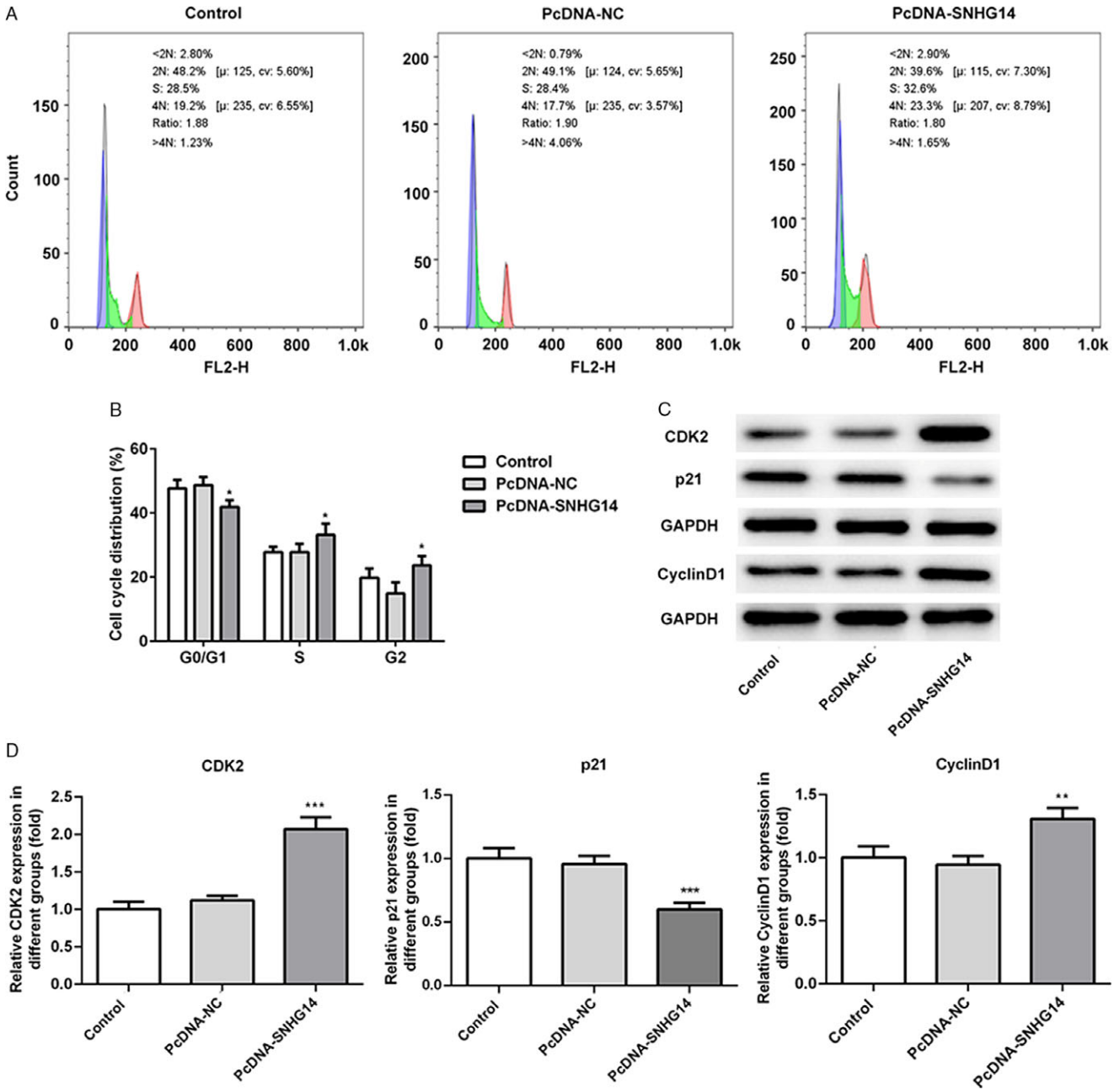

Figure 3. lncRNA SNHG14 regulated HTR-8/SVneo cell proliferation by G1/S transition. (*A, B*) Cycle distribution analyzed using flow cytometry assay. (*C, D*) CDK2, p21 and cyclin D1 levels measured by western blot assay. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus Control.

https://doi.org/10.1017/S0967199420000507 Published online by Cambridge University Press

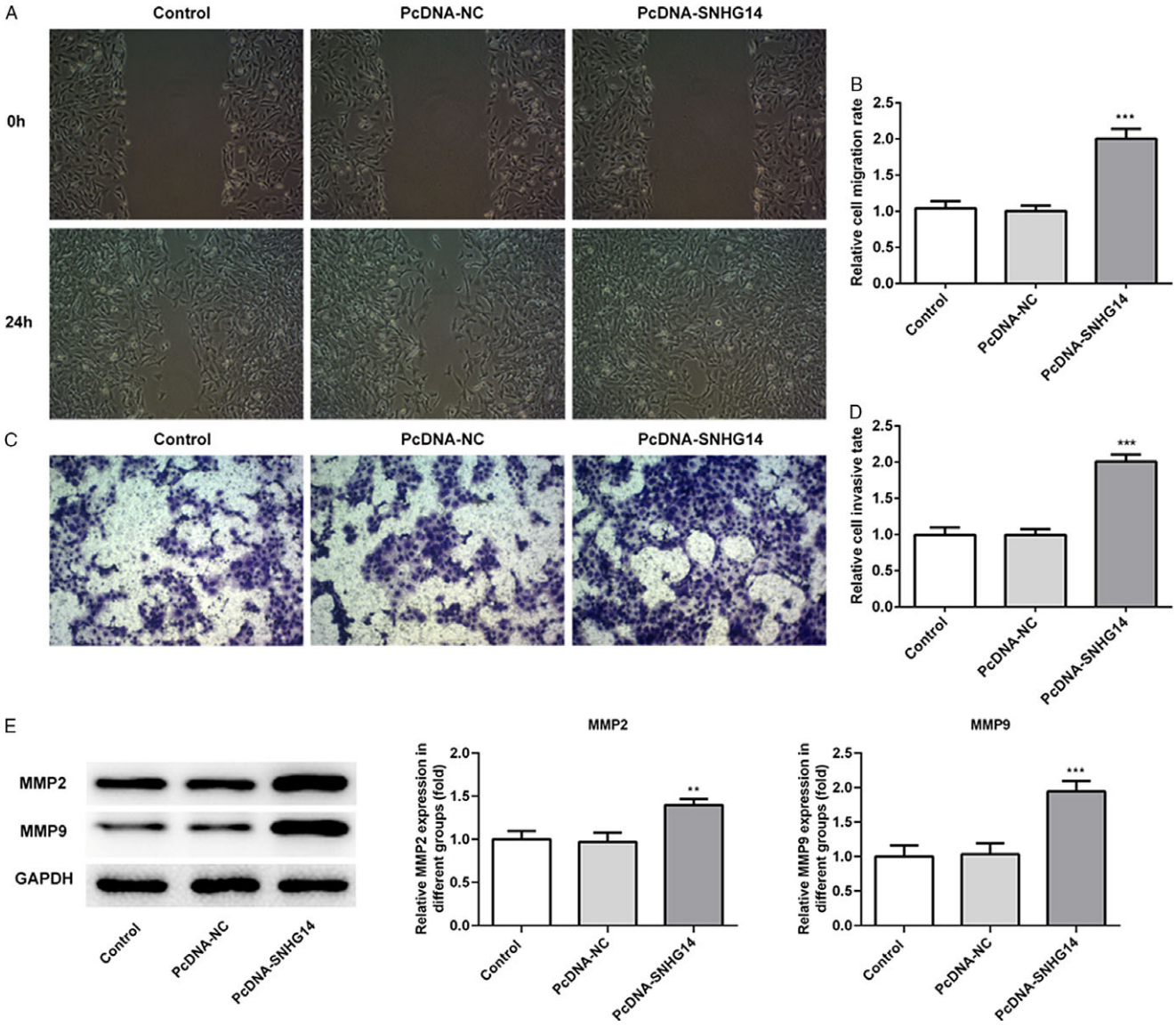

Figure 4. lncRNA SNHG14 promoted HTR-8/SVneo cells migration and invasion. (*A, B*) Wound-healing assay was performed to determine cell migration (×100). (*C, D*) Transwell assay was performed to determine cell invasion (×100). (*E*) MMP2 and MMP9 levels were measured by western blotting assay. $^{**}P < 0.01$, $^{***}P < 0.001$ versus Control.

vimentin expression (Fig. 5C) was greatly enhanced in the pcDNA-SNHG14 group. The above data suggested that SNHG14 may promote the migration and invasion of HTR-8/SVneo cells by enhancing EMT.

lncRNA SNHG14 directly targeted miR-330-5p

To further explore the relationship between lncRNA SNHG14 and miR-330-5p, we predicted miR-330-5p as the target of SNHG14. Bioinformatics analysis indicated that miR-330-5p possessed a potential binding site for SNHG14 (Fig. 6A). To verify the predicted direct binding interaction, we performed dual-luciferase assays. miR-330-5p mimics significantly suppressed the luciferase activity of the wild-type SNHG14 WT but had no effect on the luciferase activity of the mutant SNHG14 MUT (Fig. 6B). Moreover, miR-330-5p levels greatly decreased following SNHG14 overexpression in HTR-8/SVneo cells (Fig. 6C).

Downregulation of lncRNA SNHG14 suppressed cell proliferation and accelerated cell-cycle arrest by competitively binding to miR-330-5p

To further study the role of lncRNA SNHG14 and miR-330-5p in cell proliferation and the cell cycle, SNHG14 and miR-330-5p were downregulated by transfection with sh-SNHG14 and miR-330-5p inhibitor. SNHG14 and miR-330-5p mRNA levels decreased in the sh-SNHG14 group and the miR-330-5p inhibitor group, respectively (Fig. 7A, B). The inhibition effect of sh-SNHG14 on cell proliferation was clearly reversed by co-transfection with sh-SNHG14 and miR-330-5p inhibitor into HTR-8/SVneo cells (Fig. 7C).

https://doi.org/10.1017/S0967199420000507 Published online by Cambridge University Press

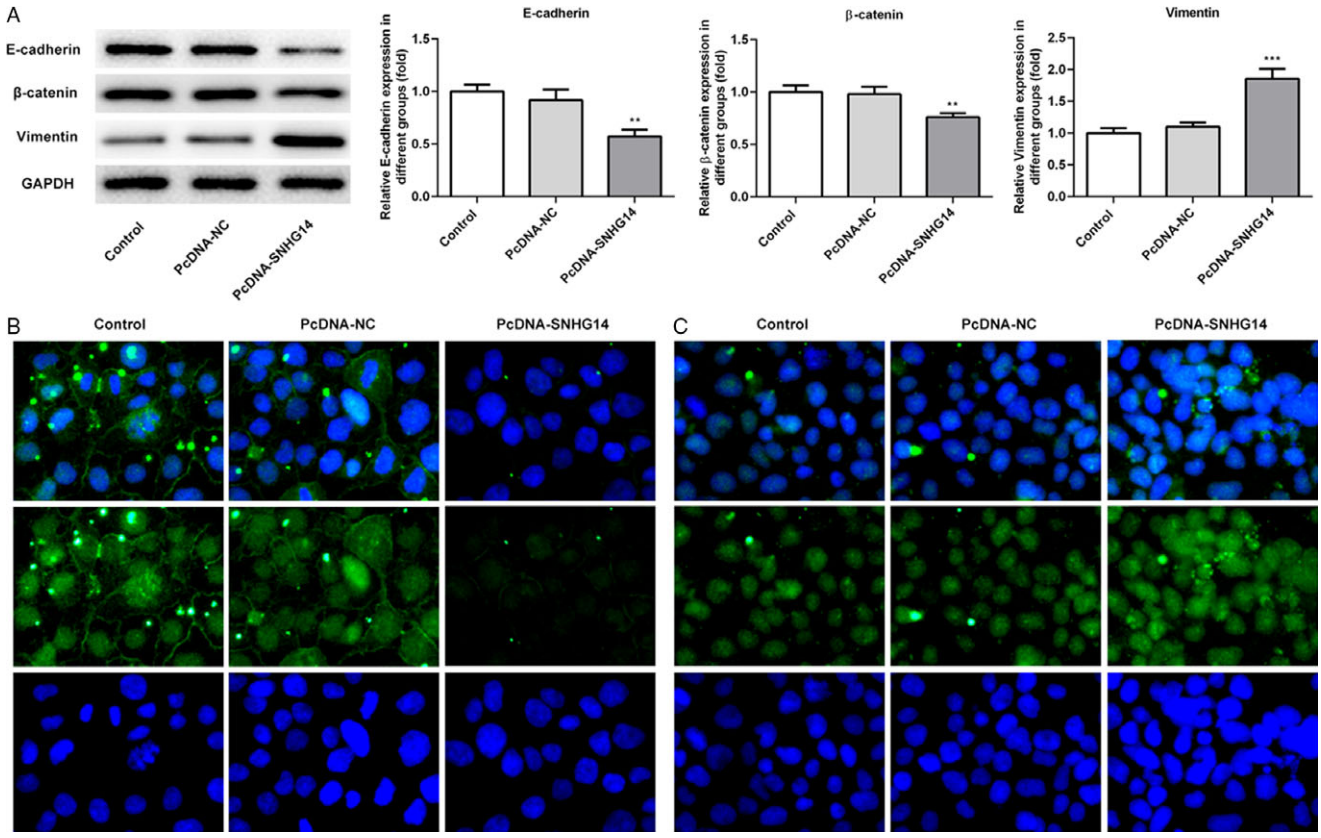

Figure 5. lncRNA SNHG14 regulated EMT in HTR-8/SVneo cells. (*A*) E-cadherin, β-catenin and vimentin levels were measured by western blotting assay. (*B, C*) E-cadherin and vimentin expression were detected by immunofluorescence assay (×200). $^{**}P < 0.01$, $^{***}P < 0.001$ versus Control.

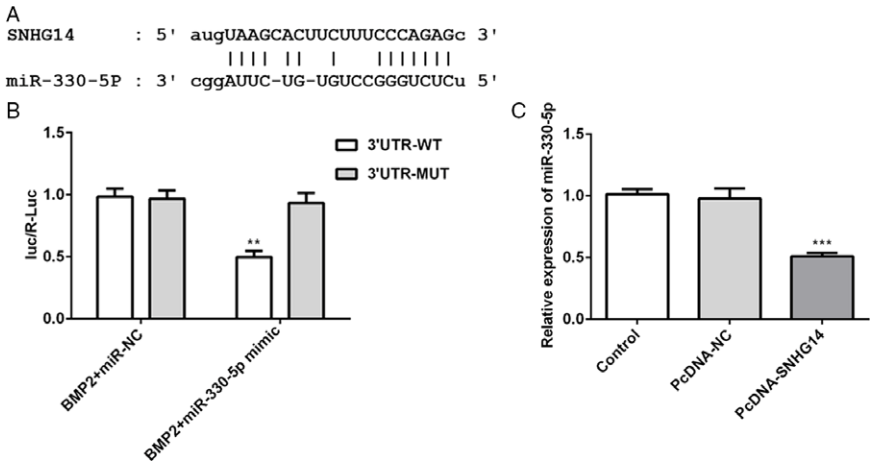

Figure 6. lncRNA SNHG14 directly targeted miR-330-5p. (*A*) Bioinformatics analysis predicted the binding site of lncRNA SNHG14 to miR-330-5p. (*B*) Luciferase reporter assay verified the binding relationship of lncRNA SNHG14 and miR-330-5p. (*C*) miR-330-5p level detected by RT-qPCR. $^{**}P < 0.01$ versus miR-NC, $^{***}P < 0.001$ versus Control.

In addition, there was an increased cell number in G0/G1 phase and a decreased cell population in G2 and S phases in sh-SNHG14-transfected cells. Furthermore, downregulation of miR-330-5p partially reversed the effect of sh-SNHG14 on the cell cycle (Fig. 7D, E).

miR-330-5p was involved in the regulatory effects of lncRNA SNHG14 on migration and invasion in HTR-8/SVneo cells

Here, we explored whether miR-330-5p was involved in sh-SNHG14-mediated effects on migration and invasion of HTR-8/SVneo cells. Downregulation of SNHG14 could significantly reduce migration activities (Fig. 8A, C) and invasion capacities (Fig. 8B, D) of HTR-8/SVneo cells, while downregulation of miR-495-3p partially reversed this effect. Similarly, protein levels of MMP2 and MMP9 were decreased in the sh-SNHG14 group, while miR-330-5p silencing significantly elevated MMP2 and MMP9 levels (Fig. 8E).

Downregulation of lncRNA SNHG14 suppressed EMT by enhancing miR-330-5p in HTR-8/SVneo cells

The effects of SNHG14 and miR-330-5p silencing on EMT were further determined. The relative expression levels of E-cadherin,

https://doi.org/10.1017/S0967199420000507 Published online by Cambridge University Press

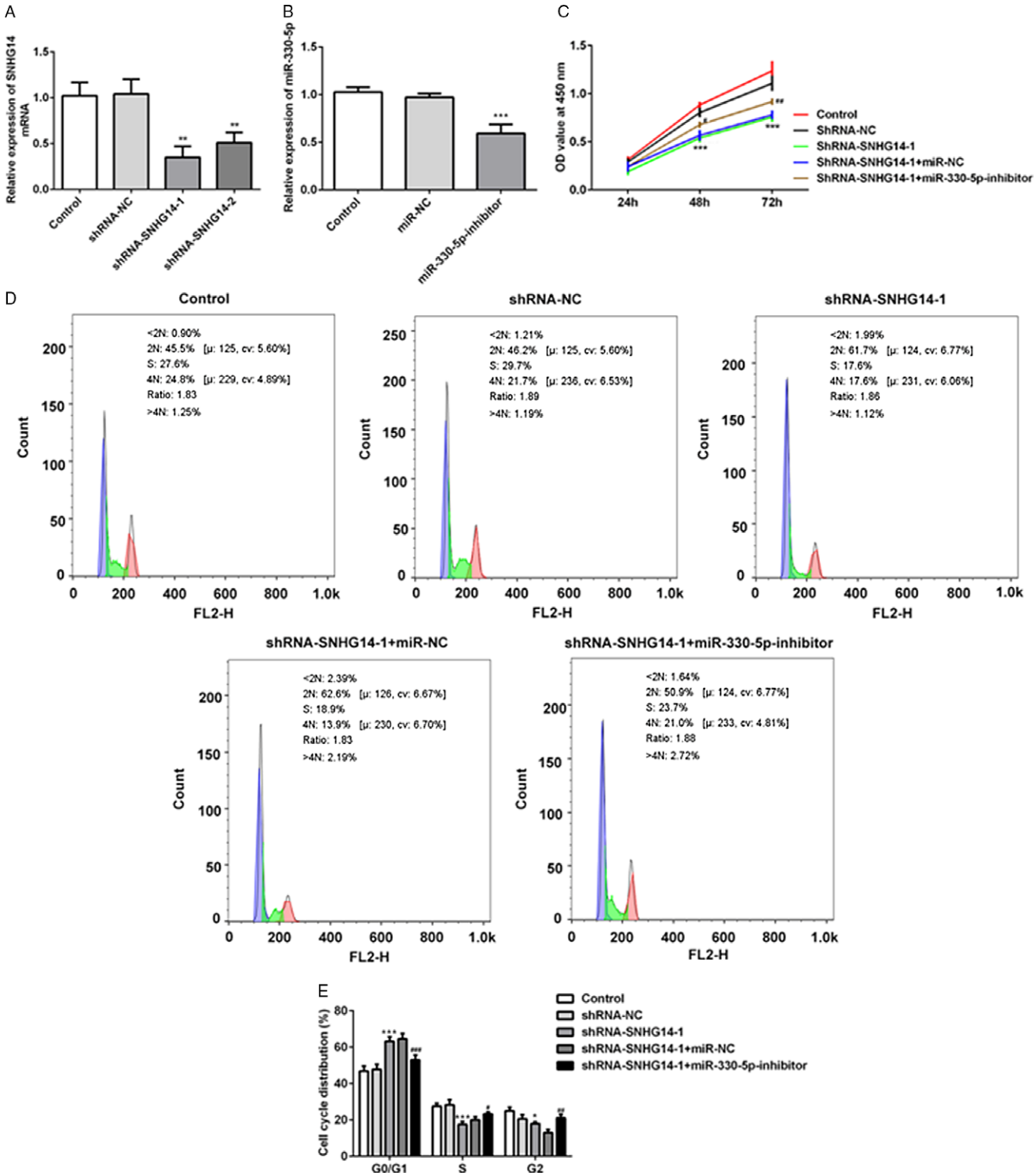

Figure 7. Downregulation of lncRNA SNHG14 suppressed proliferation and accelerated cell-cycle arrest by competitively binding to miR-330-5p. (*A*) SNHG14 expression determined by RT-qPCR. (*B*) miR-330-5p expression determined by RT-qPCR. (*C*) Cell proliferation detected by CCK-8 assay. (*D*) Percentage of cell population in G0/G1, S and G2 phases of the cell cycle was analyzed using flow cytometry assay. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus Control, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus shRNA-SNHG14-1.

β-catenin and vimentin were detected by western blot assay. Transfection with sh-SNHG14 enhanced E-cadherin and β-catenin levels but reduced the vimentin level. Furthermore, sh-SNHG14-induced effects on EMT were abolished following transfection with miR-330-5p inhibitor (Fig. 9A). In addition, immunofluorescence demonstrated that sh-SNHG14-induced upregulation in E-cadherin expression (Fig. 9B) and the downregulation in vimentin expression (Fig. 9C) were reversed after transfection with miR-330-5p inhibitor.

https://doi.org/10.1017/S0967199420000507 Published online by Cambridge University Press

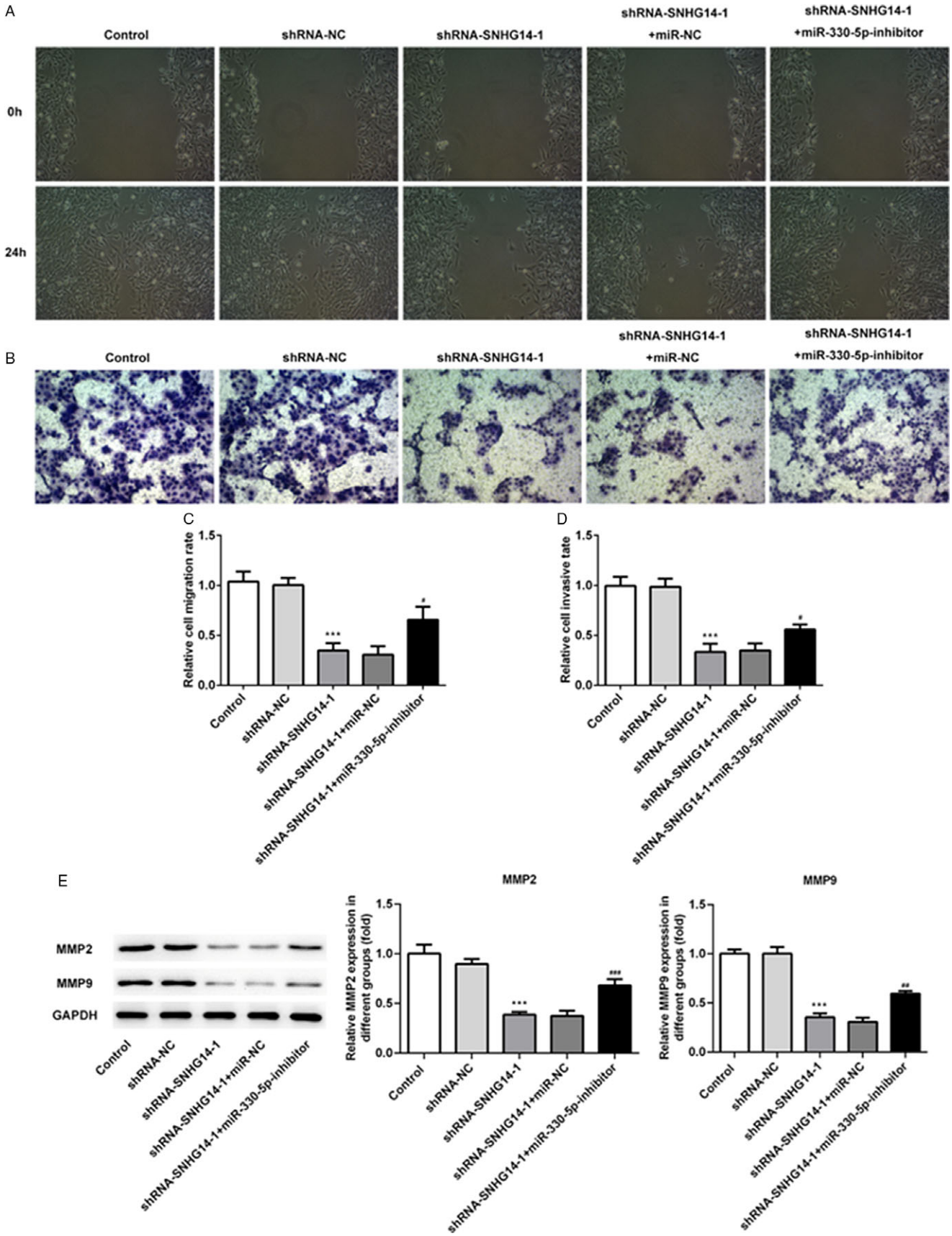

Figure 8. miR-330-5p was involved in the regulatory effects of SNHG14 on migration and invasion in HTR-8/SVneo cells. (*A, C*) Wound-healing assay was performed to determine cell migration (×100). (*B, D*) Transwell assay was performed to determine cell invasion (×100). (*E*) MMP2 and MMP9 levels were measured by western blotting assay. $^{***}P < 0.001$ versus Control, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus shRNA-SNHG14-1.

https://doi.org/10.1017/S0967199420000507 Published online by Cambridge University Press

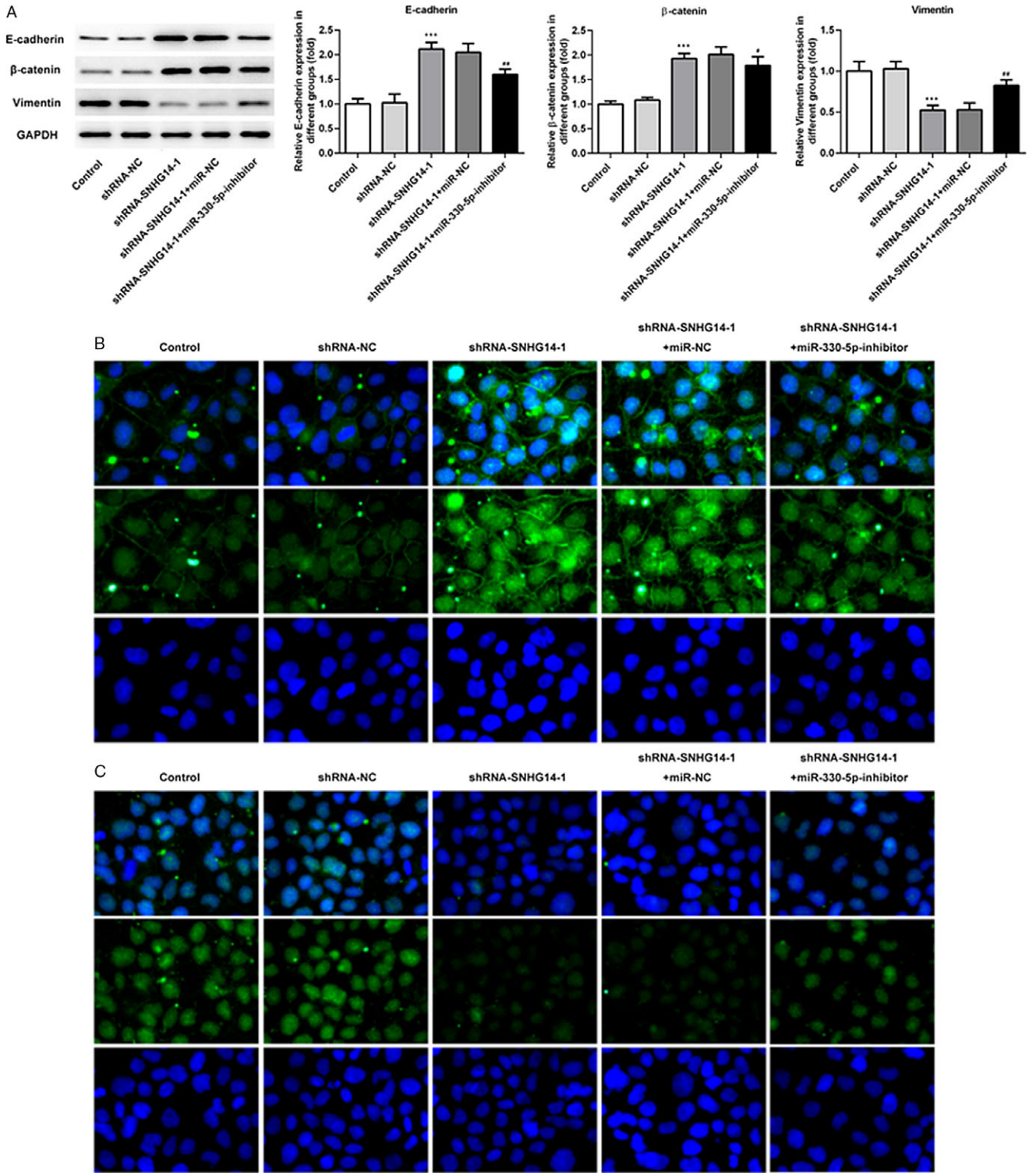

Figure 9. Downregulation of lncRNA SNHG14 suppressed EMT by enhancing miR-330-5p in HTR-8/SVneo cells. (*A*) E-cadherin, β-catenin and vimentin levels were determined by western blotting assay. (*B, C*) E-cadherin and vimentin expression were detected by immunofluorescence assay (×200). ***$P < 0.001$ versus Control, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus shRNA-SNHG14-1.

Discussion

PE is a common disease in human pregnancy and its molecular mechanism is related to multiple factors (Xie *et al.*, 2019). Abnormal trophoblastic proliferation and invasion serve indispensable roles in the development of PE (Wu *et al.*, 2018). Identification of reliable molecular targets for diagnosis and therapy of PE have therefore remained the focus of research. In the present study, we explored the potential molecular mechanisms of lncRNA SNHG14, mainly based on HTR-8/SVneo cells.

Several studies have proved the involvement of multiple lncRNAs in PE development (Li *et al.*, 2019b). However, the

https://doi.org/10.1017/S0967199420000507 Published online by Cambridge University Press

precise role of lncRNA SNHG14 in PE has still not been fully addressed. This current study confirmed that lncRNA SNHG14 was greatly downregulated in placentas of patients with PE compared with women who had normal placentas. Accumulating evidence on lncRNA SNHG14 has been mostly the investigation of its role in cancer (Ye *et al.*, 2019). In addition, lncRNA SNHG14 could play important roles in cell proliferation, cell-cycle progression and migration (Li *et al.*, 2019a). As expected, overexpression of lncRNA SNHG14 markedly promoted the proliferation and cell-cycle progression of HTR-8/SVneo cells. Furthermore, SNHG14 expression was positively associated with cell invasion, migration and EMT processes. miRNAs are also reported to be involved in trophoblast cell proliferation and invasion (Kumar *et al.*, 2013). lncRNAs could function as a 'sponge', binding to miRNAs and participating in a large array of biological processes (Thomson and Dinger, 2016). Consistent with those reports, bioinformatics analysis, luciferase reporter assay and RT-qPCR together demonstrated that lncRNA SNHG14 targeted miR-330-5p and negatively interacted with it. A recent study verified that miR-330-5p might play an essential role in human decidualization and the occurrence of PE (Tong *et al.*, 2018). Based on the present statistical analysis, downregulation of miR-330-5p partly reversed the regulation effects of lncRNA SNHG14 on proliferation and cell-cycle progression in human trophoblasts. In general, silenced SNHG14 may suppress cell invasion, migration and EMT processes by enhancing miR-330-5p.

In conclusion, this study demonstrated that lncRNA SNHG14 was markedly downregulated in placental tissues from PE patients and was involved in the development of PE. Moreover, our results strongly support evidence that in PE lncRNA SNHG14 regulates trophoblast cell proliferation, migration, invasion and EMT by targeting miR-330-5p. Collectively, findings in the current research suggest that lncRNA SNHG14 could be a novel molecular target for early diagnosis and therapy of PE.

Conflict of interest. The authors report no conflicts of interest in this work.

Ethical standards. The present study was approved and authorized by Ethic Committee of Huai'an First People's Hospital. All the patients in the study submitted the written informed consent and approved the study.

References

Bartel DP (2018). Metazoan microRNAs. *Cell* **173**, 20–51.

Dong K, Zhang X, Ma L, Gao N, Tang H, Jian F and Ma Y (2019). Downregulations of circulating miR-31 and miR-21 are associated with preeclampsia. *Pregnancy Hypertens* **17**, 59–63.

Fan M, Li X, Gao X, Dong L, Xin G, Chen L, Qiu J and Xu Y (2019). LPS induces preeclampsia-like phenotype in rats and HTR8/SVneo cells dysfunction through TLR4/p38 MAPK pathway. *Front Physiol* **10**, 1030.

Ji N, Wang Y, Bao G, Yan J and Ji S (2019). lncRNA SNHG14 promotes the progression of cervical cancer by regulating miR-206/YWHAZ. *Pathol Res Pract* **215**, 668–75.

Kumar P, Luo Y, Tudela C, Alexander JM and Mendelson CR (2013). The c-Myc-regulated microRNA-17-92 (miR-17-92) and miR-106a-363 clusters target hCYP19A1 and hGCM1 to inhibit human trophoblast differentiation. *Mol Cell Biol* **33**, 1782–96.

Li L, Zhang R and Li SJ (2019a). Long noncoding RNA SNHG14 promotes ovarian cancer cell proliferation and metastasis via sponging miR-219a-5p. *Eur Rev Med Pharmacol Sci* **23**, 4136–42.

Li Q, Zhang J, Su DM, Guan LN, Mu WH, Yu M, Ma X and Yang RJ (2019b). lncRNA TUG1 modulates proliferation, apoptosis, invasion, and angiogenesis via targeting miR-29b in trophoblast cells. *Hum Genomics* **13**, 50.

Liu DC, Song LL, Liang Q, Hao L, Zhang ZG and Han CH (2019). Long noncoding RNA LEF1-AS1 silencing suppresses the initiation and development of prostate cancer by acting as a molecular sponge of miR-330-5p via LEF1 repression. *J Cell Physiol* **234**, 12727–44.

Livak KJ and Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2(-C_T)$ method. *Methods* **25**, 402–8.

Mao XW, Xiao JQ, Li ZY, Zheng YC and Zhang N (2018). Effects of microRNA-135a on the epithelial–mesenchymal transition, migration and invasion of bladder cancer cells by targeting GSK3β through the Wnt/β-catenin signaling pathway. *Exp Mol Med* **50**, e429.

Mol B, Roberts CT, Thangaratinam S, Magee LA, de Groot C and Hofmeyr GJ (2016). Pre-eclampsia. *Lancet* **387**, 999–1011.

Redman CW and Sargent IL (2005). Latest advances in understanding preeclampsia. *Science* **308**, 1592–4.

Tang Q, Gui J, Wu X and Wu W (2019). Downregulation of miR-424 in placenta is associated with severe preeclampsia. *Pregnancy Hypertens* **17**, 109–12.

Thomson DW and Dinger ME (2016). Endogenous microRNA sponges: evidence and controversy. *Nat Rev Genet* **17**, 272–83.

Tong J, Yang J, Lv H, Lv S, Zhang C and Chen ZJ (2018). Dysfunction of pseudogene PGK1P2 is involved in preeclampsia by acting as a competing endogenous RNA of PGK1. *Pregnancy Hypertens* **13**, 37–45.

Wu D, Xu Y, Zou Y, Zuo Q, Huang S, Wang S, Lu X, He X, Wang J, Wang T and Sun L (2018). Long noncoding RNA 00473 is involved in preeclampsia by LSD1 binding-regulated TFPI2 transcription in trophoblast cells. *Mol Ther Nucleic Acids* **12**, 381–92.

Xiao J, Tao T, Yin Y, Zhao L, Yang L and Hu L (2017). miR-144 may regulate the proliferation, migration and invasion of trophoblastic cells through targeting PTEN in preeclampsia. *Biomed Pharmacother* **94**, 341–53.

Xie N, Jia Z and Li L (2019). miR-320a upregulation contributes to the development of preeclampsia by inhibiting the growth and invasion of trophoblast cells by targeting interleukin 4. *Mol Med Rep* **20**, 3256–64.

Ye T, Zhang N, Wu W, Yang B, Wang J, Huang W and Tang D (2019). SNHG14 promotes the tumorigenesis and metastasis of colorectal cancer through miR-32-5p/SKIL axis. *In Vitro Cell Dev Biol Anim* **55**, 812–20.

Zhang H, Li T, Zheng L and Huang X (2017). Biomarker microRNAs for diagnosis of oral squamous cell carcinoma identified based on gene expression data and microRNA–mRNA network analysis. *Comput Math Methods Med* **2017**, 9803018.

Zhong Y, Tuuli M and Odibo AO (2010). First-trimester assessment of placenta function and the prediction of preeclampsia and intrauterine growth restriction. *Prenat Diagn* **30**, 293–308.

Zhong Y, Zhu F and Ding Y (2019). Differential microRNA expression profile in the plasma of preeclampsia and normal pregnancies. *Exp Ther Med* **18**, 826–32.

https://doi.org/10.1017/S0967199420000507 Published online by Cambridge University Press