Tropical Journal of Pharmaceutical Research February 2023; 22 (2): 261-270
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i2.6

Original Research Article

MiR-19a promotes proliferation and inhibits apoptosis of placental trophoblasts via PTEN pathway

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Sent for review: 9 November 2022 *Revised accepted: 28 January 2023*

Abstract

Purpose: *To screen the micro-ribonucleic acids (miRNAs) in the pathological processes of preeclampsia (PE), and to assess their effects on the proliferation and apoptosis of HTR-8/SVneo cells.*
Methods: *The miRNAs in PE patients and healthy puerperae were screened out using miRNA microarray assay, and verified through low-throughput quantitative reverse transcription- polymerase chain reaction (qRT-PCR). The effects of expression level of miR-19a on proliferation and cycle of HTR-8/SVneo cells were determined using cell counting kit (CCK)-8 assay and propidium iodide (PI) staining, respectively. The influence of the expression level of miR-19a on apoptosis of HTR-8/SVneo cells was evaluated using Annexin V-fluorescein isothiocyanate (FITC)/PI staining. Subsequently, luciferase reporter gene assay, CCK-8 assay and Annexin V-FITC/PI staining were conducted, respectively.*
Results: *Serum miR-19a was significantly decreased in PE patients, and a high level of miR-19a led to increase in the proliferation of HTR-8/SVneo cells. Decreased level of miR-19a arrested HTR-8/SVneo cell cycle at G1 phase, while increase in the miR-19a level repressed the apoptosis of HTR-8/SVneo cells. The PTEN was a direct target of miR-19a, and overexpression of miR-19a promoted the activation of Akt signaling pathway, thereby significantly decreasing the expression levels of downstream phosphorylated p21/p21 (p-p21/p21), p-p27/p27 and p-glycogen synthase kinase-3 beta (GSK3β)/GSK3β. Compared with those in cells transfected with empty vectors, the pro-proliferative and anti-apoptotic effects of miR-19a weakened in HTR-8/SVneo cell lines with overexpressed PTEN.*
Conclusion: *MiR-19a targets PTEN in order to activate Akt signaling pathway, thereby promoting proliferation, and inhibiting apoptosis of HTR-8/SVneo cells. This provides new drug targets that can be investigated for future treatment of PE.*

Keywords: *PTEN, Preeclampsia, Trophoblast proliferation, Apoptosis*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Preeclampsia (PE) is an obstetric syndrome mainly pathologically characterized by hypertension and proteinuria after 20 weeks of gestation. As one of the major challenges facing modern obstetrics, it causes death of 63,000 women worldwide each year [1,2]. According to the epidemiological analyses of PE from 1990 to 2010, PE is an important cause of neonatal and maternal mortality and responsible for more than 99 % of maternal deaths in low- and middle-

income countries [3]. In developed countries such as America, PE is the leading cause of poor survival outcomes of 5 - 8 % of pregnant and lying-in women and fetuses [4].

Apoptosis is an important factor in placental development, and the imbalance between trophoblast proliferation and apoptosis may be associated with abnormalities in pregnancy [5]. At present, the specific pathogenesis of PE remains elusive, and its occurrence may be related to abnormal trophoblast apoptosis. The findings suggest that the pathological mechanism of PE may involve placental ischemia and increased apoptosis of trophoblast cells, induced by oxidative stress [6]. It also leads to trophoblast invasion and uterine spiral artery remodeling disorder, leading to placental malperfusion, and finally leading to occurrence of PE [6].

With relatively long half-life values and relatively high stability in body fluids, micro ribonucleic acids (miRNAs) may become important biomarkers for the diagnosis and prognosis of PE [7]. A multitude of studies have analyzed the expression of miRNAs in PE and normal placentas and indicated that miRNAs exert key regulatory effects on the pivotal genes in various pathological processes of PE. For example, a study by Niu *et al* [8] pointed out that the expression level of miR-30a rises significantly in the placental tissues of PE patients, and it induces apoptosis and inhibits invasion of trophoblasts by targeting IGF-1. Moreover, the research of Fu *et al* [9] showed that miR-376c can target TGF-β signaling pathway to promote the proliferation and invasion of trophoblasts.

In this present study, therefore, miRNAs were screened out from the blood of PE patients and healthy puerperae with normal labor using microarray assay of gene expression profiles, and functional validation was undertaken in HTR-8/SVneo cell lines, so as to explore the effects of miRNAs on the proliferation and apoptosis of placental trophoblasts. The main aim of this study is to further investigate the miRNAs playing key roles in the pathological processes of PE, thus providing new drug targets for the future treatment of PE.

METHODS

Collection of general data and samples from patients

Serum samples were obtained from 25 healthy puerperae and 43 PE patients to screen out the miRNAs that play an important role in the pathological processes of PE. Both PE patients and healthy puerperae were treated in our hospital from January 2019 to January 2020. Among them, PE patients were diagnosed based on blood pressure > 140 / 90 mmHg and proteinuria (the protein level in urine collected within 24 hours > 300 mg). The patients with other diseases or those treated with other drugs before collecting serum samples were excluded from the present study. This study was approved by the Ethics Committee of Jilin Province FAW General Hospital (approval no. 031). Signed informed consents were obtained from all participants before the study. The study was conducted by following the Declaration of Helsinki [10].

Microarray assay of miRNA expression profiles

The differences in the expression profiles of serum miRNAs between 3 PE patients and 3 healthy puerperae were analyzed using GeneChip miRNA 1.0 (Affymetrix, Santa Clara, CA, USA). An equal number of miRNAs were first extracted from 3 PE patients and 3 healthy puerperae and mixed separately as the miRNA pool for patient group and control group, respectively. One microgram of miRNAs was extracted from each group of miRNA pool for miRNA microarray assay. The microarrays were hybridized and glass slides were cleaned and scanned as shown in literature [11]. Subsequently, the raw data of the microarrays were analyzed using GeneSpring software (Agilent, Santa Clara, CA, USA). In the present study MiRNAs were considered to be differentially expressed at log2 (fold change) > 2 and $p < 0.05$. Finally, TargetScan (http://www.targetscan.org), an online bioinformatics tool, was employed to analyze and predict the direct targets of differentially expressed miRNAs.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNAs were extracted from the serum and tissues of patients strictly in accordance with the manufacturer's instructions on the high-purity total RNA rapid extraction kit (Bioteke Corporation, Beijing, China). Complementary deoxyribonucleic acids (cDNAs) were then synthesized from the total RNAs using the M-MLV RT kit (Promega, Wallisellen, Switzerland) at 25 °C for 10 min, 42 °C for 50 min, and 95 °C for 5 min. Subsequently, the cDNAs were amplified using qPCR through 50 cycles of denaturation at 95 °C for 10 sec, annealing at 60 °C for 20 sec and extension at 72 °C for 30 sec, with β-actin as the internal reference. The

relative fold changes of the corresponding miRNAs and messenger RNAs (mRNAs) were determined using $2^{-\Delta\Delta Ct}$ method. The experiment was repeated at least 3 times, with the primer sequences listed in Table 1.

Table 1: Primer sequences

Gene	Primer sequence (3'-5')
MiR-19a	GTTTTGCATAGTTGCACTA
	GAACATGTCTGCGTATCTC
MiR-148a	GTTCTGAGACACTCCGA
	GAACATGTCTGCGTATCTC
MiR-575	GAGCCAGTTGGACAGG
	GAACATGTCTGCGTATCTC
MiR-99a	AACCCGTAGATCCGATC
	GAACATGTCTGCGTATCTC
MiR-584	GGTTTGCCTGGGACTG
	GAACATGTCTGCGTATCTC
MiR-521	CGCACTTCCCTTTAGAG
	GAACATGTCTGCGTATCTC
PTEN	TGAGTTCCCTCAGCCGTTACCT
	GAGGTTTCCTCTGGTCCTGGTA

Cell culture and transfection

Human placental trophoblast cell lines (HTR-8/SVneo) were utilized for functional validation of miRNAs. HTR-8/SVneo cell lines were purchased from American Type Culture Collection (ATCC) cell bank (ATCC Cell Lines, Manassas, VA, USA, CRL-3271, Lot No. 64275781), and they were cultured by the methods described in literature [12]. Briefly, HTR-8/SVneo cells were cultured with RPMI-1640 medium containing 10 % fetal bovine serum in a humidified incubator with 5 % CO_2 at 37 °C. When the density of cells in a 6-well plate reached 60 %, transfection was started.

All cells were divided into 4 groups, namely miR-19a mimic group, miR-19a inhibitor group, miR-control (miR-con) group and control group. The miR-19a mimic, miR-19a inhibitor and miR-con were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The transfection doses of miR-19a mimic and miR-19a inhibitor were 50 and 100 nM, respectively, and the transfection was performed in strict accordance with the manufacturer's instructions of Lipofectamine 2000 kit. At 24 h after transfection, the cells were used for subsequent assays.

Phosphatase and tensin homolog deleted on chromosome ten (PTEN) overexpression and corresponding control vectors (Addgene, Watertown, MA, USA) were separately transfected into HTR-8/SVneo cells using Amaxa 4D-Nucleofector X Kit in strict accordance with the manufacturer's instructions, so as to overexpress PTEN.

Cell counting kit (CCK)-8 assay

The effects of miR-19a mimic and miR-19a inhibitor on the proliferation of HTR-8/SVneo cells were examined using a CCK-8 kit (Beyotime Biotechnology, Shanghai, China). The HTR-8/SVneo cells in miR-19a mimic group, miR-19a inhibitor group, miR-con group and control group were separately seeded into a 96-well plate at a density of 5×10^3 cells/well and cultured at 37 °C for 24, 48 and 72 h. After culture, each well was added with 20 µL of CCK-8 reagent, and the cells were cultured at 37 °C for 1 h. Finally, the optical density of cells was measured at 450 nm using a microplate reader.

Cell apoptosis assay

The influence of expression level of miR-19a on apoptosis of HTR-8/SVneo cells was evaluated using a Guava flow cytometer (Millipore, Billerica, MA, USA) and Annexin V-fluorescein isothiocyante (FITC)/ propidium iodide (PI) (HaiGene, Harbin, China) staining. Cells were first trypsinized, and the density was adjusted to 1×10^5 cells/mL using binding buffer. Then, they were stained with Annexin V-FITC and PI for 15 min. The resulting cells were loaded and analyzed using flow cytometry and FlowJo software. This assay was performed in triplicate.

Cell cycle assay

The effect of the expression level of miR-19a on the cycle of HTR-8/SVneo cells was examined by PI staining and flow cytometer. First, HTR-8/SVneo cells were inoculated into 12-well plates. When the density of cells was 60 %, they were transfected with 50 nM miR-19a mimic or 100 nM miR-19a inhibitor using Lipofectamine 2000 kit strictly according to the manufacturer's specifications. At 72 h after transfection, the cells were digested by trypsin and harvested.

The harvested cells were fixed in 70 % ethanol solution overnight at 4 °C. The resulting cells were centrifuged, collected and re-suspended in 500 µL of phosphate-buffered saline (PBS) containing 50 µg of RNase A. Subsequently, they were incubated with 50 µL of PI dye solution at 37 °C in the dark for 30 min. Finally, the cells were loaded and analyzed using the Guava flow cytometer.

Luciferase reporter gene assay

The direct target of miR-19a was determined by luciferase reporter gene assay. The pMIR-REPORT luciferase vector plasmid (pMIR, Ambion, Grand Island, NY, USA) was used as a

transfection tool. The 3' untranslated region (UTR) of the mutant (MUT) PTEN gene sequence was amplified using PCR and cloned into a luciferase vector as a control plasmid. Then, HTR-8/SVneo cells were co-transfected with p-wild type (WT)-UTR plasmid or p-MUT-UTR plasmid and miR-19a mimic.

Western blotting assay

After trypsinization, the cells were harvested and lysed by cell lysis buffer (Beyotime Biotechnology, Shanghai, China) on ice. Then, the cells were centrifuged using a refrigerated centrifuge at 12,000 × g and 4 °C for 12 min. Subsequently, the concentration of proteins was determined using the bicinchoninic acid (BCA) kit (Beyotime Biotechnology, Shanghai, China). Next, the proteins were isolated using 10 % sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) at 12 V and transferred onto a polyvinylidene fluoride (PVDF) membrane by the semi-dry transfer method (Millipore, Billerica, MA, USA).

The PVDF membrane was sealed with tris buffered saline (TBS) solution containing 5 % skim milk powder for 1 h, incubated with primary antibodies at the recommended concentration and 4 °C for 24 h and washed 3 times with phosphate buffered saline-tween (PBST) for 3 times (10 min/time). The membrane was incubated with secondary antibodies (1:10,000) for 1 h, and the color was developed using diaminobenzidine (DAB) solution (Solarbio, Beijing, China). Finally, protein bands were quantitatively analyzed using ImageJ software.

Statistical analysis

Data are presented as mean ± standard deviation (SD), and statistically analyzed using SPSS software (version 26.0) (SPSS Inc., Chicago, IL, USA). Comparison between multiple groups was done using One-way ANOVA followed by Post Hoc Test (Least Significant Difference). $P < 0.05$ indicated significant difference.

RESULTS

Expression levels of miR-19a

The differentially expressed miRNAs in the serum of PE patients and healthy puerperae were screened out using the microarray assay of gene expression profiles to identify the important miRNAs in the pathological processes of PE. According to the screening results, the expression profiles of miRNAs in the serum of PE patients were notably different from those of healthy puerperae. A total of 92 differentially expressed miRNAs were screened out based on the criteria of log2 (fold change > 2 and $p < 0.05$), of which 67 had notably raised expression levels and 25 had a considerably decreased expression level (Figure 1 A).

The differences in the expression levels of miRNAs in the serum between PE patients and healthy controls were verified by RT-qPCR to further validate the reliability of the high-throughput screening results. It was found that compared with those in healthy puerperae, the expression levels of miR-19a, miR-148a and miR-575 declined significantly ($p < 0.05$), while the expression level of miR-99a was remarkably increased in PE patients, with no significant differences in the expression levels of miR-584 and miR-521 (Figure 1 B).

MiR-19a promoted the proliferation of trophoblasts

Human trophoblast HTR-8/SVneo cells were transfected with miR-19a mimic, miR-19a inhibitor or miR-con to examine the effects of miR-19a on their proliferation. The results of RT-qPCR showed that there was no significant difference in the expression level of miR-19a in the cell lines between miR-con group and control group ($p > 0.05$), while the expression level of miR-19a rose significantly in miR-19a mimic group compared with that in control group, showing a statistically significant difference ($p < 0.05$). Moreover, miR-19a inhibitor group exhibited a notably lower expression level of miR-19a in cells than control group, with a statistically significant difference ($p < 0.05$ Figure 2 A).

According to the results of CCK-8 assay, the cell viability in miR-19a mimic group was significantly stronger than that in miR-con group and control group at 48 and 72 h, except 24 h, and the data were significantly different ($p < 0.05$). Besides, miR-19a inhibitor group exhibited distinctly weakened cell viability at 48 and 72 h compared with miR-con group and control group, with a statistically significant difference ($p < 0.05$ Figure 2 B). Finally, the results of cell cycle assay revealed that compared with miR-con group and control group, miR-19a inhibitor group had significantly more cells arrested in the G1 phase, while miR-19a mimic group had obviously fewer G1-phase cells, with a statistically significant difference ($p < 0.05$ Figures 2 C and D).

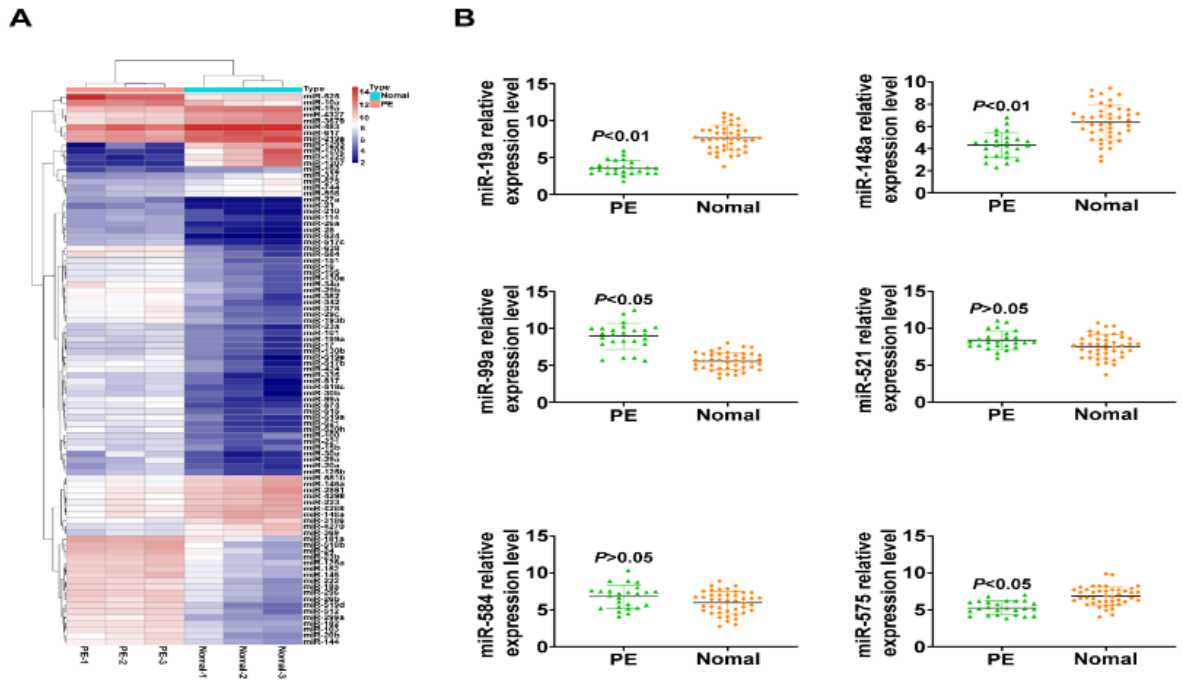

Figure 1: Expression level of miR-19a was significantly decreased in the serum of PE patients. (A) Clustering heat maps of differentially expressed miRNAs in the serum of PE patients and healthy puerperae. (B) Differentially expressed miRNAs in the serum of PE patients and healthy puerperae determined by RT-qPCR

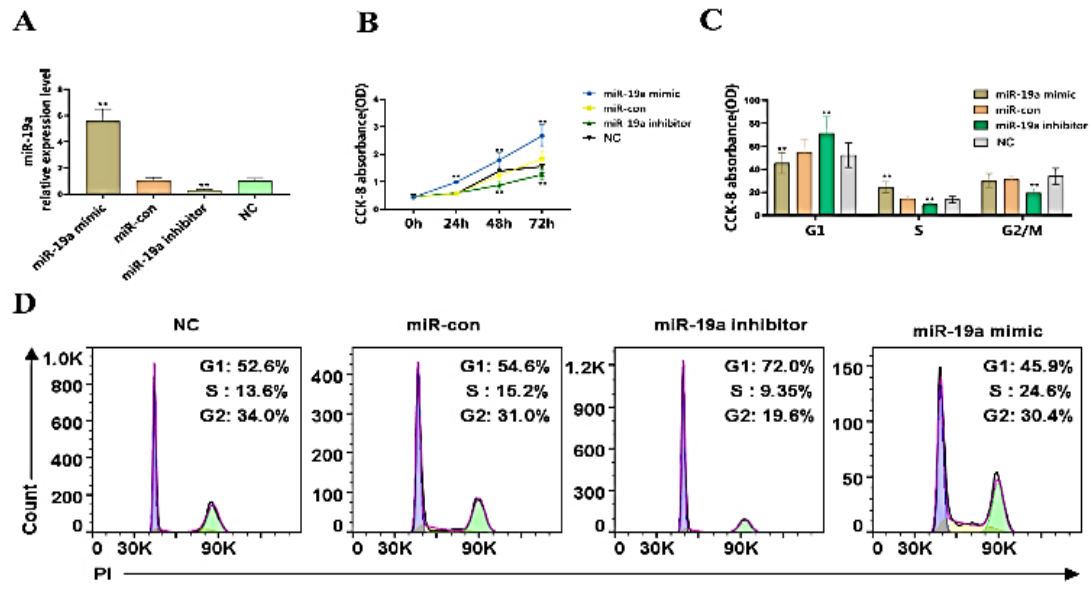

Figure 2: MiR-19a enhanced trophoblast proliferation. (A) Differences in the expression level of miR-19a among miR-19a mimic group, miR-19a inhibitor group, miR-con group and control group determined by RT-qPCR. (B) Impact of miR-19a expression level on trophoblast proliferation determined *via* CCK-8 assay. (C, D) Effect of miR-19a expression level on trophoblast cycle. ***P* < 0.05 *vs.* miR-con group

Decline in miR-19a expression level enhanced the apoptosis of trophoblasts

It was found through flow cytometry and Annexin V-FITC/PI staining that the apoptosis rate of cells was 16.8 % in control group, 20.7 % in miR-con group, 4.6 % in miR-19a mimic group, and 32.3 % in miR-19a inhibitor group (Figure 3). These results showed that compared with that in miR-con and control groups, the apoptosis rate of HTR-8/SVneo cells was significantly raised by miR-19a inhibitor.

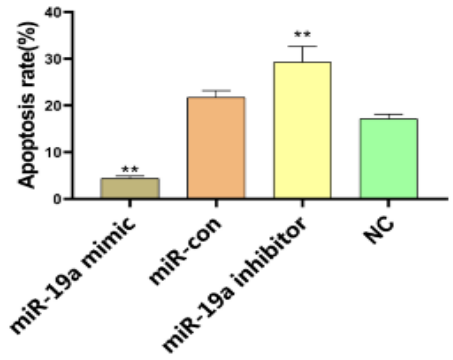

Figure 3: Decline in miR-19a expression level facilitated trophoblastic apoptosis. Effect of miR-19a expression level on the apoptosis of trophoblasts examined by flow cytometry and Annexin V-FITC/PI staining. ***P* < 0.05 *vs.* miR-con group

PTEN was the direct target of miR-19a

In this study, it was predicted using bioinformatics software, that PTEN was a potential target of miR-19a (Figure 4 A). The RT-qPCR results further revealed that the mRNA expression levels of PTEN in miR-19a inhibitor group rose significantly, but declined in miR-19a mimic group, compared with that in the miR-con group and the control group (Figure 4 B). Western blotting results showed that the PTEN protein expression level was significantly increased in the miR-19a inhibitor group and decreased in the miR-19a mimic group, compared with the miR-con group and the control group (Figure 4 C). These results suggest that PTEN may be the direct target of miR-19a.

WT-PTEN-3'UTR and MUT-PTEN-3'UTR sequences was presented in Figure 5 A. The results of co-transfection of WT-PTEN-3'UTR or MUT-PTEN-3'UTR luciferase reporter plasmid and miR-19a mimic or miR-con, showed that, compared with that in miR-con transfection group, the luciferase activity was significantly weakened in HTR-8/SVneo cells co-transfected with WT-PTEN-3'UTR and miR-19a mimic ($p < 0.05$ Figure 5 B), but had no significant change in HTR-8/SVneo cells co-transfected with MUT-PTEN-3'UTR and miR-19a mimic. All these results imply that PTEN is the direct target of miR-19a in HTR-8/SVneo cells.

MiR-19a targeted PTEN-activated protein kinase B (Akt) signaling pathway

Western blotting results showed that the protein expression levels of phosphorylated Akt (p-Akt)/Akt significantly rose in miR-19a mimic group, but notably declined in miR-19a inhibitor group compared with that in miR-con group and control group.

The effect of miR-19a expression level on the related downstream molecules of Akt signaling pathway was further assessed. The results of Western blotting assay showed that compared with those in control group and miR-con group, the protein expression levels of p-p21/p21, p-p27/p27, and p-glycogen synthase kinase-3 beta (GSK3β)/GSK3β were significantly decreased in miR-19a mimic group, but they were evidently raised in miR-19a inhibitor group (Figure 6).

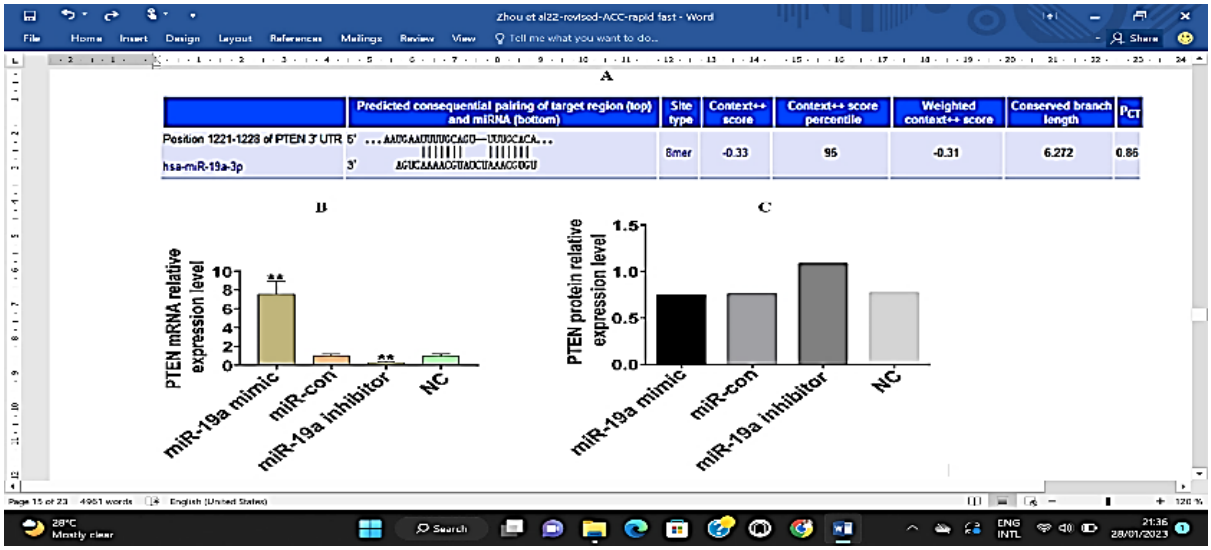

Figure 4: PTEN was a potential target of miR-19a. (A) PTEN was predicted to be the direct target of miR-19a using TargetScan software. (B) Effect of miR-19a expression level on the mRNA expression level of PTEN, as determined using RT-qPCR. (C) Effect of miR-19a expression level on the protein expression level of PTEN, as measured using Western blotting. ***P* < 0.05 *vs.* miR-con group

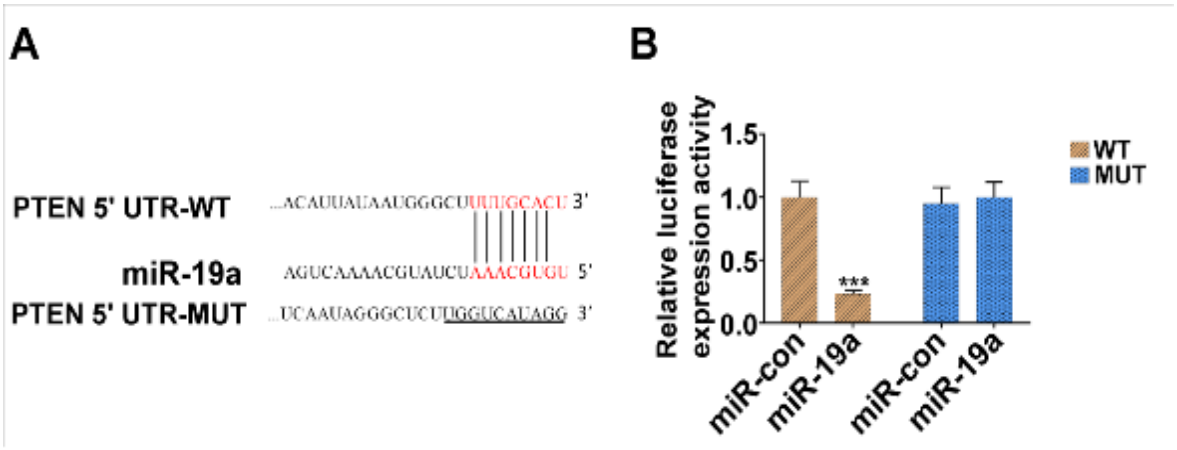

Figure 5: Direct acting relationship between miR-19a and PTEN validated by luciferase reporter gene assay. (A) WT-PTEN-3'UTR and MUT-PTEN-3'UTR sequences. (B) Direct acting relationship between miR-19a and PTEN as validated by luciferase reporter gene reporter assay. ***P* < 0.05 *vs.* miR-con group

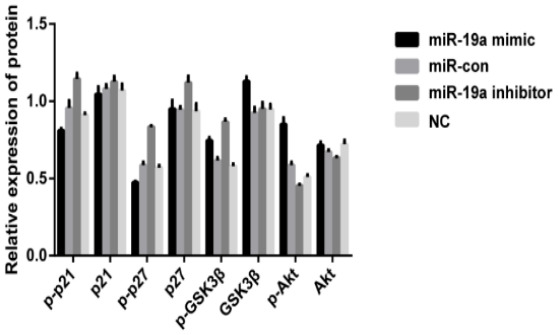

Figure 6: Effect of miR-19a expression level on the Akt signaling pathway and its downstream molecules determined by Western blotting assay

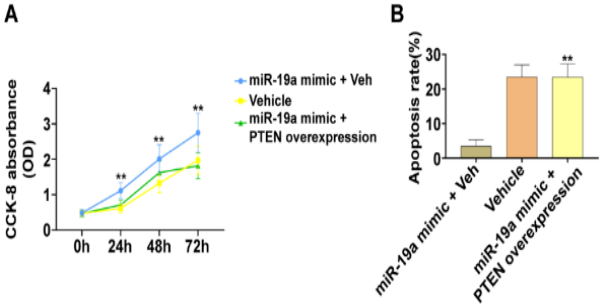

Figure 7: MiR-19a targeted PTEN to promote trophoblast proliferation. (A) Effect of overexpression of PTEN on the pro-proliferative effect of miR-19a on HTR-8/SVneo cells determined *via* CCK-8 assay. (B) Effect of overexpression of PTEN on the anti-apoptotic effect of miR-19a mimic on HTR-8/SVneo cells using Annexin V-FITC/PI staining. ***P* < 0.05 *vs.* miR-19a mimic + vehicle group

MiR-19a contributed to the proliferation of trophoblasts by targeting PTEN

The results of CCK-8 assay revealed that, compared with that in vehicle group, the cell viability clearly rose significantly in miR-19a mimic + vehicle group at 48 and 72 h, showing a statistically significant difference ($p < 0.05$). Moreover, the viability of cells in miR-19a mimic + PTEN overexpression group was significantly poorer than that in miR-19a mimic + vehicle group at 48 and 72 h, ($p < 0.05$ Figure 7 A).

According to the results of flow cytometry and Annexin V-FITC/PI staining showed that the apoptosis rates were 23.7, 3.2, and 27.3 % in vehicle group, miR-19a mimic + vehicle group and miR-19a mimic + PTEN overexpression group, respectively (Figure 7 B). These results indicate that the overexpression of PTEN notably abolishes the anti-apoptotic effect of miR-19a mimic on HTR-8/SVneo cells.

DISCUSSION

Preeclampsia (PE) is an extremely complex disease, that may cause neonatal death. Most studies have shown that the complex pathological processes of PE are significantly related to genetics, immunity, and systemic inflammation, but there has been no understanding of its exact pathogenesis [13]. The miRNAs are single-stranded, small-molecule, non-coding RNAs with a small molecular weight, and they can negatively regulate gene expression. It has been pointed out by massive studies that disturbance of the miRNA regulatory network is an important player in the progression of PE [14]. Therefore, it is urgent for researchers to further explore the function and mechanism of differentially expressed miRNAs experiencing notable changes in PE, so as to understand the molecular pathogenesis of PE.

In the present study, it was determined by microarray assay and low-throughput RT-qPCR that the expression level of serum miR-19a dropped considerably in PE patients. Then the molecular function of miR-19a was verified using *in vitro* assays. The results of CCK-8 and CFSE assays, an increase in the expression level of miR-19a significantly raised the proliferation rate of HTR-8/SVneo cells, while the decline in expression level of miR-19a obviously lowered the proliferation rate of HTR-8/SVneo cells. Moreover, the results of cell apoptosis assay revealed that a rise in the expression level of miR-19a repressed apoptosis of HTR-8/SVneo cells, whereas a decrease in the expression level of miR-19a contributed to apoptosis of HTR-8/SVneo cells. Subsequently, it was found through molecular mechanism assay that miR-19a targeted PTEN to activate the Akt signaling pathway, thereby promoting the proliferation of HTR-8/SVneo cells and repressing their apoptosis.

Currently, some studies have demonstrated that the expression level of miR-19a is significantly decreased in the placental tissues of PE patients. For instance, Zhu *et al* [15] screened differentially expressed miRNAs in the placentas of 8 patients with moderate PE and 15 patients with severe PE by microarray assay and RT-qPCR and found that miR-19a has a significantly decreased expression level in the placental tissues of PE patients. The results of study of Wang *et al* [16], showed that the expression level of miR-19a substantially falls in the placental tissues of PE patients and hypoxic trophoblast layers. Consistent with these findings, the results of the present study demonstrated that an increase in expression level of miR-19a was likely to play some role in pathogenesis of PE.

A previous study stated that miR-19a, located on chromosome 13q31, belongs to the miR-17-92 family and has physiological functions closely related to embryonic development and tumor cell proliferation and apoptosis [17]. Many studies have demonstrated that miR-19a functions by a mechanism that is prominently related to targeting PTEN. For example, miR-19a targeted PTEN in order to accelerate the proliferation of cervical cancer cells as pointed out in the study of Wang *et al* [18]. Based on the research results of Hou *et al* [19], miR-19a is able to activate PTEN by targeting the PI3K/Akt signaling pathway in bronchial smooth muscle cells, thereby promoting their proliferation. Moreover, the results of the study conducted by Sun *et al* [20] disclosed that miR-19a targets the PTEN/PI3K/Akt signaling pathway, so as to restrain the hypoxia/reoxygenation-induced apoptosis of cardiomyocytes.

According to current studies, (phosphatase and tensin homolog) PTEN is generally recognized as a tumor suppressor gene. A large number of studies report that PTEN mutation or a decline in its expression level will cause abnormal cell proliferation and prevent cell apoptosis in many different types of tumors. The decreased proliferative capacity and excessive apoptosis of placental trophoblasts may play a pivotal role in the pathological processes of PE. Therefore, it can be inferred that the increase in expression of PTEN caused by various reasons can significantly restrain the proliferation and facilitate apoptosis of placental trophoblast cells. At present, there is evidence that PTEN may serve as a drug target for treatment of PE. First, the studies of Xiao *et al* [21] and Ishioka *et al* [22] have proposed that the protein expression level of PTEN in the placental tissues of PE patients is significantly higher than that in healthy puerperae, and an increase in its expression level is accompanied by an increased apoptosis rate and a decreased proliferation rate in trophoblasts. As shown in the study of Wu *et al* [23], overexpression of lncRNA TCL6 can drive the progression of PE by modulating PTEN. According to the findings in the present study, miR-19a mimic targeted PTEN to significantly promote the proliferation of HTR-8/SVneo cells and inhibited their apoptosis, whereas miR-19a mimic and overexpression of PTEN dramatically repressed the proliferation of HTR-8/SVneo cells and promoted their apoptosis.

CONCLUSION

The findings of this study suggest that miR-19a promotes proliferation and inhibits apoptosis of trophoblasts (HTR-8/SVneo cells) in human placentas by targeting PTEN, thus providing new potential drug targets that can be investigated for the treatment of PE.

DECLARATIONS

Acknowledgements

This work was supported by the hospital-level task book of Hainan women and children's medical center (grant no. 2020YJ01); Key research and development projects of Hainan Province (no. ZDYF2022SHFZ074); Hainan Natural Science Foundation high-level Talents Project (no. 820RC771); Project supported by Hainan Province Clinical Medical Center (no. QWYH202175).

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

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

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Jing Zhou and Ning Ma contributed equally to this work.

REFERENCES

1. *Rastegari Z, Yarmohammadian MH, Mohammadi F, Kohan S. A comprehensive home-care program for health promotion of mothers with preeclampsia: protocol for a mixed method study. Reprod Health 2019; 16(1): 32.*
2. *Wang L, Liu Y, Liu S, Ling C, Li K, Xiao Q, Liang X. Levels of IL-17 and IL-35 in Chinese women as biomarkers of preeclampsia, and their pharmacological association with Traditional Chinese Medicine. Trop J Pharm Res 2021; 20(12): 2573-2579 doi: 10.4314/tjpr.v20i12.17*
3. *Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380(9859): 2095-2128.*
4. *Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005; 365(9461): 785-799.*
5. *Su M, Zhou B, Zhen M, Liu J. Aspirin protects against preeclampsia via p38MAPK signaling pathway. Trop J Pharm Res 2022; 21(12): 2555-2560 doi: 10.4314/tjpr.v21i12.8*
6. *Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biol Reprod 2003; 69(1): 1-7.*
7. *Lv Y, Lu C, Ji X, Miao Z, Long W, Ding H, Lv M. Roles of microRNAs in preeclampsia. J Cell Physiol 2019; 234(2): 1052-1061.*
8. *Niu ZR, Han T, Sun XL, Luan LX, Gou WL, Zhu XM. MicroRNA-30a-3p is overexpressed in the placentas of patients with preeclampsia and affects trophoblast invasion and apoptosis by its effects on IGF-1. Am J Obstet Gynecol 2018; 218(2): 241-249.*
9. *Fu G, Ye G, Nadeem L, Ji L, Manchanda T, Wang Y, Zhao Y, Qiao J, Wang YL, Lye S, et al. MicroRNA-376c impairs transforming growth factor-beta and nodal signaling to promote trophoblast cell proliferation and invasion. Hypertension 2013; 61(4): 864-872.*
10. *Stockhausen K. The Declaration of Helsinki: revising ethical research guidelines for the 21st century. Med J Aust 2000; 172(6): 252-253.*
11. *Jang HY, Lim SM, Lee HJ, Hong JS, Kim GJ. Identification of microRNAs and their target genes in the placenta as biomarkers of inflammation. Clin Exp Reprod Med 2020; 47(1): 42-53.*
12. *Cao C, Li J, Li J, Liu L, Cheng X, Jia R. Long non-coding RNA Uc.187 is upregulated in preeclampsia and modulates proliferation, apoptosis, and invasion of HTR-8/SVneo trophoblast cells. J Cell Biochem 2017; 118(6): 1462-1470.*
13. *Hod T, Cerdeira AS, Karumanchi SA. Molecular mechanisms of preeclampsia. Cold Spring Harb Perspect Med 2015; 5(10): a023473.*
14. *Luque A, Farwati A, Crovetto F, Crispi F, Figueras F, Gratacos E, Aran JM. Usefulness of circulating microRNAs for the prediction of early preeclampsia at first-trimester of pregnancy. Sci Rep 2014; 4: 4882.*
15. *Zhu XM, Han T, Sargent IL, Yin GW, Yao YQ. Differential expression profile of microRNAs in human placentas from preeclamptic pregnancies vs normal pregnancies. Am J Obstet Gynecol 2009; 200(6): 661.*
16. *Wang N, Li R, Xue M. Potential regulatory network in the PSG10P/miR-19a-3p/IL1RAP pathway is possibly involved in preeclampsia pathogenesis. J Cell Mol Med 2019; 23(2): 852-864.*
17. *Koga Y, Yasunaga M, Takahashi A, Kuroda J, Moriya Y, Akasu T, Fujita S, Yamamoto S, Baba H, Matsumura Y. MicroRNA expression profiling of exfoliated colonocytes isolated from feces for colorectal cancer screening. Cancer Prev Res (Phila) 2010; 3(11): 1435-1442.*
18. *Wang Y, Zhao S, Zhu L, Zhang Q, Ren Y. MiR-19a negatively regulated the expression of PTEN and*

promoted the growth of ovarian cancer cells. Gene 2018; 670: 166-173.

19. *Hou C, Chen Y, Huang X, Huang Q, Li M, Tan X. miR-19 targets PTEN and mediates high mobility group protein B1(HMGB1)-induced proliferation and migration of human airway smooth muscle cells. Plos One 2019; 14(6): e219081.*
20. *Sun G, Lu Y, Li Y, Mao J, Zhang J, Jin Y, Li Y, Sun Y, Liu L, Li L. miR-19a protects cardiomyocytes from hypoxia/reoxygenation-induced apoptosis via PTEN/PI3K/p-Akt pathway. Biosci Rep 2017; 37(6): BSR20170899.*
21. *Xiao J, Tao T, Yin Y, Zhao L, Yang L, Hu L. miR-144 may regulate the proliferation, migration and invasion of trophoblastic cells through targeting PTEN in preeclampsia. Biomed Pharmacother 2017; 94: 341-353.*
22. *Ishioka S, Ezaka Y, Umemura K, Hayashi T, Endo T, Saito T. Proteomic analysis of mechanisms of hypoxia-induced apoptosis in trophoblastic cells. Int J Med Sci 2006; 4(1): 36-44.*
23. *Wu JL, Wang YG, Gao GM, Feng L, Guo N, Zhang CX. Overexpression of lncRNA TCL6 promotes preeclampsia progression by regulating PTEN. Eur Rev Med Pharmacol Sci 2019; 23(10): 4066-4072.*