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microRNA-21 regulates the proliferation of placental cells via FOXM1 in preeclampsia

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Abstract. The present study determined the expression of microRNA (miRNA or miR)-21 and forkhead box M1 (FOXM1) in placenta and blood samples from patients with preeclampsia (PE), and investigated the relationship between miR-21 and FOXM1. A total of 32 pregnant women with PE and 28 healthy pregnant women were included in the study as the experimental and control groups, respectively. Placental tissues and peripheral blood were collected from all subjects. ELISA was performed to measure the level of FOXM1 protein in the blood. HTR8/SVneo cells overexpressing miR-21 were established by transfection with agomiR-21. Reverse transcription-quantitative PCR was performed to measure the expression of FOXM1 mRNA and miR-21 in the placenta, blood and cells, and western blotting was used to evaluate FOXM1 protein expression in the placenta. An MTT assay was also performed to assess cell viability. In addition, a dual-luciferase reporter assay was used to investigate the direct interaction between FOXM1 and miR-21. The occurrence of PE was found to be associated with reduced FOXM1 mRNA levels, and elevated FOXM1 protein expression may serve a regulatory role that when attenuated leads to the occurrence of PE. Furthermore, miR-21 may serve a regulatory role in the pathology of PE by downregulating FOXM1 expression at the transcriptional level. In HTR8/SVneo cells, the overexpression of miR-21 reduced cell viability, possibly via the reduction of FOXM1 expression. The dual-luciferase assay indicated that miR-21 directly binds to the 3'-untranslated region of FOXM1 to regulate its expression. The present study demonstrated that the expression of FOXM1 mRNA and protein is downregulated, whereas the expression of miR-21 is upregulated in the placenta and blood samples of PE patients. In conclusion, miR-21 may regulate placental cell proliferation via its effects on FOXM1 to promote the occurrence and development of PE.

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Introduction

Preeclampsia (PE) one of the main causes of maternal and infant mortality during the perinatal period, with the pathophysiological changes mainly occurring in early pregnancy (1). However, the onset of PE occurs after 20 weeks of pregnancy, with manifestations of hypertension, proteinuria and edema (2). Under serious circumstances, general convulsions and multiple organ dysfunction can be observed (2,3). The condition of PE is complex and difficult to predict as the etiology and mechanism of pathogenesis are unclear.

Most of the symptoms associated with PE are rapidly relieved following the delivery of the placenta, suggesting that the placenta is the source of the disease (4). It is generally believed that PE is caused by dysfunctional trophoblast invasion (5). Importantly, forkhead box M1 protein (FOXM1) is expressed in cytotrophoblasts in the villi and decidual tissue during early pregnancy, and possibly interacts with placental trophoblasts during early placental development (6-8). Therefore, it may be hypothesized that aberrant FOXM1 expression could induce changes in intracellular signal transduction, leading to trophoblastic dysfunction and PE. The main pathological feature of the placenta in PE patients is insufficient trophoblast invasion into the endometrium, with the depth only reaching the decidua layer (9). This in turn results in defective physiological 'vascular recasting' of the uterine spiral arterioles and a reduction in villi area and density (10). Unlike the invasion of cancer cells, trophoblast invasion is under strict paracrine regulation to prevent excessive invasion (11,12). Genes that regulate FOXM1 expression include microRNA (miRNA or miR)-365-1 and miR-374b (13,14). Furthermore, miR-21 has been reported to be upregulated in human malignant tumors (15). However, the potential relationship between miR-21 and FOXM1 in PE has not been previously reported.

In the present study, the expression of miR-21 and FOXM1 in placental tissues and blood samples from patients with PE was assessed to investigate the potential regulatory relationship between miR-21 and FOXM1.

Materials and methods

Patients. A total of 32 pregnant women with PE (age range, 22-39 years; median age, 32 years; mean age, 32±4.6 years) who received regular birth examinations prior to cesarean

section at Huaian First People's Hospital between December 2013 and March 2018 were included in the present study. In addition, 28 normal pregnant women with matched age, weeks of gestation and pre-pregnancy indices (age range, 20-41 years; median age, 33 years; mean age, 33±3.9 years) were included into the control group. Inclusion criteria were: Good health before pregnancy and no history of induced abortion. Exclusion criteria included: Age over 45 years old, history of immune diseases before pregnancy, history of ovarian or uterine diseases, hormonal disorder and long-term use of hormones or immunosuppressive drugs.

Peripheral blood samples (10-15 ml) were collected from all subjects and centrifuged at 400 x g, 4˚C for 10 min before the isolation of serum into 100-μl aliquots. During caesarean section, placental tissues were collected from the center of the placenta, 2.5 cm from the umbilical cord. All procedures performed in the present study were approved by the Ethics Committee of Nanjing Medical University. Written informed consent was obtained from all patients or their families.

Cells. Human HTR8/SVneo cells (Wuhan PriCells Biomedical Technology Co., Ltd.) in the logarithmic growth phase were seeded ($3x10^5$/well) into 24-well plates 1 day before transfection, and cultured in antibiotics-free F12/DMEM medium (SH30023.01B; HyClone; GE Healthcare Life Sciences) supplemented with 10% fetal bovine serum (SH30084.03; HyClone; GE Healthcare Life Sciences) under 37˚C and 5% CO_2 until 70% confluency was reached. For transfection, 1.5 μl agomiR-negative control (NC; agomiR-NC group; 5'-UUCUCCGAACGUGUCACGUTT-3' and 3'-TTAAGAGGCUUGCACAGUGCA-5') or agomiR-21 (miR-21 mimics group; 5'-UAGCUUAUCAGACUGAUGUUGA-3' and 3'-AUCGAAUAGUCUGACUACAACU-5'; both 20 pmol/μl; Sangon Biotech Co., Ltd.) was mixed with 50 μl Opti-Mem medium (Thermo Fisher Scientific, Inc.) in one vial. In another vial, 1 μl Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.) was mixed with 50 μl Opti-Mem medium. After 5 min, the contents of the two vials were combined, and the mixture was incubated at room temperature for 20 min. This mixture was subsequently added to the cells in their respective groups., The medium was replaced with fresh F12/DMEM medium containing 10% fetal bovine serum 6 h later. The cells were collected for downstream assays following 48 h of further culture.

Reverse transcription-quantitative PCR (RT-qPCR). Tissue samples (100 mg) were ground into powder in liquid nitrogen and lysed using 1 ml TRIzol® reagent following the manufacturer's protocol (Thermo Fisher Scientific, Inc.) for the extraction of total RNA. Serum (100 μl) or cells ($3x10^6$) were directly lysed using 1 ml TRIzol reagent. After assessing the concentration and quality of RNA using ultraviolet spectrophotometry (NanoDrop™ ND2000; Thermo Fisher Scientific, Inc.), cDNA was obtained by reverse transcription from 1 μg RNA using miRcute miRNA cDNA First-chain Synthesis kit according to the manufacturer's protocol (Tiangen Biotech Co., Ltd.) and then stored at -20˚C. SuperReal PreMix (SYBR Green) kit (Tiangen Biotech Co., Ltd.) was used to detect the expression of FOXM1 mRNA using β-actin as an internal reference. The sequences of the FOXM1 primers were forward, 5'-GGCTCCCGCAGCATCAAGCA-3' and reverse, 5'-TGTTCCGGCGGAGCTCTGGA-3'. The sequences of the β-actin primers were forward, 5'-ATCTGGCACCACACCTTCACAATGAGCTGCG-3' and reverse, 5'-CGTCATACTCCTGCTTGCTGATCCACATCTGC-3'. The reaction system (25 μl) was composed of 12.5 μl SYBR Premix Ex Taq, 0.5 μl upstream primer, 0.5 μl downstream primer, 1 μl cDNA and 10.5 μl ddH_2O. The thermocycling conditions were as follows: Initial denaturation at 95˚C for 2 min, followed by 35 cycles of denaturation at 95˚C for 30 sec, annealing at 56˚C for 30 sec and elongation at 72˚C for 1 min, final elongation at 72˚C for 2 min (iQ5 instrument; Bio-Rad Laboratories, Inc.). The $2^{-\Delta\Delta Cq}$ method (16) was used to calculate the relative expression of FOXM1 mRNA against β-actin. Each sample was tested in triplicate.

The expression of miR-21 was determined using miRcute miRNA qPCR Detection kit (SYBR Green; Tiangen Biotech Co., Ltd.) with U6 as an internal reference. The sequences of the miR-21 primers were forward, 5'-GCCCGCTAGCTTATCAGACTGATG-3' and reverse, 5'-GTGCAGGGTCCGAGGT-3'. The sequences of the U6 primers were forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'. The reaction system (20 μl) contained 10 μl miRcute miRNA pre-mix, 0.5 μl upstream primer, 0.5 μl downstream primer, 2 μl cDNA and 7 μl ddH_2O. The thermocycling conditions were as follows: Initial denaturation at 95˚C for 5 min; followed by 40 cycles of denaturation at 95˚C for 15 sec, annealing at 56˚C for 25 sec and elongation at 72˚C for 40 sec (iQ5 instrument). The $2^{-\Delta\Delta Cq}$ method (16) was used to calculate the relative expression of miR-21 against U6. Each sample was tested in triplicate.

Western blotting. Tissues (100 mg) were ground into powder, and cells ($1x10^6$) were trypsinized and collected. The samples were then lysed using radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology) for 30 min on ice. After centrifugation at 1,500 x g and 4˚C for 10 min, the supernatant was aspirated for the determination of protein concentration using a bicinchoninic acid assay kit. The samples were then mixed with 5X sodium dodecyl sulfate (SDS) loading buffer before denaturation in boiling water for 5 min. The samples (50 μg/lane) were subjected to 10% SDS-polyacrylamide gel electrophoresis at 100 V. The resolved proteins were transferred to polyvinylidene difluoride membranes on ice (100 V, 2 h) and blocked with 5% skimmed milk (ddH_2O as diluent) at room temperature for 1 h. The membranes were subsequently incubated with rabbit anti-human FOXM1 (1:2,000; cat. no. ab175798; Abcam) or β-actin (1:5,000; cat. no. ab129348; Abcam) polyclonal primary antibodies at 4˚C overnight. After extensive washing with phosphate-buffered saline (PBS) supplemented with 0.1% Tween-20 three times for 15 min, the membranes were incubated with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:3,000; cat. no. ab6721; Abcam) for 1 h at room temperature before washing with PBS with Tween-20 three times for 15 min. The membranes were visualized using an enhanced chemiluminescence detection kit (cat. no. ab65623; Abcam). Image lab v3.0 software (Bio-Rad Laboratories, Inc.) was used to acquire and analyze densitometry data. The relative expression of target protein was normalized against β-actin expression.

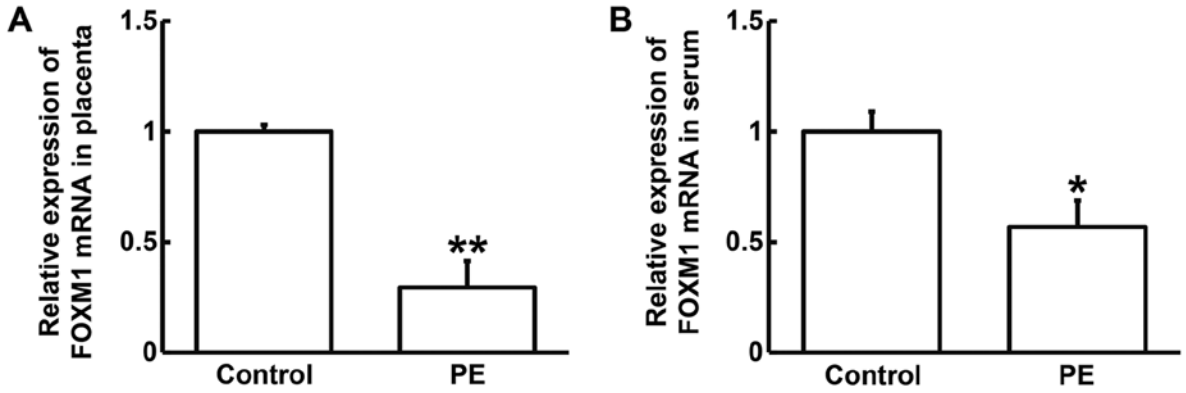

Figure 1. Association between FOXM1 mRNA expression and the occurrence of preeclampsia. Reverse transcription-quantitative PCR was used to measure the expression of FOXM1 mRNA in (A) placental tissues and (B) serum samples from healthy pregnant subjects and PE patients. *P<0.05 and **P<0.01 vs. control. FOXM1, forkhead box M1; PE, preeclampsia.

Enzyme-linked immunosorbent assay (ELISA). FOXM1 ELISA kit (cat. no. CSB-EL008828HU; Cusabio Biotech Co., Ltd.) was used to determine the concentration of FOXM1. In microplates, standards (50 μl) and samples (10 μl serum and 40 μl diluent) were added to certain wells whilst blank wells were left empty. In the wells containing standards and samples, horseradish peroxidase-labeled conjugates (100 μl) were added and the plates were then sealed for incubation at 37˚C for 1 h. After washing the plates five times, substrates A and B (50 μl of each) were added to each well. After incubation at 37˚C for 15 min, stop solution (50 μl) was added into each well, and the absorbance at 450 nm was measured within 15 min of stop solution being added.

Bioinformatics. To understand the regulatory mechanism of FOXM1, miRanda (http://www.microrna.org/microrna/home.do) (17), TargetScan (http://www.targetscan.org) (18), PiTa (http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html) (19), RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/) (20) and PICTA (http://pictar.mdc-berlin.de/) (21) were used to predict miRNAs that might regulate FOXM1.

Dual-luciferase reporter assay. Potential wild-type (WT) and mutant seed regions of miR-21 in the 3'-UTR of FOXM1 gene were first synthesized *in vitro* (Sangon Biotech Co., Ltd.) and then cloned into pMIR-REPORT luciferase reporter plasmids (Thermo Fisher Scientific, Inc.) using *Spe*I and *Hin*dIII restriction sites. Plasmids encoding either WT or mutant 3'-untranslated region (UTR) sequences (0.8 μg) were co-transfected with agomiR-21 (100 nM; Sangon Biotech Co., Ltd.) into 2x10^5 293T cells (The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences) at 37˚C for 24 h according to the manufacturer's manual. As a control, 293T cells were transfected with empty plasmids and agomiR-21 (NC group). After 24 h further incubation, the cells were lysed using the Dual-Luciferase® reporter assay kit (Promega Corporation) according to the manufacturer's protocol, and luminescence intensity was measured using the GloMax® 20/20 luminometer (Promega Corporation). Using *Renilla* luminescence activity as internal reference, the luminescence values of each group of cells were measured.

MTT assay. Cells were first seeded into 96-well plates at a density of 2x10^3 cells/well. Each condition was tested in triplicate. At 24, 48 and 72 h after transfection, 20 μl MTT (5 g/l) solution was added to each well, followed by incubation for 4 h at 37˚C. After aspiration of medium, DMSO (150 μl/well) was added to dissolve the formazan crystals. Absorbance at 490 nm was measured in each well using a microplate reader (Bio-Rad Laboratories, Inc.), and the results were used to plot cell viability curves.

Statistical analysis. Results were analyzed using SPSS 20.0 statistical software (IBM Corp.). Data are expressed as the mean ± standard deviation. Data were tested for normality using the Kolmogorov-Smirnov test. Differences among multiple groups were analyzed using one-way ANOVA, and Student-Newman-Keuls test as post-hoc test. Comparisons between two groups were carried out using Student's t-test. The χ^2 test was used to test the association between PE and miR-21 expression. P<0.05 was considered to indicate a statistically significant difference.

Results

Occurrence of PE is associated with the reduced expression of FOXM1 mRNA. To measure FOXM1 mRNA expression in pregnant women with and without PE, RT-qPCR analysis was performed. The expression of FOXM1 mRNA in placental tissues and serum samples from PE patients was found to be significantly lower compared with that in the control group (P<0.05; Fig. 1A and B). This finding suggests that the occurrence of PE is associated with FOXM1 mRNA expression.

Decreased levels of FOXM1 protein may serve a regulatory role in the occurrence of PE. Western blotting and ELISA were performed to measure FOXM1 protein expression in placental tissues and serum samples, respectively. FOXM1 protein expression in the placental tissues from PE patients was significantly lower compared with that from the control group (P<0.05; Fig. 2A). Similarly, the levels of circulating FOXM1 protein in serum from PE patients was significantly lower compared with that from the control group (P<0.05;

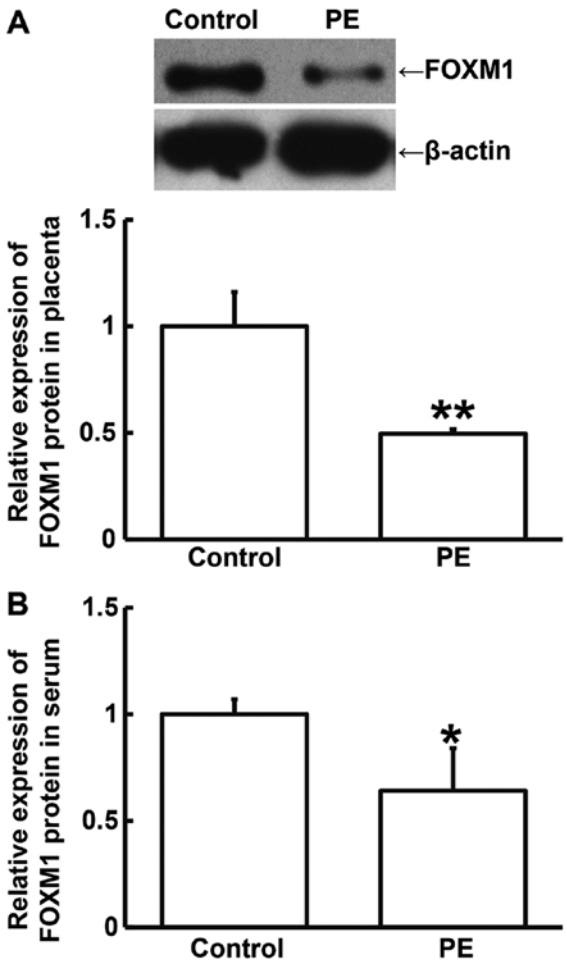

Figure 2. Comparison of relative of FOXM1 protein expression between healthy pregnant subjects and PE patients. Western blotting and ELISA were used to measure the levels of FOXM1 protein in (A) placental tissues and (B) serum samples from all subjects, respectively. *P<0.05 and **P<0.01 vs. control. FOXM1, forkhead box M1; PE, preeclampsia.

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3' aguugUAGUCAGACUAUUCGAu 5'   hsa-miR-21
             ||:|     :||||||
5' cucuaAUUAUAAAUGUAAGCUu 3'   FOXM1
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Figure 3. Prediction of direct interaction between miR-21 and the FOXM1 gene. miRanda (http://www.microrna.org/microrna/home.do), TargetScan (http://www.targetscan.org), PiTa (http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html), RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/) and PICTA (http://pictar.mdc-berlin.de/) were used to predict target genes that might be regulated by miR-21, and indicated that FOXM1 is a potential target gene of miR-21. FOXM1, forkhead box M1; miR, microRNA.

Fig. 2B). These results suggest that decreased FOXM1 protein levels may serve a regulatory role in the occurrence of PE.

miR-21 may serve a regulatory role in the pathology of PE by affecting the expression of FOXM1 at the transcriptional level. Using the listed bioinformatics tools, this present study found 1,100 miRNAs that putatively target FOXM1. For example, when using miRanda for prediction, the mirSVR score was -0.2837 and the PhastCons score was 0.6586. Since the importance of miR-21 in human diseases has been confirmed before, and the score obtained was relatively high, miR-21 was chosen for further study (Fig. 3). RT-qPCR was performed to determine the levels of miR-21 in the placental tissues and serum samples of PE patients and healthy controls. The expression of miR-21 in the placental tissues and serum samples from PE patients was significantly higher compared with those from the control group (P<0.05; Fig. 4). For further analysis, PE patients and control subjects were each divided into a positive group and a negative group. If the relative expression of miR-21, when normalized to the overall median expression levels of miR-21, in the PE patients or control subjects was >1, they were assigned to the positive group, whereas those with a relative miR-21 expression of <1 were allocated to the negative group. Analysis using the χ^2 test did not indicate an association between PE and miR-21 expression (P>0.05; Table I). However, these observations suggest that miR-21 may serve a regulatory role in the pathology of PE by regulating FOXM1 expression.

Table I. Association analysis of miR-21 expression in PE patients and control subjects using the χ^2 test.

Groups	Positive[a]	Negative[b]	Total
Control	18	10	28
PE	22	10	32
P-value			0.714393038
χ^2-value			0.133928571

[a]Relative miR-21 expression of >1 according to RT-qPCR; [b]relative miR-21 expression of <1 according to RT-qPCR analysis. miR, microRNA; PE, preeclampsia; RT-qPCR, reverse transcription-quantitative PCR.

Overexpression of miR-21 may inhibit the viability of HTR8/SVneo cells by reducing the expression of FOXM1. To assess the effect of miR-21 on the expression of FOXM1 and HTR8/SVneo cell viability, RT-qPCR, western blotting and MTT assays were performed following transfection with agomiR-21. The data showed that the expression of miR-21 in cells transfected with agomiR-21 was significantly higher compared with that in the agomiR-NC group (P<0.05; Fig. 5A). In addition, FOXM1 mRNA and protein expression in cells transfected with agomiR-21 was significantly reduced compared with that in the agomiR-NC group (P<0.05; Fig. 5B and C). The MTT assay results showed that the absorbance values of cells transfected with agomiR-21 were significantly lower compared with those in the agomiR-NC group after 48 and 72 h (P<0.05; Fig. 5D). These findings indicate that miR-21 overexpression inhibits HTR8/SVneo cell viability, possibly by reducing the expression of FOXM1.

miR-21 directly binds to the 3'-UTR of FOXM1 to regulate expression. To test whether FOXM1 is a direct target of miR-21, a dual-luciferase reporter assay was performed. The luminescence intensity of the wild-type group was found to be significantly lower compared with that in the NC group (P<0.05), whilst no significant difference was observed between the mutant group and the NC group (P>0.05; Fig. 6). This suggests that miR-21 regulates FOXM1 expression by directly binding to its 3'-UTR.

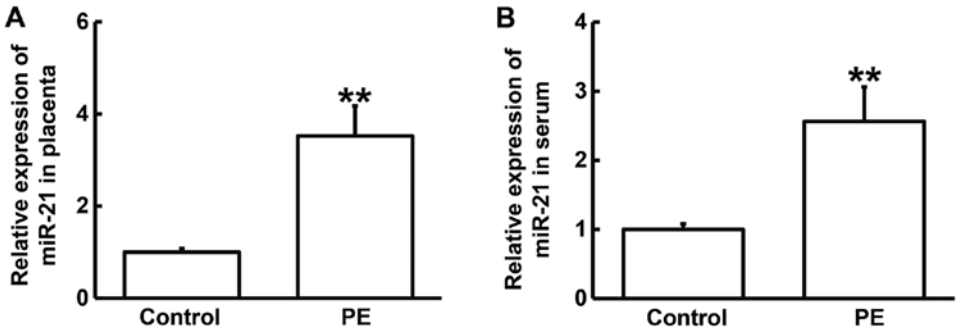

Figure 4. Comparison of relative miR-21 expression between healthy pregnant subjects and PE patients. Reverse transcription-quantitative PCR was used to measure miR-21 expression in (A) placental tissues and (B) blood samples from all subjects. **P<0.01 vs. control. FOXM1, forkhead box M1; PE, preeclampsia. miR, microRNA.

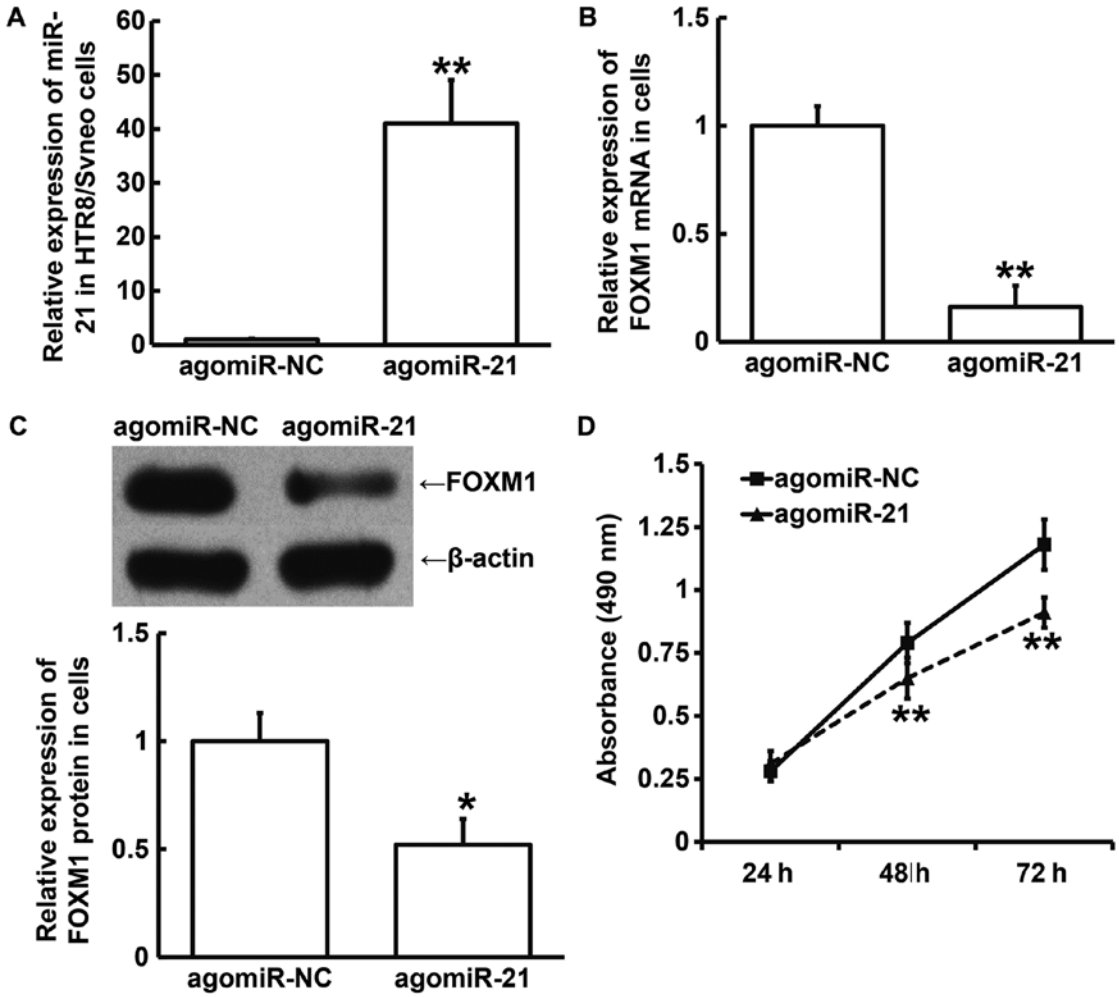

Figure 5. Effect of miR-21 overexpression on HTR8/SVneo cell viability. The expression of (A) miR-21, (B) FOXM1 mRNA and (C) FOXM1 protein in HTR8/SVneo cells transfected with agomiR-NC or agomiR-21. (D) Measurement of cell viability in HTR8/SVneo cells transfected with agomiR-NC or agomiR-21 using MTT. *P<0.05 and **P<0.01 vs. agomiR-NC. FOXM1, forkhead box M1; miR, microRNA.

Discussion

Placental hypoperfusion leads to placental ischemia, hypoxia and metabolic disorders, resulting in the production of placenta-derived toxic factors, such as IL-6, IL-8 and TNF-α (22,23). Additionally, vascular endothelial injury and imbalance of vasoactive substances occur, resulting in a series of severe pathological changes, extensive vascular endothelial injury and finally PE (24). PE is reported to have two stages. The first stage is characterized by placental hypoxia caused by diminished villous trophoblast invasion and impaired vascular remodeling. The second stage is characterized by an imbalance between the reactive oxygen species (ROS) production system and the antioxidant defense system, caused by the secretion of active polypeptides into the maternal blood circulation and deposition of ROS under the vascular endothelium (25,26).

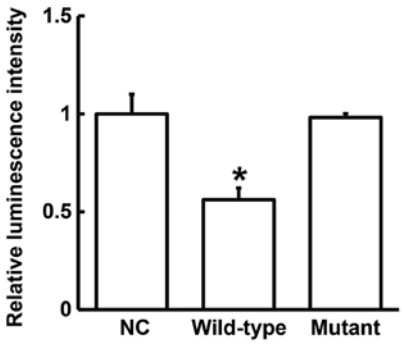

Figure 6. Identification of direct interaction between miR-21 and FOXM1 using a dual-luciferase reporter assay in 293T cells. Plasmids encoding WT or mutant 3'-UTR sequences of FOXM1, or empty plasmid were co-transfected with agomiR-21 into 293T cells. Luciferase activity was subsequently measured 24 h after transfection, and normalized to that of *Renilla*. *P<0.05 vs. agomiR-NC. NC, negative control; miR, microRNA; FOXM1, forkhead box M1; WT, wild-type; 3'-UTR, 3'-untranslated region.

This imbalance between the ROS production system and antioxidant defense system aggravates maternal systemic arteriole injury, leading to hypertension and proteinuria (27). The whole process may be associated with reductions in placental trophoblast infiltration, ischemia, hypoxia, increased apoptosis and abnormal lipid metabolism during pregnancy (28-30).

FOXM1 is widely expressed in proliferating mammalian cells and is an important mitosis-promoting factor (15,31). It serves important roles in cell proliferation, differentiation, senescence, organ formation, DNA damage repair, tumor formation and invasion (32). FOXM1 is expressed in trophoblasts and also in maternal decidual cells (12), whereby it indirectly regulates trophoblast invasion through paracrine signaling to prevent excessive invasion (11). As a result, trophoblast invasion is a tightly regulated process (12). In addition, PE is regulated by various microRNAs. For example, miR-134 has previously been found to inhibit trophoblast cell infiltration in the placenta of patients with PE by reducing integrin subunit β1 expression (33). These reports show that FOXM1 may be closely associated with the pathogenesis of PE.

The present study showed that FOXM1 mRNA and protein expression in PE patients is reduced compared with that in healthy pregnant women. Since FOXM1 may indirectly regulate trophoblast invasion in a paracrine manner as aforementioned, reductions in FOXM1 expression may reduce trophoblast invasion into the endometrium.

As mRNA regulators, miRNAs are widely involved in a number of pathophysiological processes, including tumor cell proliferation, invasion and metastasis, hypertension, diabetes mellitus and atherosclerosis (34). As biomarkers, miRNAs are released into the blood by normal or injured cells and participate in cell signaling transduction and genetic transformation (35). According to the bioinformatics analysis in the present study, miR-21 is closely associated with FOXM1, implicating it as a potential upstream regulator of FOXM1 expression. Previous studies have shown that the expression of miR-21 is aberrantly upregulated in esophageal cancer (36), liver cancer (37), cervical cancer (38), ovarian cancer (39), oral cancer (40), head and neck cancer (41), malignant glioma (42) and chronic lymphocytic leukemia (43). In addition, miR-21 has also been found to be upregulated in cell lines of lung cancer (44), colorectal cancer (45), Hodgkin lymphoma (46) and head and neck cancer (47). Therefore, the abnormal upregulation of miR-21 usually suggests the existence of human diseases. In the present study, it was found that miR-21 expression was elevated in both placenta tissues and peripheral blood samples, consistent with previous reports listed above (36-47). Upregulation of miR-21 expression in HTR8/SVneo cells by agomiR-21 transfection demonstrated that FOXM1 mRNA and protein expression was downregulated in cells overexpressing miR-21, and cell viability was also reduced. Dual-luciferase reporter assay demonstrated that FOXM1 is indeed a direct binding target of miR-21. These observations suggest that the downregulation of FOXM1 by increased miR-21 expression reduces the proliferation of trophoblasts, which attenuates their ability to infiltrate the endometrium. Therefore, miR-21 is a potential target for PE therapy, although its mechanism in trophoblast physiology requires further and more refined research.

In conclusion, the present study demonstrates that the human body regulates the viability of trophoblasts during PE by upregulating miR-21 and thereby downregulating FOXM1 mRNA and protein expression. In addition, miR-21 could possibly be a therapeutic target for PE treatment. However, the exact effect and mechanism of action of miR-21 in PE remain to be investigated further at cellular, animal and clinical levels.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

The final version of the manuscript has been read and approved by all authors. FZ, YS and HW collaborated to design the study. FZ, YS and QG were responsible for performing experiments. FZ, YS and QG analyzed the data. All authors collaborated to interpret results and develop the manuscript.

Ethics approval and consent to participate

All procedures performed in the present study were approved by the Ethics Committee of Nanjing Medical University. Written informed consent was obtained from all patients or their families.

Patient consent for publication

Written informed consent for the publication of any associated data and accompanying images were obtained from all patients or their parents, guardians or next of kin.

Competing interests

The authors declare that they have no competing interests.

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