European Review for Medical and Pharmacological Sciences 2019; 23: 7209-7216

MiR-200c regulates apoptosis of placental trophoblasts in preeclampsia rats through Wnt/β-catenin signaling pathway

X. ZHANG[1], Y.-W. GE[2], Z.-X. WANG[3], Q.-L. XU[4], R. GUO[5], H.-Y. XU[1]

[1]Department of Obstetrics, Jining No. 1 People's Hospital, Jining, China
[2]Department of Obstetrics, Rizhao Hospital of TCM, Rizhao, China
[3]Maternal and Child Center, the People's Hospital of Zhangqiu Area, Jinan, China
[4]Health Care Ward, the People's Hospital of Zhangqiu Area, Jinan, China
[5]Department of Outpatient, the People's Hospital of Zhangqiu Area, Jinan, China

Abstract. – OBJECTIVE: To investigate the influence of micro ribonucleic acid 200c (miR-200c) on the apoptosis of placental trophoblasts in a rat model of preeclampsia (PE).

MATERIALS AND METHODS: PE model in rats was established for extracting placental trophoblasts. Overexpression or knockdown of miR-200c was achieved by transfection of miR-200c mimics or inhibitor. Flow cytometry was carried out to detect the apoptotic rate of placental trophoblasts. Dual-luciferase reporter gene assay was performed to detect the interaction of miR-200c with WNT1. Western blotting was applied to determine the changes of protein levels in placental trophoblasts.

RESULTS: The expression level of miR-200c in placental trophoblasts of PE group was significantly higher than that in control group. The apoptosis rate was (22.45 ± 2.62)%, (6.58 ± 1.28)%, and (9.57 ± 1.35)% in miR-200c mimic group, miR-200c inhibitor group, and control group, respectively, showing statistically significant differences. MiR-200c overexpression downregulated the expression level of anti-apoptotic protein B-cell lymphoma 2 (Bcl-2), but upregulated expression levels of apoptotic proteins Bcl-2-associated X protein (Bax) and active Caspase-3. MiR-200c suppressed WNT1 expression through the interaction with the 3'-untranslated region (3'-UTR) of WNT1. The expressions of WNT1 and β-catenin were up-regulated after miR-200c overexpression, which was reversed by the Wnt/β-catenin pathway activator.

CONCLUSIONS: MiR-200c is involved in the development and progression of PE through the Wnt/β-catenin signaling pathway.

Key Words:
MiR-200c, Wnt/β-catenin, Preeclampsia, Rat placental trophoblasts, Apoptosis.

Introduction

The normal function of fetal trophoblasts is the most important part for a successful pregnancy. Preeclampsia (PE) is a pregnancy-specific disease that is usually associated with poor reconstruction of spiral arteries and hypoxia of the placenta due to excessive apoptosis of trophoblasts and infiltration of supertrophoblasts[1,2]. PE, characterized by new hypertension and proteinuria, is the main cause of maternal and perinatal morbidity and mortality[3]. Phipps et al[4] has found that many mechanisms are involved in the development and progression of PE. However, the precise molecular pathogenesis of PE is unknown. Recent studies[5-8] have reported that micro ribonucleic acids (miRNAs) participate in the regulation of proliferation, apoptosis, migration, and invasion of trophoblasts. Therefore, clarification of the miRNA roles in regulating the behaviors of trophoblasts may help to elucidate the development of novel biomarkers for PE diagnosis and therapeutic goals. Genetic and epigenetic changes involved in the behaviors of trophoblasts are conducive to the development of novel therapeutic targets for the treatment of PE.

MiRNAs are conserved, non-coding RNAs that regulate gene expressions through post-transcriptional repression. Mature miRNAs consisting of 21-25 nucleotides can bind to the 3'-untranslated region (3'-UTR) of the target messenger RNA (mRNA) in the manner of base pairing, resulting in instability, degradation, and translational inhibition of mRNA. Moreover, miRNAs are able to regulate various cellular processes involving in the normal development and disease onset by

Corresponding Author: Haiyan Xu, MM; e-mail: eahl13@163.com

targeting and modulating target genes. Increasing evidence has manifested that many miRNAs are expressed in human placental trophoblasts.

Wnt signaling pathway is an important pathway in regulating the proliferation, migration, and apoptosis of cells. More than 30 years ago, Nusse et al[9] identified that Wnt regulates tumorigenesis in mice. Several studies have shown that the Wnt signaling pathway can lead to various human diseases, including birth defects and cancers. A previous research[10] confirmed that the expression level of Wnt2 declines in the placenta of PE patients. However, we still need to investigate the abnormal activation of the Wnt/β-catenin signaling pathway in PE.

It is reported that miR-200 is an important regulator for various diseases. However, whether miR-200c is involved in the pathogenesis of PE and the regulation of trophoblast behaviors remains unknown. Hence, we aim to investigate the potential role of miR-200c in PE. This work indicated that miR-200c expression was significantly elevated in placental tissues of PE rats, and *in vitro* experiments revealed that miR-200c could regulate the apoptosis of trophoblasts. This investigation suggested that the miR-200c/Wnt/β-catenin axis may play an important role in the pathogenesis of PE by regulating trophoblast apoptosis.

Materials and Methods

Establishment of PE Model in Rats

One female rat and one male rat were randomly selected and placed in the same cage. At 5-6 p.m., female and male rats were raised in single tailor-made cages, respectively. On the next day, the vaginal plug was inspected, and sperm in the vaginal secretions of female rats was observed using a microscope. If both results were positive, the day was recorded as the first day of pregnancy. From the 13th day of pregnancy, rats in PE group were subcutaneously injected with 125 mg/(kg·d) chronic nitric oxide synthase inhibitor [L-nitroso-arginine methyl ester (L-NAME)] to establish PE model in rats[14]. After that, blood pressure was measured *via* a non-invasive rat tail artery blood pressure measurement system. An automatic protein analyzer was employed to detect 24 h-urine protein. Blood pressure increased by 20 mm Hg (over 115 mm Hg) after L-NAME injection, and urine protein ≥ (+) indicated the successful establishment of the PE model in rats. This study was approved by the Animal Ethics Committee of Jining No. 1 People's Hospital Animal Center.

Cell Transfection

MiR-200c/negative control (NC) mimic and miR-200c/NC inhibitor were synthesized by GenePharma (Shanghai, China). Cell transfection was carried out using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Differently-treated cells were collected for extracting total RNA. Next, 1 μL RNA solution was taken for detecting its concentration and purity on a microplate reader. The ratio of optical density at 260 and 280 nm (OD_{260}/OD_{280}) of RNA sample between 1.6-1.8 was considered to be qualified. The remaining RNA solution was sub-packaged and stored at -80°C for later use.

Complementary deoxyribonucleic acid (cDNA) was synthesized using a PrimeScriptTM Kit (TaKaRa Bio Inc., Otsu, Shiga, Japan) in accordance with the instructions for specific operating procedures. Firstly, RNA with a total mass of 2 μg was added. The volume of RNA added was calculated according to the respective concentrations. 1 μL Oligo (dT) primer (50 μM), 1 μL dNTP Mixture (10 mM), and sufficient nuclease-free double distilled water were prepared for the reaction system, with a total volume of 10 μL. The reaction system was gently mixed, heated using a PCR instrument at 65°C for 5 min and transferred onto the ice for rapid cooling. Then, 4 μL 5× PrimeScript Buffer, 1 μL PrimeScript RTase, 0.5 μL RNase inhibitor, and 4.5 μL nuclease-free water were added to the above reaction system, in a total of 20 μL. They were mixed evenly and heated at 42°C for 45 min and then at 95°C for 5 min using the PCR instrument. Lastly, the mixture was transferred onto the ice for rapid cooling, so that single-stranded complementary deoxyribose nucleic acid (cDNA) was synthesized and then stored at -20°C for later PCR amplification reaction. Primer sequences used in this study were as follows: WNT1, F: 5'-CCAGGAACAACTCCTTACTC-3', R: 5'-GCTAGCCTGTGTCCGAAGGA-3'; miR-200c, F: 5'-CCTAT-

GTAAACAGCCTCGACTG-3', R: 5'-CTGG-CGTATCGTGAGTCG-3'; U6: F: 5'-GCTTC-GGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; GAP-DH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Detection of Apoptosis Via Flow Cytometry

Culture medium was collected into a centrifuge tube, and cells were washed twice with phosphate-buffered saline (PBS) and collected. Next, cells were appropriately digested with trypsin, which was terminated by the collected culture medium. After that, cells were pipetted, collected into centrifuge tubes, and centrifuged at 1,000 rpm for 5 min. Thereafter, the supernatant was discarded, and cells were re-suspended by adding 1 mL pre-cooled PBS solution and centrifuged at 1,000 rpm for 5 min. Cells were then transferred to a 1.5 mL Eppendorf (EP) tube and washed once with PBS solution. Then, apoptosis of cells in each group was detected using an Annexin V-fluorescein isothiocyanate (FITC) Apoptosis Kit (BD, San Jose, CA, USA). Each sample was added with 0.5 mL freshly-prepared staining buffer solution, 5 μL Annexin V-FITC and 5 μL propidium iodide (PI), mixed gently and incubated in the dark at room temperature for 15 min. Next, a flow cytometer was used to measure the spectrophotometric value of each group of cells at the excitation wavelength within 1 h. The assay was repeated for 3 times.

Western Blotting

A bicinchoninic acid (BCA) protein concentration assay kit (Beyotime, Shanghai, China) was utilized to determine protein concentration. 40 μg total protein was separated by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). After blockage in 5% skim milk, membranes were incubated with primary antibody (1:2000; Abcam, Cambridge, UK) at 4°C and the corresponding horseradish peroxidase-labeled secondary antibody (1:1000; Beyotime, Shanghai, China) at room temperature for 1 h. Band exposure was developed using a chemiluminescence imaging analysis system. Lastly, ImageJ and other image analysis software were applied for gray value calculation of bands on developed images and statistical analysis.

Dual-Luciferase Reporter Gene Assay

Cells were digested, inoculated (appropriate cells were selected according to specific assays) in a 35 mm cell culture dish, and cultured in a saturated incubator at 37°C and 5% CO_2 overnight. When the cell density reached 70%, cells were co-transfected with Luciferase reporter plasmid, LacZ expression plasmid, and other plasmids for 24-36 h. Cells were washed with pre-cooled PBS (containing no calcium and magnesium ions) and lysed in 350 μL pre-cooled harvest buffer at 4°C or on ice for 10 min. During cell lysis, sufficient 1.5 mL microcentrifuge tubes were prepared, and adenosine triphosphate (ATP) buffer and luciferin buffer were mixed at a ratio of 1:3.6 to prepare into a reaction solution that was dispensed (100 μL/tube) (Cytoskeleton, Denver, CO, USA). Thereafter, an equal volume of cell lysis solution (100 μL) was sequentially applied in centrifuge tubes and mixed rapidly. OD value was read using a Luminometer. Lastly, figures were plotted based on corrected readings, and data were analyzed.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. The *t*-test was used for the differences between the two groups, and one-way analysis of variance was applied for differences among groups followed by Post Hoc Test (Least Significant Difference). For all tests, the two-sided 95% confidence interval (CI) was used, and $p<0.05$ suggested that the difference was statistically significant.

Results

Expression of MiR-200c in Models of PE and Normal Pregnancy

The qRT-PCR was performed to measure the expression level of miR-200c in placental trophoblasts extracted from PE rats. The results showed that the expression level of miR-200c in PE group was overtly higher than that in control group ($p<0.01$) (Figure 1).

Results of Cell Transfection

The relative expression level of miR-200c in placental trophoblasts transfected with miR-200c/NC mimic or miR-200c/NC inhibitor was measured *via* qRT-PCR. Cells transfected for 48 h were collected for the measurement. It was found that the expression level after transfection

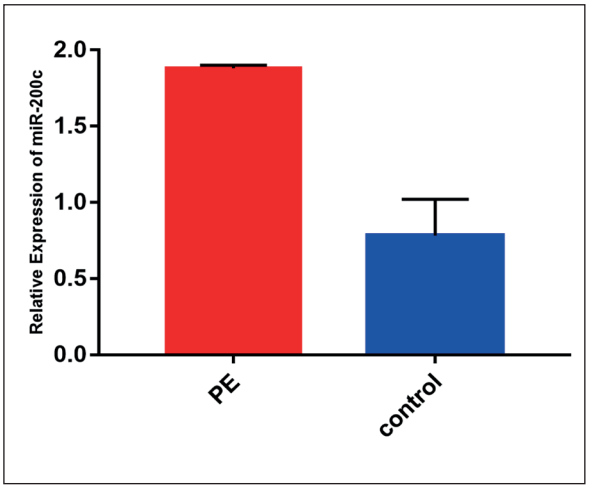

Figure 1. Expression of miR-200c in models of PE and normal pregnancy: The expression level in PE group is evidently higher than that in control group ($p<0.01$).

with miR-200c mimic increased, while it decreased after transfection with miR-200c inhibitor ($p<0.01$) (Figure 2).

Apoptosis Detected

Rat placental trophoblasts were transfected with miR-200c mimic or miR-200c inhibitor, respectively, and then, the apoptotic rate of cells was detected using the flow cytometer. The results revealed that the apoptotic rate of cells was (22.45 ± 2.62)%, (6.58 ± 1.28)%, and (9.57 ± 1.35)% in miR-200c mimic group, miR-200c inhibitor group, and control group, respectively, and the differences were statistically significant ($p<0.05$) (Figure 3A-3C).

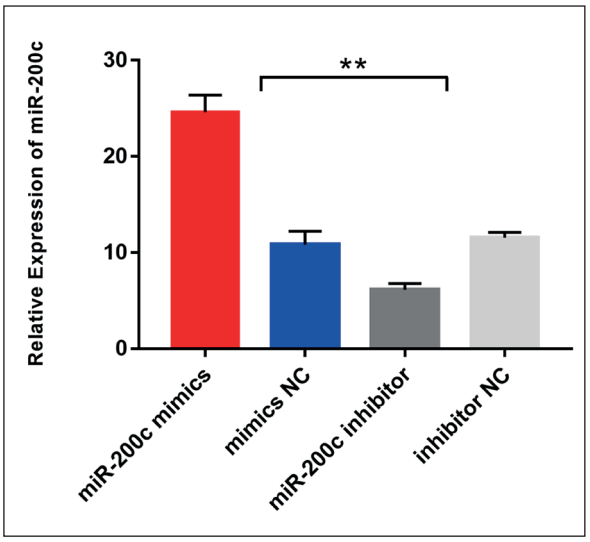

Figure 2. Expression level of miR-200c after different treatments through qRT-PCR: The expression level after transfection with miR-200c mimic is higher than that after transfection with miR-200c inhibitor ($p<0.01$).

The Western blotting assay was conducted to detect apoptosis-related proteins in each group of cells. It was discovered that miR-200c treatment up-regulated expression levels of the apoptotic proteins Bax and active caspase-3, but down-regulated the expression level of the anti-apoptotic protein Bcl-2 (Figure 3D).

Results of Dual Luciferase Reporter Gene Assay

In this study, bioinformatics analysis was employed to examine the predicted targets of miR-200c, and the results showed that the 3'UTR of WNT1 was highly-conserved to bind to miR-200c (Figure 4A). Based on results of dual-luciferase reporter gene assay, it was found that transfection of miR-200c significantly inhibited the relative luciferase activity in cells, suggesting that miR-200c suppressed WNT1 expression by interacting with the 3'-UTR of WNT1 (Figure 4B).

MiR-200c Over-Expression Inhibited the Wnt/β-Catenin Signal Transduction Pathway

Over-expression of miR-200c markedly lowered mRNA and protein expressions of WNT1 in cells (Figure 5). After 48 h of transfection with miR-200c mimic, expressions of WNT1 and β-catenin were statistically different among different groups. Compared with those in control group, expressions of WNT1 and β-catenin increased after over-expression of miR-200c, which were reversed by the Wnt/β-catenin pathway activator (Figure 6).

Discussion

PE is the primary cause of maternal and perinatal morbidity and mortality in developing countries. To reduce the risk of PE, determining its pathogenesis is the most important task. Its exact molecular mechanisms are unclear, but endothelial cell dysfunction, maternal-fetal immune balance disturbance, inflammation, and abnormal vascular recasting are believed to be associated with PE[11,12]. There is a large number of placental-inducing factors are related to PE, including shallow placenta accreta, trophoblast proliferation, and apoptosis imbalance[13]. A better understanding of the nature of the placenta can help to

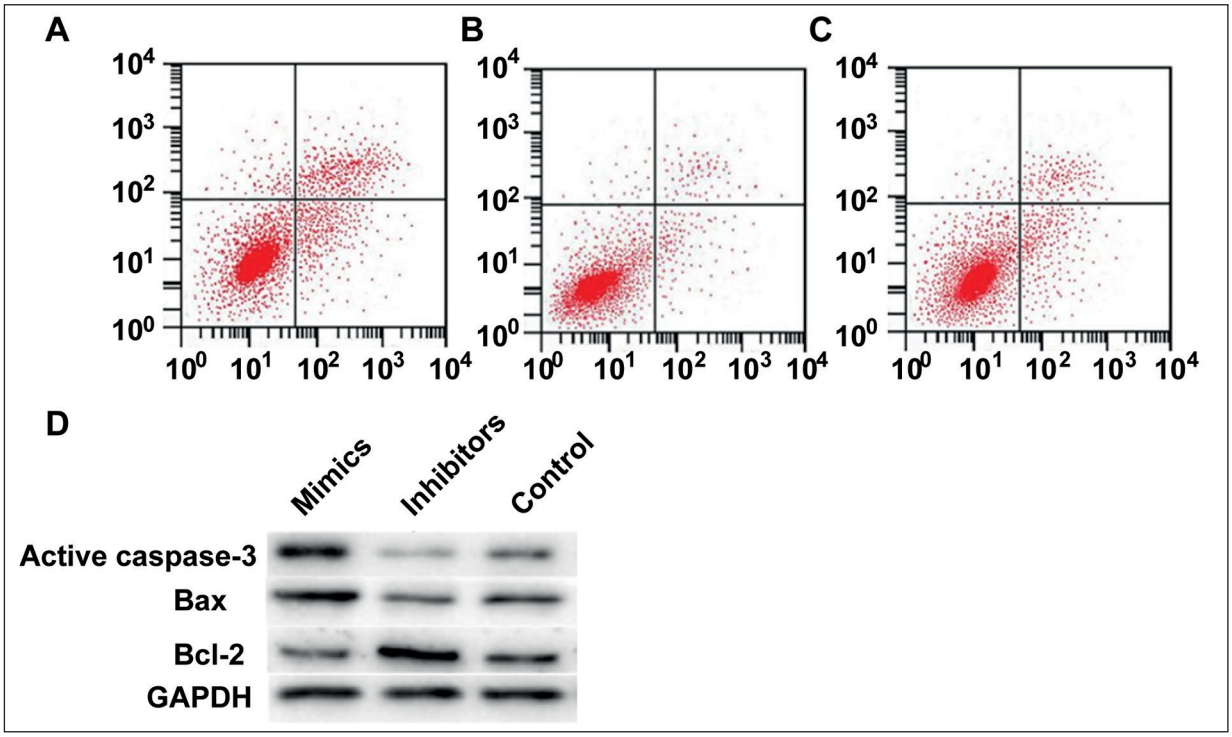

Figure 3. Apoptosis rate detected *via* flow cytometry. ***A,*** Apoptosis rate of cells in miR-200c mimic group [(22.45 ± 2.62) %]. ***B,*** Apoptosis rate of cells in miR-200c inhibitor group [(6.58 ± 1.28) %]. ***C,*** Apoptosis rate of cells in control group [(9.57 ± 1.35) %]. ***D,*** Expressions of apoptosis-related proteins detected through Western blotting. Transfection with miR-200c mimic leads to increased expressions of Bax and active caspase-3 and reduced expression of Bcl-2.

determine the factors resulting in PE. Currently, it is believed that the following factors are crucial for the development of PE, including disorder of trophoblastic differentiation, hypoxia-ischemia of the placenta, and degree of trophoblast-induced uterine artery transformation.

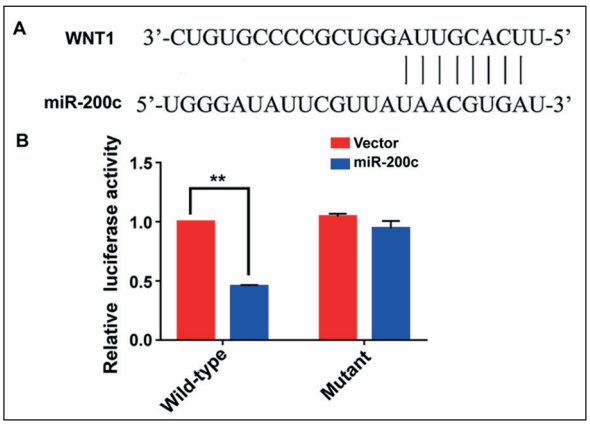

Figure 4. ***A,*** Direction targeting of miR-200c to the 3'-UTR of WNT1: the predicted binding site of miR-200c and 3'-UTR in WNT1. ***B,*** Interaction of miR-200c with WNT1 determined *via* luciferase reporter gene assay.

Trophoblasts are highly-specialized cells, of which the growth rate is faster than that of normal cells. Moreover, they can migrate and invade into the maternal myometrium, which is similar to the invading process of tumor cells into surrounding tissues. However, the migration of trophoblasts is strictly controlled by the body in both time and space, which is fundamentally different from the migration of tumor cells. There are two types of trophoblasts: cytotrophoblasts (CTBs) and STs. With further in-depth research, it is possible to artificially synthesize CTB membrane fragments to inhibit lymphocyte proliferation, leading to apoptosis of T lymphocytes, impairment of the formation of endothelial monolayer cells, thus participating in the pathogenesis of PE. CTBs are generally believed to be conducive to PE. In a normal pregnancy, the Sertoli cell matrix of the spiral artery is converted into large-volume and low-resistance blood vessel during the formation of the placenta, which ensures sufficient nutrition and oxygen requirements to the fetus. Abnormal differentiation of Sertoli cells interferes with its function, damages the migration ability of trophoblasts, and causes disturbance of

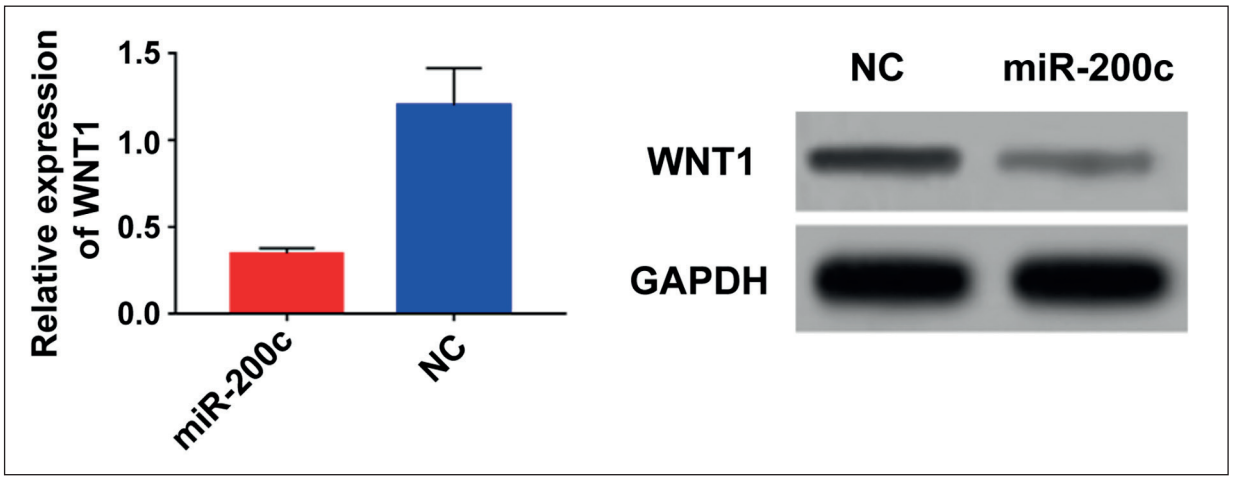

Figure 5. WNT1 expression down-regulated by miR-200c: both the mRNA and protein expression levels of WNT1 are reduced after over-expression of miR-200c.

myometrial invasion. These pathological lesions result in shallow placenta accreta and placental ischemia-hypoxia, eventually inducing PE.

Some studies have reported that many signaling pathways are involved in the regulation of the differentiation, apoptosis, and invasion of trophoblasts. Advances in understanding normal trophoblasts reveal some unique biological properties that are more similar to malignant tumors.

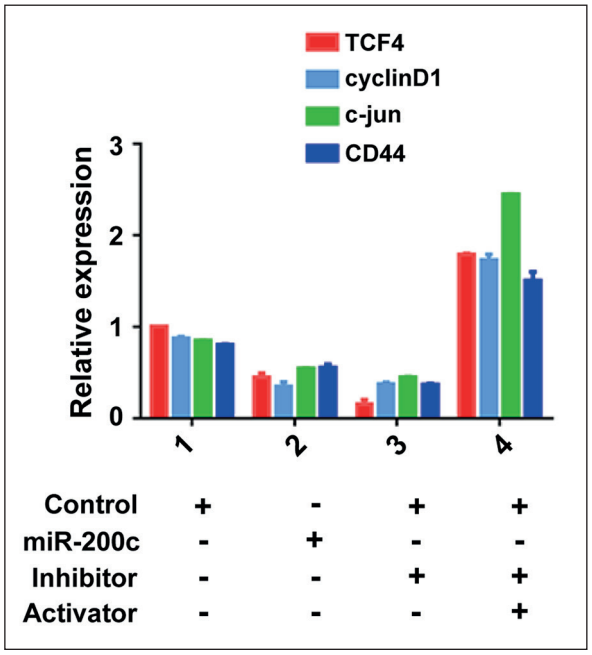

Figure 6. Cell rescue assay: after transfection with miR-200c mimic for 48 h, the expression levels of related downstream molecules of the WNT pathway decline, but such a tendency is reversed after addition of pathway activator ($p<0.05$).

The activation of the Wnt/β-catenin signaling pathway promotes the apoptosis of tumor cells.

It has been found that Wnt signaling components participate in the pathogenesis of various diseases, including pregnancy diseases. Abnormal Wnt signals may give rise to abnormal invasion and differentiation of CHM. It is discovered that APC and sFRP2 genes are hypermethylated in choriocarcinoma cells, implying that the inactivation of Wnt signaling may play a major role in the pathogenesis of trophoblastic cancer cells[14]. It is well known that the rapid production of several trophoblastic subtypes contributes to the development of mouse and human placenta[15]. The maternal uterus is then reconstituted, including stromal cell differentiation, angiogenesis, and immune changes. These key processes begin in the secretory phase of the menstrual cycle and the early stage of implantation and placental development[16]. Furthermore, Wnt signal plays a key role in organ development and tissue homeostasis. Therefore, this pathway may also play a vital role in the development and differentiation of trophoblasts.

Recently, it is found that miRNAs regulate the components of the Wnt signaling pathway. In this study, it was discovered for the first time that the expression level of miR-200c in placental trophoblasts in PE rats was higher than that in normal pregnant rats. Further cytological experiments showed that the apoptotic rate of placental trophoblasts overexpressing miR-200c increased, indicating that miR-200c may participate in the development of PE by promoting the apoptosis of placental trophoblasts. To explore the mechanism

by which miR-200c participates in the apoptosis of placental trophoblasts, we found a potential binding site of miR-200c to WNT1 through bioinformatics. Sonderegger et al[17] manifested that 14 Wnt ligands and 8 Fzd receptors are expressed in human placenta, further displaying that the Wnt-signaling pathway plays a role in the development of the placenta. According to many reports, Wnt ligands and other Wnt signaling components have been detected in the endometrium, suggesting that the Wnt pathway may be related to multiple biological functions of uterine cell types. Similarly, previously Chan et al[18] on models using different trophoblasts showed that the Wnt pathway may be closely correlated with the implantation and differentiation of trophoblasts, which is in line with our results.

Wnt signal plays a vital role in the early development of trophoblasts. Treatment of embryonic stem cells with Wnt3a induces the formation of trophectoderm stem cells that can differentiate into spongy trophoblasts[19]. Moreover, some investigations have proved that the Wnt signal plays a role in the development of extraembryonic tissues, especially placental vascularization[20]. Furthermore, the Wnt signal plays a part in trophoblast differentiation. In addition, the Wnt signal is conducive to the invasion of the trophoblast. The Wnt/β-catenin signaling pathway may result in diseases such as trophoblastic choriocarcinoma.

Conclusions

We reported for the first time that WNT1 is targeted and regulated by miR-200c, and the regulatory mechanism of miR-200c expression in the development of PE is revealed. These conclusions explain the molecular pathogenesis of PE. MiR-200c targeting to the Wnt/β-catenin signaling pathway may be potentially applied in the treatment of PE.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Lyall F, Robson SC, Bulmer JN. Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. Hypertension 2013; 62: 1046-1054.
2) Longtine MS, Chen B, Odibo AO, Zhong Y, Nelson DM. Villous trophoblast apoptosis is elevated and restricted to cytotrophoblasts in pregnancies complicated by preeclampsia, IUGR, or preeclampsia with IUGR. Placenta 2012; 33: 352-359.
3) Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science 2005; 308: 1592-1594.
4) Phipps E, Prasanna D, Brima W, Jim B. Preeclampsia: updates in pathogenesis, definitions, and guidelines. Clin J Am Soc Nephrol 2016; 11: 1102-1113.
5) Gao Y, She R, Wang Q, Li Y, Zhang H. Up-regulation of miR-299 suppressed the invasion and migration of HTR-8/SVneo trophoblast cells partly via targeting HDAC2 in pre-eclampsia. Biomed Pharmacother 2018; 97: 1222-1228.
6) Lu TM, Lu W, Zhao LJ. MicroRNA-137 Affects proliferation and migration of placenta trophoblast cells in preeclampsia by targeting ERRalpha. Reprod Sci 2017; 24: 85-96.
7) Wang ZM, Wan XH, Sang GY, Zhao JD, Zhu QY, Wang DM. MiR-15a-5p suppresses endometrial cancer cell growth via Wnt/β-catenin signaling pathway by inhibiting WNT3A. Eur Rev Med Pharmacol Sci 2017; 21: 4810-4818.
8) Jiang L, Long A, Tan L, Hong M, Wu J, Cai L, Li Q. Elevated microRNA-520g in pre-eclampsia inhibits migration and invasion of trophoblasts. Placenta 2017; 51: 70-75.
9) Nusse R, van Ooyen A, Cox D, Fung YK, Varmus H. Mode of proviral activation of a putative mammary oncogene (int-1) on mouse chromosome 15. Nature 1984; 307: 131-136.
10) Zhang Z, Zhang L, Zhang L, Jia L, Wang P, Gao Y. Association of Wnt2 and sFRP4 expression in the third trimester placenta in women with severe preeclampsia. Reprod Sci 2013; 20: 981-989.
11) Can M, Guven B, Bektas S, Arikan I. Oxidative stress and apoptosis in preeclampsia. Tissue Cell 2014; 46: 477-481.
12) Szarka A, Rigo JJ, Lazar L, Beko G, Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. BMC Immunol 2010; 11: 59.
13) Goldman-Wohl D, Yagel S. Regulation of trophoblast invasion: from normal implantation to pre-eclampsia. Mol Cell Endocrinol 2002; 187: 233-238.
14) Wong NC, Novakovic B, Weinrich B, Dewi C, Andronikos R, Sibson M, Macrae F, Morley R, Pertile MD, Craig JM, Saffery R. Methylation of the adenomatous polyposis coli (APC) gene in human placenta and hypermethylation in choriocarcinoma cells. Cancer Lett 2008; 268: 56-62.
15) Georgiades P, Ferguson-Smith AC, Burton GJ. Comparative developmental anatomy of the murine and human definitive placentae. Placenta 2002; 23: 3-19.

16) Knofler M, Pollheimer J. Human placental trophoblast invasion and differentiation: a particular focus on Wnt signaling. Front Genet 2013; 4: 190.

17) Sonderegger S, Husslein H, Leisser C, Knofler M. Complex expression pattern of Wnt ligands and frizzled receptors in human placenta and its trophoblast subtypes. Placenta 2007; 28 Suppl A: S97-S102.

18) Chen Y, Zhang Y, Deng Q, Shan N, Peng W, Luo X, Zhang H, Baker PN, Tong C, Qi H. Wnt5a inhibited human trophoblast cell line HTR8/SVneo invasion: implications for early placentation and preeclampsia. J Matern Fetal Neonatal Med 2016; 29: 3532-3538.

19) Chawengsaksophak K, de Graaff W, Rossant J, Deschamps J, Beck F. Cdx2 is essential for axial elongation in mouse development. Proc Natl Acad Sci U S A 2004; 101: 7641-7645.

20) Herr F, Horndasch M, Howe D, Baal N, Goyal P, Fischer S, Zygmunt M, Preissner KT. Human placenta-derived Wnt-5a induces the expression of ICAM-1 and VCAM-1 in CD133(+)CD34(+)-hematopoietic progenitor cells. Reprod Biol 2014; 14: 262-275.