Original Article

Up-regulated microRNA-300 in maternal whole peripheral blood and placenta associated with pregnancy-induced hypertension and preeclampsia

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Abstract: The differentially expressed microRNAs (miRs) in pregnancy-related complications have not been completely clarified. In this study, we used a nonbiased microarray approach to identify circulating miRs in maternal whole peripheral blood. Moreover, the role of post-translational regulatory mechanism of miRs in preeclampsia (PE) was investigated. Plasma and placental tissue samples were collected from 26 pregnant women who went on to develop PE and 18 healthy control subjects, and then miRs expression profiles in plasma, mRNA and protein expression of v-ets erythroblastosis virus E26 oncogene homolog 1 (ETS-1) were measured by real-time RT-PCR and western blotting analysis in plasma and placenta samples. From the microarray, we identified the expression of miR-300 showed the highest level of all the miRs, miR-300 was significantly increased in the peripheral blood form PE patients as compared to healthy control subjects. The trend for increase in miR-300 expression was mirrored within placental tissue from PE patients compared with healthy controls. In parallel, ETS-1 as a target gene could be regulated by miR-300 by targeting its 3'-UTR. Moreover, both mRNA and protein expression of ETS-1 were markedly decreased in placental tissue from PE patients compared with healthy controls. Furthermore, the association between the miR-300 levels and ETS-1 mRNA expression in placental tissue from all participants was further confirmed by linear regression analysis. The result demonstrated that miR-300 levels were significantly and negatively correlated with ETS-1 mRNA expression in placental tissue. In conclusion, miR-300 was reported to serve as an improved biomarker for PE screening, and we had identified ETS-1 as a target gene that could be regulated by miR-300.

Keywords: miR-300, ETS-1, preeclampsia, placenta

Introduction

Preeclampsia (PE) is a pregnancy-related complication characterized by gestational hypertension and proteinuria and is the major cause of maternal and fetal morbidity and mortality, affecting 2%-10% of all pregnancies around the globe [1, 2]. Increasing evidence indicates that the rapidly progressive PE can cause eclampsia, placental abruption, fetal growth restriction (FGR) and premature fetus [3, 4]. Gestational hypertension induces long-term metabolic and vascular abnormalities, which results from inadequate uteroplacental blood perfusion, ischemia and low infant birth weight [5]. Moreover, women with a history of pregnancy complications, including pregnancy-induced hypertension, PE, eclampsia and FGR, have a higher risk for subsequent ischemic heart disease stroke, and deep venous thrombosis, and metabolic syndrome [6, 7]. The current findings on the diagnosis and pathogenesis of PE remain unknown; and its prediction, prevention and management it is very urgent.

miRs belong to the family of small noncoding RNAs (18-25 nucleotides) that regulate 20-80% of the host genes and serve as important post-transcriptional gene regulators by degrading or blocking translation of target messenger RNA (mRNA) [8]. miRs as essential mediators are involved in physiological and pathological processes, including proliferation/growth, apoptosis, angiogenesis, ischemic injury and endothelial cells dysfunction [9-11]. Increasing evidence suggests an association between gestational hypertension or preeclampsia and dysregulation of microRNAs expression in the maternal...
whole peripheral blood and placenta [12]. Several studies have been found that the expression of miR-17-family miRs, miR-20a, miR-20b, miR-206 and miR-210, becomes deregulated in peripheral blood or placental tissues from preeclampsia patients [13-16]. In L-NAME-induced PE animal model, miR-126 promotes angiogenesis and blood perfusion in preeclampsia placenta and can ameliorate or prevent preeclampsia [17, 18]. Recent studies indicate that cardiovascular and cerebrovascular disease-associated miRs are observed to be associated with pregnancy-related complications, which provides basis for an alternative therapeutic approach in patients with preeclampsia [12, 19, 20]. Differentially expressed miRs target to genes encode proteins that have been implicated in the pathogenesis of preeclampsia [14]. Functional studies of the miR-34b and miR-34c family indicate that these miRs are mediators of p53-dependent suppression of endometrial proliferation, which is closely related in the pathogenesis of endometriosis and can potentially be important in preeclampsia [21, 22].

In the present study, by using miRNA microarray and real-time reverse transcription polymerase chain reaction (real-time RT-PCR) analyses, we investigated the miRs differential expression in maternal whole peripheral blood among 26 preeclampsia individuals and 18 normotensive control subjects. Moreover, the bioinformatics online predict software based evaluation of putative targets of these differentially expressed miRs were examined to elaborate the underlying molecular mechanisms and functional relationships between miRs and target genes in the progression of pregnancy-related complications, including gestational hypertension, PE and premature delivery.

Materials and methods

Patients

The studied cohort consisted of 18 normotensive control subjects and 26 preeclampsia individuals (cases corresponded to women subsequently developing preeclampsia and delivery before 35 weeks of gestation defined as early preeclampsia). Women with normal pregnancies were defined as those without medical, obstetrical or surgical complications at the time of the study and who subsequently delivered full term, singleton healthy infants weighing > 2500 g after 37 completed weeks of gestation. Preeclampsia was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg on at least two occasions 4 hours apart, developing after 20 weeks of gestation in a previously normotensive women, and proteinuria > 300 mg in a 24-hour urine specimen [23]. Samples of whole peripheral blood and placenta were collected at the Department of Gynecology and Obstetrics, China-Japan Friendship Hospital (Beijing, China) and stored at -80°C until further processing.

Cell culture

BeWo cells (Cat. No. CCL-98) and JEG-3 cells (Cat. No. HTB-144) were commercially obtained from the American Type Culture Collection (ATCC) and maintained in Ham’s F12K medium and modified RPMI-1640 medium and supplemented with 10% FBS (FBS; Life Technologies) at 37°C in a humidified incubator (Thermo, USA), 5% CO2, 95% air atmosphere. Moreover, HEK293 cells and HUVEC were obtained from the Cell Resource Center, Shanghai Institutes for Biological Sciences (SIBS, China) and were maintained in RPMI-1640 (Life Technologies, Carlsbad, CA) supplemented with 10% FBS (FBS; Life Technologies) at 37°C in a humidified incubator (Thermo, USA), 5% CO2, 95% air atmosphere.

RNA extraction

RNA extraction from whole peripheral blood (200 μL) and placenta (100 mg) was performed using the mirVana RNA Isolation Kit (Ambion, Austin, TX) according the manufacturer’s protocol. For the transfected cells, total RNA were extracted using Trizol reagent (Invitrogen, Carlsbad, CA) following the manufacturer’s protocol.

MicroRNA profiling by OpenArray

The OpenArray real-time PCR system (Applied Biosystems/Life Technologies, Paisley, UK) was used to measure miRs levels in maternal whole peripheral blood. Total RNA from patient plasma and placenta samples was processed according to manufacturer’s instructions. The array included 754 unique probes to cover all miRNA available in the version 14.0 of the miR-Base database. Briefly, reverse transcription of
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each sample was carried out with two separate primer pools in parallel, using 19.15 ng of total RNA per sample per reaction. Following reverse transcription, a preamplification step with 16 cycles was carried out and the resulting products diluted 1:20. The diluted samples, matched and grouped, were applied to OpenArray qPCR panels in a randomized fashion, for profiling of 754 miRNAs per sample. Ct values from the microarray were not subject to normalization, as suitable stable miRNAs could not be found. Therefore, all statistical analysis was performed on raw Ct values presented in the text. miRNAs with a Ct of 35 or above were not included in analysis.

Luciferase reporter gene activity assay

The 3'-UTR of ETS-1 gene containing the predicted target sites for miR-300 was obtained by PCR amplification. The fragment was inserted into the multiple cloning sites in the pMIR-REPORT luciferase microRNA expression reporter vector (Ambion, Austin, USA). HEK293 cells were co-transfected with 50 ng of luciferase reporters containing ETS-1 3'-UTR and miR-300 mimics by Lipofectamine 2000 (Invitrogen, CA, USA). We harvested the cell lysates after 24 hours transfection and measured the luciferase activity with a dual luciferase reporter assay kit according to manufacturer's instruction.

Transfection of miR-300 mimics and inhibitor

The FAM modified 2'-OMe-oligonucleotides were chemically synthesized and purified by high-performance liquid chromatography (GenePharma, Shanghai, China). The 2'-OMe-miR-300 mimics were composed of RNA duplexes with the following sequence: 5'-UAUACAAAGCCGGCAGACUCUCUCU-3'. The sequences of 2'-OMe-miR-300 inhibitor and 2'-OMe-scramble oligonucleotides were as follows: 5'-AGAGAGAGUCUGCUCGCUUGUAUA-3'; and 5'-CUUGCUGAAUGCUGCUAAUGC-3'. Cells were transfected using Lipofectamine 2000 (Invitrogen, CA, USA) at a final concentration of 100 nM. At 24 hours post-transfection, the culture medium was changed. After 24 hours, cells were harvested for analysis.

Quantitative real-time RT-PCR

Real-time RT-PCR was performed on whole peripheral blood and placenta from the healthy control and PE patients to confirm the miRNA microarray results. The miRNA from each sample were reverse-transcribed into cDNA using RT2 miRNA First Strand Kit (SABiosciences, Frederick, MD) according to the manufacturer's protocol. The real-time PCR was performed using RT2 miRNA qPCR Assay (SABiosciences) for each miRNA. The comparative C_T method (ΔΔC_T method) was used to calculate the miRNA expression, with the universally expressed small nuclear RNA U6 used as the endogenous control.

For mRNA gene expression analysis, RNA was extracted from the cell lines and placenta using TRizol (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. Synthesis of cDNA was performed on 2 μg total RNA using Moloney Murine Leukemia Virus reverse transcriptase (Promega Corporation, Madison, WI, USA) and oligo dT 15 primers (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The first strand cDNAs served as the template for the regular PCR (PCR system 9700: Applied Biosystems; Thermo Fisher Scientific, Inc.), following initial denaturation at 95°C for 3 min, 40 cycles were performed of denaturation at 95°C for 15 sec, annealing at 56°C for 20 sec and extension at 72°C for 20 sec. GAPDH as an internal control was used to normalize the data to determine the relative expression of the ETS-1. The PCR primers used in this study were as follows: ETS-1, forward 5'-TCATTTCTTTGCTGCTTGGA-3' and reverse 5'-AAGCCGACTCTCACCATCAT-3'; GAPDH, forward 5'-GCACCGTCAAGCTGAGAAC-3' and reverse 5'-TGGTGAAGACGCCAGTGGA-3'. The semiquantitative analysis was performed using Quantity One software (version 4.31; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Western blotting

The whole peripheral blood and placenta from the healthy control and PE patients were homogenized and extracted in NP-40 buffer, followed by 5-10 min boiling and centrifugation (speed, 12,000×g; duration, 10 min; temperature, 4°C) to obtain the supernatant. Samples containing 50 μg of protein were separated by 10% SDS-PAGE gel and transferred to nitrocel-
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Table 1. Clinical characteristics of the healthy control and preeclamptic patients enrolled in this study

<table>
<thead>
<tr>
<th></th>
<th>Healthy control (n = 18)</th>
<th>Preeclamptic patients (n = 26)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.6 ± 4.6</td>
<td>30.8 ± 5.2</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>113.5 ± 2.8</td>
<td>151.7 ± 3.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80.2 ± 2.1</td>
<td>106.7 ± 2.7</td>
<td>&lt; 0.001</td>
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<tr>
<td>Proteinuria (g/24 h)</td>
<td>None</td>
<td>0.9 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pregnancy body mass index (kg/m²)</td>
<td>26.2 ± 1.2</td>
<td>29.4 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>16 (88.9%)</td>
<td>6 (76.9%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>2 (11.1%)</td>
<td>20 (23.1%)</td>
<td></td>
</tr>
<tr>
<td>Fetal sex</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>8 (44.4%)</td>
<td>13 (50%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10 (55.6%)</td>
<td>13 (50%)</td>
<td></td>
</tr>
<tr>
<td>Glucose status</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Normal</td>
<td>17 (94.4%)</td>
<td>19 (73.1%)</td>
<td></td>
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<tr>
<td>DM/GDM</td>
<td>1 (5.6%)</td>
<td>7 (26.9%)</td>
<td></td>
</tr>
<tr>
<td>Gestation at delivery (weeks)</td>
<td>34.5 ± 2.5</td>
<td>38.2 ± 0.6</td>
<td>0.012</td>
</tr>
<tr>
<td>Infant birth weight (g)</td>
<td>2513 ± 435</td>
<td>3671 ± 515</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Systolic blood pressure, SBP; Diastolic blood pressure, DBP; No significant, NS.

Results

Differential miRs expression in maternal whole peripheral blood affected by pregnancy-related complications

Plasma samples were collected from 26 pregnant women who went on to develop preeclampsia and 18 healthy control subjects, whose clinical characteristics were shown in Table 1. Three pairs of healthy control and PE peripheral blood at 20 weeks of gestation were measured by miRNA microarray analysis, differential analysis identified thirty-three miRs with differential expression (based on fold change > 2 and ANOVA P-value < 0.05) between healthy control subjects and PE patients, including eighteen up-regulated miRs and fifteen down-regulated miRs in PE patients (Figure 1A). Among these thirty-three miRs, five miRs (miR-300, miR-203, miR-346, miR-582-3p and miR-381) were up-regulated and two down-regulated miRs (miR-139-5p and miR-192) with fold change > 3 and ANOVA P-value < 0.05, and the expression of miR-300 showed the highest level of all the miRs (Figure 1B). In addition, the quantitative real-time RT-PCR results showed that the levels of miR-300 were significantly increased in the peripheral blood form PE patients (n = 26) as compared to healthy control subjects (n = 16) at 20 weeks of gestation (Figure 1C). Furthermore, the level of miR-300 was approximately 2-fold greater in the peripheral blood at week 16 of gestation than that of healthy controls. The similar results showed

Statistical analysis

Data were expressed as the mean ± standard deviation. Statistical analyses were performed using SPSS 13.0 statistical software package (SPSS Inc., Chicago, IL, USA). The significance of differences between groups was estimated by Student’s t-test, one-way analysis of variance (ANOVA) and χ² test. The correlation of ETS-1 level and miR-300 level was analyzed with linear regression analysis. One-way analysis of variance was performed for comparisons among different groups. P < 0.05 was considered to indicate a statistically significant difference.
miR-300 associated with pregnancy-induced hypertension

expression levels of miR-300 were detected in severe PE patients compared with mild PE patients (P < 0.001) (Figure 3A). The up-regulation of miR-300 were achieved in the delivery before 35 weeks of gestation compared with healthy controls, and a significant difference in miR-300 expression was found between the delivery before 35 weeks of gestation and the delivery after 35 weeks of gestation (Figure 3B). Importantly, statistical analysis revealed that placental expression of miR-300 was related to low birth-weight of infants (Figure 3C). When compared to normal pregnancies, significant up-regulation of miR-300 was observed in chronic hypertension superposed on PE as compared to healthy controls, and the lower expression of miR-300 in without HP before PE group than HP with PE group (Figure 3D).

The association study of miR-300 expression and the pregnancy-related complications

Based on the results of our study, we further studied the association between miR-300 levels in placental tissues and PE. The results demonstrated that the expression of miR-300 in placenta differed significantly between the control group and PE group (P < 0.001). Higher expression levels of miR-300 were detected in severe PE patients compared with mild PE patients (P < 0.001) (Figure 3A). The up-regulation of miR-300 were achieved in the delivery before 35 weeks of gestation compared with healthy controls, and a significant difference in miR-300 expression was found between the delivery before 35 weeks of gestation and the delivery after 35 weeks of gestation (Figure 3B). Importantly, statistical analysis revealed that placental expression of miR-300 was related to low birth-weight of infants (Figure 3C). When compared to normal pregnancies, significant up-regulation of miR-300 was observed in chronic hypertension superposed on PE as compared to healthy controls, and the lower expression of miR-300 in without HP before PE group than HP with PE group (Figure 3D).

Figure 1. Unsupervised hierarchical clustering of differentially expressed miRs in maternal whole peripheral blood are measured by microRNA microarray analysis. The figure is drawn by MeV software (version 4.2.6) (A). Fold change comparison of maternal whole peripheral blood miRs expression between healthy controls and PE patients (B). The expression of miR-300 is performed by real-time RT-PCR in healthy controls (n = 18) and PE patients (n = 26) (C).
ETS-1 is a target of miR-300

miRs are non-translational and exert their effect by regulating target genes expression. We used miRanda Target database (http://www.microRNA.org) to find the potential target of miR-300. We found that ETS-1 is a target of miR-300, ETS-1 RNA contains one conserved target site of miR-320 (Figure 4A). We further examined whether ETS-1 is a direct target of miR-300. We constructed a luciferase reporter carrying the 3'-UTR of ETS-1 and transfected the reporter with either the miR-300 mimics or scramble miRNA precursor into HEK293 cells. The luciferase activity in ETS-1 wild-type cells transfected with miR-300 mimics was significantly decreased compared with control group (Figure 4B). Using HUVEC, BeWo and JEG-3 cells as the cell models, miRNA target prediction using open source software (www.TargetScan.org and www.microrna.org), over-expression and down-regulation of miR-300 was achieved by transfection of miR-300 precursor or antagomir in the HUVEC, BeWo and JEG-3 cells (Figure 4C). Moreover, we demonstrated that miR-300 regulated ETS-1 expression in HUVEC, BeWo and JEG-3 cells through translational inhibition. Overexpression miR-300 significantly suppressed ETS-1 mRNA expression in these three cell lines as compared to control group (Figure 5A). In contrast to that transfection of HUVEC, BeWo and JEG-3 cells with the miR-300 inhibitors had the opposite effect on ETS-1 mRNA expression levels (Figure 5B). Interestingly, we observed that inhibition of miR-300 resulted in up-regulating, and over-expressed miR-300 markedly decreased the protein expression of ETS-1 in HUVEC, BeWo and JEG-3 cells (Figure 5C). Thus, we demonstrated that ETS-1 was inhibited by miR-300 at both the mRNA and protein levels. The mRNA and protein expression of ETS-1 were also measured in the placental tissue. The results indicated that both mRNA and protein expression of ETS-1 were markedly decreased in placental tissue from PE patients compared with healthy control (Figure 5D and 5F). Based on these studies, we concluded that miR-300 could inhibit ETS-1 expression by targeting its 3'-UTR, which was further confirmed by linear regression analysis between the miR-300 levels and ETS-1 mRNA expression in placental tissue from all participants. The linear regression analysis demonstrated that miR-300 levels were significantly and negatively correlated with ETS-1 mRNA expression in placental tissue (Figure 5F).

Discussion

In this pilot study, we aimed to screen out specific miRs associated with pregnancy-related complications such as gestational hypertension, preeclampsia, eclampsia, placental abruption, FGR and premature fetus, which affect the fitness of mother and consequently the fetus. A nonbiased microarray approach to identify novel circulating miRs in maternal whole peripheral blood, the results demonstrated that miR-300 was significantly increased in early pregnancy and was related to gestational hypertension, low birth-weight of infants and premature fetus in clinical practice. These findings suggest that miR-300 may be an ideal biomarker candidate for non-invasive diagnostic/prognostic purposes for gestational hypertension and preeclampsia. Our study also found that miR-300 expression was observed to be higher in placental tissue from PE patients than those of the healthy controls. The further study in placental tissue and cell model, miR-300 might be involved in the regulation of ETS-1 expression in response to PE.
miR-300 associated with pregnancy-induced hypertension

Some investigators perform microRNAs expression profiling in maternal plasma or serum samples, miRNA assessment at first-trimester of pregnancy does not appear to have any predictive value for early preeclampsia [24, 25]. Cardiovascular and cerebrovascular disease-associated miRs is down-regulated in maternal whole peripheral blood, including gestational hypertension-related, miR-100-5p, miR-125b-5p and miR-199a-5p, preeclampsia-associated, miR-100-5p and miR-125b-5p, and IGF-induced, miR-100-5p, miR-125b-5p, miR-146a-5p, miR-199a-5p, miR-221-3p and miR-574-3p [12]. Moreover, there is a negative correlation between miR-942 levels and the maternal arterial pressure, and between miR-143 levels and the uterine artery Doppler pulsatility index, in agreement to their recently reported roles in the regulation of blood pressure and vascular function [24, 26, 27]. There are controversial for miR-26a-5p and miR-16-5p levels measurement in placenta from preeclamptic pregnan-

Figure 3. miR-300 is differential expression in mild and severe preeclampsia (A), the delivery before or after 35 weeks of gestation (B), birth-weight of infants less than or more than 2.5 kg (C) and with or without hypertension before PE (D).
miR-300 associated with pregnancy-induced hypertension

Figure 4. The putative binding sites of human miR-300 on wild-type and mutation-type ETS-1 3'-UTR were highlighted (A). In a dual-luciferase reporter assay, HEK293 cells were transfected with firefly luciferase reporter inserted with wild-type and mutation-type ETS-1 3'-UTR, 24 hours after transfection with scramble, miR-300 mimics and miR-300 inhibitors, relative luciferase activities are evaluated (B). Overexpression and downregulation of miR-300 was achieved by transfection of miR-300 mimics or inhibitors in HUVEC, BeWo cells and JEG-3 cells (C). Values were expressed as mean ± SD, n = 3 in each group.

acies and healthy controls, and the similar results cannot be replicated with different high-throughput profiling technologies [12, 28, 29]. Our study was also analyzing the miRs differential expression in maternal whole peripheral blood by high-throughput miRNA microarray, and found that miR-300 up-regulation was observed in peripheral blood from PE patients. Notably, placental expression of miR-300 was upregulated to a similar degree in the maternal whole peripheral blood from preeclamptic pregnancies. The similar degree of upregulation of maternal plasma and placental miR-300 observed in the present study could suggest that maternal plasma miR-300 levels may originate from the placenta. Intriguingly, our study was the screening of peripheral blood samples from three gestational time points, 16, 24 and 30 weeks, the highest level of miR-300 was found at week 24. Meanwhile, the significant up-regulation of miR-300 was first discovered at week 16, which might provide a new sight for non-invasive diagnostic/prognostic purposes. Compared with the expression in urine of healthy pregnant women, the expression levels of hsa-miR-300 is significantly down-regulated in intrahepatic cholestasis of pregnancy [30]. Based on these results, we suggest that miR-300 has different expression patterns in various tissues and diseases [30, 31].

Our results demonstrated that miR-300 levels were observed to be higher in both the circulation and the placenta, which provided a promising candidate for further study the post-transcriptional molecular mechanisms. Notably, some experimentally cell and animal model show that B lymphoma Mo-MLV insertion region 1 (Bmi1) [31], c-ros oncogene 1 receptor tyrosine kinase (ROS1) [32], ROCK1 [33] and bromodomain-containing protein 7 (BRD7) [34]. It is noteworthy that PE-induced up-regulation of miR-300 was possibly important for regulating ETS-1 expression, which was indicated by target prediction analysis in our study. A growing body of evidence suggests that ETS-1 is involved in villus CTB differentiation and is expressed in first- and third-trimester human placenta, silencing of ETS1 expression in freshly prepared CTBs markedly attenuated syncy-
miR-300 associated with pregnancy-induced hypertension

Figure 5. The mRNA expression of ETS-1 is measured by real-time RT-PCR after transfected with miR-300 mimics (A) and miR-300 inhibitors (B) in HUVEC, BeWo cells and JEG-3 cells. The protein expression of ETS-1 is measured by western blotting after transfected with miR-300 mimics and miR-300 inhibitors in HUVEC, BeWo cells and JEG-3 cells (C). The mRNA (D) and protein (E) of ETS-1 are measured by real-time RT-PCR and western blotting in placental tissue, respectively. Linear regression analysis between the miR-300 levels and ETS-1 mRNA expression in placental tissue from all participants is performed (F).

tialization [35, 36]. ETS1 is also expressed in human fetal membranes and is associated with premature rupture of the membranes and extracellular matrix remodeling of the membranes [37]. In the rat ovary during the estrous cycle and pregnancy, the expression of ETS-1 mRNA and protein fluctuated during pregnancy, increasing during early pregnancy, then decreasing during mid-pregnancy, and again increasing until parturition [38]. In our study, we found
that ETS-1 mRNA and protein levels were markedly decreased in the placental tissue from PE patients compared with healthy controls. These findings suggest that ETS-1 may participate and play an important role in the regulation of pregnancy-related complications.

In conclusion, with this pilot trial we demonstrated the feasibility to detect PE dependent miRs profiling in maternal whole peripheral blood, and circulating miR-300 was reported to serve as an improved biomarker for PE screening. Moreover, we had identified ETS-1 as a target gene that could be regulated by miR-300.

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Disclosure of conflict of interest

None.

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