

MiR-296-3p promotes the development and progression of preeclampsia *via* targeting the CEMIP

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Abstract. – OBJECTIVE: Preeclampsia (PE) is one of common pregnancy diseases, which has seriously threatened the health of the gravidas. Although upregulated miR-269-3p has been found in the placentas of the patients with PE, the regulation mechanisms of miR-296-3p remain unclear.

PATIENTS AND METHODS: In this study, the placentas of the patients and normal gravidas were used to observe the difference in miR-296-3p expression level, and HTR-8/Svneo and JAR cells were used to investigate the role of miR-296-3p in trophoblast cells. Besides, qRT-PCR, Western blot, CCK-8 assay, Dual-Luciferase reporter gene assay and transwell assay were used to explore the functions and regulation mechanisms of miR-296-3p on PE.

RESULTS: The results showed that miR-296-3p was upregulated in the PE-placentas, and increased miR-296-3p could inhibit the proliferation, invasion and migration of HTR-8/Svneo and JAR cells. Besides, miR-296-3p could directly target the 3'-UTR of CEMIP, and the phenomena induced by increased miR-296-3p, including decreased β -catenin and p-AKT and weakened proliferation, invasion and migration abilities, could be reversed by upregulating the expression level of CEMIP.

CONCLUSIONS: To summarize, this study suggests that miR-296-3p inactivates the Wnt/ β -catenin and PI3K/AKT pathways to promote the progression of PE via targeting the CEMIP.

Key Words:

Preeclampsia, MiR-296-3p, CEMIP, Wnt/ β -catenin, PI3K/AKT.

Introduction

Preeclampsia (PE) is a common pregnancy complication that mostly occurs after 20 gestational weeks¹⁻³. In clinical intervention, the

environmental disorder in the patients induced by serious symptoms contributes disastrous morbidity and mortality of both PE-patients and their fetuses⁴. Even with current therapeutic strategies, PE, with the incidence of 3%-5%, remains a major cause of perinatal deaths worldwide⁵. As expected, PE is characterized by an excess of antiangiogenic factors, hypertensive induced by uteroplacental ischemia and final multi-organ dysfunction⁶. Although the prognosis of the patients has been improved in recent years, the pathological mechanism of PE remains unclear. At present, to illustrate the pathogenesis of PE, several theories, including the impairment of the placentas induced by ischemia, abnormal apoptosis and invasion levels of trophoblastic cells and the dysfunction of vascular endothelial cells induced by oxidative stress and increased antiangiogenic factors, have been proposed^{7,8}. However, it has been widely accepted that the changes in the apoptosis level and the abilities of proliferation, invasion and migration of trophoblastic cells might be major reasons of PE.

MicroRNAs (miRNAs) are endogenous non-coding RNAs consisting with 21-25 nucleotides⁹. Increasing evidence have indicated that miRNAs play key roles in many important cellular events¹⁰. The interaction of miRNAs with 3'-UTRs involves in the translation repression of target mRNAs, contributing downregulation of the related protein in the cells¹¹. Moreover, modulating the levels of the miRNAs with abnormal expression has increasingly emerged as a promising therapeutic strategy. As possible therapeutic targets, consideration has been given to the abnormal expression of some miRNAs in the trophoblast cells, which is a hallmark feature of PE¹². Lv et al¹³ have showed that the miRNAs

are related to the abilities of the trophoblast cells such as proliferation, invasion and migration. The dysfunction of some miRNAs in the human trophoblast cells induces the expression change of some key proteins and activities of some signal pathways, to promote the development and progression of PE¹⁴.

It has been found that miR-296-3p was upregulated in the placentas of the patients with PE, while the regulation mechanisms of miR-296-3p on PE remains unclear. Therefore, this study aims to explore the functions of miR-296-3p in the progression of PE and provide some reference to PE treatment.

Patients and Methods

Clinical Sample

The study was approved by the Ethics Committee of Daqing People's Hospital, and verbal and written consent was obtained from participants. The included criteria followed the diagnostic guidelines of International Society for the Study of Hypertension in Pregnancy (ISSHP) published in 2018¹⁵.

Thirty placental samples of the patients and normal participants, from Department of Obstetrics (I), Daqing People's Hospital, from July 2016 to October 2018, were collected for detection. Inclusion criteria: the included patients did not have any history of pre-existing or chronic hypertension; the patients had severe proteinuria (urinary protein excretion, ≥ 2 g/24 hours), and the systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg on ≥ 2 occasions after the 20th week of gestation. Exclusion criteria: The patients had renal disease or transient hypertension, which seriously affect the accuracy of this study; The patients with spontaneous abortion, intrauterine fetal death, fetal chromosomal or congenital abnormalities were excluded from this study. The placentas were collected within 1 hour of cesarean birth. The tissues of the chorionic plate and basal plate were separately obtained from the placenta disc.

Cell Culture

The cell lines including HTR-8/SVneo and JAR were purchased from Procell Life Science&Technology Co., Ltd. (Waltham, MA, USA). All cells were cultured with Roswell Park Memorial Institute-1640 (RPMI-1640; Gibco, Beijing, China) contained with 10% FBS and antibiotics (100 U/mL penicillin and 100 μ g/mL

streptomycin). All cells were maintained at 37°C with 5% CO₂.

Cell Transfection

The cells seeded in 6-well plates. The transfectants including miR-296-3p inhibitor, inhibitor-NC and pcDNA-KEAP1 were synthesized and purified by Beijing Generaybiotech co., Ltd (Beijing, China). Trophoblast cells (2×10^6 cells/well) were seed in 6-well plates and incubated for 24 hours. After that, the transfectants including the miR-296-3p mimics, miR-296-3p inhibitors, pcDNA-CEMIP or empty vector were transfected into HBMECs using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). In brief, the cells were seed in 6-well plates, and the transfections were performed when the cell confluence was at 70%. 4 μ g DNA, 100 pmol RNA or 10 μ l Lipofectamine 2000 diluted by 250 μ l serum-free medium, respectively. After incubation for 5 min, the diluted DNA/RNA were mixed with diluted Lipofectamine 2000 (total volume = 500 μ l). After incubation for 20 min at room temperature, the 500 μ l of mixtures was added into each well. After that, the cells were cultured for 24-48 hours.

Quantitative Reverse Transcription Polymerase Reaction (qRT-PCR)

Total RNAs of the tissues or cells were extracted by TRIzol reagent. After that, the extracts were transcribed into cDNA by a PrimeScript[®] RT reagent Kit (Thermo Fisher Scientific, Waltham, MA, USA). All primers were synthesized and purified by Synbio Technology (Suzhou, China). According to the operation instruction of a KAPA qRT-PCR kit (Sigma-Aldrich, St. Louis, MO, USA), the reaction systems (10 μ L) were prepared for qRT-PCR. The reaction conditions followed pre-denaturation at 95°C for 30 s, 95°C for 5 s, and 60°C for 30 s for a total of 40 cycles. Besides, U6 was used as the control of miR-296-3p. The primer sequences of miR-296-3p and U6 have been shown in Table I.

Table I. Primer sequences.

Name of primer	Sequences
miR-296-3p-F	5'-TGGGAGGGCCCCCTCAA-3'
miR-296-3p-R	5'-TGGTGTCGTGGAGTCG-3'
U6-F	5'-CTCGCTTCGGCAGCAC-3'
U6-R	5'-AACGCTTACGAATTTGCGT-3'

Western Blot

The total proteins of the tissues or cells were extracted by RIPA buffer (containing 1% Phenylmethylsulfonyl fluoride, Absin Bioscience Inc, Shanghai, China), and BCA protein assay kit (Be-yotime, Shanghai, China) was used to measure the concentration of the extracts. All proteins were separated by 10% polyacrylamide gel, and then, were transferred onto the polyvinylidene difluoride (PVDF) membranes. After that, the fat-free milk (5%) was used to block the membranes for 1 hours, and then, the membranes were incubated with the primary antibodies of target proteins at 4°C overnight, and the β -actin was used for quantification of target proteins. After washing three times with TBST, the membranes were incubated with the secondary antibodies for 1.5 hours at 25 °C. Finally, a chemiluminescence detection system was used to observe the protein expression levels. The antibodies were used as follow: anti-CEMIP (1:1000, ab2853965, ThermoFisher, Waltham, MA, USA); anti-p-PI3K(1:1000, ab2816326, ThermoFisher, Waltham, MA, USA); anti- β -catenin (1:2000, ab170901, Abcam, Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti- β -actin (1:1000, sc-47,778, Santa Cruz, CA, USA).

Transwell Assay

For migration assay, 100 μ L serum-free RPMI 1640 medium with 2×10^5 cells were added to the upper chamber, and 600 μ L complete medium containing 10% FBS was added to the lower chamber. The cells were cultured in standard conditions for 24 hours. The migrated cells on the lower surface of the upper chamber were fixed in methanol for 10 min. The cells were dried at room temperature and stained with 0.1% w/v crystal violet (Cat#G1062, Solarbio, Beijing, China) for 30 min and then were washed with tap water. The migrated cells were visualized and photographed by a Leica DMi8 microscope.

For the invasion assay, the upper chamber of the Transwell chamber was coated with Matrigel and 5×10^5 cells were seeded in the upper chamber of each transwell. After incubation for 24 h, the cells were fixed by 4% paraformaldehyde, and stained with 0.4% trypan blue. After that, the cells in three randomly selected areas of chambers were counted by a microscope (Olympus, Tokyo, Japan).

CCK-8 Assay

HTR-8/Svneo cells were seeded into 96-well plates. After 48 h of transfection, the cells in each

well were added with CCK-8 solution (Amyjet, Wuhan, China), and the blank wells only added with CCK-8 solution as the controls. After incubating for 4 hours, the absorbance value was measured at 450 nm by a microplate reader (Molecular Devices, Shanghai, China).

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling (TUNEL) Assay

The *in-situ* apoptosis detection kits were used to measure the apoptosis levels of the HTR-8/Svneo and JAR cells. 4% paraformaldehyde was used to fix the cells, and 0.1% Triton X-100 was used to permeabilize the cellular membranes. After that, the cells were incubated in TUNEL mixture at 37°C for 1hour, and DAPI was used for the counterstain of nuclei. An immunofluorescent microscopy (Olympus FV1000, Tokyo, Japan) was used to observe the apoptosis level of the cells. TUNEL positive cells in 6 randomly selected fields were counted.

Dual-Luciferase Reporter Gene Assay

The DNA fragment of CEMIP containing the wild-type (CEMIP-wt) and mutant 3'-UTR of CEMIP (CEMIP-mut) were linked into the pmirGLO Luciferase reporter vectors (Promega, Madison, WI, USA). After that, the vectors of CEMIP-wt and CEMIP-mut were co-transfected into HTR-8/SVneo cells, along with miR-296-3p mimic or miR-NC, respectively. The Luciferase activities of the cells were observed by a dual-luciferase reporter assay system.

Statistical Analysis

All the experiments were performed at least three times, independently. The data were analyzed by SPSS 20.0, and the figures were charted by GraphPad Prism 8.0. Chi-squared test or ANOVA with Tukey's post hoc-test was used to calculate the difference between the groups. $p < 0.05$ means that the significant difference exists in two groups.

Results

MiR-296-3p Was Upregulated in the Placentas of the Patients with PE

The expression levels of miR-296-3p in the placentas of normal participators and the patients with PE were observed by qRT-PCR. The results

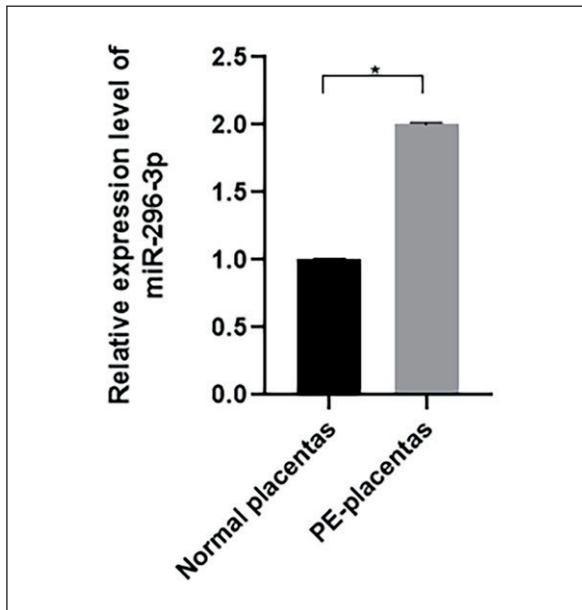


Figure 1. MiR-296-3p was upregulated in the placentas of the patients with PE.

showed that miR-296-3p was significant over-expressed in the tissues of the patients, which suggested that miR-296-3p was related with the development of PE (Figure 1. $p < 0.05$).

MiR-296-3p Could Inhibit the Proliferation of HTR-8/Svneo and JAR Cells

To make sure the functions of miR-296-3p in the progression of PE, miR-296-3p mimics were transfected into HTR-8/Svneo and JAR cells, and the proliferation ability of the cells was observed by CCK-8 and TUNEL staining assays. The results indicated that the miR-296-3p significantly suppressed the proliferation of HTR-8/Svneo and JAR cells, and the apoptosis level of the cells notably increased when miR-296-3p mimics were transfected into the cells (Figure 2 AD, $p < 0.05$).

MiR-296-3p Could Promote the Invasion and Migration of HTR-8/Svneo and JAR Cells

To confirm whether miR-296-3p promotes the progression of PE via inhibiting the invasion and migration of the trophoblast cells, the transwell assay was used to observe the effects of miR-296-3p on invasion and migration abilities of HTR-8/Svneo and JAR cells. The results showed that miR-296-3p significantly impaired the invasion and migration abilities of HTR-8/Svneo and JAR cells, which suggested miR-296-3p as a promoter of the PE (Figure 3. A-D, $p < 0.05$).

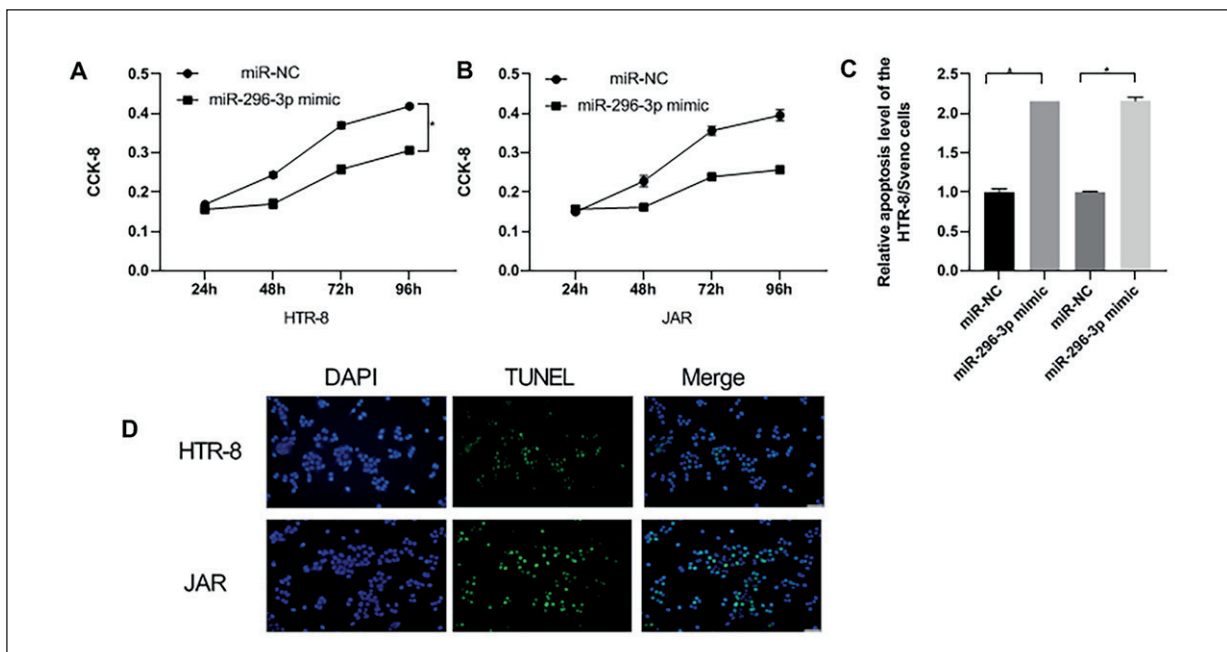


Figure 2. MiR-296-3p inhibited the proliferation of the the trophoblast cells. **A-B**, The CCK-8 values of HTR-8/Svneo and JAR cells affected by increased miR-296-3p. **C-D**, The apoptosis levels of HTR-8/Svneo and JAR cells affected by increased miR-296-3p (Scale=5 μ m).

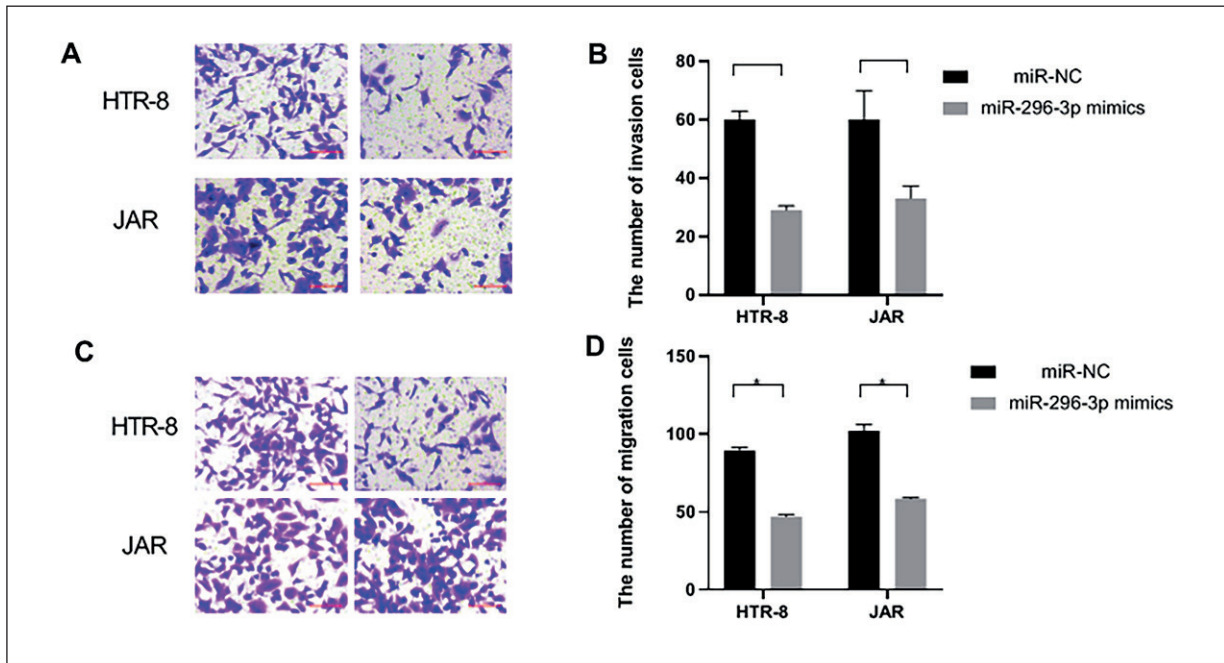


Figure 3. MiR-296-3p inhibited the invasion and migration of the trophoblast cells. **A-B**, The invasion number of HTR-8/Svneo and JAR cells affected by increased miR-296-3p. **C-D**, The migration number of HTR-8/Svneo and JAR cells affected by increased miR-296-3p (Scale=5 μ m).

MiR-296-3p Was an Upstream Factor of CEMIP and Could Directly Targeted the 3'-UTR of CEMIP

To explore the regulation mechanism of miR-296-3p on PE, the miRwalk, a database for potential target prediction of miRNAs, was used to find the downstream factors of miR-296-3p. The results indicated that CEMIP was a possible target which had low binding energy with miR-296-3p. To further validate the accuracy of the prediction result, the vectors of CEMIP-wt and CEMIP-mut were transfected into HTR-8/Svneo cells with the miR-NC or miR-296-3p mimics, respectively. It was found that miR-296-3p could decrease the luciferase activity of CEMIP-wt rather than that of CEMIP-mut (Figure 4. A, $p < 0.05$). Besides, the downregulated CEMIP was also observed in pathological tissues of PE-patients (Figure 4. B, $p < 0.05$).

CEMIP Reversed the Downregulated β -Catenin and p-AKT Induced by MiR-296-3p Overexpression in HTR-8/Svneo Cells

To further illustrate the relationship between miR-296-3p and CEMIP, the miR-296-3p mimics and CEMIP overexpressed vectors were

co-transfected into HTR-8/Svneo cells, and the expression levels of β -catenin and p-AKT were measured by Western blot. In results, the expression level of β -catenin and p-AKT decreased significantly when miR-296-3p mimics were transfected into HTR-8/Svneo cells, while the expression levels of β -catenin and p-AKT were reversed in the cells transfecting with CEMIP overexpressed vectors together (Figure 4 DH, $p < 0.05$).

CEMIP Reversed the Weakened Abilities of the Trophoblast Cells Induced by Upregulated MiR-296-3p

To prove whether CEMIP could reverse the weakened abilities of HTR-8/Svneo cells induced by upregulated miR-296-3p, the miR-296-3p mimics and CEMIP overexpressed vectors were co-transfected into HTR-8/Svneo cells, and the CCK-8 and transwell assay were used to observe the changes in the proliferation, invasion and migration of the cells. In results, the weakened proliferation, invasion and migration abilities of HTR-8/Svneo cells induced by upregulated miR-296-3p was reversed by overexpressed CEMIP (Figure 5. A-D, $p < 0.05$).

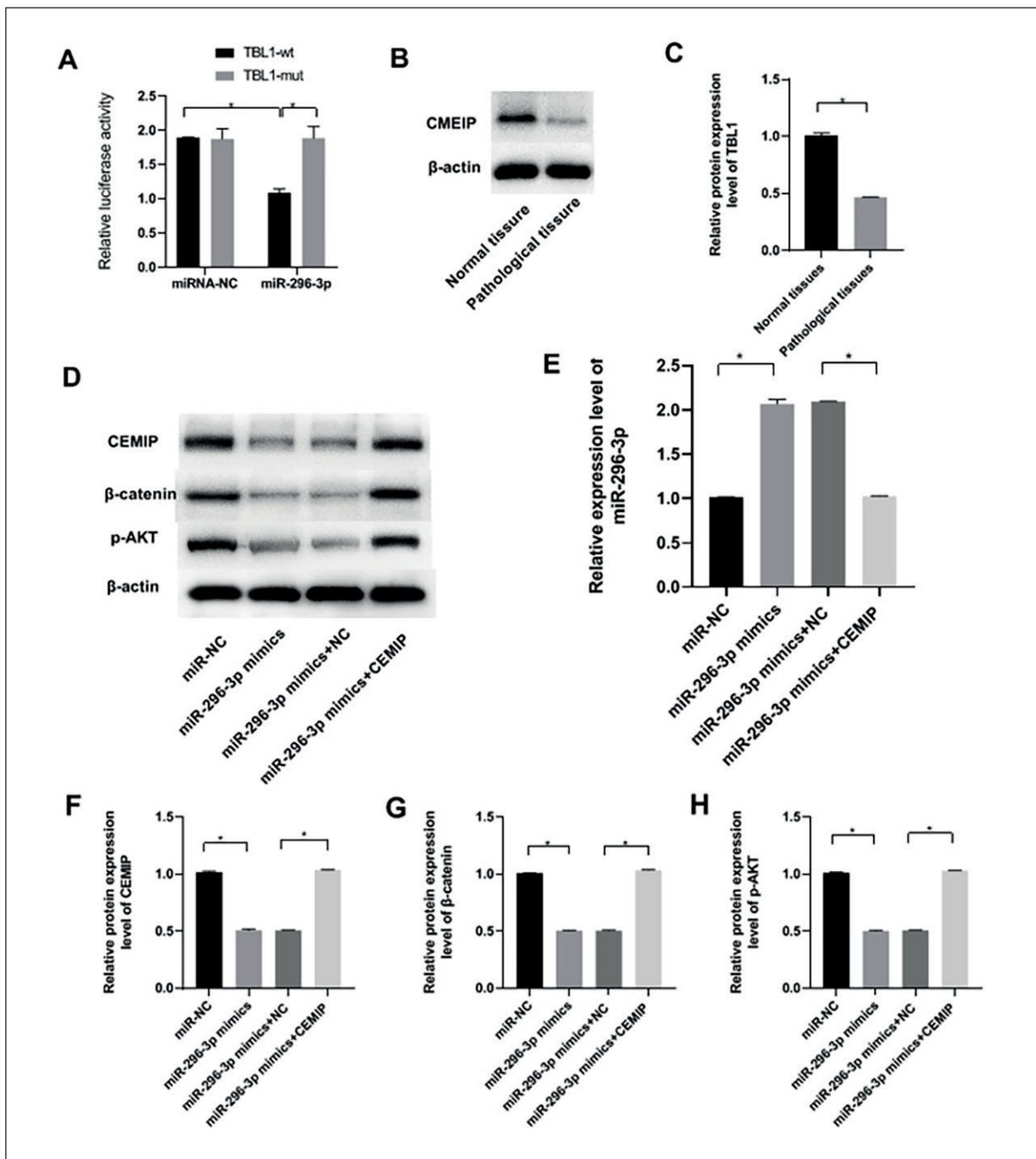


Figure 4. MiR-296-3p directly targeted the 3'-UTR of CEMIP, and decreased expression levels of CEMIP, β -catenin and p-AKT induced by miR-296-3p upregulation could be reversed by CEMIP. **A**, The relative Luciferase activities of CEMIP-wt and CEMIP-mut were tested by Dual-Luciferase reporter assay. **B-C**, The relative protein expression level of CEMIP in the tissues was measured by Western blot. **E**, Relative expression level of miR-296-3p was measured by qRT-PCR. **D, F, G, and H**, The relative protein expression levels of CEMIP, β -catenin and p-AKT in the HTR-8/Svneo were measured by Western blot.

Discussion

PE is a common disease with high morbidity and mortality, which has seriously threatened

the health of the gravidas¹⁶. Previous scholars¹⁷ have also indicated that miRNAs in cytoplasm could affect cellular biological phenotypes *via* sponging various genes to further take part

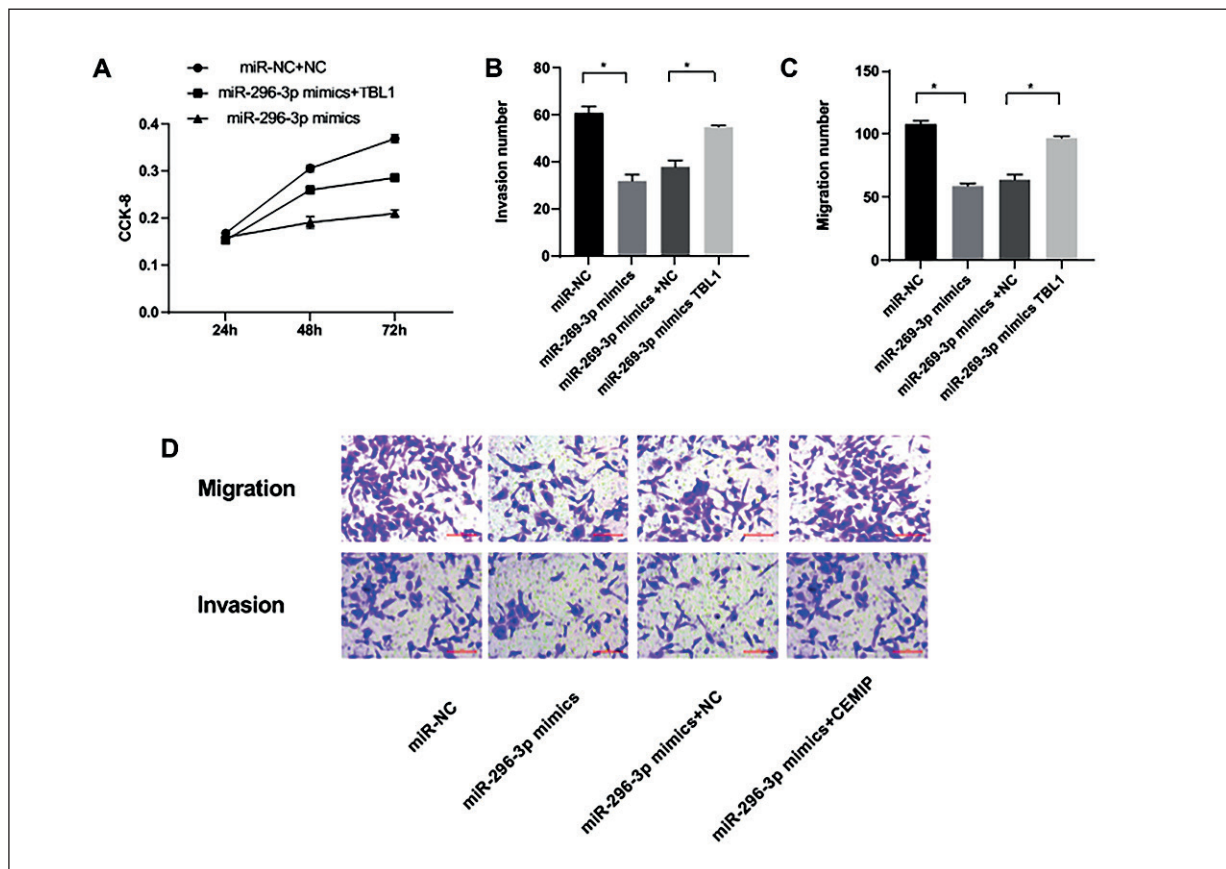


Figure 5. Weakened proliferation, invasion and migration abilities of HTR-8/Svneo cells induced by miR-296-3p upregulation could be reversed by CEMIP. **A**, The proliferation ability of HTR-8/Svneo cells was observed by CCK-8 assay. **B-C**, and **D**, The invasion and migration abilities of HTR-8/Svneo cells were observed by transwell assay (Scale=5 μ m).

in the development and progression of PE. A study¹⁸ has indicated that miR-296-3p was upregulated in the placentas of the patients with PE. Therefore, this study focused on the relationship between miR-296-3p and PE. We demonstrated that miR-296-3p did is significantly upregulated in placental tissues of patients with PE, and upregulated miR-296-3p could promote the proliferation, invasion and migration abilities of the trophoblast cells. Besides, we also found that CEMIP is a target of miR-296-3p, and it could activate Wnt/ β -catenin and PI3K/AKT pathways to reverse the effects of miR-296-3p on trophoblast cells. Those observations suggests that miR-296-3p could inactivate Wnt/ β -catenin and PI3K/AKT pathways to promote the progression of PE via targeting CEMIP.

The dysfunction of miRNAs plays different roles in many diseases, ranging from multiple pregnancy diseases to cancers, and the different profiles of the miRNAs has also been found

in PE and normal pregnant women¹⁹. Several studies^{20,21} have indicated that miR-296-3p is significantly upregulated in the serum and placentas of the patients with PE. In this study, we also found that miR-296-3p was at higher expression level in pathological tissues than that in normal tissues. MiR-296-3p has been widely accepted as a tumor suppressor, which could inhibit the proliferation, invasion and migration of multiple cancers. In fact, miR-296-3p is downregulated in lung cancer cells, while increased miR-296-3p could inhibit the proliferation and invasion of tumor cells via targeting the RABL3²². However, few studies have revealed the functions of miR-296-3p in PE. The weakened ability of the trophoblast cells contributes to failed invasion and remodeling of cytotrophoblasts to the spiral arteries, which further induced the formation of PE²³. In normal trophoblast cells, the impaired proliferation, invasion and migration abilities of

the cells was also observed when we knocked down the miR-296-3p²⁴. Therefore, it suggests that increased miR-296-3p is one reason of phenotypic changes of trophoblast cells.

CEMIP is a novel metastatic promoter and can enhance the migration ability of osteoblastic stem cells. Besides, being upregulated in many cancers, CEMIP has been found to result in the migration and invasion of some tumor cells with higher malignancy²⁵. Zhao et al²⁶ proved that CEMIP was significantly upregulated in colorectal cancer, and it could increase the phosphatase activity of phosphatase 2A (PP2A), and further induce the dephosphorylation progression to decrease the stability of Stathmin which could inhibit the progression of the cancer. However, it has been previously illustrated the functions of CEMIP in PE. In this study, we suggest CEMIP as a key factor involving the formation of PE, and the overexpressed CEMIP was also observed in the placental tissues of the patients with PE. The study has indicated that CEMIP upregulation could reduce the apoptosis level of nucleus pulposus cells, and increased CEMIP can promote the proliferation and migration of nucleus pulposus cells²⁷. Besides, we also found that CEMIP was a downstream target of miR-296-3p, and increased CEMIP could reverse the weakened proliferation, invasion and migration abilities of the trophoblast cells, which suggests that increased miR-296-3p contributes the development and progression of PE. The formation of PE is related with the changes of multiple signal pathways which further affect the cellular phenotypic functions. Xu et al²⁸ found that decreased lysyl oxidase-like protein 2 could activate the TGF- β pathway to inhibit the invasion and migration of the trophoblast cells. In this study, reduced activities of the Wnt/ β -catenin and PI3K/AKT pathways in normal trophoblast cells were observed when miR-296-3p was overexpressed, while the phenomena could be reversed by increased CEMIP. MiR-296-3p could be as an anti-tumor factor to restrict the invasion and migration of breast cancer *via* targeting FGFR1 and Wnt/ β -catenin and inhibit the progression of nasopharyngeal carcinoma cells *via* regulating the PI3K/AKT pathway^{29,30}. Moreover, CEMIP has also been found to promote the deterioration of tumor cells *via* enhancing the activities of the Wnt/ β -catenin and PI3K/AKT pathways^{31,32}. Although we have explained the regulation mechanisms of miR-269-3p in PE, it is necessary that more credible evidence of the

axes, including miR-296-3p/CEMIP/Wnt/ β -catenin and miR-296-3p/CEMIP/PI3K/AKT in PE should be obtained from *in vivo* animal models experiments.

Conclusions

In summary, it suggests that miR-296-3p could inactivate the Wnt/ β -catenin and PI3K/AKT pathways to promote the formation and development of PE *via* targeting CEMIP.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

- 1) Pierik E, Prins JR, van Goor H, Dekker GA, Daha MR, Seelen MAJ, Scherjon SA. Dysregulation of complement activation and placental dysfunction: a potential target to treat preeclampsia? *Front Immunol* 2020; 10: 3098.
- 2) Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: pathophysiology, challenges, and perspectives. *Circ Res* 2019;124: 1094-1112.
- 3) Voutetakis A, Pervanidou P, Kanaka-Gantenbein C. Aspirin for the prevention of preeclampsia and potential consequences for fetal brain development. *JAMA Pediatr* 2019; 173: 619-620.
- 4) Wright D, Wright A, Nicolaidis KH. The competing risk approach for prediction of preeclampsia. *Am J Obstet Gynecol* 2020; 223: 12-23.e7.
- 5) Chiarello DI, Salsoso R, Toledo F, Mate A, Vázquez CM, Sobrevia L. Foetoplacental communication via extracellular vesicles in normal pregnancy and preeclampsia. *Mol Aspects Med* 2018; 60: 69-80.
- 6) Phipps EA, Thadhani R, Benzing T, Karumanchi SA. Pre-eclampsia: pathogenesis, novel diagnostics and therapies. *Nat Rev Nephrol* 2019; 15: 275-289.
- 7) Tenório MB, Ferreira RC, Moura FA, Bueno NB, de Oliveira ACM, Goulart MOF. Cross-talk between oxidative stress and inflammation in preeclampsia. *Oxid Med Cell Longev* 2019; 2019: 8238727.
- 8) Abbas Y, Turco MY, Burton GJ, Moffett A. Investigation of human trophoblast invasion in vitro. *Hum Reprod Update* 2020; 26: 501-513.
- 9) Si W, Shen J, Zheng H, Fan W. The role and mechanisms of action of microRNAs in cancer drug resistance. *Clin Epigenetics* 2019; 11: 25.
- 10) Reh TA, Hindges R. MicroRNAs in retinal development. *Annu Rev Vis Sci* 2018; 4: 25-44.

- 11) Bayraktar R, Van Roosbroeck K, Calin GA. Cell-to-cell communication: microRNAs as hormones. *Mol Oncol* 2017; 11: 1673-1686.
- 12) Lip SV, Boekschoten MV, Hooiveld GJ, van Pampus MG, Scherjon SA, Plösch T, Faas MM. Early-onset preeclampsia, plasma microRNAs, and endothelial cell function. *Am J Obstet Gynecol* 2020; 222: 497.e1-497.e12.
- 13) Lv Y, Lu C, Ji X, Miao Z, Long W, Ding H, Lv M. Roles of microRNAs in preeclampsia. *J Cell Physiol* 2019; 234: 1052-1061.
- 14) Lykoudi A, KoliAlexi A, Lambrou GI, Braoudaki M, Siristatidis C, Papaioanou GK, Tzetis M, Mavrou A, Papantoniou N. Dysregulated placental microRNAs in early and late onset preeclampsia. *Placenta* 2018; 61: 24-32.
- 15) Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, Hall DR, Warren CE, Adayi G, Ishaku S; International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice. *Hypertension* 2018; 72: 24-43.
- 16) Chaemsaitong P, Pooh RK, Zheng M, Ma R, Chaiyasit N, Tokunaka M, Shaw SW, Seshadri S, Choolani M, Wataganara T, Yeo GSH, Wright A, Leung WC, Sekizawa A, Hu Y, Naruse K, Saito S, Sahota D, Leung TY, Poon LC. Prospective evaluation of screening performance of first-trimester prediction models for preterm preeclampsia in an Asian population. *Am J Obstet Gynecol* 2019; 221: 650.e1-650.e16.
- 17) Chen X, Xie D, Zhao Q, You ZH. MicroRNAs and complex diseases: from experimental results to computational models. *Brief Bioinform* 2019; 20: 515-539.
- 18) Murphy MS, Casselman RC, Tayade C, Smith GN. Differential expression of plasma microRNA in preeclamptic patients at delivery and 1 year postpartum. *Am J Obstet Gynecol* 2015; 213: 367.e1-9.
- 19) Zhou C, Zou QY, Li H, Wang RF, Liu AX, Magness RR, Zheng J. Preeclampsia downregulates microRNAs in fetal endothelial cells: roles of miR-29a/c-3p in endothelial function. *J Clin Endocrinol Metab* 2017; 102: 3470-3479.
- 20) Choi SY, Yun J, Lee OJ, Han HS, Yeo MK, Lee MA, Suh KS. MicroRNA expression profiles in placenta with severe preeclampsia using a PNA-based microarray. *Placenta* 2013; 34: 799-804.
- 21) Mayor-Lynn K, Toloubeydokhti T, Cruz AC, Chegini N. Expression profile of microRNAs and mRNAs in human placentas from pregnancies complicated by preeclampsia and preterm labor. *Reprod Sci* 2011; 18: 46-56.
- 22) Jia Y, Li T, Huang X, Xu X, Zhou X, Jia L, Zhu J, Xie D, Wang K, Zhou Q, Jin L, Zhang J, Duan T. Dysregulated DNA methyltransferase 3A upregulates IGFBP5 to suppress trophoblast cell migration and invasion in preeclampsia. *Hypertension* 2017; 69: 356-366.
- 23) Chen L, Shi K, Andersen TL, Qiu W, Kassem M. KIAA1199 is a secreted molecule that enhances osteoblastic stem cell migration and recruitment. *Cell Death Dis* 2019; 10: 126.
- 24) Ge T, Wu HC, Zhou YY, Shen SM, Zhu LG, You GX. MiR-296-3p may affect the proliferation and migration of non-small cell lung cancer cells via regulating RABL3. *Eur Rev Med Pharmacol Sci* 2019; 23: 5823-5830.
- 25) Li L, Pan Y, Mo X, Wei T, Song J, Luo M, Huang G, Teng C, Liang K, Mao N, Yang J. A novel metastatic promoter CEMIP and its downstream molecular targets and signaling pathway of cellular migration and invasion in SCLC cells based on proteome analysis. *J Cancer Res Clin Oncol* 2020; 146: 2519-2534.
- 26) Zhao L, Zhang D, Shen Q, Jin M, Lin Z, Ma H, Huang S, Zhou P, Wu G, Zhang T. KIAA1199 promotes metastasis of colorectal cancer cells via microtubule destabilization regulated by a PP2A/stathmin pathway. *Oncogene* 2019; 38: 935-949.
- 27) Cui S, Zhang L. Circ_001653 silencing promotes the proliferation and ECM synthesis of NPCs in IDD by downregulating miR-486-3p-mediated CEMIP. *Mol Ther Nucleic Acids* 2020; 20: 385-399.
- 28) Xu XH, Jia Y, Zhou X, Xie D, Huang X, Jia L, Zhou Q, Zheng Q, Zhou X, Wang K, Jin LP. Downregulation of lysyl oxidase and lysyl oxidase-like protein 2 suppressed the migration and invasion of trophoblasts by activating the TGF- β /collagen pathway in preeclampsia. *Exp Mol Med* 2019; 51: 1-12.
- 29) Sun WM, Tao W, Li JC, Zhu DM, Miao Y. MicroRNA-296 functions as a tumor suppressor in breast cancer by targeting FGFR1 and regulating the Wnt/ β -catenin signaling pathway. *Eur Rev Med Pharmacol Sci* 2019; 23: 10422-10432.
- 30) Deng X, Liu Z, Liu X, Fu Q, Deng T, Lu J, Liu Y, Liang Z, Jiang Q, Cheng C, Fang W. MiR-296-3p negatively regulated by nicotine stimulates cytoplasmic translocation of c-Myc via MK2 to suppress chemotherapy resistance. *Mol Ther* 2018; 26: 1066-1081.
- 31) Duong HQ, Nemazany I, Rambow F, Tang SC, Delaunay S, Tharun L, Florin A, Büttner R, Vandaele D, Close P, Marine JC, Shostak K, Chariot A. The endosomal protein CEMIP links WNT signaling to MEK1-ERK1/2 activation in Selumetinib-resistant intestinal organoids. *Cancer Res* 2018; 78: 4533-4548.
- 32) Shen F, Zong ZH, Liu Y, Chen S, Sheng XJ, Zhao Y. CEMIP promotes ovarian cancer development and progression via the PI3K/AKT signaling pathway. *Biomed Pharmacother* 2019; 114: 108787.