Full text open access online (Since 2001)

PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330
Int J Hum Genet, 26(2): 76-87 (2026)
DOI: 10.31901/24566330.2026/25.02.926

MicroRNA-146b Suppresses Trophoblast Migration, Invasion, and EMT in Preeclampsia Through Regulation of PTEN

Hui Zhang,[1#] Wenban Qin [2#], Liange Xia[3], Yanhua Nong[4], Meiqi Huang[5] and Mujun Li[6*]

[1]Department of Reproductive Center, The First Affiliated Hospital of Guangxi Medical University, 530000, Nanning, China
[2]Department of Oncology, Chongzuo People's Hospital, 532200, Chongzuo, China
[3]Pharmacy Department of Chongzuo People's Hospital, 532200, Chongzuo, China
[4]Obstetrics Department of Chongzuo People's Hospital, 532200, Chongzuo, China

KEYWORDS Preeclampsia Pathogenesis. Cell Migration. Cell Invasion. MicroRNA-146b.

ABSTRACT This study aimed to clarify miR-146b's role in preeclampsia (PE). Placental tissues were obtained from 20 PE individuals and 20 normotensive pregnant participants, and all cell experiments were performed using HTR-8/SVneo cells. miR-146b's was detected, and its role in cell behaviours was evaluated. The target gene was identified. Our findings revealed that miR 146b was upregulated in placental specimens of women diagnosed with PE. Furthermore, miR 146b inhibition markedly enhanced the migratory, invasive, and EMT phenotypes of HTR 8/SVneo cells. miR-146b was shown to target PTEN as its downstream effector, and the miR-146b/PTEN axis was revealed to govern the activation process of the PI3K/AKT cascade. Collectively, miR 146b may participate in the pathogenesis of preeclampsia via modulating trophoblast biological functions, highlighting its potential as a diagnostic biomarker and therapeutic target for PE.

INTRODUCTION

A unique complication unique to pregnancy, preeclampsia (PE) manifests as hypertension (with or without proteinuria) that generally arises from the 20th week of gestation onwards (Rana et al. 2019; Ives et al. 2020; Filipek and Jurewicz 2018). PE is considered a key factor underlying maternal mortality and morbidity, with its global prevalence ranging from 2% to 8% among all pregnant populations (Ma'ayeh and Constantine 2020; Roberts 2024; Allard et al. 2024). PE is a multi-system syndrome whose pathogenic mechanisms encompass genetic and environmental elements, including aberrant systemic immune reactions, oxidative stress, placental formation and dysfunction, inflammatory responses, and heredity factors (Laivuori et al. 2003; Oudejans et al. 2004). Nevertheless, the pathogenic mechanisms responsible for PE have not yet been fully elucidated. At present, defective trophoblast invasion and excessive apoptosis are thought to drive PE development, ultimately causing incomplete spiral artery remodeling and insufficient uteroplacental blood flow (Gingras-Charland et al. 2019). In uncomplicated pregnancies, trophoblasts undergo differentiation into extravillous trophoblasts with strong invasive potential, which migrate to the decidual stroma to degrade the wall of uterine spiral arteries and substitute the resident endothelial cells (Phipps et al. 2019). If migration and invasion are reduced or apoptosis is increased during this process, it can lead to impaired placental formation and implantation, leading to PE (El-Sayed 2017; Ramos et al. 2017). Accordingly, sustaining normal trophoblast function is vital for preventing PE development.

As a group of endogenous non coding RNAs, microRNAs (miRNAs) are capable of modulating cell proliferation, apoptosis, migration, angiogenesis, and differentiation (Rupaimoole and Slack 2017). They control gene expression via regulating stability and mobility

*Address for correspondence:
Mujun Li
Department of Reproductive Center,
The First Affiliated Hospital of Guangxi Medical University,
No. 6 Shuangyong Road, Nanning City,
Guangxi Zhuang Autonomous Region
E-mail: huanghefu1615@163.com,lmj1699@vip.163.com
#These authors contributed equally to this work

of target messenger RNA (Gebert and MacRae 2019). It is well known that variations in miRNA levels can lead to altered expression of targeted genes. Although abnormal miRNA expression generally presents with a mild pattern, this slight dysregulation is capable of causing marked fluctuations in expression of target genes, which can significantly influence the pathogenic process and further disrupt multiple bodily physiological functions (Zhang et al. 2019). miRNAs exert regulatory effects on gene expression by controlling target mRNA stability and translational efficiency in a range of cellular pathways (Lim et al. 2005). Existing research suggests that miRNAs participate in PE pathogenesis and hold potential as both diagnostic biomarkers and therapeutic tools for this syndrome (Shu et al. 2019). Notably, prior investigations have detected upregulated miR 146b levels in dairy cows affected by PE-like pregnancy disorders (Markkandan et al. 2018). However, whether its expression is upregulated in human preeclampsia requires further evaluation.

PTEN acts as a lipid phosphatase and can dephosphorylate PIP3. PIP3 is the second intracellular messenger of insulin, epidermal growth factor (EGF), and other cytokines essential in controlling cell growth. It primarily activates other kinases in the signalling system, such as protein kinase B (PKB) and silk/threonine kinase (AKT), which promote cell division and prevent apoptosis. By dephosphorylating PIP3, PTEN blocks signal transduction, thereby inhibiting the growth of normal cells. In PTEN-cells, PIP3 and AKT kinase levels increase and prevent apoptosis. PTEN regulates the PIP3 and AKT/PKB signalling pathways to control two crucial processes in cell growth, that is, cell division and cell survival. This molecule also exerts a regulatory role in angiogenesis: it controls VEGF expression through the PI3K/AKT signalling cascade, thereby inhibiting neovascularization. PTEN is the primary tumor suppressor gene involved in tumour development (Lee et al. 2018; Li et al. 2020). Besides, recent researches have reported a reduced level of PTEN expression in PE (Li et al. 2022; Wu et al. 2019).

Previous investigation has revealed that miR-146b controls prostate cancer cell proliferation via binding to PTEN (Gao et al. 2018). Meanwhile, aberrant expression of PTEN is implicated in trophoblastic diseases such as PE. These observations verify that miR 146b and PTEN are aberrantly expressed in PE; nevertheless, the precise biological function of miR 146b in PE and how it may regulate PTEN during PE pathogenesis remain largely unknown. Accordingly, our research intended to unravel the intrinsic mechanism of miR-146b in PE pathogenesis and identify novel candidate targets that can be used for the diagnosis and therapy of this disorder.

MATERIAL AND METHODS

Sample Collection

Forty pregnant individuals (20 PE cases and 20 healthy pregnancies) from Chongzuo People's Hospital were recruited for this study. All pregnant women underwent rigorous screening for the clinical features of PE, with other confounding etiologies excluded (ACOG et al. 2019). The placental surface tissues of the pregnant woman were obtained and rinsed thrice with normal saline treated with diethyl coke to flush out the blood. The tissues were rapidly frozen in liquid nitrogen. In addition, these tissues were extracted within 15 minutes of the cesarean section. The Ethics Committee of Chongzuo People's Hospital reviewed and approved this study (Approval No.: 2019010), with all participants signing written informed consent forms.

Cell Culture and Transfection

Human extravillous trophoblast HTR 8/SV-neo cells were obtained from ATCC (Manassas, VA, USA) and grown in Gibco RPMI 1640 medium containing 10 percent fetal bovine serum (FBS, Hyclone). Six well plates were used for cell seeding, followed by incubation at 37 ! in a 5% CO, atmosphere for 48 hours. miR 146b inhibitor, inhibitor NC, MiR-146b mimic and mimic NC, were acquired from Riobo. Subsequently, 100/nM of miR 146b mimic, inhibitor, or negative control oligonucleotides were transfected into the cells. Recombinant pcDNA3.1 PTEN was generated by inserting full length PTEN, and empty pcDNA3.1 served as the negative control. Lipofectamine 2000 reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) was utilized for cell transfection.

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

We used TRIzol reagent for total RNA extraction, followed by reverse transcription of the isolated RNA into cDNA using the PrimeScript 1st Strand cDNA Synthesis Kit (Takara, Dalian, China). We performed qRT PCR using the SYBR® Premix Ex Taq™ Kit (Takara) on an ABI7300 real time PCR platform (Applied Biosystems, Foster City, CA, USA). Relative gene expression levels were quantified using the 2"ÄÄCt algorithm, and GAPDH was used as the internal control. The sequences of the specific primers are summarized in Table 1.

Wound-healing Assay

Transfected cells were plated into 6-well plates (1×10v cells/well) and grown to full confluence. A sterile pipette tip was used to create a uniform scratch in the cell monolayer, followed by rinsing with serum free medium to remove non adherent cells. Each well was supplemented with serum free RPMI 1640 medium, and cells were then incubated at 37/ °C with 5% CO, for 24/ hours. Images were acquired at 0 and 24 hours with a light microscope, and cell migration was subsequently evaluated.

Transwell Invasion

The invasive potential of the cells was measured via Matrigel precoated Transwell chambers (BD Biosciences). Post transfection, cells were resuspended in serum free medium (5×10u cells) and added to the upper chamber, with the lower chamber containing 600 ìL DMEM supplemented with 10 percent FBS as a chemoattractant. Following 72/ hours of culture at 37/ °C under 5% humidified conditions, the cells invading the bottom of the membrane were fixed in 4% paraformaldehyde for 30/ minutes. Afterwards, the resulting cells were stained using 0.1 percent crystal violet for 30 minutes. Subsequently, the number of invasive cells was then visualized and quantified via microscopy.

Luciferase Reporter Assay

The interaction was determined with the double-luciferase reporter enzyme method. Lipofectamine™ 2000 was utilized to co-transfect cells with PTEN-WT or PTEN-MUT and 20/ nmol/L miR 146b mimics. At 48/ h post transfection, cells were lysed, and luciferase activity was measured using the Pierce™ dual luciferase reporter assay kit (Thermo Fisher Scientific, USA).

Table 1: Primer information in this study

Primer name	Sequences
miR-146bforward	5'-TACCCATCCTGGGCCTCAA-3'
miR-146breverse	5'-CCAGTGGGCAAGATGTGGGCC-3'
PTENforward	5′-TGGATTCGACTTAGACTTGACCT-3′
PTENreverse	5′-GGTGGGTTATGGTCTTCAAAAGG-3′
GAPDHforward	5′-GGAGCGAGATCCCTCCAAAAT-3′
GAPDHreverse	5′-GGCTGTTGTCATACTTCTCATGG-3′

Western Blot

Total protein was isolated via RIPA lysis buffer (Beyotime, Shanghai, China), and its concentration was quantified using a BCA kit according to the manufacturer's protocols. After extraction, proteins were separated via SDS PAGE electrophoresis and transferred electrophoretically to PVDF membranes supplied by Millipore (Billerica, MA, USA). Following blocking, PVDF membranes were incubated with primary antibodies overnight at 4/ °C: mouse anti-E-cadherin, mouse anti-N-cadherin, mouse anti-GAPDH (1:1000), and rabbit anti-PTEN (1:1000), all supplied by Abcam (Cambridge, MA, USA). After TBST washing, membranes were incubated with HRP-labeled secondary antibodies (goat anti-rabbit or anti-mouse, 1:5000; Abcam) at room temperature for 1/ hour. Immunoreactive signals were visualized using ECL reagents (Yeasen, Shanghai, China) and documented by a Bio-Rad ChemiDoc XRS Plus imaging system. Densitometric quantification of band intensities was performed using the Image-Pro Plus 6.0, with GAPDH serving as the housekeeping control to normalize expression levels.

Statistical Analysis

All statistical assessments were conducted with GraphPad Prism 8.0. The experimental results are shown as mean ± SD. Two-group comparisons were performed using Student's t-test, and multiple-group comparisons by one-way ANOVA. $P < 0.05$ indicated statistical significance.

RESULTS

Placental Tissues From Preeclamptic Patients Show Markedly Increased Expression of miR 146b

We first validated miR 146b expression in placental villous tissues from PE patients. qRT PCR results demonstrated significantly elevated miR 146b expression in PE tissues relative to normal controls (Fig. 1A). Moreover, the AUC value was above 0.80, implying that miR 146b possesses diagnostic value for PE (Fig. 1B).

MiR-146b Inhibits Migration, Invasion and EMT of HTR-8/SVneo Cells

The miR 146b inhibitor notably reduced endogenous miR 146b expression in transfected cells (Fig. 2A). Wound healing assay results revealed that HTR 8/SVneo cells exhibited obviously faster migratory capacity following miR 146b inhibitor transfection relative to the inhibitor-NC group, indicating that miR 146b negatively regulates trophoblast cell migration (Fig. 1C). Consistently, Transwell experiments revealed a notable elevation in the number of invasive HTR-8/SVneo cells following miR 146b downregulation (Fig. 1C). Western blotting was utilized to evaluate EMT marker proteins. As illustrated in Figure 2D, cells transfected with miR 146b inhibitor displayed upregulated N cadherin and vimentin, alongside downregulated E cadherin. Collectively, our findings imply that miR 146b participates in regulating the key processes of PE at the cellular level.

PTEN is a Downstream Target of miR-146b

PTEN was chosen as the candidate gene because it has potential complementary sequences to miR-146b (Fig. 3A). Luciferase reporter experiments were conducted to assess how miR 146b influences PTEN expression. As shown by the assay results, the ectopic expression of miR 146b led to a notable reduction in luciferase activity of the PTEN/WT luciferase construct relative to the miR NC control. However, the inhibitory effect was abolished when the putative PTEN binding site was mutated (Fig. 3B). Moreover, western blot analysis revealed that ectopic miR 146b expression suppressed PTEN protein levels, and the miR 146b inhibitor elevated PTEN expression (Fig. 3C).

Targeting PTEN by miR-146b Downregulation Enhances the Migratory, Invasive Capabilities and EMT Progression of HTR-8/SVneo Cells

The researchers found that cells transfected with pcDNA-PTEN significantly overexpressed PTEN (Fig. 4A). Suppression of miR 146b expression remarkably strengthened the migratory and invasive capacities of HTR 8/SVneo trophoblast cells. However, treatment of miR-146b

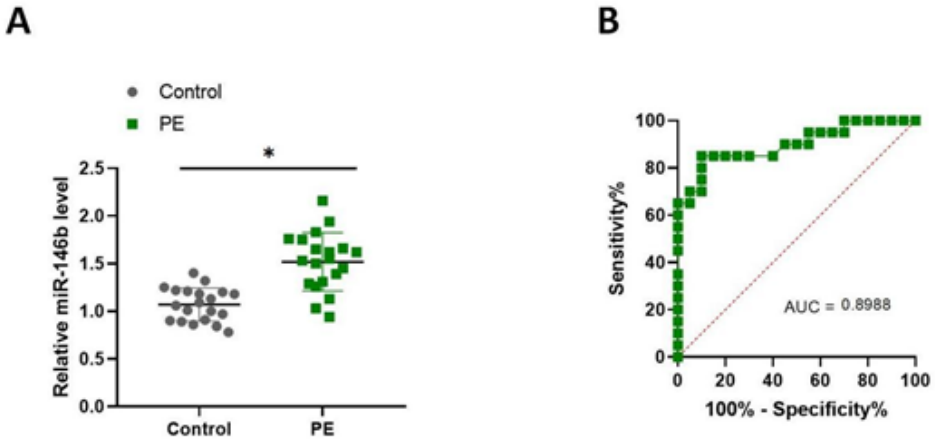

Fig .1. Differential expression of miR 146b between PE and normal placental tissues
(A) RT qPCR of miR 146b expression in 20 PE placental tissues and corresponding normal tissues. (B) The area under the ROC curve of TCL6 expression in PE pregnancies
***: P<0.05.**

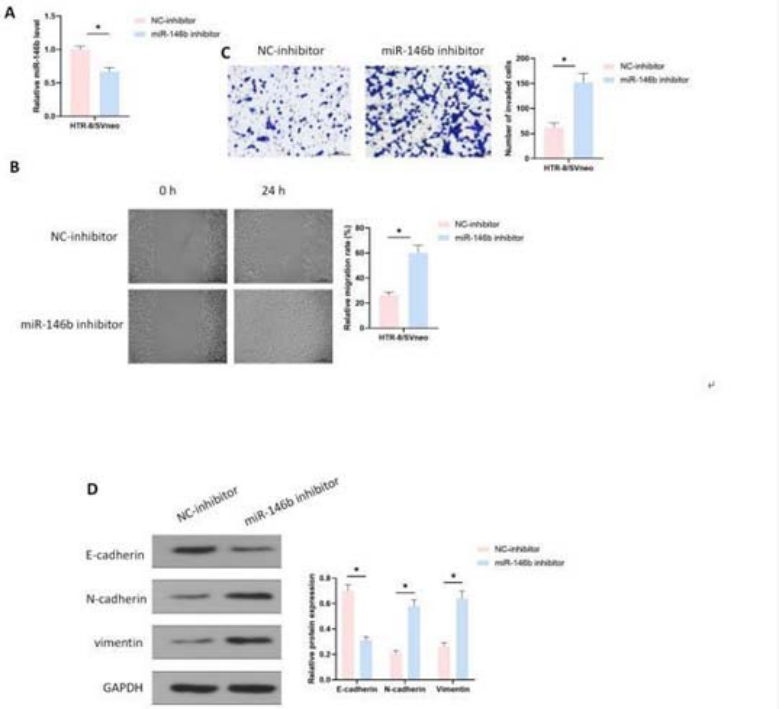

Fig. 2. Effect of miR-146bdown-regulation on migration, invasion, and EMT of HTR-8/SVneo cells
(A) The knockdown efficiency of miR 146b inhibitor was verified using qRT PCR. (B, C) Transfection with miR 146b inhibitor enhanced the migratory and invasive ability of HTR 8/SVneo cells. (D) Western blot of the protein levels of EMT markers (N cadherin, Vimentin, E cadherin).
***P<0.05, **P<0.01 compared with control**

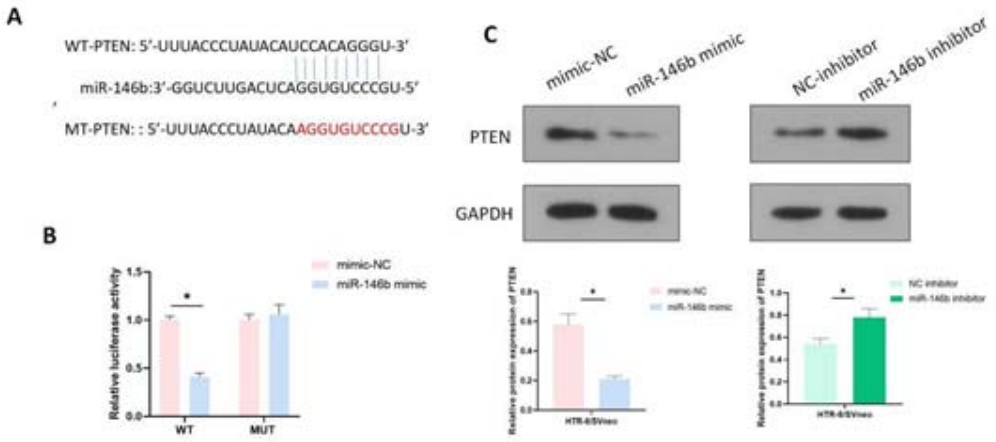

Fig. 3. miR 146b directly modulates the expression of PTEN
(A) Schematic of wild-type and mutant miR-146b for luciferase assay. (B) Upregulation of miR 146b inhibited the activity of wild type PTEN reporter plasmid (PTEN/WT luc) in comparison with miR NC, whereas mutation of the target site abrogated this inhibitory action in Dual luciferase reporter assay. (C) miR-146b mimics reduced PTEN protein expression, while miR-146b inhibitor increased PTEN protein levels in HTR-8/SVneo cells.
***: P<0.05**

down-regulated cells with pcDNA-PTEN partially dampened these effects (Fig. 4B and C). Moreover, suppression of miR 146b expression resulted in prominent upregulation of E cadherin, alongside distinct reductions in N cadherin and vimentin expression in HTR 8/SVneo cells compared with controls. Conversely, co-transfection with pcDNA-PTEN partially reversed these effects on E-cadherin, N-cadherin, and vimentin expression (Fig. 4D). Taken together, the findings presented above demonstrate that miR 146b and PTEN mutually inhibit each other's function, and they have contrasting impacts on the migratory and invasive capacities, and EMT progression of trophoblast cells.

MiR-146b/PTEN Axis Regulates the Activation of PI3K/AKT

PTEN exerts its tumour-suppressive role, which is tightly linked to PI3K/AKT signalling cascade (Li et al. 2016). Accordingly, the research group carried out additional experiments to explore whether miR-146b and PTEN modulate PI3K/AKT pathway activity. As indicated by Western blot results, the miR 146b upregulation markedly elevated phosphorylated AKT levels, whereas total AKT expression did not exhibit any significant difference. Nevertheless, co expression of PTEN significantly reversed this promoting effect induced by miR 146b overexpression (Fig. 5). These observations confirmed that miR-146b/PTEN contributes significantly to the activation ofPI3K/AKT signalling cascade in trophoblast cell progression.

Placental Tissues From Preeclamptic Patients Show Markedly Increased Expression of miR 146b

We first validated miR 146b expression in placental villous tissues from PE patients. qRT PCR results demonstrated significantly elevated miR 146b expression in PE tissues relative to normal controls (Fig. 1A). Moreover, the AUC value was above 0.80, implying that miR 146b possesses diagnostic value for PE (Fig. 1B).

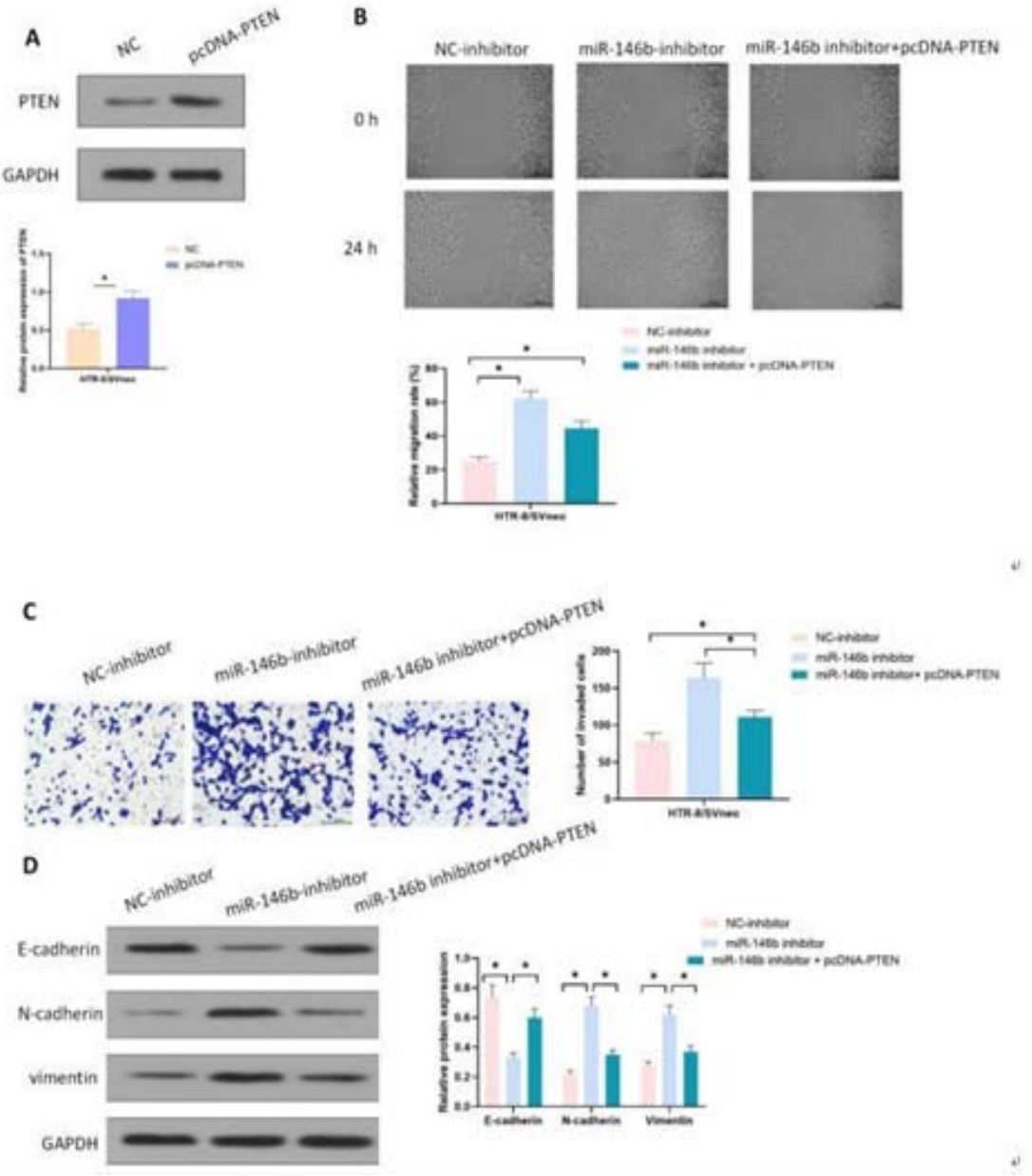

Fig. 4. MiR 146b downregulated PTEN at the molecular level. (A) PTEN expression was up-regulated after pcDNA-PTEN transfection. (B) and (C) Overexpression of PTEN abolished the enhanced migratory and invasive potential induced by miR 146b inhibitors in HTR 8/SVneo cells. (D) Expression changes of N-cadherin, vimentin and E-cadherin after transfection with miR-146b inhibitors were recovered by overexpression of PTEN
***P < 0.05**

MiR-146b Inhibits Migration, Invasion and EMT of HTR-8/SVneo Cells

The miR 146b inhibitor notably reduced endogenous miR 146b expression in transfected cells (Fig. 2A). Wound healing assay results revealed that HTR 8/SVneo cells exhibited obviously faster migratory capacity following miR 146b inhibitor transfection relative to the inhibitor-NC group, indicating that miR 146b negatively regulates trophoblast cell migration (Fig. 1C). Consistently, Transwell experiments revealed a notable elevation in the number of invasive HTR-8/SVneo cells following miR 146b downregulation (Fig. 1C). Western blotting was utilized to evaluate EMT marker proteins. As illustrated in Figure 2D, cells transfected with miR 146b inhibitor displayed upregulated N cadherin and vimentin, alongside downregulated E cadherin. Collectively, our findings imply that miR 146b participates in regulating the key processes of PE at the cellular level.

PTEN is a Downstream Target of miR-146b

PTEN was chosen as the candidate gene because it has potential complementary sequences to miR-146b (Fig. 3A). Luciferase reporter experiments were conducted to assess how miR 146b influences PTEN expression. As shown by the assay results, the ectopic expression of miR 146b led to a notable reduction in luciferase activity of the PTEN/WT luciferase construct relative to the miR NC control. However, the inhibitory effect was abolished when the putative PTEN binding site was mutated (Fig. 3B). Moreover, western blot analysis revealed that ectopic miR 146b expression suppressed PTEN protein levels, and the miR 146b inhibitor elevated PTEN expression (Fig. 3C).

Targeting PTEN by miR-146b Downregulation Enhances the Migratory, Invasive Capabilities and EMT Progression of HTR-8/SVneo Cells

The researchers found that cells transfected with pcDNA-PTEN significantly overexpressed PTEN (Fig. 4A). Suppression of miR 146b expression remarkably strengthened the migratory and invasive capacities of HTR 8/SVneo trophoblast cells. However, treatment of miR-146b down-regulated cells with pcDNA-PTEN partially dampened these effects (Fig. 4B and C). Moreover, suppression of miR 146b expression resulted in prominent upregulation of E cadherin, alongside distinct reductions in N cadherin and vimentin expression in HTR 8/SVneo cells compared with controls. Conversely, co-transfection with pcDNA-PTEN partially reversed these effects on E-cadherin, N-cadherin, and vimentin expression (Fig. 4D). Taken together, the findings presented above demonstrate that miR 146b and PTEN mutually inhibit each other's function, and they have contrasting impacts on the migratory and invasive capacities, and EMT progression of trophoblast cells.

MiR-146b/PTEN Axis Regulates the Activation of PI3K/AKT

PTEN exerts its tumour-suppressive role, which is tightly linked to PI3K/AKT signalling cascade (Li et al. 2016). Accordingly, the research group carried out additional experiments to explore whether miR-146b and PTEN modulate PI3K/AKT pathway activity. As indicated by Western blot results, the miR 146b upregulation markedly elevated phosphorylated AKT levels, whereas total AKT expression did not exhibit any significant difference. Nevertheless, co expression of PTEN significantly reversed this promoting effect induced by miR 146b overexpression (Fig. 5). These observations confirmed that miR-146b/PTEN contributes significantly to the activation ofPI3K/AKT signalling cascade in trophoblast cell progression.

DISCUSSION

miRNA expression patterns exhibit dynamic changes and are capable of reflecting variations in the internal and external milieus of the cell. Accordingly, miRNAs can be utilized as promising biomarkers to reflect particular disease states or pathophysiological events at various phases of disease development (Ingman et al. 2000; Haberman et al. 2019). Dysregulation of miRNAs has been demonstrated to be directly implicated in the progression of PE and to regulate the migratory ability and EMT process of human trophoblast cells (Yang et al., 2019). Previous research has also demonstrated that miR-146b is

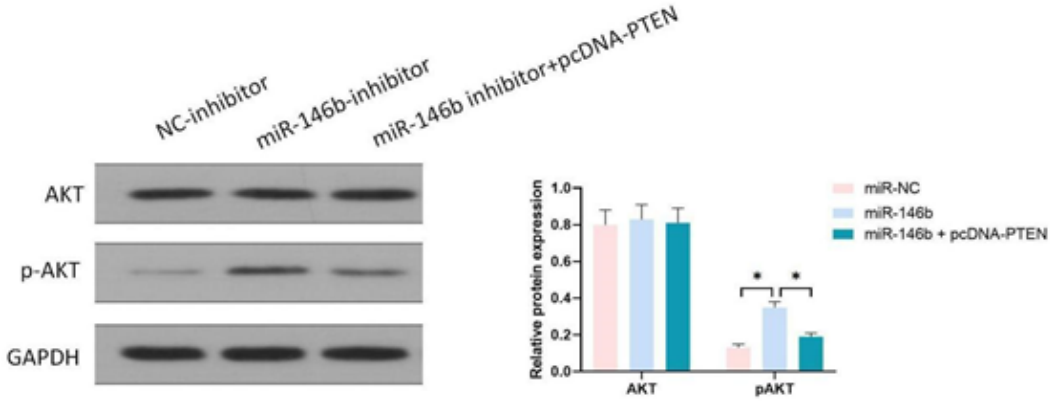

Fig. 5. Western blot of p-AKT and total AKT in HTR-8/SVneo cells. *P < 0.05

highly expressed in cows suffering from PE (Markkandan et al., 2018). Nevertheless, no existing report has described the expression profile and molecular mechanism of miR 146b in PE. We observed markedly higher expression levels of miR 146b in PE patient tissues relative to normal tissues. Furthermore, ROC curve analysis supports the utility of miR 146b as a candidate diagnostic biomarker for PE.

Relevant research has shown that the development of PE is linked to several abnormal physiological changes, including the aberrant invasion of trophoblast cells, impaired function of endothelial cells, and disrupted angiogenesis. Dysregulation of trophoblast invasion can also lead to PE. Typically, the uterine placental arteries are invaded by endovascular trophoblast cells and remolded into dilated, inelastic tubes without vasomotor control. However, dysregulation of trophoblast invasion, disturbed remodelling, maintenance of high uterine placental vascular resistance, and intrauterine growth restriction are associated with PE. From the perspective of abnormal trophoblast invasion, this research aims to investigate the potential regulatory mechanism by which dysregulated miR 146b affects trophoblast migration and invasion.

Epithelial-mesenchymal transition in trophoblasts describes the phenotypic conversion of these cells from an epithelial status to a mesenchymal status. This transition is accompanied by elevated migratory and invasive potential. This enables trophoblasts to immobilise the placenta in the maternal tissue and reshape the maternal spiral arteries. Accordingly, impaired migratory and invasive capacities of trophoblasts are considered to be implicated in the pathogenesis of PE (Kakkar et al., 2013). In this investigation, we observed that miR 146b regulates the migratory and invasive abilities of HTR 8/SVneo cells, suggesting that miR 146b could facilitate PE development by weakening trophoblast migration, invasion, and EMT.

PTEN was confirmed to be a direct downstream target of miR 146b, which supports the negative regulatory interaction between their expression levels in the examined trophoblast cell lines. Additionally, previous research has indicated that miR 146b participates in regulating cell apoptosis, cell cycle progression, invasive ability, and migratory capacity. This work expands these observations to trophoblast cells, showing that PTEN is able to partially rescue all miR 146b mediated cellular effects. In some cancer studies, miR-146b was reported to participate in regulating cancer cell migration and invasion through targeting PTEN (Ramírez Moya et al. 2018). This study confirmed that miR 146b expression presents an inverse regulatory correlation with PTEN abundance. After further evaluation of the effects of PTEN and miR-146b on PE, the researchers found that the upregulation of PTEN following overexpression of the PTEN + miR-146b mimic negated the promoting

effects of the miR-146b mimic on cell proliferation, metastasis, and EMT. It is illustrated by our outcomes that PTEN functions as a vital downstream effector targeted by miR 146b, and trophoblast phenotypes are modulated by miR 146b via controlling PTEN expression. In summary, miR 146b exerts essential regulatory functions during the initiation and progression of PE.

As an upstream lipid kinase of the PI3K/AKT/mTOR cascade, PI3K is physiologically triggered by receptor tyrosine kinases and G protein-coupled receptors, thereby promoting the conversion of PIP, to PIP*ƒ* via phosphorylation. As a second messenger, PIP3 promotes the amplification of PI3K signalling through the recruitment of lipid-binding domain–containing proteins, including AKT and PDK1. These proteins can interact with and stimulate more than 100 effector factors by phosphorylating and activating AKT on Thr-308. Phosphorylation of Ser 473 is required to maximise AKT function, which depends on mTOR regulated by PIP3. PTEN participates in multiple cellular events, including cell growth and proliferation. In this pathway, PTEN acts as a lipid phosphatase, leading to the dephosphorylation of 30 PIP3 phosphate groups. This converts the phosphorylated PIP3 generated under the action of PI3K to PIP2, thus inhibiting the activation of PI3K signalling. Mutation or loss of PTEN reduces the regulation of PIP3, and over-activation of the PI3K cascade results in the binding of activated AKT. The activation of AKT leads to further activation of G1 cyclin-dependent kinase, thus facilitating the G1/S cell cycle transition, which promotes cell proliferation and affects VEGF-mediated angiogenesis.

As an important cellular signalling axis, the PI3K/Akt pathway exerts vital functions in cell proliferation and apoptotic progression. Emerging evidence indicates that reduced activity of the PI3K/AKT/mTOR cascade is tightly correlated with PE pathogenesis. As a critical signalling pathway, the PI3K/AKT/mTOR axis governs multiple aspects of ovarian function, such as the dormancy and activation of PFs, oocyte survival and activation, and granulosa cell proliferation and differentiation. Dysregulation of these signalling cascades facilitate the pathological development of PE. The PI3K/AKT signalling cascade is widely known for its involvement in regulating cellular functions, including cell migration, invasion, and EMT (Xu et al. 2020). Hepatitis B virus x (HBx) inhibited apoptosis by activating Akt pathway via EGFR in vitro trophoblast cell line JEG-3. In addition, retinol-binding protein 4 (RBP4) overexpression promoted the proliferative capacity of HTR8/SVneo trophoblast cells through suppressing the PI3K/Akt signalling cascade, and HTR8/SVneo cell apoptosis increased when RBP4 downregulation activated the pathway. In PE, low levels of PI3K/AKT activation and AKT phosphorylation are maintained, leading to decreased trophoblast migratory and invasive phenotypes (Chen et al., 2019). In the current investigation, AKT phosphorylation was significantly regulated by the miR-146b/PTEN axis, suggesting that this axis participates in the PI3K/AKT signalling cascade in the process of trophoblast development. Although the outcomes of this study align with previous research, it is still uncertain whether this axis mediates trophoblast migration, invasion, and EMT in PE by acting on the PI3K/AKT pathway, which necessitates further experimental verification.

CONCLUSION

Our findings revealed remarkable upregulation of miR 146b in placental specimens derived from PE individuals. Conversely, reduced miR 146b expression markedly improved trophoblast migration, invasion as well as EMT progression. PTEN was confirmed to be the downstream target gene of miR-146b, and the mutual inhibition contributes to the joint regulation of trophoblast cell behaviour. The miR 146b/PTEN axis serves as a vital mediator that modulates the activation of the PI3K/AKT signalling cascade. However, this study is limited to the cellular level and lacks in vivo experiments. Collectively, it can be concluded that miR 146b may mediate trophoblast cell functions via the PTEN/PI3K/AKT axis, consequently playing a role in the pathological process of PE. Nevertheless, further in-depth investigations are still required to elucidate its regulatory role in animal models, thereby providing more robust evidence for its contribution to PE pathogenesis.

RECOMMENDATIONS

Future studies should focus on performing in vivo experiments to clarify miR-146b's role in preeclampsia pathophysiology. Investigate other molecules in the miR-146b/PTEN axis and its interaction with the PI3K/AKT pathway. Optimise and validate miR-146b as a diagnostic biomarker. Explore the development of targeted therapies based on this axis to improve patient outcomes.

AUTHORS' CONTRIBUTION

Hui Zhang and Mujun Li conceptualised manuscript contents and prepared the original draft. Wenban Qin and Liange Xia participated in manuscript writing, Yanhua Nong and Meiqi Huang reviewed and supervised manuscript writing. Each author participated sufficiently in the work and has approved the manuscript in its submitted form.

CONFLICT OF INTERESTS

None.

FUNDING

Financial support for this work was provided by Chongzuo Technology Plan Project (Chongke 2022QN1223) and Guangxi Key Laboratory of Biomedical Clinical Transformation (Chongke 20220625) and ability improvement of Guangxi key laboratory of biomedical clinical transformation (Chongke 2022ZY1002).

REFERENCES

Allard M, Grosch S, Jouret F, Masson V, Surinder T, Masset C 2024. Prevention of preeclampsia and its complications. *Rev Med Liege,* 79(5-6): 448-454. (French).

American College of Obstetricians and Gynecologists 2019. ACOG Practice Bulletin No. 202: Gestational Hypertension and Preeclampsia. *Obstet Gynecol,* 133(1): 1.

Chen J, Yue C, Xu J, Zhan Y, Zhao H, Li Y, Ye Y 2019. Downregulation of receptor tyrosine kinase-like orphan receptor 1 in preeclampsia placenta inhibits human trophoblast cell proliferation, migration, and invasion by PI3K/AKT/mTOR pathway accommodation. *Placenta*, 82: 17-24.

El-Sayed AAF 2017. Preeclampsia: A review of the pathogenesis and possible management strategies based on its pathophysiological derangements. *Taiwan J Obstet Gynecol,* 56(5): 593-598.

Filipek A, Jurewicz E 2018. Preeklampsja – choroba kobiet w ci¹¿y [Preeclampsia - a disease of pregnant women]. *Postepy Biochem,* 64(4): 232-229. (Polish).

Gao S, Zhao Z, Wu R, Wu L, Tian X, Zhang Z 2018 MiR-146b inhibits autophagy in prostate cancer by targeting the PTEN/Akt/mTOR signaling pathway. *Aging*, 10(8): 2113-2121.

Gebert LFR, MacRae IJ 2019. Regulation of microRNA function in animals. *Nat Rev Mol Cell Biol*, 20(1): 21-37.

Gingras-Charland ME, Côté AM, Girard P, Grenier A, Pasquier JC, Sauvé N 2019. Pre-eclampsia educational tool impact on knowledge, anxiety, and satisfaction in pregnant women: A randomized trial. *J Obstet Gynaecol Can*, 41(7): 960-970.

Haberman Y, Karns R, Dexheimer PJ, Schirmer M, Somekh J, Jurickova I, Braun T, Novak E, Bauman L, Collins MH et al. 2019. Ulcerative colitis mucosal transcriptomes reveal mitochondriopathy and personalized mechanisms underlying disease severity and treatment response. *Nat Commun*, 10(1): 38. doi: 10.1038/s41467-018-07841-3. https://doi.org/10.1186/s13046-020-01562-6

Ingman M, Kaessmann H, Pääbo S, Gyllensten U 2000. Mitochondrial genome variation and the origin of modern humans. *Nature*, 408(6813): 708-713.

Ives CW, Sinkey R, Rajapreyar I, Tita ATN, Oparil S 2020. Preeclampsia-Pathophysiology and clinical presentations: JACC State-of-the-Art Review. *J Am Coll Cardiol,* 76(14): 1690-1702.

Kakkar T, Singh V, Razdan R, Digra SK, Gupta A, Kakkar M 2013. Placental laterality as a predictor for development of preeclampsia. *J Obstet Gynaecol India*, 63(1): 22-25.

Laivuori H, Lahermo P, Ollikainen V, Widen E, Häivä-Mällinen L, Sundström H, Laitinen T, Kaaja R, Ylikorkala O, Kere J 2003. Susceptibility loci for preeclampsia on chromosomes 2p25 and 9p13 in Finnish families. *Am J Hum Genet,* 72(1): 168-177.

Lee YR, Chen M, Pandolfi PP 2018. The functions and regulation of the PTEN tumour suppressor: new modes and prospects. *Nat Rev Mol Cell Biol*, 19(9): 547-562.

Li M, Peng J, Shi Y, Sun P 2020. miR-92a promotes progesterone resistance in endometriosis through PTEN/AKT pathway. *Life Sci*, 242(117190): 18.

Li Y, Tsang CK, Wang S, Li XX, Yang Y, Fu L, Huang W, Li M, Wang HY, Zheng XF 2016. MAF1 suppresses AKT-mTOR signaling and liver cancer through activation of PTEN transcription. *Hepatology,* 63(6): 1928-1942.

Li Z, Zhou X, Gao W, Sun M, Chen H, Meng T 2022. Circular RNA VRK1 facilitates pre-eclampsia progression via sponging miR-221-3P to regulate PTEN/Akt. *J Cell Mol Med*, 26(6): 1826-1841.

Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM 2005. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature*, 433(7027): 769-773.

Ma'ayeh M, Costantine MM 2020. Prevention of preeclampsia. *Semin Fetal Neonatal Med,* 25(5): 101123.

Markkandan K, Ahn K, Lee DJ, Kim TI, Dang C, Hong SE, Yoon HB, Lim HJ, Hong CP 2018. Profiling and

identification of pregnancy-associated circulating microRNAs in dairy cattle. *Genes Genomics*, 40(10): 1111-1117.

Oudejans CB, Mulders J, Lachmeijer AM, van Dijk M, Könst AA 2004. The parent-of-origin effect of 10q22 in pre-eclamptic females coincides with two regions clustered for genes with down-regulated expression in androgenetic placentas. *Mol Hum Reprod*, 10(8): 589-598.

Phipps EA, Thadhani R, Benzing T, Karumanchi SA 2019. Pre-eclampsia: Pathogenesis, novel diagnostics and therapies. *Nat Rev Nephrol*, 15(5): 275-289.

Ramírez-Moya J, Wert-Lamas L, Santisteban P 2018. MicroRNA-146b promotes PI3K/AKT pathway hyperactivation and thyroid cancer progression by targeting PTEN. *Oncogene*, 37(25): 3369-3383.

Ramos JGL, Sass N, Costa SHM 2017. Preeclampsia. *Rev Bras Ginecol Obstet*, 39(9): 496-512.

Rana S, Lemoine E, Granger JP, Karumanchi SA 2019. Preeclampsia: Pathophysiology, challenges, and perspectives. *Circ Res,* 124(7): 1094-1112.

Roberts JM 2024. Preeclampsia epidemiology(ies) and pathophysiology(ies). *Best Pract Res Clin Obstet Gynaecol,* 94: 102480.

Rupaimoole R, Slack FJ 2017. MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov*, 16(3): 203-222.

Shu C, Yan D, Chen C, Mo Y, Wu L, Gu J, Shah NK, He J, Dong S 2019. Metformin exhibits its therapeutic effect in the treatment of pre-eclampsia via modulating the Met/H19/miR-148a-5p/P28 and Met/H19/miR-216-3p/EBI3 signaling pathways. *Int Immunopharmacol*, 74(105693): 13.

Wu JL, Wang YG, Gao GM, Feng L, Guo N, Zhang CX 2019. Overexpression of lncRNA TCL6 promotes preeclampsia progression by regulating PTEN. *Eur Rev Med Pharmacol Sci*, 23(10): 4066-4072.

Xu J, Xiao Y, Liu B, Pan S, Liu Q, Shan Y, Li S, Qi Y, Huang Y, Jia L 2020. Exosomal MALAT1 sponges miR-26a/26b to promote the invasion and metastasis of colorectal cancer via FUT4 enhanced fucosylation and PI3K/Akt pathway. *J Exp Clin Cancer Res*, 39(1): 54.

Yang Y, Li H, Ma Y, Zhu X, Zhang S, Li J 2019. MiR-221-3p is down-regulated in preeclampsia and affects trophoblast growth, invasion and migration partly via targeting thrombospondin 2. *Biomed Pharmacother*, 109: 127-134.

Zhang F, Liu J, Xie BB 2019. Downregulation of microRNA-205 inhibits cell invasion and angiogenesis of cervical cancer through TSLC1-mediated Akt signaling pathway. *J Cell Physiol*, 234(10): 18626-18638.

Paper received for publication in July, 2024
Paper accepted for publication in March, 2026