Upregulated LncZBTB39 in Pre-eclampsia and Its Effects on Trophoblast Invasion and Migration via Antagonizing the Inhibition of miR-210 on THSD7A Expression

Short Title: Upregulation of LncZBTB39 in Preeclamptic Placentas

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Abstract

Objective

Pre-eclampsia (PE) is a major cause of maternal morbidity and mortality, but its etiology remains to be elucidated. Accumulating evidence suggests that placental long noncoding RNAs (lncRNAs) might contribute to the pathogenesis of pre-eclampsia.

Study Design

In the present study, the expression levels of lncRNAs in human placenta were first determined by microarray analysis and then validated by secondary RT-qPCR and FISH. LncZBTB39 expression manipulation in HTR8/SVneo trophoblast cells was achieved by shRNA and plasmid transfection. Then, the invasion and migration of lncZBTB39-deficient and lncZBTB39-overexpressing trophoblast cells were evaluated by transwell assays and wound-healing assays, respectively. MMP2 activity was measured by gelatin zymography. The downstream target genes of lncZBTB39 were then identified by a transcriptomic microarray, followed by RT-qPCR validation.

Results

We found that lncZBTB39 was upregulated in PE-complicated human placentas, and overexpression of lncZBTB39 inhibited invasion and migration, as well as MMP2 activity in HTR8/SVneo cells, while downregulation of lncZBTB39 enhanced invasion, migration and MMP2 activity. In addition, THSD7A expression was elevated by lncZBTB39 overexpression but reduced in lncZBTB39-deficient cells; moreover, lncZBTB39 antagonized the inhibitory effects of miR-210 on THSD7A expression.

Conclusion

PE-complicated placentas are associated with upregulated lncZBTB39, which negatively regulates trophoblast invasion and migration, most likely by preserving the expression of THSD7A mRNA through sponging miR-210. The results of this study not only provide novel evidence that lncRNAs regulate trophoblastic activities but also suggest that lncZBTB39 may be a potential interventional target for PE.

Keywords: invasion; lncZBTB39; pre-eclampsia; trophoblast;

Introduction

Pre-eclampsia (PE) is a pregnancy-specific complication and the leading cause of maternal morbidity and mortality during the perinatal period; it affects 4-5% of pregnancies worldwide[1]. PE can affect virtually every maternal organ system, although the condition is alleviated by the cessation of pregnancy and delivery of the placenta, an effective cure for PE is still currently lacking[2]. Several etiologies have been proposed for PE, such as abnormal trophoblastic invasion [3], immunological factors[4], endothelial cell activation[5], and genetic factors[6], while the "two-stage" theory is widely accepted[7].

Successful pregnancy requires extensive remodeling of the spiral arterioles within the decidua basalis. At the early stages of pregnancy, cytotrophoblast cells differentiate into extravillous trophoblast cells (EVT), which gradually migrate into and invade the uterine spiral arteries, subsequently replacing the vascular endothelial and muscular linings and enlarging the vessel diameter, allowing adequate perfusion across the utero-placenta interface[8]. However, this process is incomplete in PE, as trophoblastic invasion or migration is impaired. According to findings from histological studies, PE placentas are associated with superficial implantation and an insufficient recasting of the uterine spiral artery, and the lumen diameter is only half the size of vessels in a normal pregnancy[9].

Long non-coding RNAs (lncRNAs) are a type of RNA that is longer than 200 nucleotides and does not harbor an open reading frame (ORF). In addition, lncRNAs do not encode a protein[10]. Previously, lncRNAs were deemed "transcriptional noise," but accumulating evidence has shown that lncRNAs exert versatile biological functions, and therefore may play important regulatory roles in cellular migration, invasion[11], X chromosome inactivation[12], gene imprinting[13], and differentiation[14].

As the results, lncRNAs has been reported participated in the pathogenesis of various human diseases, including myocardial infarction[15], diabetic nephropathy[16] and solid organ tumors [17, 18]. However, the involvement of lncRNAs in PE development and its underlying mechanisms remain largely unknown. In the present study, we report

an association between the upregulation of a novel lncRNA, lncZBTB39, which is involved in regulating trophoblast viability and invasion via TAC3 and THSD7A, respectively, and PE placentas.

Materials and Methods

Study population and sampling

PE complicated (n=25) and normotensive pregnancies (n=25) were admitted into the Department of Obstetrics at The First Affiliated Hospital of Chongqing Medical University for elective caesarian delivery that was not due to maternal medical issues and were recruited into this study (Table 1). PE was diagnosed according to the guidelines of the American College of Obstetrics and Gynecology[19]. This work was approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University (2017-026), and written informed consent was obtained from all subjects. All procedures were performed in accordance with the principles stated in the Declaration of Helsinki.

Human placenta sampling

$0.5cm_3$ placental tissue was collected from the maternal surface of the placenta immediately after delivery, and rinsed with ice-cold sterile saline, then immersed in RNAlater (Invitrogen, Lithuania) or snap-frozen in liquid nitrogen and stored at -80 °C for subsequent use.

Cell lines

HTR8/SVneo cells were kindly provided by Dr. Charles Graham (Queen's University, Canada), and maintained in RPMI1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, PAN-Biotech, Germany). Cells were cultured in a humidified atmosphere consisting of 5% CO_2 at 37 °C.

Transfection

Three shRNAs targeting lncZBTB39 and a scrambled shRNA (shNC) were designed (Table 2) and synthesized by Genomeditech (China); pHB-EF1-MCS-GFP plasmid containing the lncZBTB39 sequence (pEGFP-lncZBTB39) and empty vector control (pEGFP-NC) were purchased from Hanbio (China); miR-210 mimics were purchased from GenePharma (China); The shRNAs, plasmids or miR-210 mimics were

transfected into HTR8/SVneo trophoblast cells according to the manufacturer's protocol[20].

Isolation of primary human trophoblasts (PHTs)

PHT cells were isolated from human term placenta as previously described[21]. Briefly, immediately after delivery, the placental tissue was sectioned into 2-mm$_3$, and the connective tissue and blood clots were removed. Dispase II (Roche, Swiss) and DNAse (Roche, Swiss) were consecutively added onto the placental tissue for 1 hr and 15 min, respectively, at 37 °C. The suspension was then filtered (70 μm) and centrifuged at 300 g for 7 min. The precipitate was resuspended in 40 mL of platelet lysis solution (Gibco, USA) and washed twice with DMEM/F12 supplemented with 10% FBS (Gibco, USA). The suspension was layered onto the Percoll gradient (60%, 50%, 40%, 30%, and 20%), followed by centrifugation at 1000 g for 20 min. The 20-40% Percoll (Bio-Rad, USA) layer was then collected and resuspended, followed by 300 g centrifugation for 7 min. The pellet was resuspended and then seeded into petri dishes[22, 23].

RT-qPCR

Total RNA was isolated using RNAiso plus (TaKaRa, Japan), according to the manufacturer's instruction[24]. The cDNA was reversely transcribed from 1 μg of total RNA by using the PrimeScript™ RT reagent Kit (TaKaRa, Japan). RT-qPCR was performed by the use of SYBR Green PCR Kit (Roche, Germany), and the melting curve analysis was performed to ensure amplification specificity. 40 PCR cycles were performed in a Bio-Rad CFX Connect Real-Time System (USA). The initial enzyme activation and template denaturation at 95 °C for 10 min was followed by denaturation at 95 °C for 10 s, annealing at 57 °C for 30 s, and an extension phase at 72 °C for 10 s. Ct values were used for quantification. All assays were 95-105% efficient. The primers were synthesized by Invitrogen (Carlsbad, USA) (Table 3).

Transwell invasion assay

The transwell invasion assay was performed using previously established methods[25]. Briefly, $5\times10_4$ HTR8/SVneo cells were re-suspended in serum-free RPMI 1640 and then added to the upper chamber of each insert (8-μm pore size; Millipore, USA), which was pre-coated with 60 μL of Matrigel (Millipore, USA). The lower chamber was

loaded with 600 μL of RPMI 1640 medium containing 10% FBS. After 24 hrs of incubation, the invaded cells were fixed with ice-cold methanol, stained with crystal violet, and then imaged using a microscope (ZEISS, Germany). Five fields of view in each well were randomly selected for statistical analysis.

Wound-healing migration assay

The migration assay was performed as previously described[26]. Briefly, $2 \times 10_5$ HTR8/SVneo cells were incubated in RPMI 1640 supplemented with 10% FBS for 24 hrs, and then a scratch was made in the cell monolayer with a sterile 200-μL pipette tip. Images were captured immediately and 24 hrs later by using a microscope (EVOS, Life Technology, USA); three view fields at the lesion border were randomly selected and analyzed by using Image J software (NIH, USA).

Gelatin zymography

The gelatin zymographic analysis was performed as previously described[27]. The cell culture medium was centrifuged at 13000 g for 15 min, and the supernatant was then collected for gel electrophoresis. Next, the gel was washed with TBS-T for 30 min at RT, followed by incubation with the digestion solution at RT for 30 min. The digestion solution was replaced with fresh solution prior to incubation at 37 °C for 48 hours on a shaking platform at 50 rpm. Gels were stained with Coomassie blue for 1 hour at RT, and the de-stained gels were then scanned using the Quantity One System (Bio-Rad, USA).

Fluorescence *in situ* hybridization (FISH)

FISH was performed using a fluorescent *in situ* hybridization kit (Ribobio, China), according to the manufacturer's instructions. Briefly, cells grown on coverslips were fixed with 4% paraformaldehyde for 10 min, and then permeabilized with 0.5% Triton X-100 (in PBS) at 4 °C for 5 min. Next, the samples were blocked with prehybridization buffer for 30 min at 37 °C, followed by an overnight hybridization with probes for lncZBTB39 (Ribobio, China) in hybridization buffer at 37 °C in the dark. On the next day, cells were sequentially washed with 4×, 2×, and 1× saline sodium citrate buffers and then counterstained with DAPI (Ribobio, China) for 10 min at room temperature

(RT) in the dark; images were acquired by a fluorescence microscope (Life Technologies, USA).

Transcriptome microarrays in human placentas

Total RNA was extracted using TRIzol/chloroform, and then purified with Agencourt Ampure magnetic beads (Beckman Coulter, USA). Target preparation for microarray processing was performed according to the instructions provided in the GeneChip® WT PLUS Reagent Kit. After hybridization with Affymetrix Human Gene 1.0ST Array chips, the microarrays were washed and stained with streptavidin-phycoerythrin on the Affymetrix Fluidics Station 450 (Affymetrix Inc., USA), and then scanned using an Affymetrix® GeneChip Command Console, which was installed in a GeneChip® Scanner 3000 7G (Affymetrix Inc., USA).

Transcriptome microarrays in HTR8/SVneo cells

Total RNA was extracted from cells using RNAiso Plus (TaKaRa, Japan). An Agilent Gene Expression array (Agilent Human 4X 44K, USA) was used to investigate the transcriptomic changes in HTR8/SVneo cells after the manipulation of lncZBTB39 expression. Differentially expressed genes were identified by examining fold changes in expression. Gene Ontology (GO) biological process and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the Database for Annotation, Visualization and Integrated Discovery.

Statistical analysis

Human placenta microarray data were analyzed using the RMA algorithm with default settings and global scaling as the normalization method with Partek® Genomics Suite 6.6. Values are presented as log2 RMA signal intensities. The cellular microarray data were analyzed using GenePix pro V6.0 software (Molecular Devices, LLC, USA) and standardized using the Agilent GeneSpring GX v11.5.1 Software (Agilent, USA) for further analysis. Data are presented as means±SEM, and the significance of differences was tested using Student's unpaired two-tailed t-tests or two-way repeated measures ANOVA with Bonferroni's correction; $P<0.05$ was considered significant.

Results

PE placentas are associated with an upregulation of lncZBTB39 expression

According to the microarray data, 24 lncRNAs were differentially expressed (cutoff fold changes >1.5 and <-1.5; $P<0.05$) between PE and normal placentas; 20 were upregulated and 4 were downregulated (Figure 1A). Further validation in another set of placenta samples using RT-qPCR suggested that the expression of lncZBTBT39 was significantly elevated in PE-complicated human placentas (Figure 1B). To further confirm the subcellular localization of lncZBTBT39 in trophoblasts, FISH of lncZBTB39 was conducted in HTR8/SVneo cells and PHT cells. LncZBTB39 was expressed at high levels in HTR8/SVneo and PHT cells but was mainly localized in the cytoplasm (Figure 1C).

LncZBTB39 inhibits trophoblast migration and invasion

We next evaluated the effects of lncZBTB39 on regulating trophoblast invasion and migration to investigate its role in PE development. First, lncZBTB39-overexpressing and knockdown HTR8/SVneo cells were established. The RT-qPCR results confirmed a greater than 50-fold increase in lncZBTB39 expression in lncZBTB39-overexpressing HTR8/SVneo cells, while lentivirus-mediated delivery of the shRNA reduced lncZBTB39 expression in HTR8/SVneo cells by more than 30% (Figure 2A). Next, the lncZBTB39-overexpressing and lncZBTB39-KD HTR8/SVneo cells were subjected to migration and invasion assays. Knockdown of lncZBTB39 strongly promoted HTR8/SVneo cell migration, while overexpression of lncZBTB39 resulted in a significant loss of trophoblast cell motility (Figure 2B). Furthermore, the matrigel-based transwell assay showed that the invasion of lncZBTB39- overexpressing HTR8/SVneo cells was significantly compromised compared to that of wildtype (*WT*) HTR8/SVneo cells. In contrast, sh-lncZBTB39-transfected cells exhibited a sharp increase in invasiveness (Figure 2C). Consistent with these findings, gelatin zymography results revealed the significant suppression of MMP-2 activity in trophoblasts overexpressing lncZBTB39, while lncZBTB39 knockdown significantly increased MMP-2 activation (Figure 2D).

LncZBTB39 modulates gene expression patterns in trophoblasts

LncZBTB39-overexpressing and lncZBTB39-KD cells were subjected to an mRNA microassay to further investigate the downstream target genes of lncZBTB39. As shown

in Figure 3A and B, 3533 mRNAs were upregulated and 3346 mRNAs were downregulated (cutoff fold change=log2; $P<0.05$) in response to lncZBTB39 overexpression, while 199 mRNAs were upregulated and 207 mRNAs were downregulated (cutoff fold change=log2; $P<0.05$) in lncZBTB39-KD HTR8/SVneo cells. Among these genes, 7 mRNAs exhibited consistent changes in expression in response to alterations in lncZBTB39 expression, including C1orf168, PMCH, LAMA1, ST8SLA4, THSD7A, LPHN3, and NR6A1. Further validation by RT-qPCR confirmed that THSD7A transcription is regulated by lncZBTB39 (Figure 3C).

LncZBTB39 preserves THSD7A expression by sponging miR210

The levels of the THSD7A mRNA were significantly increased in PE placentas (Figure 4A), showing a similar trend to that of lncZBTB39 expression. Unexpectedly, the elevation of THSD7A expression observed in response to lncZBTB39 transfection was further diminished by additional miR210 mimics in a dose-dependent manner (Figure 4B).

LncZBTB39 regulates miR210 expression in trophoblasts

We next investigated the relationship between LncZTBT39 and miR210 in HTR8/SVneo cell. The RT-PCR results showed that overexpression of lncZBTB39 up-regulated miR210 expression in HTR8/SVneo cell(Figure 4C), while Knockdown of lncZBTB39 strongly decreased miR210 expression in HTR8/SVneo cell(Figure 4C).

Discussion

For many years, the pathogenesis of pre-eclampsia has been recognized as complex but involving essentially two stages: abnormal placentation and the development of the maternal syndrome (associated with endothelial dysfunction)[7]. The main pathophysiological predisposition to PE occurs between weeks 8 and 18 of pregnancy, associated with deficient maternal spiral artery remodeling leading to an abnormal uteroplacental circulation, ultimately leading to uterine hypoperfusion and higher-than-normal velocity of blood flow to the intervillous space. As a consequence, abnormal perfusion of the intervillous space of the placenta occurs often associated with oxidative and hemodynamic stress, leading to the release of pro-inflammatory and antiangiogenic factors into the maternal circulation. This then leads to the second stage of PE, namely,

endothelial and vascular smooth muscle dysfunction and the onset of clinical hypertension and proteinuria. However, increasingly there is scientific opinion that this 'theory' of pre-eclampsia pathogenesis is too simplistic. With the derivation of aberrant placentation considered to be immunological origin, the development of PE is more complex and divided into "six stages"[28]. Due to defective immunotolerance, arising from a short interval between first coitus and conception increases the risk of poor placentation and pre-eclampsia, which revised to be the first stage. Immediately, it is associated with adverse effects on health and growth of embryo (second stage). The third stage is equivalent to the first stage of "two stage theory", when defective placentation extends over about 10 weeks. Stages 4-6 all occur in the second half of pregnancy, which is equivalent to the second stage of "two stage theory". There is more detailed development of clinical syndromes in "six stage model" than "two stage theory", which can explain etiology and symptomology of PE more effectively. However, the key of these two theories has a common pathway, that of abnormal function of the trophoblast, uterine spiral artery recasting, and the "shallow implantation" of the placenta during its formation.

Accumulating evidence has suggested that various lncRNAs participate in regulating the invasive and migratory function in cancer cells[11, 29] , might contribute to the behavior of trophoblasts. Indeed, lncRNAs including H19[30], SPRY4-IT1[31, 32], MEG3[33], Uc.187[34] and MALAT1[35] have been recently found to be implicated in the pathogenesis of PE. In the present study, we firstly identified the upregulation of a novel lncRNA, lncZBTB39, expression in PE placentas through transcriptomic screening, suggesting that it might be associated with the pathogenesis of PE. It is generally considered that the lower shallow reconstruction of maternal blood vessels by trophoblasts causes PE development[8]. Therefore, we then investigated the effect of lncZBTB39 on the biological functions of trophoblast cells. Our results suggested that lncZBTB39 represses trophoblast invasion and migration, possibly by inhibiting the activity of the MMP-2 protein.

In order to identify the underlying mechanism, transcriptome chip assay was performed once again to identify the downstream mRNAs regulated by lncZBTB39 in lncZBTB39-overexpressing and knockdown HTR8/SVneo cells. Seven candidate

genes were found might be regulated by lncZBTB39 in trophoblast, but further validation elucidated that only THSD7A is certainly regulated by lncZBTB39.

However, THSD7A overexpression in HUVECs inhibits cell migration and disrupts tube formation, while suppression of THSD7A expression enhances HUVEC migration and tube formation[36], indicating that THSD7A overexpression might also play a role in PE development. Given that the THSD7A gene is located on Chromosome 7 (p21.3) (remote from the site of the lncZBTB39 gene), lncZBTB39 may regulate THSD7A expression through a mechanism other than direct binding. It is reported that miR210 is involved in the etiology of PE by modulating THSD7A[37], suggesting that lncZBTB39 might regulate THSD7A expression in trophoblast cells by antagonizing miR210[38]. Our results provide evidence that lncZBTB39 and THSD7A are expressed in the human placenta and trophoblasts, and lncZBTB39 negatively modulate miR210-induced THSD7A downregulation.

Although we have described an increased expression of lncZBTB39 in PE term placenta, the expression pattern of placental lncZBTB39 throughout pregnancy remains unknown. It is recognized that the gestational age of collection of placental tissue at delivery was lower in the pre-eclamptic group and this may be a minor confounding variable. Further examination of lncZBTB39 in early-gestation placental tissue prospectively collected from PE patients is required to determine its potential in the prediction and/or diagnosis of PE[39]. In addition, measuring lncZBTB39 in other types of biospecimen, such as placental syncytiotrophoblast extracellular vesicles (STBEVs) in maternal peripheral blood, is warranted to validate its feasibility in clinical use as a predictive test[40].

In conclusion, PE placenta is associated with upregulation of lncZBTB39, which impairs trophoblast invasion and migration. Moreover, lncZBTB39 preserves transcription of target gene THSD7A by antagonizing miR210. Thus, further explorations of the biological functions and regulatory network of lncZBTB39 are likely to provide in-depth insights into PE development.

Acknowledgments

Conflict of interest

None of the authors have conflicts of interest to declare.

References

1.   Phipps EA, Thadhani R, Benzing T, Karumanchi SA. Pre-eclampsia: pathogenesis, novel diagnostics and therapies. Nat Rev Nephrol 2019;15:275-89.
2.   Peter ST, Scholl TO, Schluter MD, et al. Oxidative stress early in pregnancy and pregnancy outcome. Free Radic Res 2008;42:841-8.
3.   Nelson DB, Ziadie MS, McIntire DD, Rogers BB, Leveno KJ. Placental pathology suggesting that preeclampsia is more than one disease. Am J Obstet Gynecol 2014;210:66 e1-7.
4.   Erlebacher A. Immunology of the maternal-fetal interface. Annu Rev Immunol 2013;31:387-411.
5.   Mackenzie RM, Sandrim VC, Carty DM, et al. Endothelial FOS expression and pre-eclampsia. BJOG 2012;119:1564-71.
6.   Manten GT, Van Der Hoek YY , Marko Sikkema J, et al. The role of lipoprotein (a) in pregnancies complicated by pre-eclampsia. Med Hypotheses 2005;64:162-9.
7.   Redman C, Sargent IL. Placental Stress and Pre-eclampsia: A Revised View. Placenta 2009;30:38-42.
8.   Pijnenborg R, Vercruysse L, Hanssens M. The Uterine Spiral Arteries In Human Pregnancy: Facts and Controversies. Placenta 2006;27:939-58.
9.   Basak S, Duttaroy AK. Effects of fatty acids on angiogenic activity in the placental extravillious trophoblast cells. Prostaglandins Leukot Essent Fatty Acids 2013;88:155-62.
10. Struhl K. Transcriptional noise and the fidelity of initiation by RNA polymerase II. Nat Struct Mol Biol 2007;14:103-5.
11. Khaitan D, Dinger ME, Mazar J, Crawford J, Perera R. The melanoma-upregulated long noncoding RNA SPRY4-IT1 modulates apoptosis and invasion. Cancer Res 2011;71:3852-62.
12. Tian D, Sun S, Lee JT. The long noncoding RNA, Jpx, is a molecular switch for X chromosome inactivation. Cell 2012;143:390-403.

13. Mancini D, Steele SJ, Levorse JM. Elongation of the Kcnq1ot1 transcript is required for genomic imprinting of neighboring genes. Genes Dev 2006;20:1268-82.

14. Guttman M, Donaghey J, Carey BW, et al. lincRNAs act in the circuitry controlling pluripotency and differentiation. Nature 2011(7364):295-300.

15. Micheletti R, Plaisance I, Abraham BJ, et al. The long noncoding RNA Wisper controls cardiac fibrosis and remodeling. Sci Transl Med 2017;9.

16. Bai X, Geng J, Li X, et al. Long Noncoding RNA LINC01619 Regulates MicroRNA-27a/Forkhead Box Protein O1 and Endoplasmic Reticulum Stress-Mediated Podocyte Injury in Diabetic Nephropathy. Antioxid Redox Signal 2018;29:355-76.

17. Xu S, Kong D, Chen Q, Ping Y, Pang D. Oncogenic long noncoding RNA landscape in breast cancer. Mol Cancer 2017;16:129.

18. Mitra R, Chen X, Greenawalt EJ, et al. Decoding critical long non-coding RNA in ovarian cancer epithelial-to-mesenchymal transition. Nat Commun 2017;8:1604.

19. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol 2013;122:1122-31.

20. Qu B, Han X, Tang Y, Shen N. A novel vector-based method for exclusive overexpression of star-form microRNAs. PloS one 2012;7:41504.

21. Li L, Schust DJ. Isolation, purification and in vitro differentiation of cytotrophoblast cells from human term placenta. Reprod Biol Endocrinol 2015;13:71.

22. Donker RB, Mouillet JF, Nelson DM, Sadovsky Y. The expression of Argonaute2 and related microRNA biogenesis proteins in normal and hypoxic trophoblasts. Mol Hum Reprod 2007;13:273-9.

23. Mouillet JF, Chu T, Nelson DM, Mishima T, Sadovsky Y. MiR-205 silences MED1 in hypoxic primary human trophoblasts. FASEB J 2010;24:2030-9.

24. Lin Y, Chen H, Hu Z, et al. miR-26a inhibits proliferation and motility in bladder cancer by targeting HMGA1. FEBS Lett 2013;587:2467-73.

25. Deng Q, Chen Y, Yin N, et al. N-acetylglucosaminyltransferase V inhibits the invasion of trophoblast cells by attenuating MMP2/9 activity in early human pregnancy. Placenta 2015;36:1291-9.

26. Xiong J, Liu Y, Luo S, et al. High expression of the long non-coding RNA HEIRCC promotes Renal Cell Carcinoma metastasis by inducing epithelial-mesenchymal transition. Oncotarget 2017;8:6555-63.

27. Wang Q, Rajshankar D, Laschinger C, Talior VI, Mcculloch C. Importance of Protein-tyrosine Phosphatase- Catalytic Domains for Interactions with SHP-2 and Interleukin-1-induced Matrix Metalloproteinase-3 Expression. J Biol Chem 2010;285:22308-17.

28. Redman C. The six stages of pre-eclampsia. Pregnancy Hypertens 2014;4:246.

29. Eißmann M, Gutschner T, Hämmerle M, Günther S, Diederichs S. Loss of the abundant nuclear non-coding RNA MALAT1 is compatible with life and development. RNA Biol 2012;9:1076-87.

30. Gao W, Liu M, Yang Y, et al. The imprinted H19 gene regulates human placental trophoblast cell proliferation via encoding miR-675 that targets Nodal Modulator 1 (NOMO1). RNA Biol 2012;9:1002-10.

31. Zuo Q, Huang S, Zou Y, et al. The Lnc RNA SPRY4-IT1 Modulates Trophoblast Cell Invasion and Migration by Affecting the Epithelial-Mesenchymal Transition. Sci Rep 2016;6:37183.

32. Zou Y, Jiang Z, Yu X, et al. Upregulation of long noncoding RNA SPRY4-IT1 modulates proliferation, migration, apoptosis, and network formation in trophoblast cells HTR-8SV/neo. PLoS One 2013;8:e79598.

33. Zhang Y, Zou Y, Wang W, et al. Down-Regulated Long Non-Coding RNA MEG3 and its Effect on Promoting Apoptosis and Suppressing Migration of Trophoblast Cells. J Cell Biochem 2015;116:542-50.

34. 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;118:1462-70.

35. Chen H, Meng T, Liu X, et al. Long non-coding RNA MALAT-1 is downregulated in preeclampsia and regulates proliferation, apoptosis, migration and invasion of JEG-3 trophoblast cells. Int J Clin Exp Pathol 2015;8:12718-27.

36. Wang C, Su P, Du X, et al. Thrombospondin type I domain containing 7A (THSD7A) mediates endothelial cell migration and tube formation. J Cell Physiol 2010;222:685-94.

37. Tong C, Morrison A, Mattison S, et al. Impaired SIRT1 nucleocytoplasmic shuttling in the senescent heart during ischemic stress. FASEB J 2013;27:4332-42.

38. Luo R, Wang Y, Xu P, et al. Hypoxia-inducible miR-210 contributes to preeclampsia via targeting thrombospondin type I domain containing 7A. Sci Rep 2016;6:19588.

39. Kusanovic JP, Romero R, Chaiworapongsa T, et al. A prospective cohort study of the value of maternal plasma concentrations of angiogenic and anti-angiogenic factors in early pregnancy and midtrimester in the identification of patients destined to develop preeclampsia. J Matern Fetal Neonatal Med 2009;22:1021-38.

40. Alexandre L, Alfonso LT, Eilstein N, et al. Microfluidic platform to restore the angiogenic balance in preeclampsia. Pregnancy Hypertension 2018;13:S104.

Table 1: Clinical characteristics of participants.

Category	Normal pregnancy (n=25)	Pre-eclampsia (n=25)
Maternal age	30.73±4.234	30.89±6.235
BMI (kg/m$_2$)	26.8±1.904	30.55±4.791*
Length of gestation (weeks)	39±0.6164	36.99±3.516*
Smoking	None	None
Alcohol uptake	None	None
Placental weight (g)	547.3±60.88	525.8±63.10
Neonatal birth weight (g)	3425±420.9	2832±799.1*
Systolic blood pressure (mmHg)	111.5±11.72	156.6±15.02**
Diastolic blood pressure (mm Hg)	69.53±8.543	97.47±11.57**
Proteinuria level (g/24h)	<300mg(negative)	>300mg(positive)

Values are shown as the mean ± SD, *$P < 0.01$; **$P < 0.001$, two-tailed t-test.

Table 2. shRNAs sequence

shRNAs	Sequence 5'-3'
sh-LncZBTB39-1	Forward： GATCCGGATCATGCTGCTATTCACAGTTCAAGAGACTGTGAATAGCAGCATGATCCTTTTTTG Reverse： AATTCAAAAAAGGATCATGCTGCTATTCACAGTCTCTTGAACTGTGAATAGCAGCATGATCCG
sh-LncZBTB39-2	Forward： GATCCGAAACGTAAGTACCCTCTTCTTTCAAGAGAAGAAGAGGGTACTTACGTTTCTTTTTTG Reverse: AATTCAAAAAAGAAACGTAAGTACCCTCTTCTTCTCTTGAAAGAAGAGGGTACTTACGTTTCG
sh-LncZBTB39-3	Forward: GATCCGCCATCCAGAACTAGGAGGAATTCAAGAGATTCCTCCTAGTTCTGGATGGCTTTTTTG Reverse: AATTCAAAAAAGCCATCCAGAACTAGGAGGAATCTCTTGAATTCCTCCTAGTTCTGGATGGCG

Table 3. Sequence of primers for RT-PCR.

Gene	Sequence 5'-3'
Lnc-DERA-4:6	Forward：GTTGACCTCACCCAGGTAAT
	Reverse：CGTACACGTTCTACTCTGTCATC
LncZBTB39	Forward：TCTGTAAGGAGCCACAGGA
	Reverse：CAAGAGATAGGGAGGGAGAAGA
LncMMP10-2	Forward：CCAGATGTGGAGTGCCTGAT
	Reverse：CTTGACCCTCAGAGACCTTGG
ST8SIA4	Forward：GAAAGGCTGGCTCTTCAATCT
	Reverse：ACCACTGACACATCTCGTTCT
THSD7A	Forward：GTGGAGGGATGGACTACACTG
	Reverse：TGCCAATCGCAAACTTTGAAAC
LPHN3	Forward：GATGGGGAGGCAAATCTGACA
	Reverse：CCGTAGGGTGTAAGGGTTCAAT
NR6A1	Forward：GATCGGGCTGAACAACGAAC
	Reverse：TCACAGGAGATGATCCCATAGTG
C1orf168	Forward: ATTGGAGGCACACAGTCAACT
	Reverse：CTGGGACTCACTACTGGAACA
LAMA1	Forward：TTAGCCACCGGGAACCTAAAG
	Reverse：GCCATAGCAGATACACATGCCT
PMCH	Forward: TTCAGCATCCAAGTCCATAAGA
	Reverse：GTTCCAGGGAAGGAGCAATAA
β-actin	Forward：TGGCACCCAGCACAATGAA
	Reverse：CTAAGTCATAGTCCGCCTAGAAGCA

Figure 1. PE placentas are associated with upregulation of lncZBTB39.

(A) Hierarchical clustering analysis of lncRNAs that are differentially expressed (> 1.5-fold, *P*<0.05, n=4) in normal and PE placentas; **(B)** RT-qPCR validation for lncZBTB39, lncDERA, and lncMMP10. **P*<0.05, n=12; **(C)** FISH of lncZBTB39 in HTR8/SVneo cells and PHT; scale bar, 200 μm.

Figure 2. LncZBTB39 regulates trophoblast migration and invasion.

(A) Levels of lncZBTB39 expression in HTR8/SVneo cells transfected with sh-lncZBTB39, shNC, pEGFP-lncZBTB39, or pEGFP-NC, as well as wildtype HTR8/SVneo cells (control), ***P<0.001, *P<0.05, n=3; **(B)** wound-healing assays in lncZBTB39-knockdown, lncZBTB39-overexpression and wildtype HTR8/SVneo cells. Images were captured 0 and 24 hours after scratching, and the cell migration rate was calculated using ImageJ software, ***P<0.001, n=3; **(C)** Representative images of matrigel transwell assays in lncZBTB39-knockdown, lncZBTB39-overexpression and wildtype HTR8/SVneo cells. Scale bar, 200 μm. Five view-fields were randomly selected for quantification, *P<0.05, ***P<0.001, n=3; **(D)** Gelatin zymography assay for MMP2 in lncZBTB39-knockdown and lncZBTB39-overexpression HTR8/SVneo cells. *P<0.05, n=3.

Figure 3. LncZBTB39 regulates THSD7A expression expression in trophoblasts.

(A) Altered gene expression in pEGFP-lncZBTB39-transfected HTR8/SVneo cells (overexpression/control, fold change=log2, *P*<0.05, n=3); (B) Altered gene expression in sh-lncZBTB39-transfected HTR8/SVneo cells (knockdown/control, fold change=log2, P<0.05, n=3); (C) RT-qPCR validation of genes, of which expression was affected by lncZBTB39 manipulations. *P<0.01, # P<0.05, n=6.

Figure 4. LncZBTB39 modulates THSD7A expression in HTR8/SVneo cells via antagonizing miR-210

(A) THSD7A mRNA levels in normal and PE-complicated human placentas, *P<0.05，n=6; (**B**) THSD7A mRNA levels in HTR8/SVneo cells transfected with pEGFP-Nc or pEGFP-lncZBTB39 along with different doses of miR-210 mimics. *P<0.05 vs. pEGFP-Nc, # P<0.05 vs. 0 nM, n=3; (**C**) miR210 levels in HTR8/SVneo cells transfected with pEGFP-Nc or pEGFP-lncZBTB39. *P=0.0045 vs. pEGFP-Nc, # P<0.001 vs. sh-Nc, n=3.