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miR-145–5p promotes trophoblast cell growth and invasion by targeting FLT1

Yan Lv[a,b,1], Xun Lu[c,1], Chunyan Li[b], Yuru Fan[b], Xiaohong Ji[b], Wei Long[b], Li Meng[b,***], Lan Wu[b], Luyao Wang[b], Mingming Lv[a,**], Hongjuan Ding[b,*]

[a] Department of Breast, Women's Hospital of Nanjing Medical University, The Affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing, China

[b] Department of Obstetrics, Women's Hospital of Nanjing Medical University, The Affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing, China

[c] Milken School of Public Health, George Washington University, Washington, DC, USA

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ABSTRACT

Objective: We aimed to explore the expression level and biological function of miR-145–5p in preeclampsia (PE).

Methods: The differentially expressed miRNA/mRNA between normal placentas and PE placentas were screened using the GSE84260 and GSE73374 datasets from the Gene Expression Omnibus Database. The expression of miR-145–5p in PE placentas was detected by qRT-PCR. The CCK-8 assay, wound healing and transwell were carried out to determine the cell growth, migration and invasion when miR-145–5p was overexpressed or inhibited. The real-time quantitative PCR (qRT-PCR), Western Blot and dual-luciferase reporter assays were conducted to preliminarily explore possible mechanisms.

Results: A total of 33 miRNAs were found significantly differentially expressed in PE patients, 19 were significantly upregulated and 14 were significantly downregulated. The relative miR-145–5p expression was lower in PE placentas than normal placentas. The viability and invasion were suppressed when miR-145–5p was inhibited in trophoblasts cells, while miR-145–5p overexpression promoted the effectiveness. In addition, mRNA and protein expression of FLT1 in HTR-8/SVneo cell was also downregulated with miR-145–5p overexpression, suggesting that FLT1 is the target gene of miR-145–5p. Consistent with miR-145–5p overexpression, the mRNA and protein expression of FLT1 also were upregulated with miR-145–5p interference. Furthermore, the expression of miR-145–5p was regulated by the Hypoxic conditions.

Conclusions: In conclusion, the results showed miR-145–5p may participate in PE development by affecting the proliferation and invasion of trophoblast cells. This is a new perspective to understand the etiology and pathogenesis of PE, which may provide a new breakthrough for the early prediction and diagnosis of PE.

1. Introduction

Pre-eclampsia (PE) refers to abnormal elevated blood pressure during pregnancy, with proteinuria or maternal organ dysfunction or fetal growth restriction (Mol et al., 2016). PE is a unique disease during pregnancy, which can cause multiple organ damage, cerebral edema, placental abruption, stillbirth and other adverse pregnancy outcomes [1,2]. PE is one of the highest incidences of diseases leading to the morbidity and mortality in pregnant women and fetuses [2]. The pathogenesis and theory of PE are numerous. Studies have shown that the pathogenesis is related to placental function, immune factors, angiogenesis, genetic factors and nutritional condition [3]. The placental factors are consistently considered to be the most important factor in the pathogenesis of PE [4]. In the PE, abnormalities of trophoblast

* Corresponding author. Department of Obstetrics, Women's Hospital of Nanjing Medical University, The Affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, No.123, Tianfeixiang, Mochou Rd., Nanjing, 210004, China.

** Corresponding author. Department of Breast, Women's Hospital of Nanjing Medical University, The Affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, No.123, Tianfeixiang, Mochou Rd., Nanjing, 210004, China.

*** Corresponding author. Department of Obstetrics, Women's Hospital of Nanjing Medical University, The Affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, No.123, Tianfeixiang, Mochou Rd., Nanjing, 210004, China.

E-mail addresses: menglidoctor@hotmail.com (L. Meng), jinanmingming@126.com (M. Lv), dinghongjuan_226@163.com (H. Ding).

[1] Yan Lv and Xun Lu contributed equally to this paper.

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function and poor recasting of the spiral arteries cause placental ischemia and hypoxia, abnormal oxidative stress, etc., and eventually lead to the appearance of clinical symptoms of preeclampsia [5].

MicroRNAs (miRNA) are a class of single-stranded small RNAs that are ~22 nucleotides in length and do not translate proteins [6]. The production of miRNAs requires transcription, nuclear maturation, export, and cytoplasmic processing. Large primary transcripts are cleaved by the microprocessor into pre-miRNAs [7,8] in the nucleus and exported into the cytoplasm, which is mediated by the karyopherin Exportin-5 and RAN-GTP [9]. The RNase III endonucleases Dicer, double-stranded RNA binding protein, and Argonaute proteins are involved in the formation of mature miRNAs in the cytoplasm [6]. The regulation of miRNA activity and stability is a complex process by alternative processing, sequence editing and post-translational modification of Argonaute proteins [10]. The miRNA binds to the 3′-untranslated region (3′-UTR) of the mRNA through the 5′-end "seed sequence" to achieve post-transcriptional regulation [11], including mRNA degradation and inhibition of mRNA translation [12]. Abnormal expression of miRNAs are observed in a variety of cancers, and are involved in the regulation of cellular functions, immune responses, angiogenesis, and the development of various organ systems [13].

In recent years, a number of studies have found differential expression of miRNAs in the placenta and circulation of PE patients, such as miRNA-210, miRNA-155, miR-195 [14–16]. These differentially expressed miRNAs play important roles in regulating trophoblast function, oxidative stress, expression of angiogenesis-related factors, and mesenchymal stem cell function [14–16]. Through bioinformatics analysis, we screened a variety of differentially expressed miRNAs in PE and we focused on one of the miRNAs and conducted a preliminary study of its function and regulatory mechanisms in PE.

2. Materials and methods

2.1. Patients' information

After approved by the Ethics Committee of the Affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University (Nanjing Maternity and Child Health Care Hospital) and the patients, 18 patients with PE who were delivered by cesarean section at the Affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University (Nanjing Maternity and Child Health Care Hospital) and 18 normal singleton pregnant women by cesarean from 2016 to 2018 were selected as research objects. 1.0 g tissue was collected in the calcified area of the maternal surface of the placenta and immediately frozen in liquid nitrogen. None of these patients had a history of chronic hypertension, poor lifestyle habits, history of heart, liver and kidney disease. PE is defined as a blood pressure of more than 140/90 mmHg or mild hypertension with more than 3 g protein/24 h urine.

2.2. RNA extraction and polymerase chain reaction

Total RNA, including miRNA, was extracted by conventional methods by adding 1 ml of TRIzol (Invitrogen Technologies Co, USA) reagent to the samples. The quantity and purity of the extracted RNA were detected using NanoDrop technology (Agilent, Santa Clara, CA, USA). The OD 260/280 absorbance ratios were between 1.8 and 2.0 for all the samples. RNA integrity was determined using 1% agarose gel electrophoresis. Reverse transcription of cDNA was performed with the Thermo Fisher K1662 Reverse Transcription Kit. The method to quantify miR-145–5p was performed by stem-loop RT-PCR (primers purchased from Guangzhou Ruibo Biotechnology Co., Ltd.). 1 μg RNA and primers were put at 65 °C for 5 min to form highly target-specific stem-loop structure, then reverse transcriptase, RNase inhibitor, dNTPs and 5x buffers (from Thermo Fisher K1662 Reverse Transcription Kit) were added for reverse transcription. All steps are in accordance with the manufacturer's instructions. The real-time quantitative PCR (qRT-PCR) reaction with SYBR Green (Life Tech) was performed according to the standard procedure, and the 384-well plate standard quantitation cycle procedure was used on the ViiA7 real-time PCR System-Life Tech to determine the cycle threshold (CT). After the completion of qRT-PCR amplification, the relative fold change was calculated based on the 2-ΔΔCt method. GAPDH was used as an endogenous control for mRNA expression and U6 was used as an endogenous control for miRNA expression. The sequence of primers used for qRT-PCR was showed in Table 2. The primers of miR-145–5p/U6 used for qRT-PCR were purchased from Guangzhou Ruibo Biotechnology Co., Ltd.

Table 1
Clinical characteristics of study patients. Data are presented as the means ± SD. *P < 0.05,**P < 0.01, ***P < 0.001.

	Normal (n = 18)	PE (n = 18)
Maternal age (years)	31.06 ± 4.02	32.94 ± 4.64
Maternal BMI (Kg/m^2)	23.65 ± 3.26	23.74 ± 4.53
Gestational weeks	39.25 ± 1.27	38.45 ± 1.16
SBP (mmHg)	117.63 ± 8.34	164.77 ± 16.28***
DBP (mmHg)	73.76 ± 8.27	104.71 ± 12.36***
24 h urine protein (g)	< 0.30 g	> 0.30 g*
Birth weight (g)	3124.25 ± 216.87	2456.17 ± 1132.05*

2.3. Cell culture and transfection

The HTR-8/SVneo trophoblast cell line derived from placental trophoblast (purchased from ATCC) was selected as the research object. These cells were cultured in RPMI 1640 complete medium containing 10% fetal calf serum at 37 °C temperature and atmosphere of 5% CO_2. MiRNA-145–5p mimic and inhibitor (purchased from Guangzhou Ruibo Biotechnology Co., Ltd.) were used to achieve overexpression and silencing of miRNA in trophoblast cells, and negative controls were established respectively. The sequences of miR-145–5p mimic provided by the company are 5′-GUCCAGUUUUCCCAGGAAUCCCU-3′ and 3′-AGGGAUUCCUGGGAAAACUGGAC-5'. The sequences of miR-145–5p inhibitor are AGGGAUUCCUGGGAAAACUGGAC by 2′OMe decoration. The sequences of mimic NC are 5′-UUUGUACUACACAAAAGUACUG-3′ and 3′-AAACAUGAUGUGUUUUCAUGAC-5'. The sequences of inhibitor NC are CAGUACUUUUGUGUAGUACAAA by 2′OMe decoration. Cell transfection was performed with 5 μl of Lipofectamine 2000 according to the manufacturer's protocol. The total cell culture system was 2 ml per well in a six-well plate at a final concentration of 50 μM miRNA mimic and 100 μM miRNA inhibitor. RNA was extracted 48 h after transfection, and then qRT-PCR reaction was performed. In addition, the expression level of miR-145–5p of trophoblast cells was treated with $CoCl_2$ (800 μM) for 16 h was also measured to establish an anoxic mode.

2.4. Cell proliferation assay

The cell proliferation ability was measured by the cell count kit-8 (CCK-8) (Dojindo, Japan). 100 μl of the transfected cell suspension was inoculated in a 96-well plate and the density was 1×10^3 cells per well in a six-well plate. 10 μl of CCK-8 reagent was added into per well at 0, 24, 48, and 72 h and incubated for 2 h. The absorbance at a wavelength of 450 nm was measured using a multi-function microplate reader.

2.5. Cell migration assay

The ability of trophoblasts migration was assessed by the wound healing experiment. When the transfected trophoblast cells were overgrown in the six-well plate, a 0.1 mm scar was drawn in the middle of each well. The necrotic cells were removed and then the six-well plate was cultured in serum-free RPMI1640 medium, which was set as 0 h. The area between the scratches was calculated by Image J software after 48 h, and the migration ability of the cells was determined by the

Table 2
The sequence of primers used for qRT-PCR.

Gene name	Forward primer	Reverse primer
GAPDH	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC
FLT1	TTTGCCTGAAATGGTGAGTAAGG	TGGTTTGCTTGAGCTGTGTTC
ACVR2A	GTTTGCCGTCTTTCTTATCTCCT	GTCACCATAACACGGTTCAACA
SERPINE1	AGTGGACTTTTCAGAGGTGGA	GCCGTTGAAGTAGAGGGCATT
SMAD4	CTCATGTGATCTATGCCCGTC	AGGTGATACAACTCGTTCGTAGT
TGFB2	CCCCGGAGGTGATTTCCATC	GGGCGGCATGTCTATTTTGTAAA

ratio of (scratch area at 0 h-scratch area at 48 h)/scratch area at 0 h.

2.6. Cell invasive assay

Transwell assay was used to assess the invasive ability of trophoblasts. 200 μg/ml matrigel (BD Biosciences, USA) was previously added into a Transwell plate (8 μm PET, Millipore). After transfection, 50×10^3 HTR-8/SVneo cells were seeded in a Transwell chamber and incubated for 48 h. Then the Transwell chamber was fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The invading cells were examined using a digital microscope at 200× magnification and counted by using Image J software. Each experiment was repeated three times.

2.7. Western blot

The transfected cells were lysed using RIPA (Servicebio, China) supplemented with protease inhibitor and centrifuged at 12000 rpm, 4 °C for 10 min. The supernatant was collected to obtain a total protein solution. The protein concentration was measured using a BCA protein concentration assay kit (Servicebio, China) according to the manufacturer's protocol. 5% concentrated gel was prepared for SDS-PAGE protein electrophoresis. The electrophoresis conditions were 75 V voltage for concentrated gel and 120 V voltage for separation gel. PVDF membrane of 0.45 μm (Millipore) was used and the membrane transfer conditions were 300 mA for 1 h. The transferred membrane was blocked with 5% skim milk for 1 h at room temperature and incubated overnight with diluted VEGFR1 (1:1000) and GAPDH (1:2000) primary antibody at 4 °C. After washed three times with TBST for 5 min each time, the transferred membrane was incubated with secondary antibody (1:3000 dilution) for 30 min at room temperature. The same method was used to wash three times before exposure. The Alpha software processing system was used to analyze the density values of the target bands.

2.8. Dual-luciferase reporter assays

The 3′UTR of FLT1 was cloned into the pmirGLO plasmid (W.T.FLT1-3′UTR). For mutated plasmid of FLT1-3′UTR, miR-145–5p target sequence (the "seed" binding sequence) were deleted (M.T.FLT1-3′UTR). For luciferase assays, HTR-8 cells were pre-seeded into 12-well plates 1 day before transfection. Then miR-145–5p mimic or mimic NC were cotransfected with W.T.FLT1-3′UTR or M.T.FLT1-3′UTR using lipo2000 according to the manufacturer's protocol. 48 h post-transfection, cell lysates were prepared using reporter lysis buffer (Vazyme Biotech, Nanjing, China). The Dual-Luciferase Reporter Assay was performed following the kit's instruction (Vazyme Biotech, Nanjing, China) using a Synergy H4 Hybrid Reader (Biotek, Winooski, VT, USA).

2.9. Statistical analysis

The average of three independent experimental data was calculated and expressed as mean ± standard deviation (SD). Differences between the two sets of experimental data were analyzed by Student's *t*-test using SPSS 23.0 software. $P < 0.05$ was used as a significant difference.

3. Result

3.1. Identification of important miRNA based miRNA-mRNA integrated analysis

The RNA expression data of PE patients (GSE84260 and GSE73374) were downloaded from Gene Expression Omnibus (GEO). The differentially expressed miRNA/mRNA between normal placentas and PE placentas were screened. A total of 33 miRNAs were found significantly differentially expressed in PE patients. Among these differentially expressed miRNAs, 19 were significantly upregulated and 14 were significantly downregulated (Fig. 1A). Gene Ontology and pathway analysis were then undertaken, we combined the genes in the GO and Pathway analysis with significant difference ($P < 0.05$) to obtain the most important differentially expressed genes in PE. TargetScan and miRanda were used predict potential target genes of miR-145–5p in those important differentially expressed genes above. Then, in order to decipher the regulation network of these specific miRNAs, we integrated these potential target mRNA with differentially expressed miRNA. The differentially expressed miRNA and their predicted target genes were showed in Fig. 1B, and the largest number of targeted genes associated with preeclampsia was miR-145–5p. The results demonstrated that the expression level of miR-145–5p in PE placental tissues is significantly abnormally expressed and may be involved in the occurrence and development of preeclampsia.

3.2. miR-145–5p was down-regulated in placental tissue from patients with PE

To investigate the role of miR-145–5p in the development of PE, we first studied the expression level of miR-145–5p in placental tissues of 18 normal and 18 PE patients. qRT-PCR results showed that the expression level of miR-145–5p was significantly decreased in PE placenta ($P < 0.05$) (Fig. 2). Table 1 shows the clinical characteristics of the study group (n = 18) and PE (n = 18).

3.3. Over-expressed miR-145–5p could induce the proliferation and invasion of trophoblast HTR-8/SVneo cells

We transfected HTR-8/SVneo cells with miR-145–5p mimic and its negative control to investigate the effect of miR-145–5p on trophoblast cell function. After transfection of miR-145–5p mimic for 48 h, miR-145–5p expression was significantly increased in trophoblast cells by qPCR (Fig. 3A). The results of CCK8 showed that the proliferation of HTR-8/SVneo trophoblasts was significantly enhanced at 24 h, 48 h and 72 h after transfection($P < 0.05$) (Fig. 3B). Moreover, the invasion ability of HTR-8/SVneo trophoblast cells was also significantly increased ($P < 0.05$) after 48 h of transfection by transwell chamber experiments (Fig. 3C and D). However, there was no significant difference in the area of the scratch after overexpression of miR-145–5p ($P > 0.05$) (Fig. 3E and F), indicating that miR-145–5p had no significant effect on the migration ability of trophoblasts.

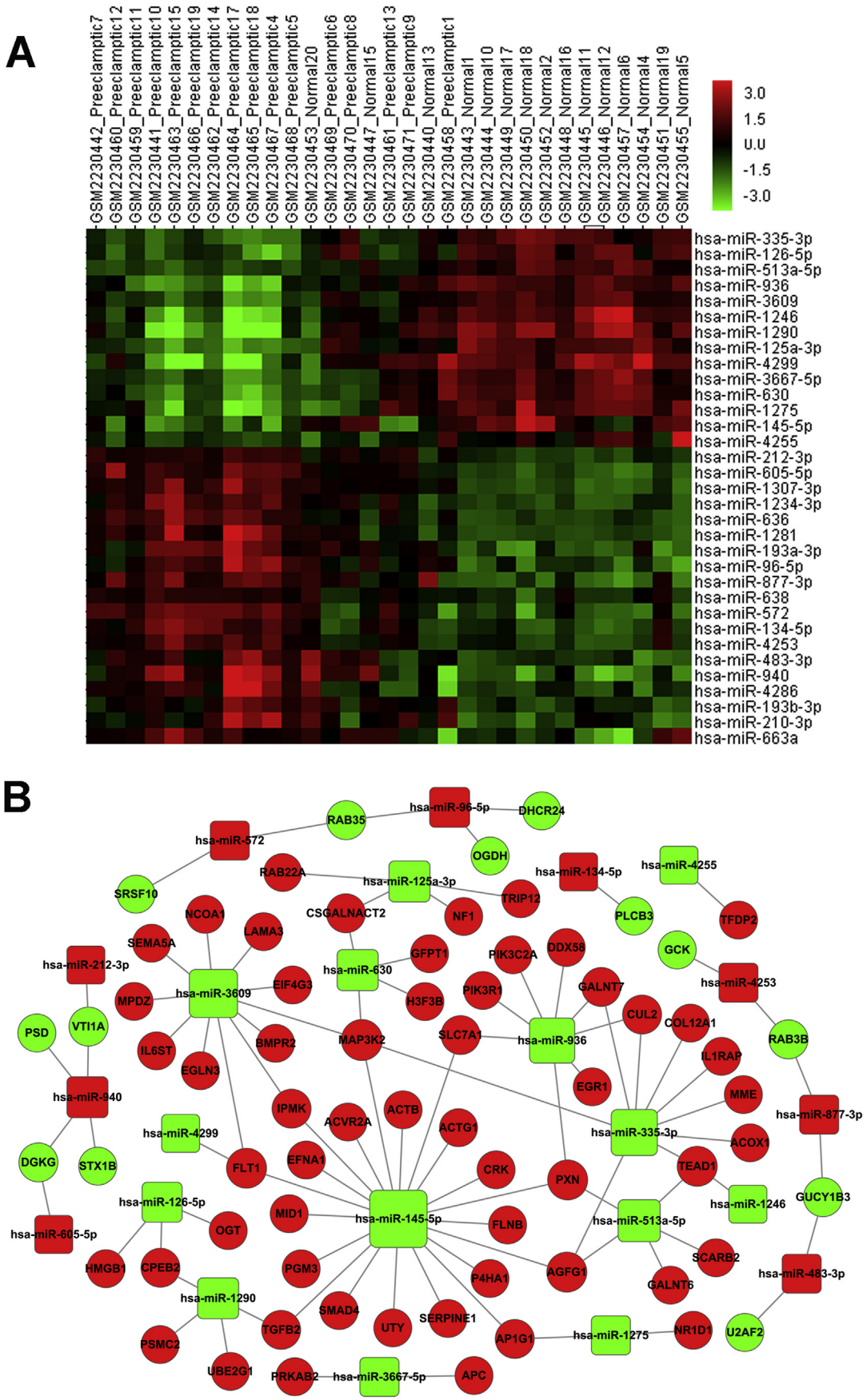

Fig. 1. Identification of important miRNA based miRNA-mRNA integrated analysis. (A) Heatmap of differentially expressed miRNAs between normal placentas and PE placentas. (B) miRNA-mRNA integrated analysis.

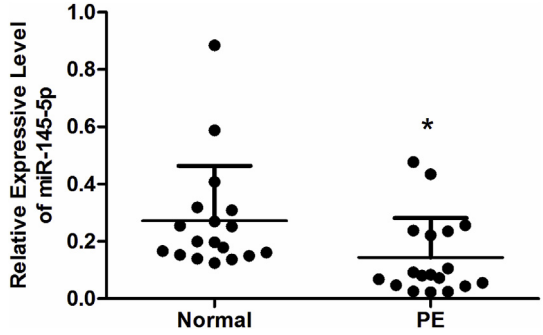

Fig. 2. The expression level of miR-145–5p in placental tissues of normal and PE patients by qPCR (n = 18) (*P < 0.05).

3.4. Downregulation of miR-145–5p could suppress the proliferation and invasive capacity of the trophoblast HTR-8/SVneo cells

After transfecting of miR-145–5p inhibitor, the expression of miR-145–5p in trophoblast cell was down-regulated by 80–90% (P < 0.05) (Fig. 4A). The lower expression of miR-145–5p significantly inhibited the proliferation of trophoblast cell at 24 h, 48 h, and 72 h compared with the control group (Fig. 4B). Moreover, the number of HTR-8/SVneo trophoblast cell passing through the Transwell chamber was significantly reduced after transfection of the miR-145–5p inhibitor (P < 0.05) (Fig. 4C and D). On the other hand, transfection of miR-145–5p inhibitor had no significant effect on scratch healing that demonstrated trophoblast migration (P > 0.05) (Fig. 4E and F).

3.5. Mechanism of miR-145–5p regulation of cell function

In order to study the mechanism of miR-145–5p, five mRNAs, which are related to cell proliferation, differentiation, angiogenesis, vascular permeability, coagulation function, pre-eclampsia susceptibility and cancer, of the results of miRNA-mRNA integration analysis were further studied. The expression levels of these five mRNAs FLT1, ACVR2A, SERPINE1, SMAD4, TGFB2 in the trophoblast cells after transfection were determined by qPCR. The results showed that the expression of FLT1 was significantly down-regulated and the expression of TGFB2 was up-regulated after overexpression of miR-145–5p (P < 0.05) (Fig. 5A). Moreover, the expression of FLT1 was significantly increased and the expression of SMAD4 was decreased after transfection with miR-145–5p inhibitor (P < 0.05) (Fig. 5B). There was no difference in the expression of ACVR2A and SERPINE1 (P > 0.05) (Fig. 5A and B). These results indicate that FLT1 may be a target of miR-145–5p. Further effects of miR-145–5p on FLT1 protein expression were detected by Western blot. The results were consistent with qPCR. The expression of FLT1 protein was down-regulated after transfection with miR-145–5p mimic (P < 0.05) (Fig. 5C) and the expression of FLT1 protein was significantly increased after transfection with miR-145–5p inhibitor (P < 0.05) (Fig. 5D).

TargetScan and miRanda were used to predict potential target sites of miR-145–5p in FLT1 mRNA. There were two regions on the FLT1 that miR-145–5p could bind (Fig. 6A and B). For mutated plasmid of FLT1-3′UTR, two regions on the FLT1 were all deleted (Fig. 6C). Then the dual-luciferase assay was performed to confirm the relationship between miR-145–5p and FLT-1. As is shown in Fig. 6D, the relative luciferase intensity was significantly decreased in HTR8 cells co-transfected with miR-145–5p mimic and W.T. FLT1-3′UTR reporter plasmid for 48 h compared with that co-transfected mimic NC with W.T. FLT1-3′UTR. There was no significant change in fluorescence intensity after mutation of binding site. Fig. 6E shows that the expression of miR-145–5p was significantly decreased under hypoxic conditions formed after $CoCl_2$ treatment (P < 0.05).

4. Discussion

PE is one of the common complications during pregnancy, which can lead to a variety of adverse pregnancy outcomes. Currently, the

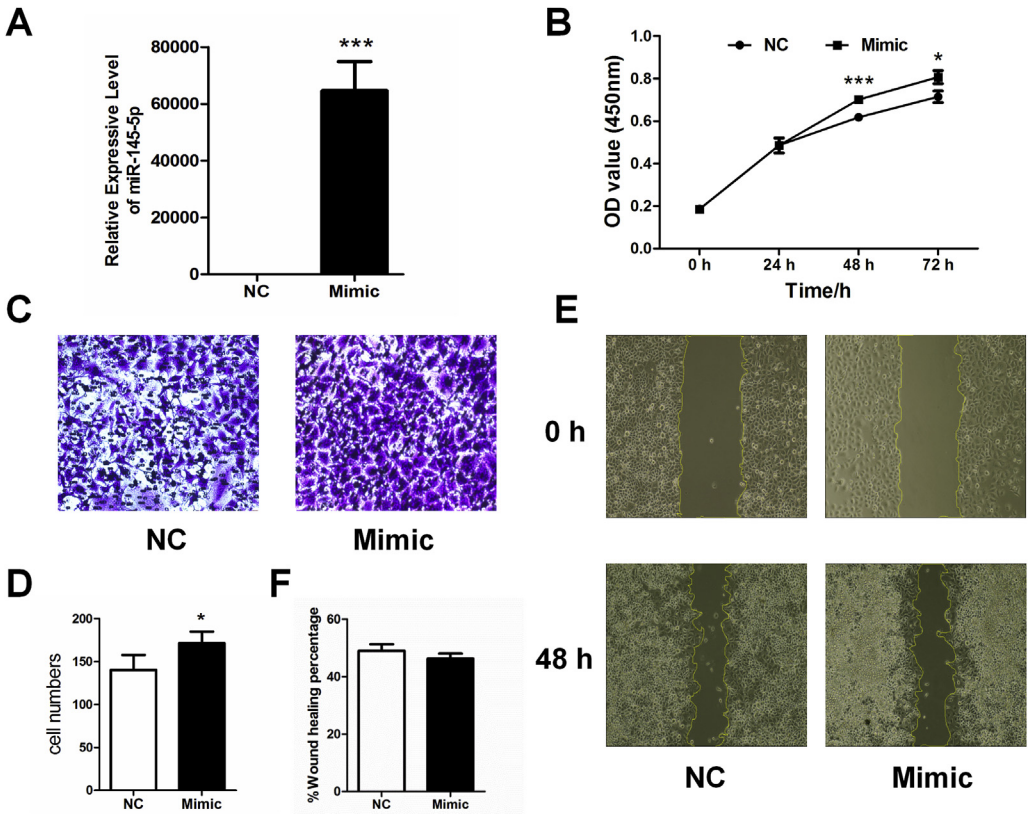

Fig. 3. The roles of over-expressed miR-145-5p in affecting trophoblast HTR-8/SVneo cells function. (A) The expression level of miR-145–5p in trophoblast cells was measured by qPCR analysis after being treated with miR-145–5p mimic for 48 h. (B) The proliferation of HTR-8/SVneo trophoblasts was assessed by CCK8 at 0 h, 24 h, 48 h and 72 h after transfection. (C–D) Transwell chamber experiment was applied to evaluate the invasion ability of HTR-8/SVneo trophoblast cells after 48 h of transfection. (E–F) The area of the wound healing experiment after overexpression of miR-145–5p at 0 h and 48 h was measured by Image J. The migration ability of the cells was determined by the ratio of (scratch area at 0 h-scratch area at 48 h)/scratch area at 0 h. (*P < 0.05, ***P < 0.001).

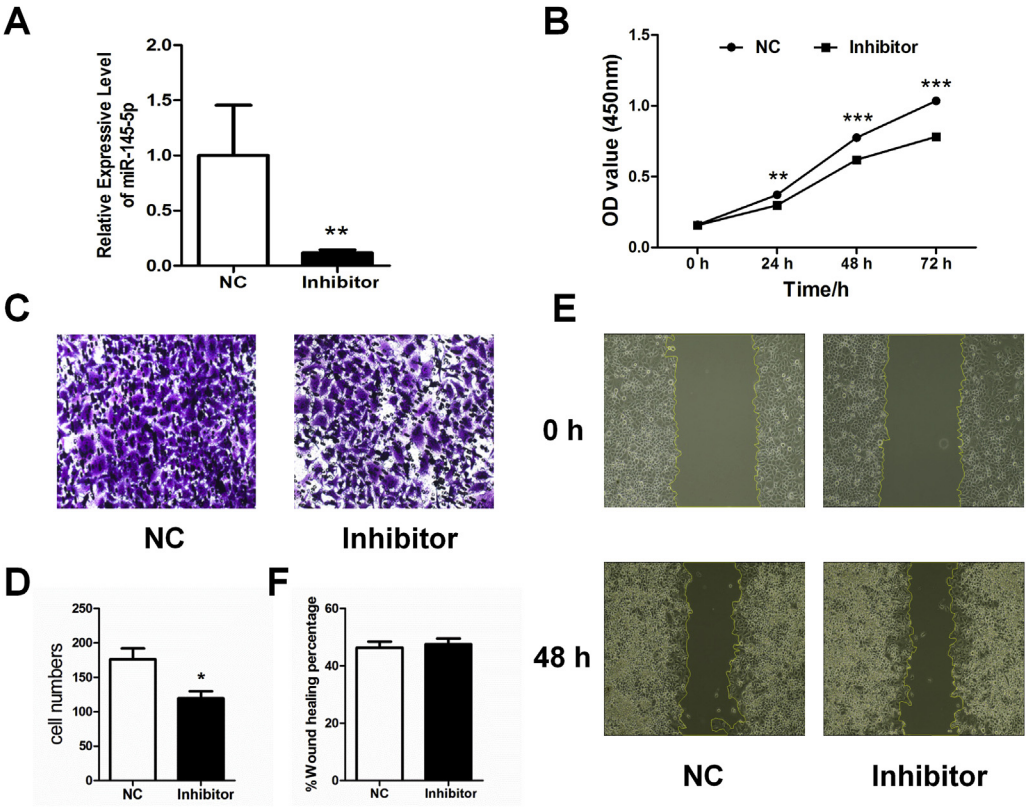

Fig. 4. The roles of decreased expression of miR-145-5p in affecting trophoblast HTR-8/SVneo cells function. (A) The expression level of miR-145–5p in trophoblast cells after being transfected of miR-145–5p inhibitor was measured by qPCR analysis for 48 h. (B) The proliferation of HTR-8/SVneo trophoblasts was assessed by CCK8 at 0 h, 24 h, 48 h and 72 h after transfection. (C–D) Transwell chamber experiment was applied to evaluate the invasion ability of HTR-8/SVneo trophoblast cells after 48 h of transfection. (E–F) The area of the wound healing experiment after decreased expression of miR-145–5p at 0 h and 48 h was measured by Image J. The migration ability of the cells was determined by the ratio of (scratch area at 0 h-scratch area at 48 h)/scratch area at 0 h. (*P < 0.05, **P < 0.01, ***P < 0.001).

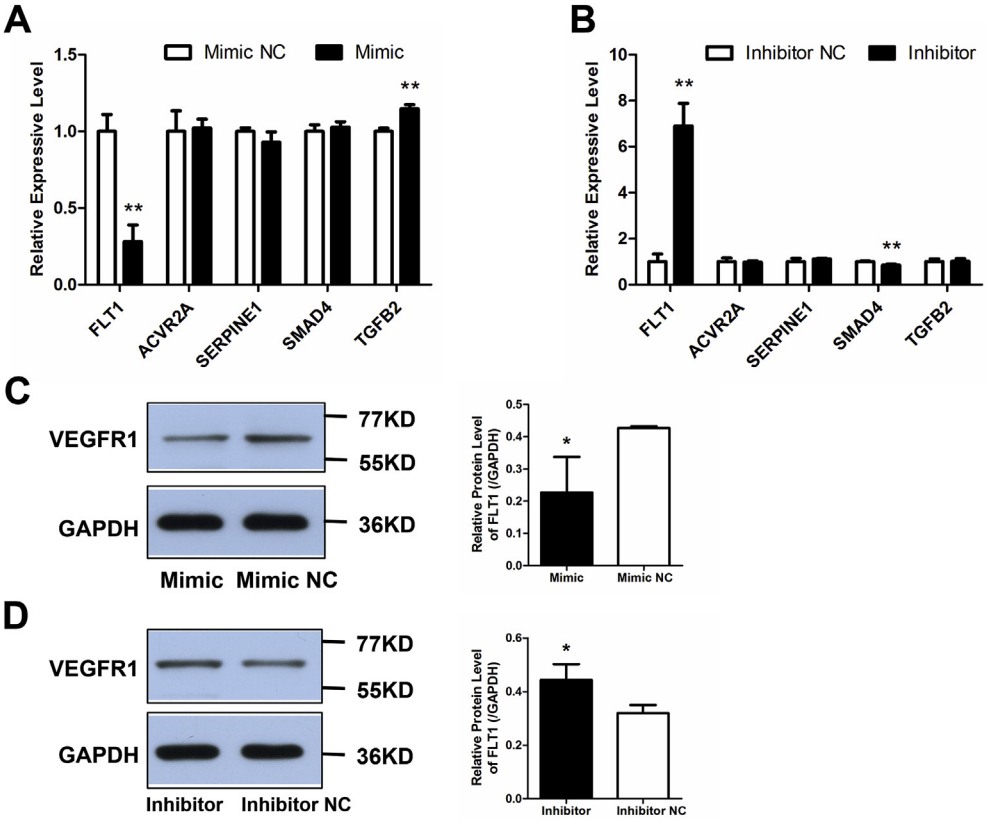

Fig. 5. Mechanism of miR-145-5p regulation of cell function. (A) The expression levels of five mRNAs FLT1, ACVR2A, SERPINE1, SMAD4, TGFB2 in HTR-8/SVneo trophoblast cells after being transfected of miR145–5p mimic for 48 h was determined by qPCR. (B) The expression levels of five mRNAs FLT1, ACVR2A, SERPINE1, SMAD4, TGFB2 in the trophoblast cells after being treated with miR145–5p inhibitor for 48 h was measured by qPCR analysis. (C) The effect of overexpression miR-145–5p on VEGFR1 protein, which is the protein product of the FLT1 gene, was detected by Western blot. (D) The effect of being transfected of miR-145–5p inhibitor on VEGFR1 protein, which is the protein product of the FLT1 gene, was detected by Western blot. (*P < 0.05, **P < 0.01).

pathogenesis and etiology of PE are not clear. Through bioinformatics analysis and detecting tissue expression level, we found that miR-145–5p is differentially lower expressed in the placenta of PE patients. In order to study the role of miR-145–5p in the pathogenesis of PE, we performed cell function experiments and found that miR-145–5p can enhance the proliferation and invasion of trophoblast cells, but has no significant effect on migration. Moreover, miR-145–5p may play a role in PE by negatively regulating the expression of FLT1.

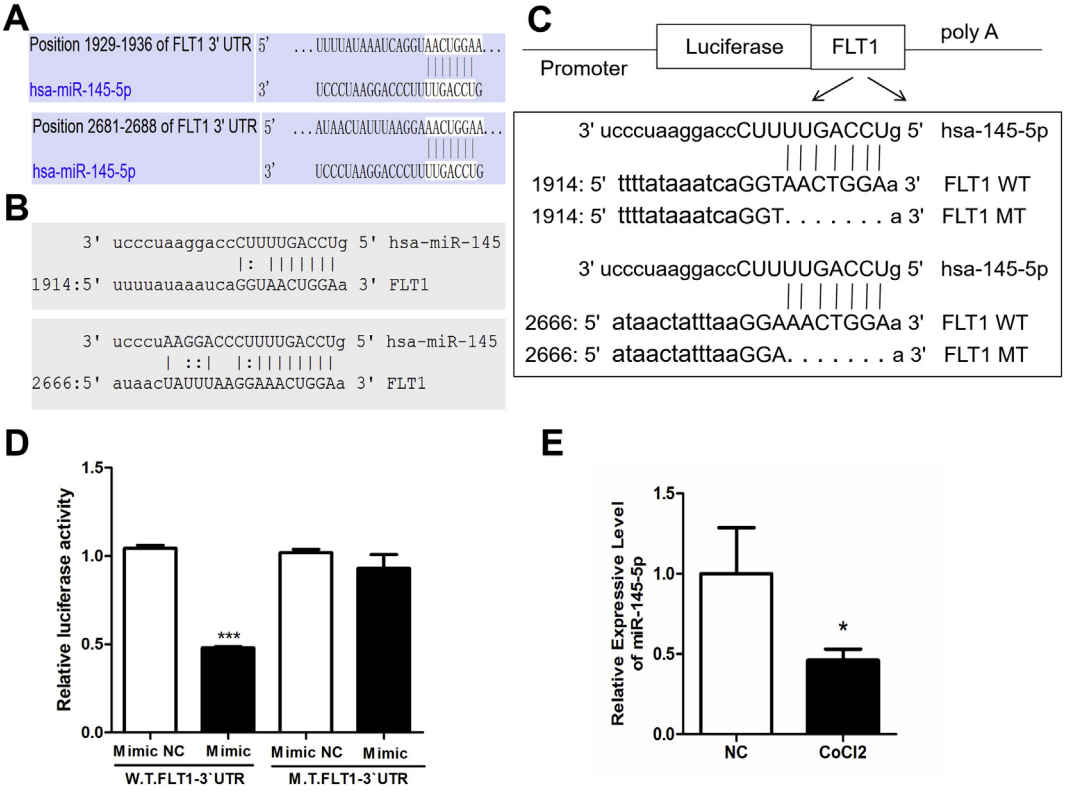

Fig. 6. (A). Two regions on FLT1 3′UTR that miR-145–5p can bind identified using TargetScan. (B). Two regions on FLT1 3′UTR that miR-145–5p can bind identified using miRanda. (C) Schematic Map of Binding Sites of Wild and Mutant Plasmids of FLT1 3′UTR. (D) Analysis of luciferase intensity in cells co-transfected with mimic miR-145–5p or mimic NC and plasmid containing wild FLT1-3′UTR or mutant FLT1-3′UTR. All results are from three independent experiments. (E) The expression of miR-145–5p was measured by qPCR analysis after being treated with $CoCl_2$ (800 μM) for 48 h (*P < 0.05, ***P < 0.001).

During the placental implantation and developmental stages, the intravascular extravillous trophoblast penetrates and remodels the parental spiral arteries, transforming the uterine spiral arteries into low-resistance, high-flow blood vessels that supply enough blood to the placenta bed [17]. The proliferation, migration and invasion of extravillous trophoblast cells are critical during the process. The dysregulation of these functions can lead to a decrease in placental perfusion and repeated episodes of ischemia/reperfusion. Ultimately, maternal systemic inflammatory response occurs [18]. Down-regulated expression of miR-145–5p may lead to placental dysplasia by inhibiting the proliferation and invasion of trophoblasts and participate in the pathogenesis of PE. A large number of studies have shown that miR-145–5p acts as a tumor suppressor during the pathogenesis of cancer, which can inhibit the proliferation and invasion of various tumor cells and promote tumor cell apoptosis [19,20]. These studies, as well as our studies, suggest that miR-145–5p is a beneficial factor for the body. However, the effect of miR-145–5p on trophoblast function in our study seems to be opposite to that in tumor cells, which may be due to differences in the regulation of different physiological activities of different diseases.

Through searching database, we discovered FLT1 may bind to miR-145–5p and both qPCR and Western blot have confirmed that miR-145–5p can regulate the expression of FLT1. In the pathogenesis of PE, the relationship between abnormal placental function and maternal multiple organ dysfunction are thought to be several anti-angiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFlt1) and soluble endoglin, effect on the mother's blood vessels and result in maternal systemic endothelial dysfunction [21,22]. sFlt1 can exert anti-angiogenic effects by interacting with vascular endothelial growth factor (VEGF), which is currently recognized as a factor involved in the pathogenesis of PE [21]. FLT1 and sFlt1 are alternate transcripts of the same mRNA FLT1. In addition, sFLT1 can also be produced by cleavage protein after translation. FLT1, a full-length form of the VEGF receptor, can be cleaved by an activated protein kinase into an N-terminal extracellular fragment and a C-terminal intracellular fragment [23]. The N-terminal extracellular fragment of FLT1 may bind to VEGF and placental growth factor and inhibit VEGF-induced angiogenesis, confirming that it is functionally equivalent to sFLT1 [23]. Down-regulated expression of miR-145–5p in PE may increase the expression of FLT1 and thereby produce more sFlt1. It also can inhibit the proliferation and invasion of trophoblast cells. All these both result in poor remodeling of the spiral arteries in the placenta and lead to decreased placental perfusion and ischemia and hypoxia change. Studies on hypoxia-induced cardiomyocyte injury and neuronal injury after acute cerebral ischemia-reperfusion have also shown that down-regulation of miR-145–5p expression can aggravate ischemia-anoxia-induced cell damage [24,25].

In conclusion, our study found that miR-145–5p may affect the proliferation and invasion of trophoblast cells by regulating the expression of FLT1. This indicates that miR-145–5p may participate in the process of spiral artery remodeling of the placenta and the development of PE. This is a new perspective to understand the etiology and pathogenesis of PE, which may provide a new breakthrough for the early prediction and diagnosis of PE.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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