

miR-148a targets XBP1 to regulate trophoblast apoptosis induced by plasma reticulum stress in preeclampsia

Na Li, MM^{a,*}, Jinqiong Li, MM^a, Jiantao Jia, PhD^b, Xia Yao, MM^a, Yan Zhu, MM^a

Abstract

To study the relationship between miR-148a and preeclampsia (PE), and clarify that miR-148a can regulate the endoplasmic reticulum stress (ERS) of placental trophoblasts by targeting the ERS protein X box binding protein 1 (XBP1).

Fifty patients with hypertension during pregnancy, patients with mild PE, patients with severe PE, and normal pregnant women were selected, and their placental tissues were collected. RT-polymerase chain reaction was used to detect the expression of miR-148a in placental tissues, and Western blot was used to detect XBP1 in placental tissues. Compare the expression differences of miR-148a and XBP1 in each group, and analyze the correlation between the expressions of the two.

Compared with the Neg-miR group, MTT experiment result in pre-miR-148a group was decreased. MTT experiment result in anti-miR-148a group was increased. Cell cycle test result in pre-miR-148a group [G1 (%)] was increased. Cell cycle test result in anti-miR-148a group [S (%)] was increased. Apoptosis test result in pre-miR-148a group [early apoptotic cells (%), late apoptotic cells (%)] was increased. Apoptosis test result in anti-miR-148a group [early apoptotic cells (%), late apoptotic cells (%)] was decreased. XBP1 expression result in pre-miR-148a group was increased. XBP1 expression result in anti-miR-148a group was decreased. Compared with the normal population, XBP1 is expressed in hypertension, mild eclampsia, severe eclampsia increased. GRP78, CHOP, and caspase-12 expression result in pre-miR-148a group was increased. GRP78, CHOP, and caspase-12 expression result in anti-miR-148a group was decreased.

miR-148a can regulate the ERS-mediated apoptosis by targeting XBP1, thereby intervening in the occurrence and development of PE.

Abbreviations: ERS = endoplasmic reticulum stress, miRNA = microRNA, PCR = polymerase chain reaction, PE = preeclampsia, XBP1 = X box binding protein 1.

Keywords: endoplasmic reticulum, miR-148a, preeclampsia, trophoblast apoptosis, X box binding protein 1

1. Introduction

Preeclampsia (PE) is the most common complication of pregnancy, with an incidence of about 3% to 5% of total pregnancy, and it is one of the important causes of maternal and

fetal death.^[1] At present, there is no specific treatment for PE, and termination of pregnancy is the only method. The lack of in-depth understanding of the pathogenesis of PE is the main factor limiting the level of clinical diagnosis and treatment of PE. Studies have shown that insufficient endometrial spiral artery remodeling is closely related to the occurrence and development of PE.^[2] The excessive apoptosis of placental trophoblasts is the main cause of insufficient endometrial spiral artery remodeling.^[3] Excessive apoptosis of trophoblast cells can increase the number of syncytiotrophoblast microparticles released into the maternal blood, induce systemic inflammatory response, cause systemic vascular endothelial damage, and trigger various clinical signs of PE.^[4] Therefore, inhibiting excessive apoptosis of placental trophoblast cells is an important strategy in anti-PE therapy.^[5]

Endoplasmic reticulum stress (ERS) can mediate the apoptosis of placental trophoblast cells and play an important role in the occurrence and development of PE. The research on ERS not only helps to further understand the molecular mechanism of PE-induced apoptosis of placental trophoblasts, but also provides new explanations for the clinical prevention and treatment of PE. ERS can mediate the apoptosis of placental trophoblast cells and play an important role in the occurrence and development of PE. Research on ERS not only helps to further understand the molecular mechanism of placental trophoblast cell apoptosis caused by PE, but also serves as a preparatory link for the clinical prevention and treatment of PE. Studies have found that the key ERS protein X box binding protein 1 (XBP1) is highly expressed

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The study was approved by the Heji Hospital. Informed consent was obtained.

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^a Heji Hospital, Changzhi City, Shanxi Province, China, ^b Changzhi Medical College, Changzhi City, Shanxi Province, China.

* Correspondence: Na Li, Heji Hospital, No. 271 Taihang East Street, Changzhi City 046000, Shanxi Province, China (e-mail: lina_65@21cn.com).

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in the placenta tissue of PE pregnant women, and there is a binding site between miR-148a and XBP1. The 2 have a targeting relationship. Therefore, we propose the following scientific hypothesis: miR-148a can regulate the apoptosis of cytoplasmic reticulum mediated by ERS by targeting XBP1, thereby intervening in the occurrence and development of PE. Apoptosis and necrosis are 2 common ways of cell death, and they have completely different characteristics.^[5] Apoptosis is the main manifestation of programmed cell death and an active physiological response mechanism. Necrosis is a passive reaction and degeneration process of cells to severe damage. In order to confirm this hypothesis, in this study, we intend to use ERS as the starting point, miR-148a targets the effect of XBP1 on the apoptosis of cytokines, and provides experimental evidence and theoretical support for the clinical prevention and treatment of PE.

2. Materials and methods

2.1. Research object

Case inclusion: From January 2020 to December 2020, 50 patients with hypertension during pregnancy, patients with mild PE, patients with severe PE, and normal pregnant women admitted to our hospital were selected. The diagnostic criteria of hypertension during pregnancy, mild PE, and severe PE refer to the 9th edition textbook of Obstetrics and Gynecology. Pregnant women suffering from essential hypertension, heart disease, chronic kidney disease, hepatitis, diabetes, infectious diseases during pregnancy or other pregnancy complications are not included in this trial. Sample collection: Save 30 to 50g of placental tissue for testing during delivery and store at -20°C . The study was approved by the Heji Hospital. Informed consent was obtained.

To analyze the differences in the expression of miR-148a and XBP1 in placental tissues of patients with hypertension during pregnancy, patients with mild PE, patients with severe PE, and normal pregnant women. Spearman method analyzes the correlation between miR-148a and XBP1. Collect maternal gestational age, blood pressure, urine protein, plasma albumin, fetal weight and other clinical indicators, and analyze their relationship with miR-148a and XBP1.

2.2. Verification of the targeting relationship between miR-148a and XBP1

Using trophoblast genomic DNA as template, construct wild-type XBP1 3'-UTR (XBP1-wt) plasmid and mutant XBP1 3'-UTR. Collect the pair of trophoblast cells, adjust the cell density to 2×10^5 cells/well with 1640 medium (10% fetal bovine serum + 1% double antibody), and combine XBP1-wt, mutant XBP1 3'-UTR and Neg-miR or pre-miR-148a was co-transfected into the cells, and the cells were incubated at 37°C and 5% CO_2 for 48 hours. The cell luciferase activity was measured, and the Renilla plasmid fluorescence value was used as an internal reference.

2.3. In vitro culture and transfection of trophoblasts

The trophoblast cell HTR-8/SVneo is routinely resuscitated, and the cells are cultured in RPMI1640 medium containing 1% double antibody + 10% fetal bovine serum, and incubated at

37°C and 5% CO_2 . The cells in the logarithmic growth phase were collected and seeded in a 6-well culture plate with 2×10^5 cells/well. After the cells were placed in a 37°C , 5% CO_2 constant temperature incubator for 24 hours, the cells were randomly divided into 3 groups. There are 3 wells in each group, namely Neg-miR group, pre-miR-148a group, and anti-miR-148a group. Each group was transfected with Neg-miR, pre-miR-148a, and anti-miR-148a. The cells were kept in a 37°C , 5% CO_2 constant temperature incubator for 24 hours, and the transfection was completed.

2.4. Cell proliferation activity

Collect the transfected cells and place the cells in a constant temperature culture at 37°C and 5% CO_2 . After culturing for 24 hours, 36 hours and 72 hours, the supernatant was discarded by centrifugation. Add $20 \mu\text{L}$ of 5 mg/mL MTT solution to each well, continue to incubate at 37°C and 5% CO_2 for 4 hours, centrifuge and discard the supernatant. Add $100 \mu\text{L}$ of DMSO, shake until the crystal is fully solution, and measure the OD value at 450 nm with an automatic microplate reader.

2.5. Apoptosis and cycle

Collect the transfected cells, wash them twice with PBS, add $500 \mu\text{L}$ of binding buffer to resuspend the cells, add the corresponding apoptosis or cell cycle reagents to treat the cells, refer to the kit instructions for specific methods. Incubate at room temperature and dark for 15 minutes, and detect on flow cytometer.

2.6. miR-148a detection

RT-polymerase chain reaction (PCR) method was used to detect the expression of miR-148a in placenta tissue. The total RNA was extracted according to the instructions of the Trizol kit, and the total RNA was reverse transcribed to cDNA, and then RT-PCR (SYBR Green method) was performed, using U6 as the internal control.

2.7. XBP1 detection

Western blot was used to detect the expression of XBP1 protein in placental tissue, extract tissue protein, and determine the protein concentration of the sample. Take an appropriate amount of samples for SDS-PAGE gel electrophoresis, transfer to nitrocellulose membrane, place the membrane in 5% blocking solution at 4°C for 4 hours, and then add XBP1 monoclonal antibody (1:2000) and secondary antibody (1:500) in sequence. Incubate, and perform electrochemiluminescence detection according to the ECL kit instructions. The images of bands were quantified with Image-Pro Plus 6.0.

2.8. Endoplasmic reticulum stress

RT-PCR and western blot were used to detect the mRNA, protein expression and phosphorylation of GRP78, CHOP, caspase-12, and other ERS marker molecules. RT-PCR and western blot were used to detect the mRNA, protein expression and phosphorylation of related proteins in the IRE1-XBP1 signaling pathway.

Table 1**MTT experiment results in different group ($\bar{x} \pm s$).**

Group	0 h	24 h	36 h	72 h
Neg-miR	0.246 ± 0.004	0.392 ± 0.009	0.564 ± 0.008	0.750 ± 0.039
pre-miR-148a	0.242 ± 0.025	0.341 ± 0.035	0.473 ± 0.012*	0.587 ± 0.040*
anti-miR-148a	0.248 ± 0.005	0.438 ± 0.016	0.646 ± 0.021*	0.822 ± 0.039*

* $P < .05$ vs Neg-miR.**2.9. Statistical analysis**

Data were described as the means ± standard deviation and analysed statistically with SPSS version 13.0. One-way ANOVA was used to detect the differences in changes between the groups of various treatments after establishing if the data were normally distributed and equivalency of variances. P value (the probabilities) less than .05 were considered statistically significant.

3. Results**3.1. MTT experiment results**

MTT is often used to detect cell proliferation ability. According to the measured absorbance value to judge the number of living cells, the greater the measured absorbance value, the stronger the cell viability.

As shown in Table 1, compared with the Neg-miR group, MTT experiment result in pre-miR-148a group (36 hours and 72 hours) was decreased ($P < .05$). Compared with the Neg-miR group, MTT experiment result in anti-miR-148a group (36 hours and 72 hours) was increased ($P < .05$).

3.2. Cell cycle test results

The cell cycle refers to the time it takes for a generation of cells. From the end of one cell division to the end of the next division is a cycle. The cell cycle reflects the rate of cell proliferation. Flow cytometry can be used to determine the DNA content of cells, and then according to the distribution of DNA content. Cell cycle and apoptosis analysis can be performed.

As shown in Table 2, compared with the Neg-miR group, cell cycle test result in pre-miR-148a group [G1 (%)] was increased

Table 2**Cell cycle test results ($\bar{x} \pm s$).**

Group	G1 (%)	S (%)	G2 (%)
Neg-miR	45.22 ± 1.59	29.96 ± 0.89	19.28 ± 1.89
pre-miR-148a	51.06 ± 1.39*	24.57 ± 1.63	21.65 ± 0.37
anti-miR-148a	41.18 ± 0.24	38.41 ± 2.94*	15.14 ± 2.79

* $P < .05$ vs Neg-miR.**Table 3****Apoptosis test results ($\bar{x} \pm s$).**

Group	Normal living cells (%)	Early apoptotic cells (%)	Late apoptotic cells (%)	Necrotic cell (%)
Neg-miR	93.9 ± 0.95	4.3 ± 0.17	1.8 ± 0.82	0.0 ± 0
pre-miR-148a	89.6 ± 0.56	6.7 ± 0.47*	3.7 ± 0.45*	0.0 ± 0
anti-miR-148a	97.40 ± 0.60	2.60 ± 0.60*	0.00 ± 0.00*	0.0 ± 0

* $P < .05$ vs Neg-miR.

($P < .05$). Compared with the Neg-miR group, cell cycle test result in anti-miR-148a group [S (%)] was increased ($P < .05$).

3.3. Apoptosis test results

As shown in Table 3, compared with the Neg-miR group, apoptosis test result in pre-miR-148a group [early apoptotic cells (%), late apoptotic cells (%)] was increased ($P < .05$). Compared with the Neg-miR group, apoptosis test result in anti-miR-148a group [early apoptotic cells (%), late apoptotic cells (%)] was decreased ($P < .05$) (Fig. 1).

3.4. Dual luciferase test results

Transfection refers to the process by which eukaryotic cells acquire new genetic markers due to the incorporation of foreign DNA. The probability of integration of foreign DNA into the chromosome is very small, about $1/10^4$ of the transfected cells can be integrated, usually some selectable markers are required to obtain a stable transfected homologous cell line (Table 4).

3.5. PCR test results

PCR is a method of enzymatically synthesizing specific DNA fragments in vitro. It consists of several steps of high-temperature denaturation, low-temperature annealing (renaturation), and temperature-appropriate extension to form a cycle, which is carried out in cycles, so that the target DNA can be rapidly amplified. It has the characteristics of strong specificity, high sensitivity, simple operation and time saving.

It can be used not only for basic research such as gene isolation, cloning and nucleic acid sequence analysis, but also for disease diagnosis or any place where DNA and RNA are present. PCR is also known as cell-free molecular cloning or in vitro primer-directed enzymatic amplification technology of specific DNA sequences (Figs. 2–9).

3.6. Western blot results

As shown in Figure 10, compared with the Neg-miR group, XBP1 expression result in pre-miR-148a group was increased ($P < .05$). Compared with the Neg-miR group, XBP1 expression result in anti-miR-148a group was decreased ($P < .05$).

As shown in Figure 11, compared with the normal population, XBP1 is expressed in hypertension, mild eclampsia, and severe eclampsia increased ($P < .05$).

As shown in Figure 12, compared with the Neg-miR group, GRP78, CHOP, and caspase-12 expression result in pre-miR-148a group was increased ($P < .05$). Compared with the Neg-miR group, GRP78, CHOP, and caspase-12 expression result in anti-miR-148a group was decreased ($P < .05$).

4. Discussion

Recent studies have found that ERS is closely related to trophoblast cell apoptosis. The endoplasmic reticulum is an organelle in eukaryotic cells, which regulates protein synthesis and folding and aggregation after synthesis. It is also a place to regulate intracellular Ca^{2+} levels. When its function is disordered, there will be disturbances in the balance of Ca^{2+} concentration and the aggregation of misfolded and unfolded proteins. Can cause ERS.^[6,7] Short-term and moderate ERS can maintain the homeostasis of the endoplasmic reticulum and help protect cell survival. However, if there

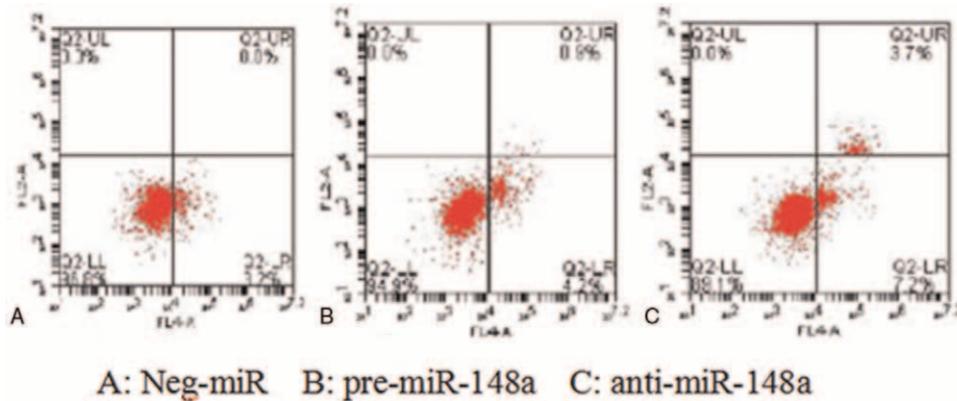


Figure 1. Apoptosis test results.

Table 4

Dual luciferase test results ($\bar{x} \pm s$).

Group	Firefly luciferase	Renilla Luciferase	Ratio
PRL-TK empty + pGL3-blank	52392.54 ± 2628.68	60051.89 ± 8191.43	0.88 ± 0.12
PRL-TK empty + pGL3-empty + Pcdna3.1-WT XBP1 empty	47589.21 ± 4158.47	55940.30 ± 1363.19	0.85 ± 0.06
PRL-TK empty + pGL3-empty + Pcdna3.1-WT XBP1	55477.88 ± 7154.93	56593.74 ± 289.43	0.98 ± 0.12
PRL-TK empty + pGL3-empty + Pcdna3.1-MUT XBP1	53201.55 ± 831.50	53681.83 ± 1521.96	0.99 ± 0.04
PRL-TK-Neg-miR-148a + pGL3 + Pcdna3.1	53483.62 ± 2832.92	58083.16 ± 3840.15	0.92 ± 0.04
PRL-TK-pre-miR-148a + pGL3 + Pcdna3.1 WT XBP1	55298.67 ± 2650.73	34629.31 ± 3384.46	1.60 ± 0.13
PRL-TK-pre-miR-148a + pGL3 + Pcdna3.1 MUT XBP1	55482.32 ± 4154.90	59424.53 ± 1446.75	0.93 ± 0.05

XBP1 = X box binding protein 1.

is high-intensity or long-term stimulation, the dysfunction of the endoplasmic reticulum cannot be corrected, which will cause damage to the endoplasmic reticulum, that is, start the apoptosis program, cause cell dysfunction or even death.^[8] A large number of studies have confirmed that continuous or excessively strong ERS can activate the apoptotic pathway, thereby causing ERS-related trophoblast cell apoptosis.^[9] It

has been clear that regulating trophoblast cell apoptosis is one of the important measures to treat PE.

Our previous studies showed that when the expression of XBP1 in the placental tissues of PE pregnant women and normal pregnant women was different, we found that the expression level of XBP1 mRNA in the placental tissues of PE pregnant women was significantly higher than that of normal pregnant

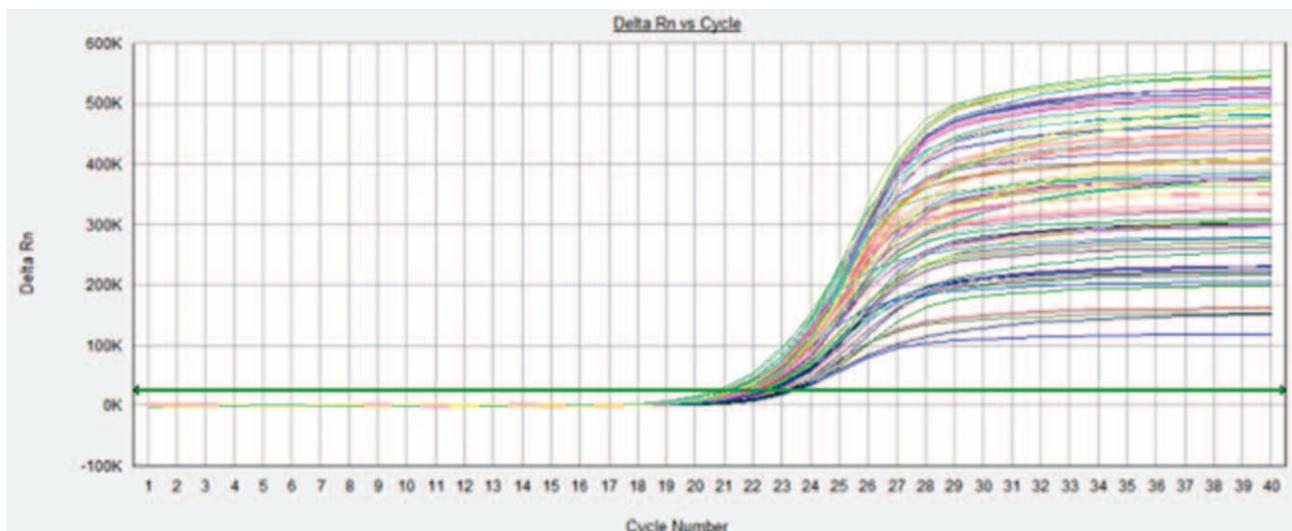


Figure 2. XBP1 mRNA amplification curve. XBP1 = X box binding protein 1.

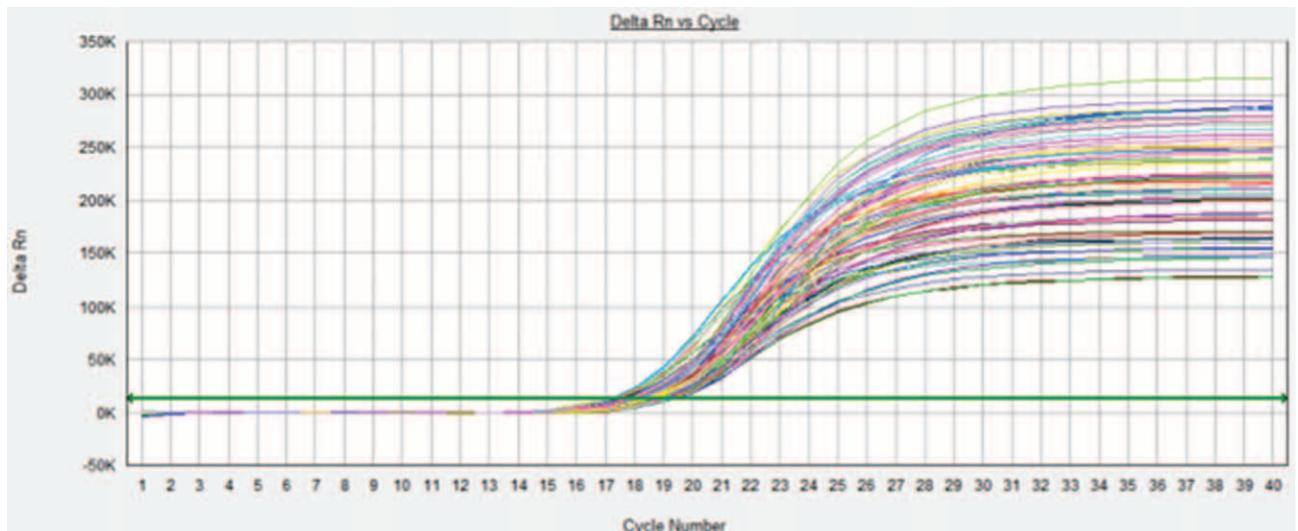


Figure 3. β -Actin mRNA amplification curve.

women ($P < .05$). And it was found that the key ERS protein XBP1 was highly expressed in the placenta tissue of PE pregnant women, and there was a binding site between miR-148a and XBP1.

When ERS occurs, cells respond through 3 main signal transduction pathways, which induce apoptosis: IRE1XBP1 pathway, ATF6 pathway and PERK pathway.^[10] XBP1 belongs to the cyclic adenylyl response element binding protein/activating factor family member, and is also a member of the ERS signal network Important transcription factor.^[11] Research by Gu et al^[12] showed that inhibiting XBP1 expression can significantly inhibit the activation of IRE1-XBP1 pathway, thereby inhibiting cell apoptosis caused by ERS. The above studies suggest that XBP1 is an important target in the mechanism of apoptosis induced by ERS.

MicroRNA (miRNA) is a type of endogenous single-stranded noncoding miRNA molecules with a length of 19-23 nt, which can be combined with the 3'-untranslated region (UTR) of downstream target genes. Realize the regulation of the post-transcriptional level of cell genes, and then participate in the processes of cell proliferation, apoptosis and signal transduction.^[13] The view that abnormal expression or function of miRNA can mediate the development of PE has been generally accepted.^[14,15] miR-148a is a newly discovered miRNAs located on human 7q15.2 chromosome.^[16-18] Xu^[19] found that miR-148a is closely related to ERS regulation when screening miRNAs related to ERS,^[12] suggesting that miR-148a is an important regulator of ERS, and its mechanism of action may be related to targeting XBP1. However, this study also has limitations. In the future, animal studies will be added to

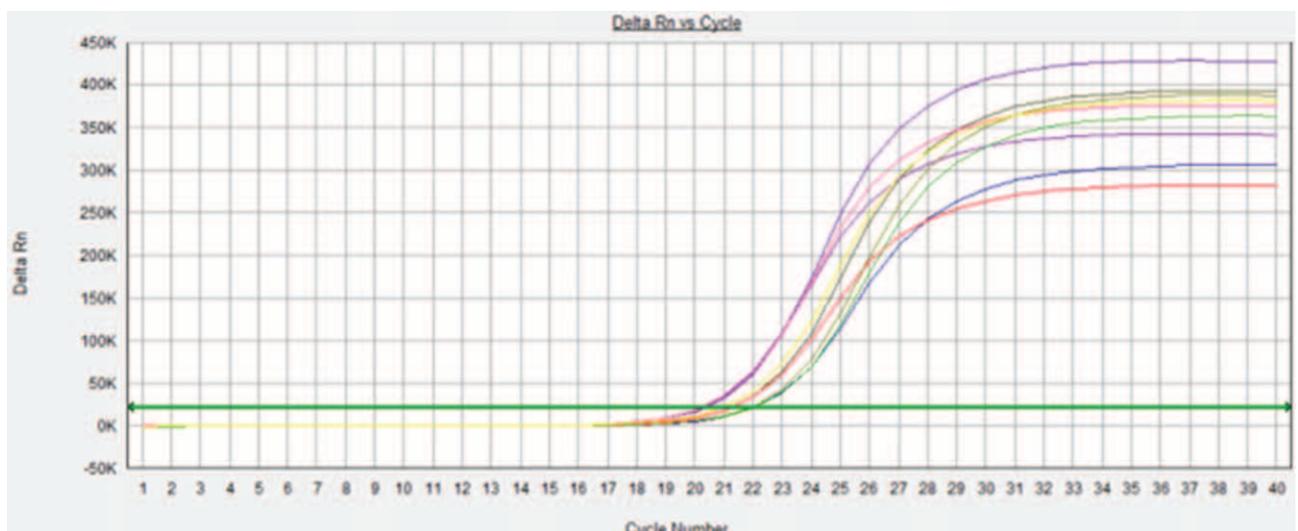


Figure 4. miR-148 RNA amplification kinetic curve.

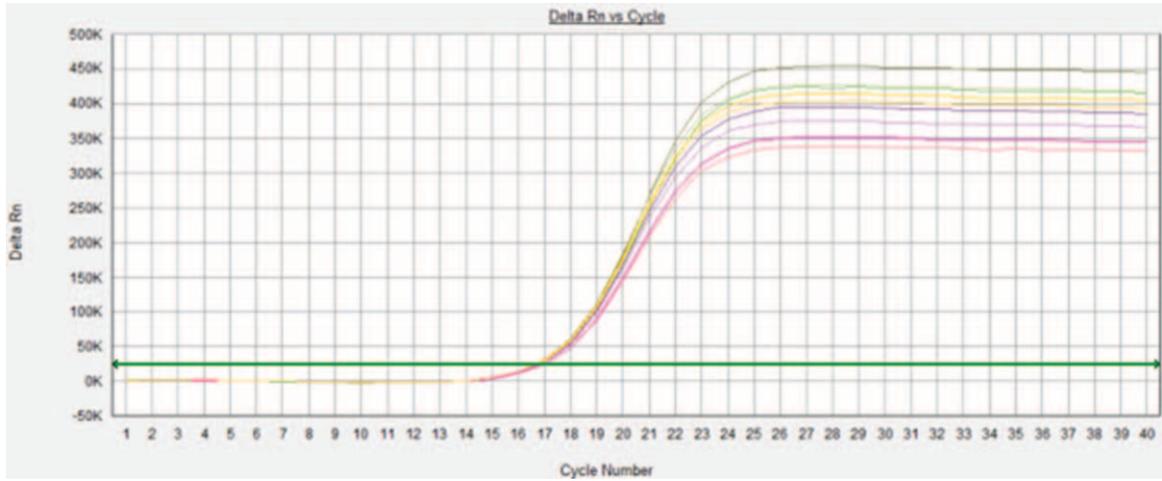


Figure 5. U6 RNA amplification kinetic curve.

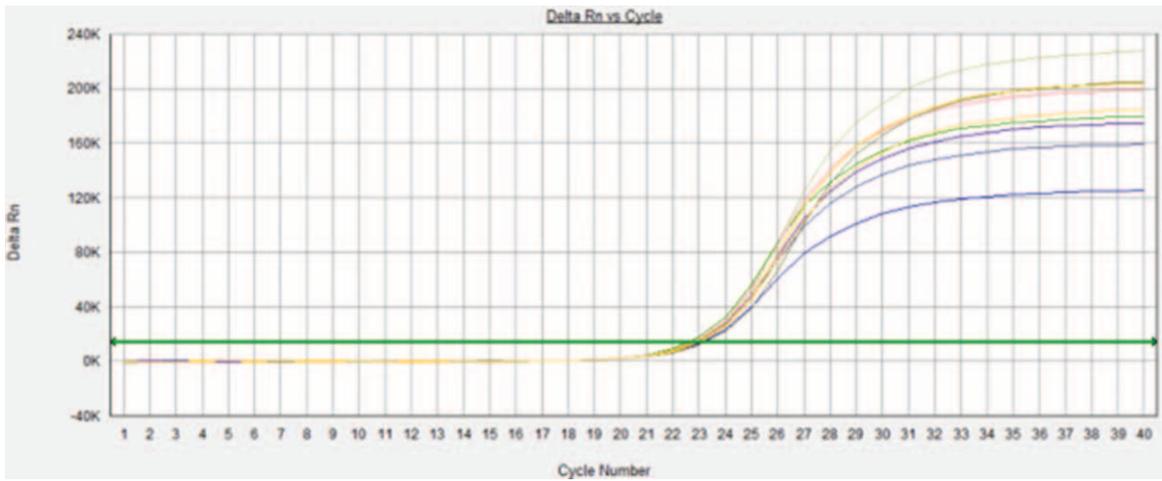


Figure 6. GRP78 mRNA amplification curve.

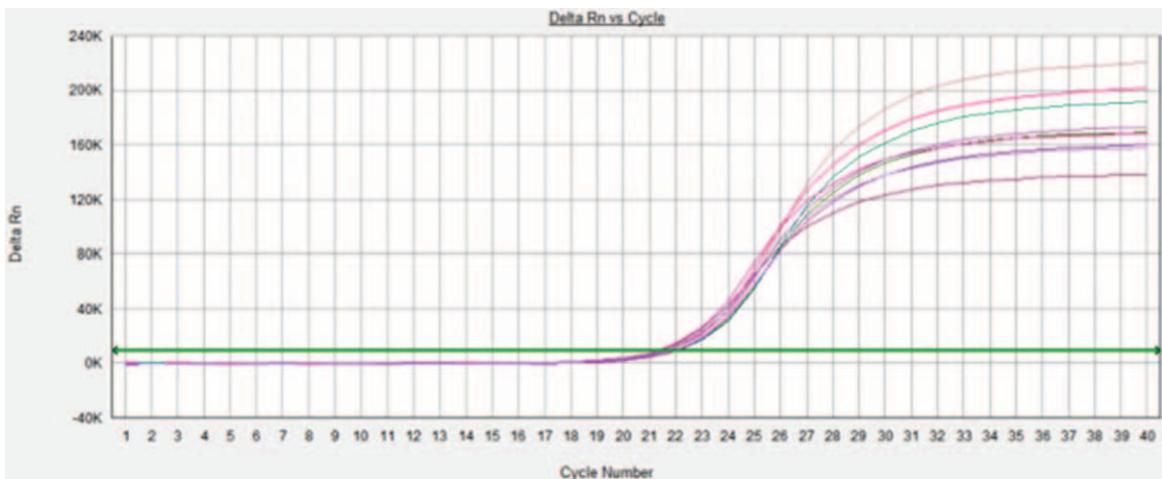


Figure 7. CHOP mRNA amplification curve.

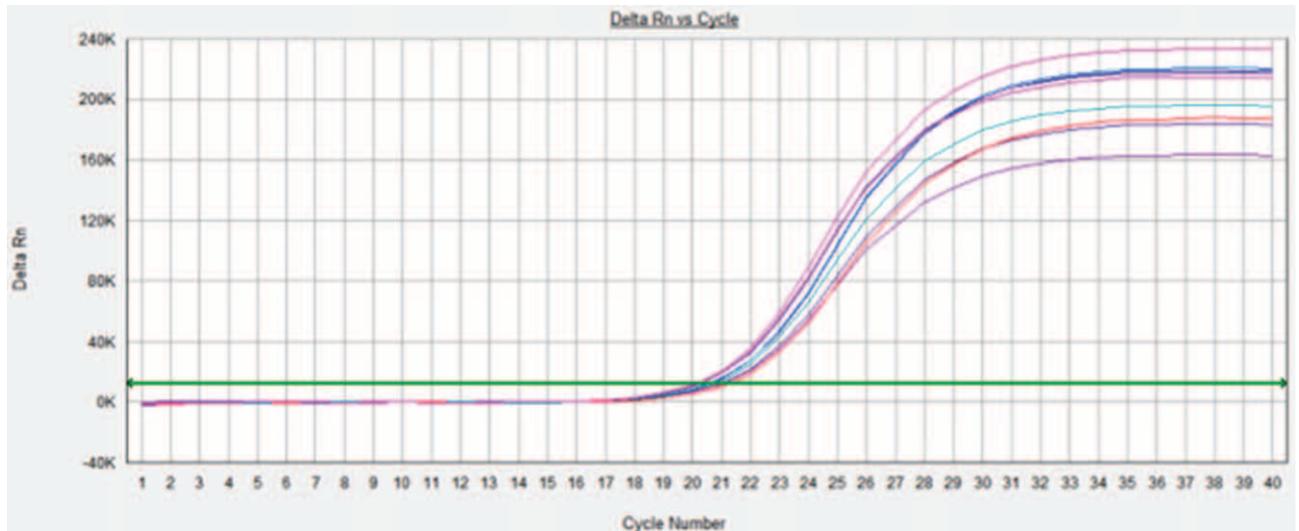


Figure 8. Caspase-12 mRNA amplification curve.

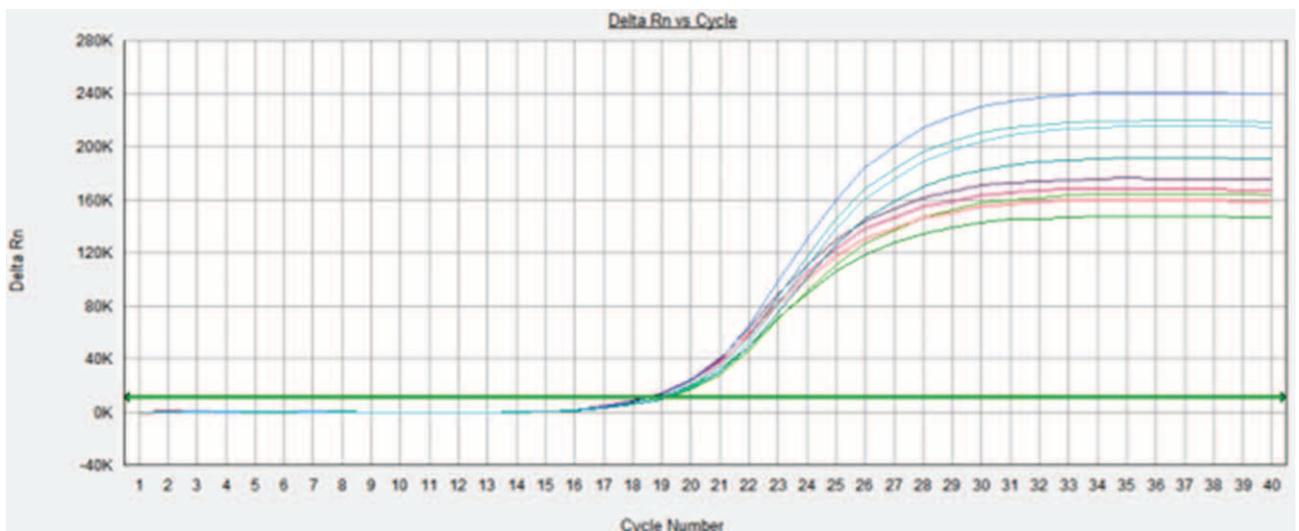


Figure 9. beta-Actin mRNA amplification curve.

confirm that miR-148a targets XBP1 to interfere with the occurrence and development of PE in vivo.

miR-148a can regulate ERS-mediated apoptosis by targeting XBP1, thereby intervening in the occurrence and development of PE.

Author contributions

NL is responsible for the guarantor of integrity of the entire study, study concepts & design, literature research, experimental studies, data acquisition, data analysis, statistical analysis, manuscript editing; JQL is responsible for the study design, definition of intellectual content, experimental studies, manuscript editing; JTJ is responsible for the clinical studies, data acquisition, data analysis, statistical analysis, manuscript review; XY is responsible for the literature research, experimental studies, data acquisition, data analysis, statistical analysis; YZ is responsible for the experimental studies, manuscript preparation & editing. All authors read and approved the final manuscript.

Conceptualization: Na Li, Jinqiong Li.

Data curation: Na Li, Jiantao Jia, Xia Yao.

Formal analysis: Na Li, Xia Yao.

Methodology: Na Li, Jinqiong Li, Xia Yao, Yan Zhu.

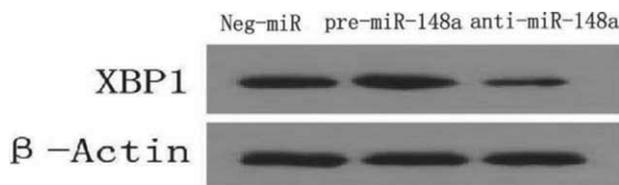


Figure 10. XBP1 expression in Neg-miR, pre-miR-148a, and anti-miR-148a groups. XBP1 = X box binding protein 1.

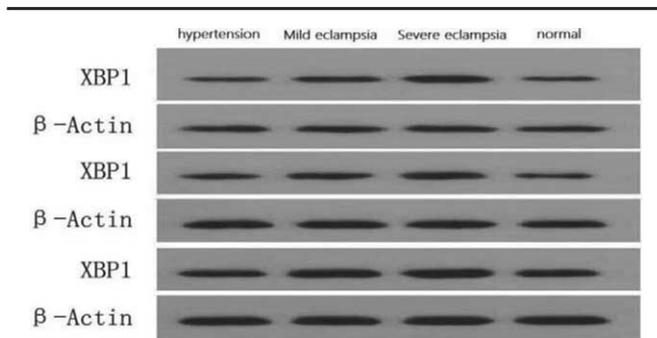


Figure 11. XBP1 is expressed in hypertension, mild eclampsia, severe eclampsia, and normal population. XBP1 = X box binding protein 1.

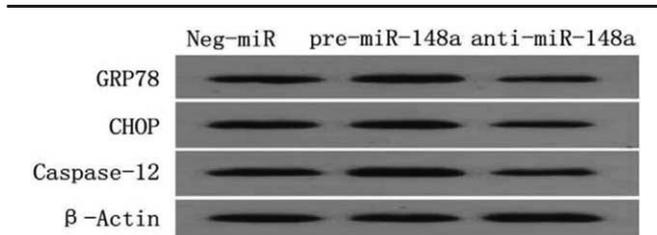


Figure 12. GRP78, CHOP, and caspase-12 expression in Neg-miR, pre-miR-148a, and anti-miR-148a groups.

Resources: Jiantao Jia, Xia Yao.

Writing – original draft: Yan Zhu.

Writing – review & editing: Na Li, Jinqiong Li, Jiantao Jia, Yan Zhu.

References

- [1] Harmon AC, Cornelius DC, Amaral LM, et al. The role of inflammation in the pathology of preeclampsia. *Clin Sci (Lond)* 2016;130:409–19.
- [2] Tong C, Feng X, Chen J, et al. G protein-coupled receptor 30 regulates trophoblast invasion and its deficiency is associated with preeclampsia. *J Hypertens* 2016;34:710–8.

- [3] Tannetta D, Masliukaite I, Vatish M, Redman C, Sargent I. Update of syncytiotrophoblast derived extracellular vesicles in normal pregnancy and preeclampsia. *J Reprod Immunol* 2017;119:98–106.
- [4] Kohli S, Ranjan S, Hoffmann J, et al. Maternal extracellular vesicles and platelets promote preeclampsia via inflammasome activation in trophoblasts. *Blood* 2016;128:2153–9.
- [5] Lu J, Zhou WH, Ren L, Zhang YZ. CXCR4, CXCR7, and CXCL12 are associated with trophoblastic cells apoptosis and linked to pathophysiology of severe preeclampsia. *Exp Mol Pathol* 2016;100:184–91.
- [6] Korner CJ, Du X, Vollmer ME, et al. Endoplasmic reticulum stress signaling in plant immunity – at the crossroad of life and death. *Int J Mol Sci* 2015;16:26582–98.
- [7] Brown M, Strudwick N, Suwara M, Pajeroska-Mukhtar KM. An initial phase of JNK activation inhibits cell death early in the endoplasmic reticulum stress response. *J Cell Sci* 2016;129:2317–28.
- [8] Marton M, Kurucz A, Lizák B, Margittai É, Bánhegyi G, Kapuy O. A systems biological view of life-and-death decision with respect to endoplasmic reticulum stress-the role of PERK pathway. *Int J Mol Sci* 2017;18:58.
- [9] Zhang H, Zhang MH. miRNA-106 regulates endoplasmic reticulum stress-induced trophoblast apoptosis in gestational diabetes by regulating endoplasmic reticulum protein 44. *Chin Med Clin* 2017;17:1589–91.
- [10] Xu C, Baillymaitre B, Reed JC. Endoplasmic reticulum stress: cell life and death decisions. *J Clin Invest* 2005;115:2656–64.
- [11] Zhao WJ, Zhu HF, Zhou JH, Li XZ, Liu YN. Effects of endoplasmic reticulum stress on the transcription of XBP1 promoter by ATF6. *Chin Med J Chin People's Lib Army* 2012;37:317–21.
- [12] Gu XF, Deng XF, Ma QF, Miao YU, Arshad M, Zhou LK. RNF13 regulates endoplasmic reticulum stress-mediated apoptosis through the IRE1/XBP-1 pathway. *Adv Mod Biomed* 2016;16:801–5.
- [13] Zhou Y, Song Y, Shaikh Z, et al. MicroRNA-155 attenuates late sepsis-induced cardiac dysfunction through JNK and β -arrestin 2. *Oncotarget* 2017;8:4731747329.
- [14] Salomon C, Guanzon D, Scholz-Romero K, et al. Placental exosomes as early biomarker of preeclampsia: potential role of exosomal microRNAs across gestation. *J Clin Endocrinol Metab* 2017;102:3182–94.
- [15] Yang X, Zhang J, Ding Y. Association of microRNA-155, interleukin 17A, and proteinuria in preeclampsia. *Medicine* 2017;96:e6509.
- [16] Xu X, Fan Z, Kang L, et al. Hepatitis B virus X protein represses miRNA-148a to enhance tumorigenesis. *J Clin Invest* 2013;123:630–45.
- [17] Chen Y, Min L, Zhang X, et al. Decreased miRNA-148a is associated with lymph node metastasis and poor clinical outcomes and functions as a suppressor of tumor metastasis in non-small cell lung cancer. *Oncol Rep* 2013;30:1832–40.
- [18] Li HP, Huang HY, Lai YR, et al. Silencing of miRNA-148a by hypermethylation activates the integrin-mediated signaling pathway in nasopharyngeal carcinom. *Oncotarget* 2014;5:7610–24.
- [19] Xu P. Screening of MicroRNAs Related to Endoplasmic Reticulum Stress and Verification of its Targets. *Huazhong Agricultural University*:2012.