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RESEARCH ARTICLE

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LncRNA LINC00534 regulates cell proliferation and migration via the miR-494-3p/PTEN axis in HTR-8/SVneo cells

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Abstract

Background: LncRNA LINC00534 has been found to be differentially expressed in placental tissue samples of preeclampsia (PE), but the exact mechanism is still unclear.

Methods: In vitro assays were carried out in HTR-8/SVneo cells using various methods, including cell counting kit-8 (CCK-8), transwells, flow cytometry, and Western blotting (WB) and quantitative polymerase chain reaction. RNA pull-down and bioinformatics analysis were applied to examine other potential underlying mechanisms involved.

Results: We found that there was a high expression of LINC00534 in the placental tissues of patients with PE. LINC00534 overexpression (OE) significantly inhibited cell proliferation and migration as well as accelerated cell apoptosis in HTR8/SVneo cells. The knockdown of LINC00534 produced an opposite trend. Mechanistically, LINC00534 promoted the expressions of PTEN (Phosphatase and tensin homolog) through decreasing miR-494-3p. Further rescue studies showed that LINC00534 played a role by targeting mir-494-3p, which controlled the growth and migration of HTR-8/SVneo trophoblast cells via regulating PTEN/PI3K/AKT (Phosphatidylinositol3-kinase/protein kinase B). Moreover, lncRNA pull-down assay identified 198 potential bound proteins for LINC00534. Those proteins were mostly involved in RNA processing and modification, posttranslational modification, protein turnover, and chaperones.

Conclusion: Overall, by suppressing HTR8/SVneo cell growth and migration via the miR-494-3p/PTEN axis and other mechanisms, LINC00534 offers new insight into PE pathogenesis.

Xuejing Song, Xinxin Zhang and Qing Xia contributed equally to this paper.

KEYWORDS
LINC00534, LncRNA, miR-494-3p, preeclampsia, PTEN

1 | INTRODUCTION

In pregnancy, preeclampsia (PE) is a multisystemic vascular syndrome with hypertension and proteinuria, generally observed after 20 weeks.[1] The incidence of PE worldwide is about 2%–8%. It is a leading cause of death and morbidity for mothers and their babies.[2] The only way to treat PE is to deliver the placenta.[3,4] Currently, PE's pathogenesis is not fully understood, and the placenta may be the breakthrough to determine the pathological mechanism of PE.

The placenta is a unique organ that facilitates the exchange of material between the mother and fetus, and it is crucial for the development of the human embryo and fetus. During the initial stage of implantation, trophoblast cells invade the endometrium to help reshape the spiral arterioles in the villi space, thus increasing the diameter of blood vessels, reducing blood flow resistance, and ensuring adequate blood flow to meet the requirements of the fetoplacental unit.[5,6] In PE, abnormal trophoblast invasion damages spiral artery remodeling, resulting in reduced placental perfusion, which leads to placental ischemia and a series of complications.[7] Recently, studies have increasingly focused on trophoblast cell-related signaling pathways and key mechanisms, in order to further explore the progression of PE.[1] It has been generally accepted that the pathogenesis of PE is related to the dysfunction of extravillous trophoblast (EVT), including proliferation, apoptosis, migration, and invasion.

In the human genome, only 2% of RNA is protein-encoded mRNA. Non-coding RNAs (ncRNAs) account for 98% of all RNAs, including long non-coding RNAs (lncRNAs) with over 200 nucleotides[8] and microRNAs (miRNAs) with around 22 nucleotides.[9,10] As suggested by the competitive endogenous RNA hypothesis, long non-coding RNAs can bind to miRNAs, regulate target gene expression, and thus function in a variety of physiological processes.[11,12] For example, LINC01133 regulates the Wnt/β-catenin signaling pathway by inhibiting miR-106a-3p; thus, inhibiting the progression of gastric cancer.[13] In the breast cancer, the expression level of lncRNA HOX transcript antisense RNA (HOTAIR) tends to be increased, which inhibits miR-20a-5p, promotes the expression of target gene HMGA2, and affects cell proliferation, invasion, and apoptosis.[14] LncRNA SND1-IT1 can up-regulate POU2F1 to accelerate osteosarcoma proliferation and migration by sponging miR-665.[15]

Studies have shown that lncRNAs, such as uc.294,[16] NR_002794,[17] CRNDE,[18] and TUG1,[19] are involved in PE development by affecting trophoblast functions. In the previous study, we detected lncRNAs specifically expressed in the placenta tissues of PE patients by using microarray assays.[20] We discovered that LINC00534 is an interspecific lncRNA with high-transcriptional activity and differential expression in PE, and its function has not yet been reported. Therefore, we attempted to examine the function and potential mechanisms of LINC00534 in PE by measuring the biological functions of EVT HTR-8/SVneo cells.

2 | MATERIALS AND METHODS

2.1 | Ethics approval and consent to participate

This study was approved by the Institutional Review Board of the Women's Hospital of Nanjing Medical University (No. [2017] 91) and in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Patients provided written informed consent for sample collection.

2.2 | Clinical sample collection

Researchers obtained the placental tissues from patients aged 28–37 years with PE and normal mothers at the Women's Hospital of Nanjing Medical University in Jiangsu Province from 2020 to 2021. There were 5 parturients in each group. Samples of placental tissues were collected immediately following delivery and stored in liquid nitrogen for future research. PE was defined as hypertension (≥140/90 mmHg) with proteinuria (urinary protein 300 mg/day). All the participants had conceived naturally and had no history of diabetes, cardiovascular disease, hypertension, hyperthyroidism, alcoholism, smoking, kidney disease, intrauterine fetal death, chemical dependence, and fetal chromosomal or congenital abnormalities.

2.3 | Cell culture

The cell line used was the human HTR-8/SVneo trophoblast cell line, an immortalized EVT cell line in early pregnancy purchased from the Chinese Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, Shanghai, China). HTR-8/SVneo cells were cultured with RPMI1640 medium added with 10% FBS in a cell incubator at 37°C with 5% carbon dioxide. The fresh medium was changed every 2 days according to the cell status.

2.4 | Plasmids and oligonucleotides transfections

Specific plasmids and oligonucleotides were designed to regulate the expressions of LINC00534 and miR-494-3p. The OE plasmids (NR_051989.1) and specific small interfering RNAs (siRNAs) for LINC00534 were derived from Wuhan GeneCreate Biological Engineering Co., Ltd. In addition, synthetic miR-494-3p mimics and its negative control oligonucleotides were purchased from Guangzhou RiboBio Co., Ltd. Lipofectamine 2000 (Invitrogen) was used to co-transfect plasmids and oligonucleotides into cell lines. HTR-8/SVneo cells were plated in a 6-well cell culture dish and the degree of fusion was 70%–80% before transfection. The transfected

cells were cultured at 37°C in a 5% carbon dioxide incubator and the medium was changed 6 h after transfection.

2.5 | Cell proliferation assay

The proliferation capacity of trophoblast cells was measured using a Cell Counting Kit-8 (CCK-8, Bioscience Technology Co. Ltd.) assay. The transfected HTR-8/SVneo cells were seeded into 96-well plates. These cells were cultured in six replication wells with 3000 cells and 100 μl medium each, and then they adhered to the wall about 6 h later. Further, a 10 μl solution of CCK-8 was added to the medium at 0, 24, and 48 h after the cells had been incubated. A multifunctional microplate reader was used to measure the absorbance at 450 nm after adding CCK-8 for 2 h.

2.6 | Cell migration assays

Migration of HTR-8/SVneo cells was tested in Transwell chambers. In brief, the upper chamber was filled with 200 μl of medium (including 20,000 cells), and the lower chamber was filled with 600 μl of medium containing 20% FBS. After 24 h, the upper sides were fixed with methanol for 15 min and stained for 20 min using 0.1% crystal violet solution. After washing 3 times with PBS, optical microscopes were used to examine the number of cells that penetrated the filter membrane. Photographs and counts were taken of three randomly selected visual fields.

2.7 | Flow cytometric analysis of cell apoptosis

Overexpression or knockdown vectors were transiently transfected into HTR-8/SVneo cells for 48 h. The collected old medium was washed twice with PBS. Afterward, the cells were harvested using trypsin without ethanoldiamine tetraacetic acid (EDTA). Annexin V-FITC Apoptosis Detection Kit (Solarbio) was used to detect apoptosis. A flow cytometer (CytoFLEX S, Beckman Coulter) equipped with CytExpert/FlowJo software was used to analyze the cells. Cells were sorted into survival cells, necrotic cells, early apoptotic cells, and late apoptotic cells. Apoptotic cells were also assessed for further comparisons.

2.8 | Quantitative real-time reverse-transcription-polymerase chain reaction (qRT–PCR)

The Trizol Reagent (TaKaRa) was used to extract and purify total RNA from tissues or cells. By using the One Drop OD-1000+ Spectrophotometer (One drop Technologies), the quality and quantity of total RNA were confirmed. For lncRNA and mRNA analysis, cDNA was synthesized by the PrimeScript™ RT reagent kit (Takara) and was subjected to quantitative PCR with Applied Biosystems™ SYBR Reagent (Thermo Fisher Scientific). The miRNA expression levels were determined using the Bulge-Loop miRNA qPCR Primer Set (RiboBio). All reactions were performed on the ViiA7 real-time PCR System-Life Tech (Applied Biosystems). The initial denaturation steps were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The relative expression levels were calculated via the $2^{-\Delta Ct}$ method. Relative expression levels of lncRNA and mRNAs were normalized to the endogenous control glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and relative expression levels of miRNAs were normalized to the endogenous control U6. Three replicates were amplified each time to verify the results. The primer sequences for LINC00534 were as follows: forward: 5′-TCTCACCTCAGCCTCGCAAG-3′; reverse: 5′-AGTTCAAGACCAGCCTGTTCAAG-3′. Primer-blast was used to test the specificity of the primers for LINC00534.

2.9 | Transcriptome sequencing of RNA

RNA was isolated from HTR-8/SVneo cells treated with siRNAs for LINC00534 and control for 48 h. Three replicates per group were performed. Each sample was tested for purity, and RNA sequencing was performed by BGI. Then, a volcano plot and heatmap were drawn, and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were conducted.

2.10 | Western blotting (WB) analysis

WB was used to quantify the protein levels in samples. HTR-8/SVneo cells were transfected with LINC00534 OE plasmids or siRNAs for 48 h. A RIPA buffer supplemented with PMSF (Beyotime Institute of Biotechnology) and phosphatase inhibitors (Servicebio) was used to extract proteins. After separation by 10% SDS-PAGE (Servicebio), the samples were transferred to nitrocellulose membranes. Further, after blocking with 5% skim milk (Guangzhou Saiguo Biotech Co., LTD) for 2 h, membranes were incubated with primary antibodies against PI3K (p110β) (CST, 1:1000, #3011), ERK1 (Abcam, 1:1000, ab32537), NF−κB p65 (CST, 1:1000, #8242), STAT3 (Abcam, 1:2000, ab68153), PTEN (Abcam, 1:5000, ab32199), and β-ACTIN (Proteintech, 1:1000, 66009-1-Ig) overnight at 4°C. The membranes were washed three times for 10 min each and incubated with secondary antibodies the following day.

2.11 | RNA pull-down

Briefly, biotin-labeled 869 nt of LINC00534 and antisense (Ribobio) were purified and incubated with streptavidin agarose beads (Thermo Fisher Scientific). HTR-8/SVneo cell lysates were collected and incubated with the RNA-beads complex. The captured beads were divided into two parts. One part was washed and boiled at 95°C in SDS loading buffer (Beyotime Biotechnology). Afterwards, boiled samples were separated and visualized by using a silver staining kit

(Thermo Fisher Scientific). The other part of LINC00534 captured beads was sent to BGI for mass spectrometry assay.

2.12 | Statistical analysis

At least two or three independent experiments were conducted for in vitro culture-related studies, and data were expressed as mean ± standard deviation (SD). GraphPad Prism 8.0 (GraphPad Software) and Image J software were used to analyze statistics and images, respectively. An analysis of Student's *T*-test was employed to determine differences between the two groups. $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | LINC00534 was upregulated in the placenta tissues of PE patients

Using the public LNCipedia database website, five transcripts of LINC00534 were identified (Figure 1A). We here studied the longest transcript of LINC00534, with a length of 869 nt (Figure 1B). Compared with five normal controls, a significant difference in blood pressure and proteinuria in the placentas of five cases of PE were found (Table 1). LINC00534 levels were tested by RT-qPCR. The result showed that LINC00534 was significantly upregulated in the placentae of PE cases ($p < 0.05$) (Figure 1C).

3.2 | LINC00534 repressed the proliferation and migration, and promoted apoptosis in extravillous trophoblast HTR-8/SVneo cells

Firstly, we examined the effects of LINC00534 on trophoblast cell proliferation, migration, and apoptosis. After 48 h of transfection, the LINC00534 level was significantly upregulated after the introduction of the LINC00534 OE vector into trophoblast cells (Figure 1D). Based on the CCK-8 assay, we found that OE of LINC00534 repressed cell proliferation (Figure 1E). By flow cytometry, we detected that OE of LINC00534 promoted cell apoptosis level (Figure 1F). The mRNA expression of anti-apoptotic B-cell lymphoma-2 (Bcl-2) was downregulated in cells transfected with the LINC00534 OE vector compared with the control (Figure 1G). On the contrary, after LINC00534 was knocked down, its expression level decreased, with siRNA sequence 1 (si-LINC-1) being the most significant (Figure 1H), thus, si-LINC-1 was utilized in subsequent research to test trophoblast functions. Knockdown of LINC00534 promoted cell proliferation (Figure 1I), repressed apoptosis (Figure 1J), and increased the expressions of Bcl-2 (Figure 1K).

We evaluated cell migration through the Transwell assay. These results showed that OE of LINC00534 repressed the migration of HTR-8/SVneo trophoblast cells (Figure 2A), while knockdown of LINC00534 promoted cell migration (Figure 2B). Following LINC00534 OE, matrix metalloproteinase-2 (MMP2) and matrix metalloproteinase-9 (MMP9) mRNA expression levels were reduced compared to the control group, and the corresponding expression levels were decreased when LINC00534 was knocked down (Figure 2C).

These results prove that LINC00534 can inhibit the proliferation and migration of EVT HTR-8/SVneo cells and promote apoptosis, suggesting that up-regulation of LINC00534 may impair the proliferation, migration, and apoptosis of EVT cells, leading to placental dysplasia.

3.3 | Mechanisms of LINC00534 function detected by RNA sequencing

To further understand the mechanism of LINC00534, RNA sequencing was conducted in the control group and the si-RNA (si-LINC-1) group. The DEGseq database was applied to analyze the differentially expressed genes in the experimental group and the control group. On filter condition of $|\log2FC| \geq 1$, Qvalue ≤0.05, 522 differentially expressed genes were detected. The difference in genes between the two groups was represented by a volcano map. The red dots represent 171 genes that were significantly increased in the experimental group, the green dots represent 351 genes that were significantly decreased, and the gray dots indicate genes with no statistically significant differences (Figure 3A). A clustering heat map was used to represent the difference in genes between the two groups. Red represents the increased genes in the experimental group, and the green represents the decreased genes in the experimental group (Figure 3B). GO enrichment analysis was conducted on the differentially expressed genes from the aspects of cellular component, biological process, and molecular function, and

FIGURE 1 Effects of LINC00534 on cell proliferation and apoptosis in HTR8/SVneo trophoblast cells. (A) Five transcripts of LINC00534 were recorded in the public database LNCipedia. (B) Sequences of 869 nt of LINC00534. (C) Expression of LINC00534 in placentae tissues of patients with PE. Boxplot of LINC00534 expression levels in PE placentae ($n = 5$) compared to normal placentae ($n = 5$). (D) RT–qPCR was conducted to verify the transfection efficiency of LINC00534 OE in HTR8/SVneo cells. (E) CCK-8 assay was taken to evaluate the effects of OE of LINC00534 on the proliferation of HTR8/SVneo cells. (F) Apoptosis level was analyzed using Annexin-V assay coped with flow cytometry on cells overexpressing LINC00534. (G) Effects of LINC00534 OE on mRNA expression of Bcl-2. (H) Knockdown efficiency of LINC00534 was tested using RT-qPCR. (I) Effects of knockdown of LINC00534 on cell proliferation detected by CCK-8 assay. (J) Apoptosis level was analyzed using Annexin-V assay coped with flow cytometry on cells knocking down LINC00534. (K) Effects of LINC00534 knockdown on mRNA expression of Bcl-2. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. OE–NC: negative control of LINC00534; OE-LINC: OE of LINC00534; si-NC: negative control of siRNA; si-LINC: knockdown of LINC00534. The values are shown as the mean ± SD of three independent experiments.

(A)

Transcript ID	Gene ID	Location (hg38)	strand	transcript size
LINC00534:1	LINC00534	chr8:90221488-90388145	+	793
LINC00534:2	LINC00534	chr8:90221488-90387988	+	772
LINC00534:3	LINC00534	chr8:90221488-90387959	+	869
LINC00534:4	LINC00534	chr8:90221517-90569318	+	620
LINC00534:5	LINC00534	chr8:90221538-90387952	+	812

(B)

>NR_051989.1 (LINC00534) length=869 nt

GTCTTTTCTGGCAGATTACTTCTGTGAGCAACTGGGTCTCTCAGTTCTTTGGGAGCACCCTGGGAAAC
AGTGTGGCTATGACTTGGAGCTGCTCACCAGTTCCAGTCATTCTGTGGTTGAGAGCTGCTCCCTATGG
ATGTTAATTCTTCTCTATTTTAGATTTTCCACAGGTGTGGGCCCCATTAGCTCTGGCCACCTGAGAAAG
CCCTCAGGCAAAGAGTCGCAGGTGAAAGCCAGCAGTTAGAGGTTCACAGAAATGAGTGCAGAAGCAT
CTTCAGTTGGAGAGCCATTGCCTCTTGCTGTGAAATATTTGGGAATCTTCAGAAACCATGAAGAATAAC
CTGCTAAAGCTGAGCTGGGACTCTGCCTGGATATAACTTATGGCCTATTCTATATGGAAAGTTGGTTTT
CTCACAGTTCCACTCCCTTTCCAGATATCAGCAAATGGCCCTCCTAGCCCAGAAGTCTCCAGAGAGGG
TTTTATTTTGTCAGTCAAGCTGGAATGCAGTGGCGTGAACATTGCTTACTGCAGCTTCAATCTCCTGAG
GTCAAACAATCTTCTCACCTCAGCCTCGCAAGCAGCTAGGACTACAGGCATGTGCCACCACGCCCCAC
TAATTTTTTAATTTTTCTTTTGTGGAGACGCAGTCTTGTTATCTTGAACAGGCTGGTCTTGAACTCTTGGA
GTCAAGCAATCCTTCTGCCTCAGACTCTCAAAGTGCTGGGATTACAGGCATGAGCCACCACATTCAGCC
AGGAAAAGTTTTTGTACATTTAAAAAATTATAGGATATTTGTGTATATAGCTTTGCTGGTTTGACTTCCTAG
AATAGCTCCCAGGAACACAAAGTACTTAATTGATTAGTATAA

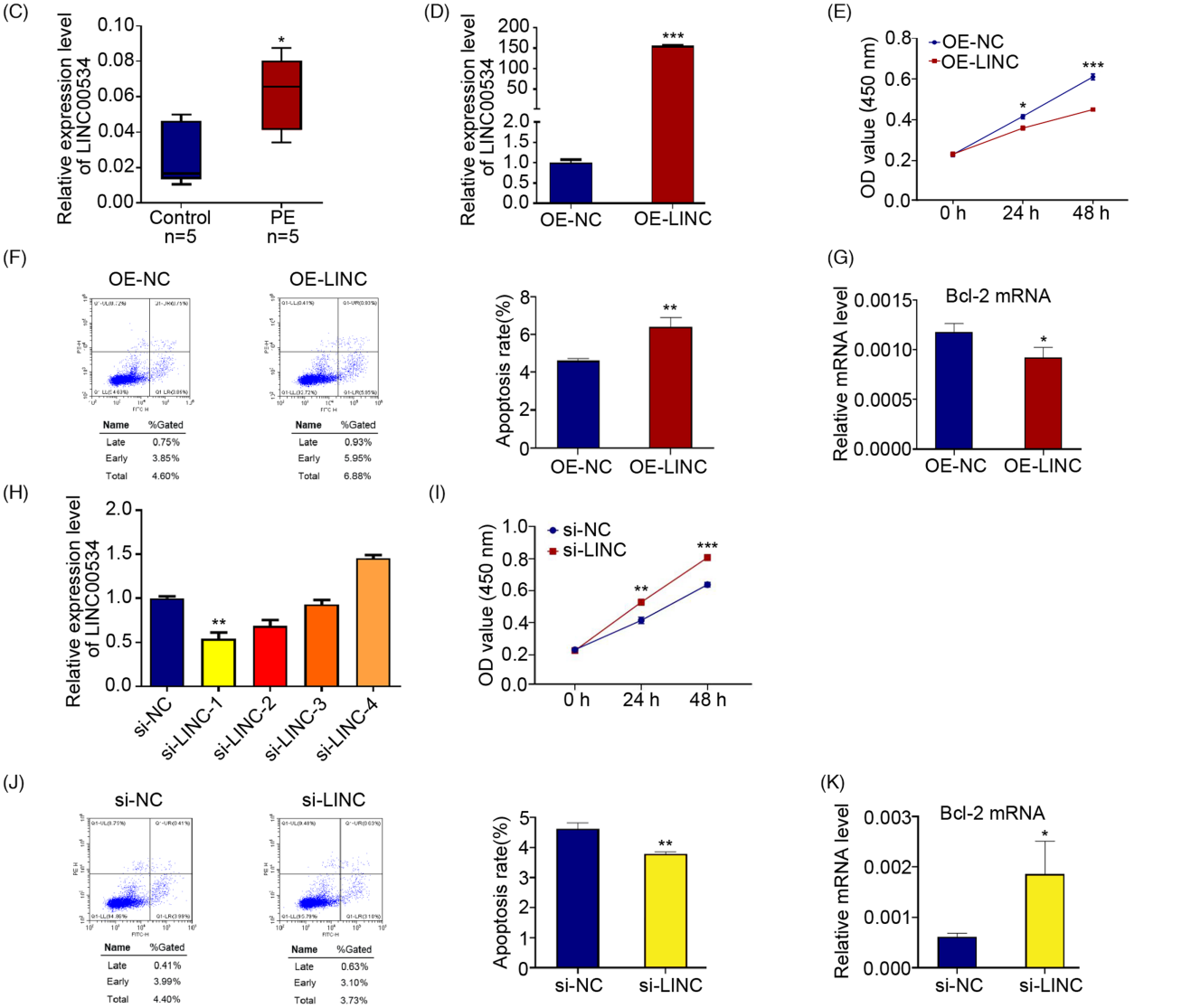


TABLE 1 Clinical characteristics of patients with normal and PE pregnancies

Variable	Control ($n = 5$)	PE ($n = 5$)	p Value
Maternal age (year)	31.2 ± 4.8	32.0 ± 5.0	>0.05*
Systolic blood pressure (mmHg)	111.2 ± 20.2	146.0 ± 18.0	<0.01**
Diastolic blood pressure (mmHg)	69.2 ± 10.8	97.8 ± 16.8	<0.01**
Proteinuria (g/day)	N/A	4.0 ± 2.4	<0.001***
Gestational age(week)	37.8 ± 2.3	36.8 ± 1.3	>0.05*
Birth weight(g)	2878 ± 162	2256 ± 274	<0.001***

Note: The values are shown as the mean ± SD.

Abbreviation: N/A, not applicable.

*$p < 0.05$.; **$p < 0.01$.; ***$p < 0.001$, preeclampsia compared with normal pregnancy.

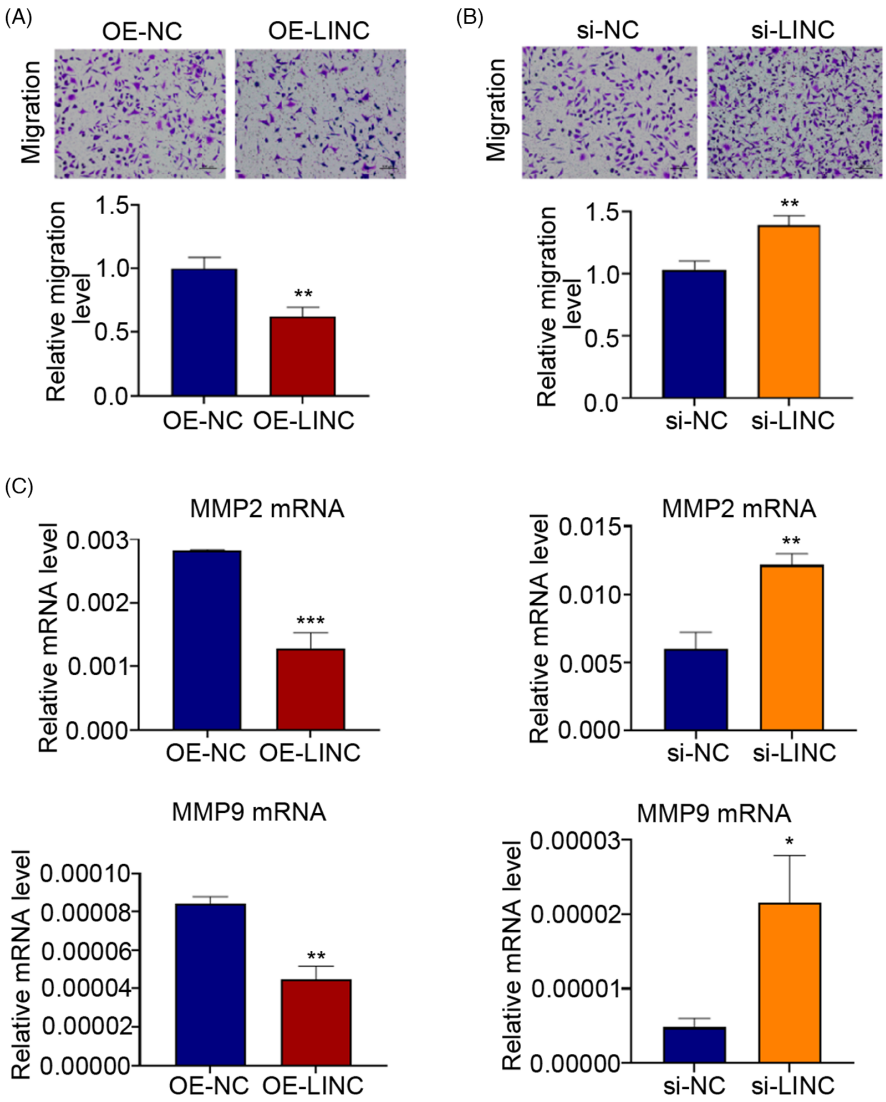


FIGURE 2 Effects of LINC00534 on the migrative abilities of trophoblast cells. Migration of HTR-8/SVneo trophoblast cells was tested by a Transwell assay after LINC00534 overexpression (OE) (A) or knockdown (B). (C) Effects of LINC00534 OE or knockdown on mRNA expressions of MMP-2 and MMP-9. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. OE-NC: negative control of LINC00534; OE-LINC: OE of LINC00534; si-NC: negative control of siRNA; si-LINC: knockdown of LINC00534.

the involved GO enrichment pathways were classified. In terms of biological functions, differentially expressed genes were mostly related to cell proliferation, apoptosis, and cell adhesion (Figure 3C). KEGG enrichment pathway analysis was used to search for differentially expressed gene-related pathways. A bubble diagram of the KEGG enrichment pathway suggested that the PI3K-AKT (Phosphatidylinositol3-kinase/protein kinase B) signaling pathway was the LINC00534 differential enrichment pathway (Figure 3D).

3.4 | LINC00534 upregulated its target gene PTEN (phosphatase and tensin homolog), inhibited the PI3K/AKT signaling pathway

According to the results of RNA sequencing, the PI3K/AKT signaling pathway was regulated by LINC00534. Numerous studies have shown that PTEN is a regulatory gene of the PI3K/AKT signaling pathway.[21,22] RT–qPCR data demonstrated that knockdown of

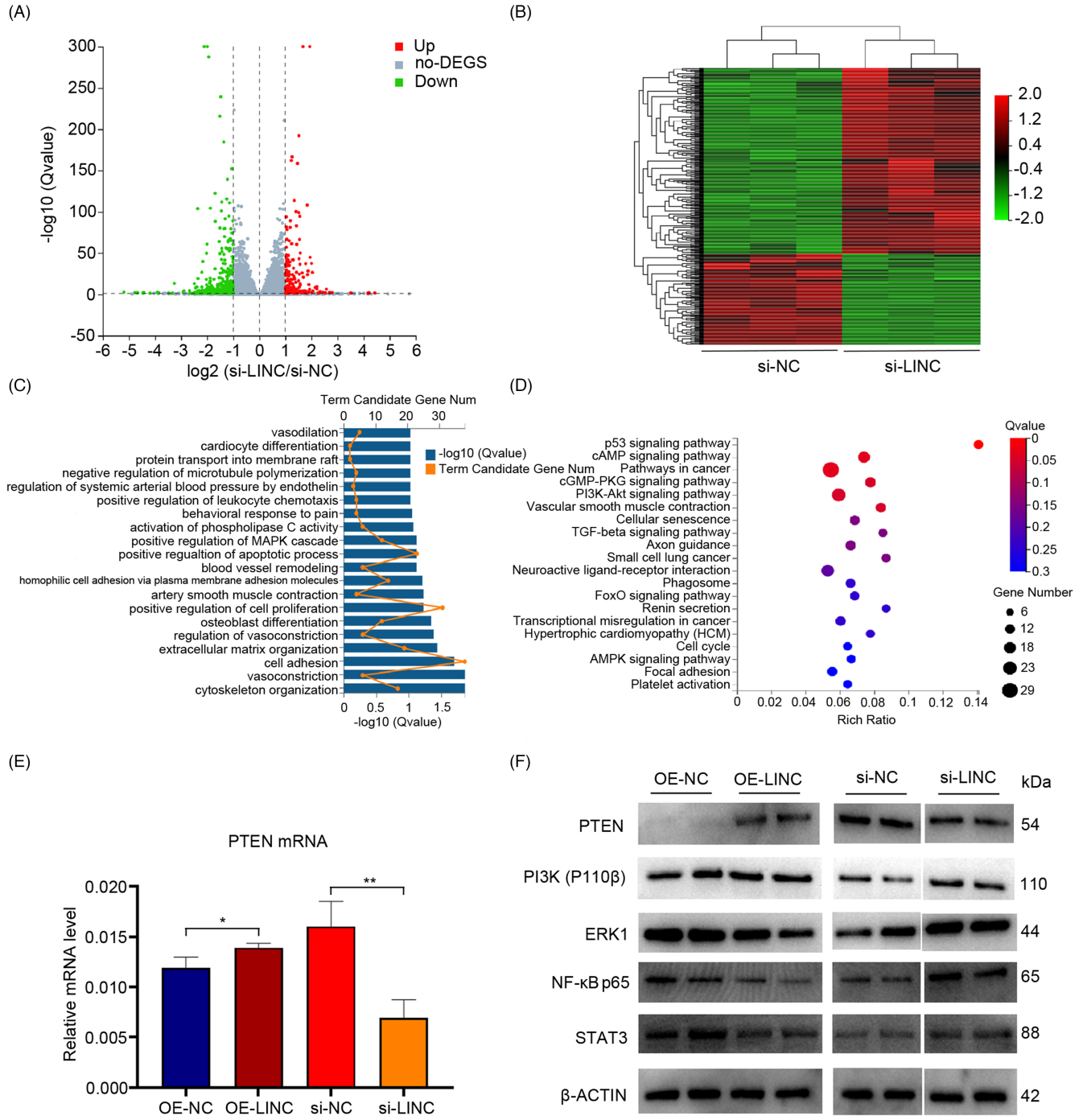


FIGURE 3 Function mechanisms of LINC00534 detected by RNA sequencing, qRT–PCR and Western blotting. (A) Volcano plot. (B) Heat map. (C) Results of GO analysis. GO: Gene Ontology. (D) Results of KEGG enrichment analysis. KEGG: Kyoto Encyclopaedia of Genes and Genomes. (E) Effects of LINC00534 overexpression (OE) or knockdown on mRNA expression of PTEN. (F) Effects of LINC00534 OE or knockdown on protein expressions of PI3K, PTEN, ERK1, NF–κB, and STAT3. The values are shown as the mean ± SD of three independent experiments; $^{**}p < 0.01$. OE–NC: negative control of LINC00534; OE–LINC: OE of LINC00534; si–NC: negative control of siRNA; si–LINC: knockdown of LINC00534.

LINC00534 reduced the mRNA expression of PTEN (Figure 3E). WB analysis showed a dramatic upregulation in PTEN together with downregulation in its downstream targets (PI3K), following LINC00534 OE, while LINC00534 knockdown had an opposite effect (Figures 3F). We also detected that the protein expression levels of ERK1, NF–κB, and STAT3 showed the same tendency (Figure 3F).

3.5 | MiR-494-3p counteracted LINC00534 overexpression-induced anti-proliferative and anti-migrative effects

In addition, we speculated that LINC00534 functioned as a miRNA sponge in trophoblast cells. Based on StarBase and DIANA Tools, miR-494-3p and miR-139-5p were predicted to bind with LINC00534

(Figure 4A). As hypothesized, LINC00534 OE remarkably decreased the miR-494-3p level and LINC00534 knockdown had an opposite effect, while the expression of miR-139-5p showed no significant difference (Figure 4B). Post 48 h of transfection, the transfection efficiency was tested, which verified that the miR-494-3p level was greatly upregulated in trophoblast cells following treatment with the miR-494-3p mimic (Figure 4C). PTEN was known to be the target gene of miR-494-3p; RT–qPCR showed that OE of miR-494-3p could reduce the mRNA expression of PTEN (Figure 4D).

We then examined the relationship between LINC00534 and miR-494-3p by using CCK-8 and Transwell migration assay. By transfecting miR-494-3p mimic, the decreased proliferation viability caused by LINC00534 OE was reversed (Figure 4E). Besides, miR-494-3p promotion significantly reduced the suppressive effects of LINC00534 elevation on cell migration (Figure 4F). These results presented that miR-494-3p promotion abolished the inhibitory effect of LINC00534 OE on trophoblast cell proliferation and migration.

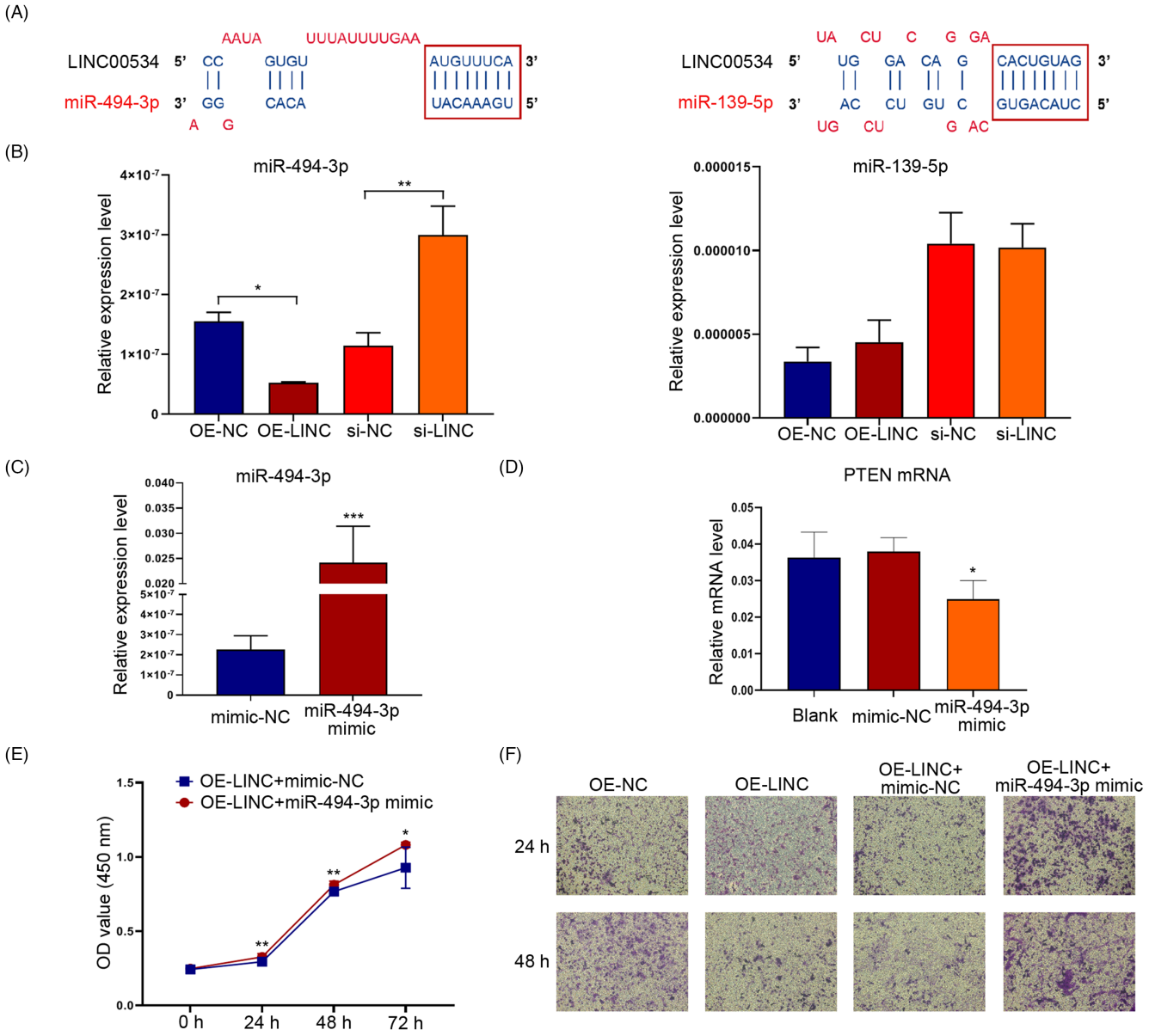


FIGURE 4 The targeted relationship between LINC00534 and miR-494-3p in HTR8/SVneo cells. (A) The potential binding site between LINC00534 and miR-494-3p (or miR-139-5p) was predicted by StarBase and DIANA Tools, and the binding site was shown. (B) RT–qPCR was used to check miR-494-3p (or miR-139-5p) level following overexpression (OE) and knockdown of LINC00534. (C) Transfection efficiency in HTR8/SVneo cells after transfection with miR-494-3p mimic was validated by RT–qPCR. (D) The effects of miR-494-3p mimic on the mRNA expression level of PTEN. (E) CCK-8 assay was conducted to analyze the effects of miR-494-3p promotion on the roles of overexpressing LINC00534 in HTR8/SVneo cells. (F) Transwell assay was applied to assess the effects of miR-494-3p promotion on the anti-migration effects of overexpressing LINC00534 in HTR8/SVneo cells. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. OE–NC: negative control of LINC00534; OE–LINC: OE of LINC00534; si–NC: negative control of siRNA; si–LINC: knockdown of LINC00534.

In addition, miR-494-3p promotes the PI3K/AKT pathway by targeting PTEN, and PTEN loss is one of the most common abnormalities in PE.[23] We hypothesize that LINC00534 may affect PTEN expression through targeting miR-494-3p and then regulate the PI3K/AKT signaling pathway in trophoblast cells; thus, it participates in the development of PE.

3.6 | RNA pull down assay suggests other potential involved mechanisms

RNA pull down assay was performed to identify the bound proteins of LINC00534. Two bands at 61 and 84 kDa were obviously enriched in the LINC00534 group (Figure 5A). After mass spectrometry, a total of 198 proteins were identified in the LINC00534 group. The sizes of most proteins were 30–60 kDa (Figure 5B). Through GO and KEGG pathway analysis, the potential bound proteins of LINC00534 were mostly involved in binding, organelle, cellular process, RNA processing and modification, posttranslational modification, protein turnover and chaperones (Figure 5C,D). According to the matched protein mass, several proteins such as heat shock protein HSP90-alpha (HS90A), endoplasmic reticulum chaperone BiP (GRP78, glucose regulated protein-78) and heat shock cognate 71 kDa protein (HSP7C) were predicted to be the mostly potential bound proteins of LINC00534 (Figure 5E). Further WB assay should be applied to test their interactions.

4 | DISCUSSION

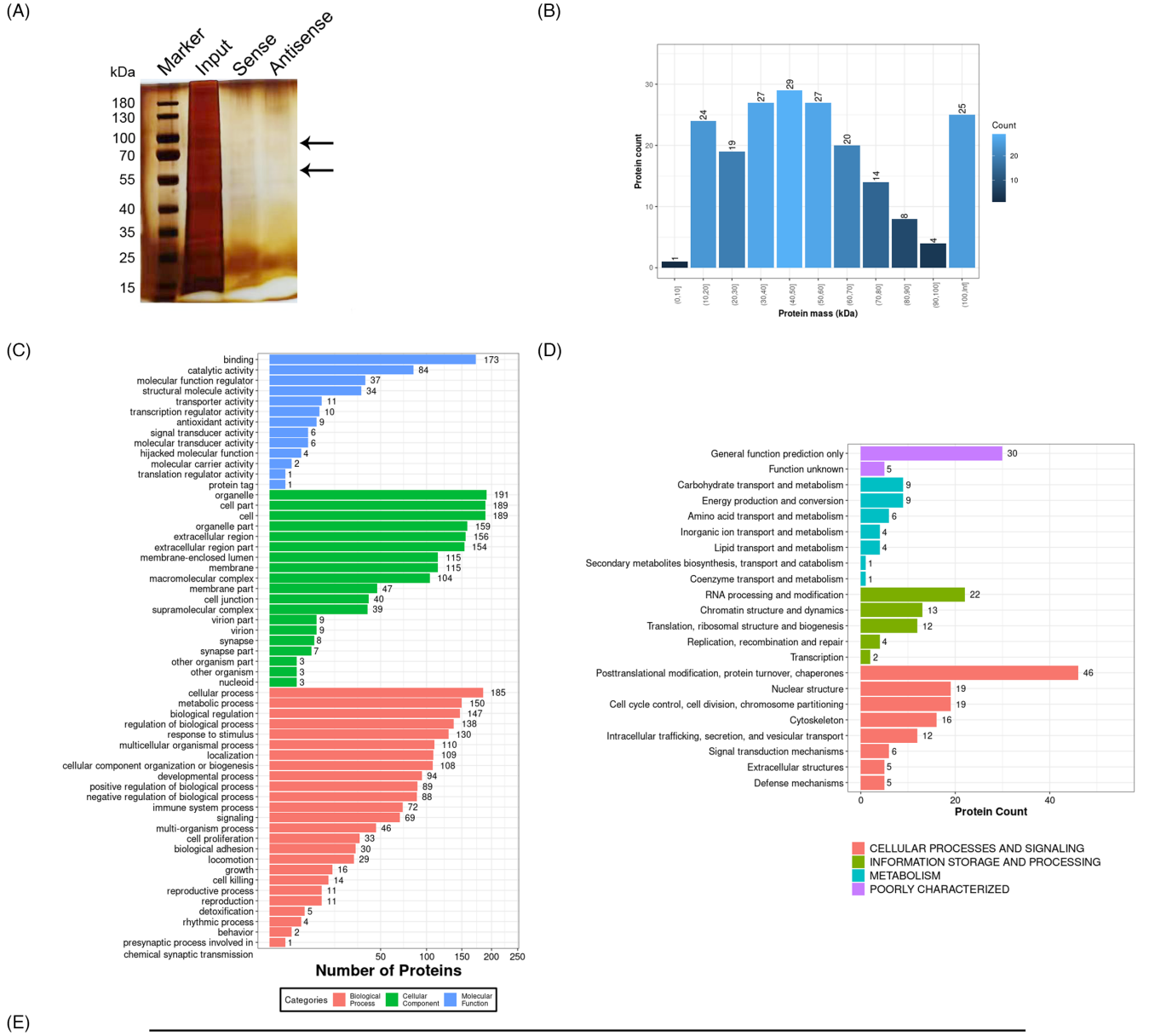
PE is a global obstetric problem. Because its pathogenesis is still not known, there is neither accurate prediction and prevention methods, nor effective diagnosis and treatment measures.[1] The study of pathophysiological mechanisms in PE is highly significant for its diagnosis and treatment.

During pregnancy, trophoblast cells were differentiated into two cell types: EVTs and intravillous trophoblasts. EVTs migrate from the villi of the uterus to attach the placenta to the decidual stroma cells.[24] The poor migration and invasion of EVTs into the decidua of the mother leads to improper remodeling of spiral arteries during pregnancy. This is thought to be one of the significant cause of PE. Placentas from pregnancies complicated by PE had an increased level of apoptosis in EVTs.[25] Dysregulated EVT proliferation and apoptosis are known to increase the risk of PE.

The aberrant expression of lncRNAs is closely related to the development of PE. In numerous studies, lncRNAs are implicated in PE through their effects on proliferation, migration, invasion, and apoptosis of trophoblasts.[26,27] LINC00534 is a differential lncRNA screened from the placental tissue of PE patients. It has not yet been studied, and only one literature has reported that it may be significantly up-regulated in platelets of patients with colorectal cancer.[28] This study showed that LINC00534 was increased in the PE placental tissues compared with normal tissues, suggesting that LINC00534 may be involved in the development of PE. Our analysis illustrated that OE of LINC00534 reduced the proliferation and migration of EVT HTR-8/SVneo cells and promoted their apoptosis. The MMP family includes MMP2 and MMP9. MMPs are involved in migration and invasion of cells. It has been reported that reduction of MMP2 and MMP9 expressions in trophoblast cells is related to PE.[29] In our research, OE of LINC00534 could decrease the mRNA expression levels of BCL-2, MMP2, and MMP9, while knockdown of LINC00534 could increase the corresponding levels. These results suggest that LINC00534 may be involved in the pathogenesis of PE by inhibiting the proliferation, migration, and apoptosis of EVT cells, leading to superficial placental implantation, which can lead to the development of PE in early pregnancy.[30]

The results of sequencing analysis showed that compared to the control group, the differentially expressed genes in the cells after knocking down LINC00534 were mostly related to cell proliferation, apoptosis, and adhesion. KEGG enrichment pathway analysis revealed that the PI3K/AKT signaling pathway was its enrichment pathway. PI3K/AKT signaling pathway was known to be widely involved in regulating cell biological behavior.[31] Many researches have explored that the PI3K/AKT signaling pathway is related to the progression of PE. Syntaxin2 (STX2) activates the PI3K/AKT pathway through membrane recruitment of p85 (a regulatory subunit of PI3K), promotes trophoblast cell proliferation, migration, and invasion, and participates in the mechanism of PE.[32] Decrease of cAMP-dependent protein kinase inhibitor-b (PKIB) promotes PE by inhibiting the migration, invasion, and vascularization of HTR-8/SVneo trophoblast cells by reducing phosphorylated AKT.[33] The PI3K/AKT pathway was also associated with the expression of sFlt-1, which plays a crucial role in PE.[34] From the WB data, OE of LINC00534 significantly inhibited the phosphorylation of p110β (the catalytic subunits of PI3K) and AKT protein, while knockdown of LINC00534 showed the opposite effect, indicating that LINC00534 can negatively regulate the activation of the PI3K/AKT signaling pathway.

On human chromosome 10, PTEN is a tumor suppressor gene associated with the PI3K/AKT pathway.[22] Its expression can reduce the activation of the PI3K/AKT signaling pathway.[21] Aberrant expression of PTEN has been shown to be related with trophoblastic diseases, such as epithelioid trophoblastic tumor,[35] hydatidiform mole,[36] and early spontaneous abortion.[37] Compared with the normal placenta, the expression of PTEN in PE patients was significantly increased ($p < 0.01$), suggesting that the increase in PTEN may be related to the development of PE.[23] Studies have shown that PTEN can reduce the proliferation and invasion of trophoblast cells by inactivating the PI3K/AKT signaling pathway, thereby participating in the biological process of PE.[38] Both RT–qPCR and WB experiments showed that LINC00534 could promote the expression of the PTEN gene and protein. The results of these experiments show that LINC00534 may regulate the proliferation, migration, and apoptosis of trophoblasts through the PTEN/PI3K/AKT signaling pathway, and contribute to the pathogenesis of PE.



(E)

Protein_ID	Protein_Mass	Description	Abundance	iBAQ
sp|P13639|EF2_HUMAN	95276.9474	Elongation factor 2	1107014.51	21288.74
sp|P07900|HS90A_HUMAN	**84606.67999**	**Heat shock protein HSP 90-alpha**	**2229746.96**	**57173.00**
sp|P08238|HS90B_HUMAN	83212.10066	Heat shock protein HSP 90-beta	1298506.40	32462.66
sp|P19338|NUCL_HUMAN	76568.35695	Nucleolin	2076582.69	45143.10
sp|P38646|GRP75_HUMAN	73634.77131	Stress-70 protein, mitochondrial	1241177.52	28864.59
sp|P11021|BIP_HUMAN	**72288.43496**	**Endoplasmic reticulum chaperone BiP**	**5296542.81**	**135808.79**
sp|P11142|HSP7C_HUMAN	**70854.22227**	**Heat shock cognate 71 kDa protein**	**5799986.48**	**156756.39**
sp|P0DMV8|HS71A_HUMAN	70009.03579	Heat shock 70 kDa protein 1A	829351.02	23037.53
sp|P02768|ALBU_HUMAN	69321.49456	Albumin	3103644.23	73896.29
sp|P10809|CH60_HUMAN	61016.38048	60 kDa heat shock protein, mitochondrial	2666903.04	74080.64
sp|P07237|PDIA1_HUMAN	57080.67209	Protein disulfide-isomerase	2097906.89	53792.48

FIGURE 5 Potential bound proteins of LINC00534 detected by RNA pull down assay. (A) Silver staining of the potential bound proteins of biotinylated LINC00534. (B) The sizes distribution of the identified 198 proteins. GO (C) and KEGG pathway (D) analysis of the identified 198 proteins. (E) List of the most potential interacted proteins according to the matched protein mass identified in the silver staining.

Numerous studies have shown that exploring the mechanism of lnc-RNA-miRNA–mRNA in the ceRNA bioinformatics network may provide new research targets for PE.[39] For example, lncRNA KCNQ1OT1 can target miR-146A-3p functions in HTR-8/SVneo cells through the CXCL12/CXCR4 pathway.[40] Interfering with the expression of lncRNA NEAT1 can up-regulate miR-411-5p and inhibit the expression of PTEN, and then promote the proliferation, migration, and invasion of trophoblast cells; thus slowing down the development of PE.[41] By predicting the site, we detected that there may be binding sites between LINC00534 and miR-139-5p or miR-494-3p, and RT–qPCR experiments confirmed the negative correlation between miR-494-3p and LINC00534. As reported, miR-494-3p was implicated in numerous cancers including non-small cell lung cancer,[42] osteosarcoma[43] and glioma,[44] in endocrine diseases such as diabetes mellitus,[45] and in cardiovascular system diseases.[46] Among them, Ou Yuhua et al. proved that miR-494-3p can attenuate the invasion and migration of trophoblasts by regulating the HTRA1/notch-1 pathway, and participate in the progression of PE disease.[47] Our results demonstrated that miR-494-3p is a target of LINC00534 and is negatively regulated by LINC00534 in HTR-8/SVneo cells. Functional experiments also revealed that OE of miR-494-3p could rescue the inhibition of proliferation and migration of LINC00534 in trophoblast cells. Literature reviews have suggested that miR-494-3p stimulates the activation of the PI3K/AKT signaling pathway by targeting PTEN.[48] Therefore, we hypothesized that LINC00534 might affect the expression of PTEN by targeting miR-494-3p, thereby regulating the PI3K/AKT signaling pathway. With respect to this hypothesis, more in-depth exploration is needed in future research.

We also checked whether LINC00534 functioned through interacting with proteins. Potential bound proteins HS90A and HSP7C were reported to play roles in chaperones, but their roles in trophoblasts have not been proved. BiP (GRP78) was a master regulator of endoplasmic reticulum.[49] The status of BiP (GRP78) is associated with the invasive properties of cytotrophoblastic cells, syncytialisation and apoptosis in PE.[50–52] Moreover, GRP78 is an upstream regulator of PI3K/AKT signaling,[53] while GRP78 has also been shown to be a downstream target of AKT.[54] Therefore, the complex function mechanisms of LINC00534-bound proteins needed further investigations.

In summary, our findings indicate that LINC00534/miR-494-3p can inhibit the proliferation and migration of HTR-8/SVneo trophoblast cells and promote their apoptosis through the PTEN/PI3K/AKT pathway. Our work clearly has some limitations, but it may provide some new insights into the pathogenesis of PE.

AUTHOR CONTRIBUTIONS

Conceptualization: JYL and WL; Methodology and Investigation: XJS, XXZ, and QX; Software and Formal analysis: CYL, YRZ, YH, LM, and CHW; Writing: XXZ, QX, JYL, and WL; Visualization: JYL and WL; Project Administration: JYL; Resources and Funding Acquisition: WL and XJS.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

CONSENT FOR PUBLICATION

Patients signed informed consent regarding publishing their data.

CODE AVAILABILITY

Not applicable.

DATA AVAILABILITYSTATEMENT

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

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REFERENCES

1. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: pathophysiology, challenges, and perspectives. *Circ Res*. 2019;124(7):1094-1112.
2. Ives CW, Sinkey R, Rajapreyar I, Tita ATN, Oparil S. Preeclampsia-pathophysiology and clinical presentations: JACC state-of-the-art review. *J Am Coll Cardiol*. 2020;76(14):1690-1702.
3. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. *Nat Rev Nephrol*. 2014;10(8):466-480.
4. Jena MK, Sharma NR, Petitt M, Maulik D, Nayak NR. Pathogenesis of preeclampsia and therapeutic approaches targeting the placenta. *Biomolecules*. 2020;10(6):953.
5. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "great obstetrical syndromes" are associated with disorders of deep placentation. *Am J Obstet Gynecol*. 2011;204(3):193-201.
6. Brosens I, Robertson WB, Dixon HG. The physiological response of the vessels of the placental bed to normal pregnancy. *J Pathol Bacteriol*. 1967;93(2):569-579.
7. Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? *J Clin Invest*. 1997;99(9):2152-2164.
8. Kapranov P, Cheng J, Dike S, et al. RNA maps reveal new RNA classes and a possible function for pervasive transcription. *Science*. 2007;316(5830):1484-1488.
9. Bartel DP. Metazoan MicroRNAs. *Cell*. 2018;173(1):20-51.

10. Hutvagner G, Zamore PD. A microRNA in a multiple-turnover RNAi enzyme complex. *Science*. 2002;297(5589):2056-2060.
11. Chan JJ, Tay Y. Noncoding RNA: RNA regulatory networks in cancer. *Int J Mol Sci*. 2018;19(5):1310.
12. Xing C, Sun SG, Yue ZQ, Bai F. Role of lncRNA LUCAT1 in cancer. *Biomed Pharmacother*. 2021;134:111158.
13. Yang XZ, Cheng TT, He QJ, et al. LINC01133 as ceRNA inhibits gastric cancer progression by sponging miR-106a-3p to regulate APC expression and the Wnt/beta-catenin pathway. *Mol Cancer*. 2018;17(1):126.
14. Zhao W, Geng D, Li S, Chen Z, Sun M. LncRNA HOTAIR influences cell growth, migration, invasion, and apoptosis via the miR-20a-5p/HMGA2 axis in breast cancer. *Cancer Med*. 2018;7(3):842-855.
15. Jin XM, Xu B, Zhang Y, et al. LncRNA SND1-IT1 accelerates the proliferation and migration of osteosarcoma via sponging miRNA-665 to upregulate POU2F1. *Eur Rev Med Pharmacol Sci*. 2019;23(22):9772-9780.
16. Song X, Li C, Li J, et al. The long noncoding RNA uc.294 is upregulated in early-onset pre-eclampsia and inhibits proliferation, invasion of trophoblast cells (HTR-8/SVneo). *J Cell Physiol*. 2019;234(7):11001-11008.
17. Ma Y, Liang X, Wu H, Zhang C, Ma Y. Long noncoding RNA NR_002794 is upregulated in preeclampsia and regulates the proliferation, apoptosis and invasion of trophoblast cells. *Mol Med Rep*. 2019;20(5):4567-4575.
18. Zhu H, Kong L. LncRNA CRNDE regulates trophoblast cell proliferation, invasion, and migration via modulating miR-1277. *Am J Transl Res*. 2019;11(9):5905-5918.
19. Xu Y, Wu D, Hui B, et al. A novel regulatory mechanism network mediated by lncRNA TUG1 that induces the impairment of spiral artery remodeling in preeclampsia. *Mol Ther*. 2022;30(4):1692-1705.
20. Long W, Rui C, Song X, et al. Distinct expression profiles of lncRNAs between early-onset preeclampsia and preterm controls. *Clin Chim Acta*. 2016;463:193-199.
21. Carnero A, Blanco-Aparicio C, Renner O, Link W, Leal J. The PTEN/PI3K/AKT signalling pathway in cancer, therapeutic implications. *Curr Cancer Drug Targets*. 2008;8(3):187-198.
22. Noorolyai S, Shajari N, Baghbani E, Sadreddini S, Baradaran B. The relation between PI3K/AKT signalling pathway and cancer. *Gene*. 2019;698:120-128.
23. 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.
24. Ridder A, Giorgione V, Khalil A, Thilaganathan B. Preeclampsia: the relationship between uterine artery blood flow and trophoblast function. *Int J Mol Sci*. 2019;20(13):3263.
25. Whitley GS, Dash PR, Ayling LJ, Prefumo F, Thilaganathan B, Cartwright JE. Increased apoptosis in first trimester extravillous trophoblasts from pregnancies at higher risk of developing preeclampsia. *Am J Pathol*. 2007;170(6):1903-1909.
26. Jiang S, Chen Q, Liu H, et al. Preeclampsia-associated lncRNA INHBA-AS1 regulates the proliferation, invasion, and migration of placental trophoblast cells. *Mol Ther Nucleic Acids*. 2020;22:684-695.
27. Zhang Q, Wang Z, Cheng X, Wu H. lncRNA DANCR promotes the migration an invasion and of trophoblast cells through microRNA-214-5p in preeclampsia. *Bioengineered*. 2021;12(2):9424-9434.
28. Ye B, Li F, Chen M, et al. A panel of platelet-associated circulating long non-coding RNAs as potential biomarkers for colorectal cancer. *Genomics*. 2022;114(1):31-37.
29. Oh SY, Hwang JR, Choi M, et al. Autophagy regulates trophoblast invasion by targeting NF-kappaB activity. *Sci Rep*. 2020;10(1):14033.
30. Fisher SJ. Why is placentation abnormal in preeclampsia? *Am J Obstet Gynecol*. 2015;213(4 Suppl):S115-S122.
31. Duan C, Bauchat JR, Hsieh T. Phosphatidylinositol 3-kinase is required for insulin-like growth factor-I-induced vascular smooth muscle cell proliferation and migration. *Circ Res*. 2000;86(1):15-23.
32. Li Y, Sun XL, Ma CL, et al. STX2 promotes trophoblast growth, migration, and invasion through activation of the PI3K-AKT pathway in preeclampsia. *Front Cell Dev Biol*. 2021;9:615973.
33. Liu C, Wang H, Yang M, et al. Downregulation of cAMP-dependent protein kinase inhibitor-b promotes preeclampsia by decreasing phosphorylated Akt. *Reprod Sci*. 2021;28(1):178-185.
34. Park JK, Jeong JW, Kang MY, et al. Inhibition of the PI3K-Akt pathway suppresses sFlt1 expression in human placental hypoxia models in vitro. *Placenta*. 2010;31(7):621-629.
35. Cho EJ, Chun SM, Park H, Sung CO, Kim KR. Whole transcriptome analysis of gestational trophoblastic neoplasms reveals altered PI3K signaling pathway in epithelioid trophoblastic tumor. *Gynecol Oncol*. 2020;157(1):151-160.
36. Wang YX, Zhao JR, Xu YY, Wu WB, Zhang HJ. miR-21 is overexpressed in Hydatidiform mole tissues and promotes proliferation, migration, and invasion in Choriocarcinoma cells. *Int J Gynecol Cancer*. 2017;27(2):364-374.
37. Tokyol C, Aktepe F, Husniye Dilek F, Yilmazer M. Comparison of placental PTEN and beta1 integrin expression in early spontaneous abortion, early and late normal pregnancy. *Ups J Med Sci*. 2008;113(2):235-242.
38. Li Z, Zhou X, Gao W, Sun M, Chen H, Meng T. Circular RNA VRK1 facilitates pre-eclampsia progression via sponging miR-221-3P to regulate PTEN/Akt. *J Cell Mol Med*. 2022;26(6):1826-1841.
39. Zhang Z, Wang P, Zhang L, et al. Identification of key genes and Long noncoding RNA-associated competing endogenous RNA (ceRNA) networks in early-onset preeclampsia. *Biomed Res Int*. 2020;2020:1673486-1673489.
40. Chen FR, Zheng LM, Wu DC, Gong HM, Cen H, Chen WC. Regulatory relationship between lncRNA KCNQ1OT1 and miR-146a-3p in preeclampsia. *Zhonghua Fu Chan Ke Za Zhi*. 2020;55(8):535-543.
41. Fan X, Lou J, Zheng X, et al. Interference with lncRNA NEAT1 promotes the proliferation, migration, and invasion of trophoblasts by upregulating miR-411-5p and inhibiting PTEN expression. *Immunopharmacol Immunotoxicol*. 2021;43(3):334-342.
42. Wu C, Yang J, Li R, Lin X, Wu J, Wu J. LncRNA WT1-AS/miR-494-3p regulates cell proliferation, apoptosis, migration and invasion via PTEN/PI3K/AKT signaling pathway in non-small cell lung cancer. *Onco Targets Ther*. 2021;14:891-904.
43. Liu S, Duan K, Zhang X, et al. Circ_0081001 down-regulates miR-494-3p to enhance BACH1 expression and promotes osteosarcoma progression. *Aging (Albany NY)*. 2021;13(13):17274-17284.
44. Qiu G, Tong W, Jiang C, et al. Long noncoding RNA WT1-AS inhibit cell malignancy via miR-494-3p in glioma. *Technol Cancer Res Treat*. 2020;19:1-9.
45. Guo H, Yan Z, Hu Y, Huang X, Pan C. Complement C7 is specifically expressed in mesangial cells and is a potential diagnostic biomarker for diabetic nephropathy and is regulated by miR-494-3p and miR-574-5p. *Diabetes Metab Syndr Obes*. 2021;14:3077-3088.
46. Liu H, Zhang Y, Yuan J, et al. Dendritic cellderived exosomal miR4943p promotes angiogenesis following myocardial infarction. *Int J Mol Med*. 2021;47(1):315-325.
47. Ou Y, Zhu L, Wei X, et al. Circular RNA circ_0111277 attenuates human trophoblast cell invasion and migration by regulating miR-494/HTRA1/Notch-1 signal pathway in pre-eclampsia. *Cell Death Dis*. 2020;11(6):479.
48. Lin H, Huang ZP, Liu J, et al. MiR-494-3p promotes PI3K/AKT pathway hyperactivation and human hepatocellular carcinoma progression by targeting PTEN. *Sci Rep*. 2018;8(1):10461.

49. Chen S, Zhao R, Wu T, et al. An endogenous retroviral LTR-derived Long noncoding RNA lnc-LTR5B interacts with BiP to modulate ALV-J replication in chicken cells. *Front Microbiol*. 2021;12:788317.
50. Du L, He F, Kuang L, Tang W, Li Y, Chen D. eNOS/iNOS and endoplasmic reticulum stress-induced apoptosis in the placentas of patients with preeclampsia. *J Hum Hypertens*. 2017;31(1):49-55.
51. Fradet S, Pierredon S, Ribaux P, et al. Involvement of membrane GRP78 in trophoblastic cell fusion. *PLoS One*. 2012;7(8):e40596.
52. Laverriere A, Landau R, Charvet I, et al. GRP78 as a marker of pre-eclampsia: an exploratory study. *Mol Hum Reprod*. 2009;15(9):569-574.
53. Lin YG, Shen J, Yoo E, et al. Targeting the glucose-regulated protein-78 abrogates Pten-null driven AKT activation and endometrioid tumorigenesis. *Oncogene*. 2015;34(43):5418-5426.
54. Gray MJ, Mhawech-Fauceglia P, Yoo E, et al. AKT inhibition mitigates GRP78 (glucose-regulated protein) expression and contribution to chemoresistance in endometrial cancers. *Int J Cancer*. 2013;133(1):21-30.

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