

Original Research

Investigation into the Role of Forkhead Box A1 (FOXA1) in Late-Onset Preeclampsia of a Prospective Cohort Study and Its Actions on Trophoblast Invasion and Migration

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Academic Editor: Michael H. Dahan

Submitted: 25 August 2023 Revised: 19 October 2023 Accepted: 2 November 2023 Published: 29 December 2023

Abstract

Background: The primary objective was to investigate how Forkhead Box A1 (FOXA1) contributes to late-onset preeclampsia (LOPE) and its impact on trophoblast invasion and migration. **Methods:** The prospective cohort study included 15 pregnant women with LOPE (gestational age of $\geq 34^{+0}$ weeks), and 18 normal pregnant women. FOXA1 expression in placental tissues was determined by immunofluorescent and immunohistochemical (IHC) staining. FOXA1 mRNA and protein expression in HTR-8/SVneo was determined by real-time quantitative polymerase chain reaction (qPCR) and western blot, respectively. Flow cytometry was utilized to analyze cell apoptosis/cycle of HTR-8/SVneo cells. Additionally, the Transwell/wound healing assays were employed to assess invasion/migration of HTR-8/SVneo cells. Student's *t*-test was employed to compare measurement data of normal distribution between two groups. **Results:** In placental tissues of women with LOPE, FOXA1 exhibited downregulation when compared to the normal controls. No significant differences were observed in pregnancy duration, maternal age, delivery times, or 1- and 5-minute Apgar scores between the two groups. However, the LOPE group had a significantly shorter gestational week at delivery, higher systolic and diastolic blood pressure, the presence of 24-hour proteinuria, lower neonatal birth weight, and lower placental weight. FOXA1 overexpression altered the cell cycle of trophoblasts, increasing the population in the S phase and decreasing it in the G2/M phase, with no effect on the G0/G1 phase. It did not affect trophoblast apoptosis. Furthermore, FOXA1 overexpression enhanced trophoblast invasive ability and migration. However, FOXA1 overexpression did not affect the mRNA expression levels of N-cadherin, vimentin, and fibronectin in trophoblast cells. **Conclusions:** In summary, our findings indicate that FOXA1 was underexpressed in the placental tissues of women with LOPE. Furthermore, the overexpression of FOXA1 led to significant changes in the trophoblast cell cycle and substantially enhanced trophoblast invasion and migration capabilities.

Keywords: FOXA1; late-onset preeclampsia; trophoblasts; immunohistochemistry; invasion; migration

1. Introduction

Preeclampsia (PE) is characterized by hypertension and proteinuria occurring at 20 weeks of gestation in women, and affects approximately 6% of pregnant women worldwide [1–4]. PE can result in maternal and neonatal mortality and morbidity [5]. The detailed etiology of PE remains an area of intense research. Studies have suggested that both the mother and fetus play a role in the development of PE [6,7]. PE can be categorized as mild, moderate, and severe. Chronologically, PE can be classified as “early-onset” (occurring before 34⁺⁰ weeks of gestation) and “late-onset” (after 34⁺⁰ weeks) PE, also known as late-onset preeclampsia (LOPE) [8,9]. During the first trimester of pregnancy, invading extravillous trophoblasts

(EVTs) can regulate the remodeling of maternal spiral arteries, leading to a loss of myogenic tone and impaired placental perfusion [10–12]. The resulting placental hypoxia can lead to fetal growth restriction and/or the release of placental soluble factors into the maternal circulation, causing elevated maternal blood pressure [10–12]. Therefore, understanding the cellular behaviors of EVT's may help unravel the etiology of PE and develop better strategies for its management.

Forkhead Box A1 (FOXA1) is a forkhead box transcriptional factor primarily found in mammary luminal epithelial cells. FOXA1 can facilitate the binding of estrogen receptor alpha (ER α) to chromatin, and is crucial for regulating mammary epithelial differentiation and ductal



morphology [13]. Studies have shown that FOXA1 is involved in the pathophysiology of various cancers, such as prostate, breast, colorectal, gastric, and others [14–16]. The role of FOXA1 in cellular invasive behaviors remains controversial. FOXA1 can promote colorectal cancer cell invasion [17], and studies by Lou *et al.* [18] demonstrated that FOXA1 can enhance epithelial ovarian carcinoma progression by promoting proliferation and invasion. On the contrary, FOXA1 can inhibit hepatocellular carcinoma invasion by suppressing PIK3R1 [19], and Peng *et al.* [20] showed that FOXA1 has the ability to inhibit the invasion of nasopharyngeal carcinoma cells. In the context of PE, Wang *et al.* [21] showed that miR-20a-mediated repression of FOXA1 could inhibit the invasive and proliferative abilities of JEG-3 cells. However, as far as we know, the role of FOXA1 in PE remains unclear.

This study firstly investigated FOXA1 expression in placental tissues of patients with LOPE. Additionally, *in vitro* experiments explored the impact of FOXA1 overexpression on trophoblast proliferation, apoptosis, invasion, and migration. Overall, these findings provide novel insights into understanding the role of FOXA1 in the pathophysiology of LOPE.

2. Materials and Methods

2.1 Study Population

This was a prospective cohort study that included 15 pregnant women who developed LOPE after reaching a gestational age of 34 weeks or more, and 18 healthy pregnant women who delivered at full term (37 weeks or more), conducted between 2012 and 2018, at the First Affiliated Hospital of Hainan Medical College. All participants were carrying single pregnancies, and placental samples were obtained through elective cesarean section. Ethical approval for the study was granted by the Ethics Committee of the First Affiliated Hospital of Hainan Medical College. All participants provided written informed consent.

2.2 Sample Collection

After performing a cesarean section, placental tissues were collected and subsequently incubated in 10% formalin for 48 hours. Next, the tissues underwent a dehydration process using alcohol, and diaphanization using xylene before being embedded in paraffin.

2.3 Immunofluorescence Staining of Placental Tissues

The placental tissues were permeabilized for 5 minutes with phosphate buffered saline (PBS) containing 0.2% Triton X-100 (#X100, Sigma, St. Louis, MO, USA) and 10% goat serum (#50197Z, Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, they were incubated overnight at 4 °C with an anti-FOXA1 antibody (Abcam, Cambridge, MA, USA) or an anti-cytokeratin7 (CK7) antibody (Abcam, Cambridge, MA, USA). Following PBS washes, the sections were incubated with a fluorescein

isothiocyanate (FITC)-conjugated or Cy5-conjugated secondary antibody (Abcam, Cambridge, MA, USA) for 1 hour at 37 °C. After additional PBS washes, the nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, #D9542, Sigma, St. Louis, MO, USA). The expression of the target proteins was observed using a confocal laser-scanning microscope (Olympus, Tokyo, Japan).

2.4 Immunohistochemical (IHC) Analysis of Placental Tissues

The IHC analysis of FOXA1 was conducted following the methods described in a previous study [22]. The IHC staining score for placental trophoblasts was determined based on the intensity of staining and the percentage of positive cells. Depending on the staining intensity, it was categorized as follows: “intense staining” (dark brown), “moderate staining” (brown yellow), “weak staining” (light yellow), or “non-staining” (no staining), corresponding to scores of 3, 2, 1, and 0 points, respectively. Additionally, based on the percentage of positive cells, it was divided into the following groups: 0, <10%, 10%–50%, 50%–80%, and >80%, with corresponding scores of 0, 1, 2, 3, and 4 points. The final score was calculated using the formula: “number of positive cells score” × “staining degree score”. A score of “>8 points” indicated low expression, while a score of “≥8 points” indicated high expression.

2.5 Trophoblast Culture and Infection

Human HTR-8/SVneo cells were obtained from Shanghai Institute of Cell Biology (Shanghai, China) in 2017. The cell lines were verified by CK7 immunofluorescent staining, and have been tested for mycoplasma contamination and the test outcome is negative. The cell lines were cultured in HERA CELL 150 medium (Thermo, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS) (#10099141; Thermo Fisher Scientific). The cells were maintained in a humidified incubator (HERA CELL 150; Thermo Fisher Scientific) with 5% CO₂ at 37 °C. The green fluorescent protein (GFP) plasmid (pSIN-EF2-Pur-FLAG-ENNSPC syl) was obtained from IGE Biotech (Guangzhou, Guangdong, China), while the FOXA1 plasmid was constructed and verified through restrictive enzyme analysis and DNA sequencing (the detailed map of the FOXA1-overexpressing plasmid is shown in **Supplementary Fig. 1**). The DNA sequencing confirmed the successful construction of the FOXA1 plasmid. When the HTR-8/SVneo cells reached 40–50% confluence, they were infected with retrovirus-carrying the FOXA1 plasmid. After 48 hours of infection, cells were collected for further assays.

2.6 Reverse Transcription and Real-Time PCR Analysis (qPCR)

Total RNA from 1×10^6 cells was prepared by TRIzol extraction (#15596026; Invitrogen, Carlsbad, CA,

USA). 1 µg mRNA was reversely transcribed into cDNA, and the reverse transcription reaction was performed using 15 mins at 42 °C and 5 sec at 85 °C. Transcripts were quantified by qPCR on the Mx3000P qPCR system (Agilent Technologies, Santa Clara, USA). The cycling conditions were as follows: 95 °C for 30 sec, 40 cycles of 95 °C for 5 sec and 60 °C for 30 sec, followed by 95 °C for 15 sec, 60 °C for 60 sec and 95 °C for 15 sec. The forward and reverse PCR primers (5'-3') were as follows: FOXA1 (human): forward (F): 5'-GGAAGGGCATGAAACCAGC-3', reverse (R): 5'-CGTTGGCATAGGACATGT-3'; N-cadherin (human): F: 5'-GCCCAAGACAAAGAGACCCA-3', R: 5'-ACCCAGTCTCTTCTGCCT-3'; vimetin (human): F: 5'-AGCTCTTGACCTTGAACGCA-3', R: 5'-CGTGAGGTCAGGCTTGAAA3'; fibronectin (human): F: 5'-AAGAGGCAGGCTCAGCAAAT-3', R: 5'-TCGCAGTTAAACCTCGGCT-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (human): F: 5'-CGA-GAT-CCCTCCAAAATCAA-3', R: 5'-TGTGGTCATGAGTCCTTCCA-3'. For qPCR normalization, GAPDH was used as the internal control, and $2^{-\Delta\Delta C_t}$ method was used for calculating the relative mRNA expression.

2.7 Western Blot

Protein extraction from HTR-8/SVneo cells (1×10^6) infected with the retrovirus carrying the plasmid was performed using radio immunoprecipitation assay (RIPA) buffer (#89901; Thermo Fisher Scientific, Waltham, MA, USA). A total of 30 µg of proteins were separated on a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred to nitrocellulose membranes (#88025; Thermo Fisher Scientific, Waltham, MA, USA) at 300 mA for 1 hour. These membranes were subsequently blocked with 5% bovine serum albumin (BSA; #A8531, Sigma, St. Louis, MO, USA) in Tris buffered saline with Tween 20 for 1 hour at room temperature. Following this, they were incubated overnight at 4 °C with the following primary antibodies: rabbit anti-FOXA1 (diluted at 1:1000, Abcam, Cambridge, MA, USA) and mouse monoclonal anti-human GAPDH (diluted at 1:10,000, Abcam, Cambridge, MA, USA) antibodies. Afterward, the membranes were incubated with secondary antibodies for 1 hour at room temperature. Finally, the membranes were visualized using the ECL kit (Thermo Fisher Scientific, Waltham, MA, USA). GAPDH was used as the reference for normalizing the expression of the target protein.

2.8 Flow Cytometry

Cell apoptosis was assessed using the FITC-Annexin V/propidium iodide (PI) Apoptosis Detection kit (Thermo Fisher Scientific, Waltham, MA, USA). The transfected cells were collected, ensuring that suspension cells were also included, and washed twice with cold PBS. Subse-

quently, the cells were resuspended in $1 \times$ Binding Buffer and then incubated with FITC -Annexin V/PI in the dark for 15 minutes. A flow cytometer (BD Bioscience, Oxford, UK) was used to analyze the rates of cell apoptosis. For cell cycle analysis, the PI Cell Cycle analysis kit (BD Biosciences, Franklin Lakes, NJ, USA) was employed.

2.9 Transwell Invasion Assay

The Transwell invasion assay was used to assess cell invasion. In brief, the transfected cells were seeded onto the top chamber of a Transwell (8 µm) that was coated with Matrigel (Corning, New York, NY, USA). The upper chamber was filled with Roswell Park Memorial Institute (RPMI) 1640 medium (#11875119; Thermo Fisher Scientific, Waltham, MA, USA) without FBS, while the lower chamber contained RPMI 1640 medium supplemented with 10% FBS (#10099141; Thermo Fisher Scientific, Waltham, MA, USA). After an additional 24 hours of incubation, the invaded cells were fixed using 4% paraformaldehyde (#158127; Sigma, St. Louis, MO, USA) for 30 minutes and then stained with Giemsa (#G4507; Sigma, St. Louis, MO, USA). Cells that had invaded the lower membrane surface were counted in ten random fields ($10 \times$ magnification).

2.10 Wound Healing Assay

Cell migration was evaluated using a wound healing assay [21]. Briefly, transfected HTR-8/SVneo cells were seeded in six-well plates (#140675; Thermo Fisher Scientific, Waltham, MA, USA) and cultured for 24 hours. Subsequently, an artificial wound was created using a 200 µL pipette tip. The width of the wound was measured by ImageJ software at 0, 6, and 9 hours, respectively.

2.11 Statistical Analysis

For all statistical analyses, SPSS 22.0 software (IBM Corp., Armonk, NY, USA) was utilized. The normal distribution of the data were assessed by Kolmogorov-Smirnov test. Student's *t*-test was employed to compare measurement data of normal distribution between two groups. The data were presented as means \pm standard deviation (SD). $p < 0.05$ was considered statistically significant.

3. Results

3.1 Placental Tissue Morphology

To determine the FOXA1 expression in placental tissues of patients with LOPE, we performed immunofluorescence double-staining of placental tissue, captured under a laser confocal fluorescence microscope (Fig. 1). In the image from the normal group, the placental villi appear well-matured, with a relatively uniform matrix and a plump shape. Endothelial cells are intact, and the nuclei between syncytiotrophoblasts are neatly arranged. Additionally, a few red blood cells can be observed within the villi capillary lumens. In contrast, the LOPE group exhibits disordered nuclei in the trophoblasts, along with vascular reduc-

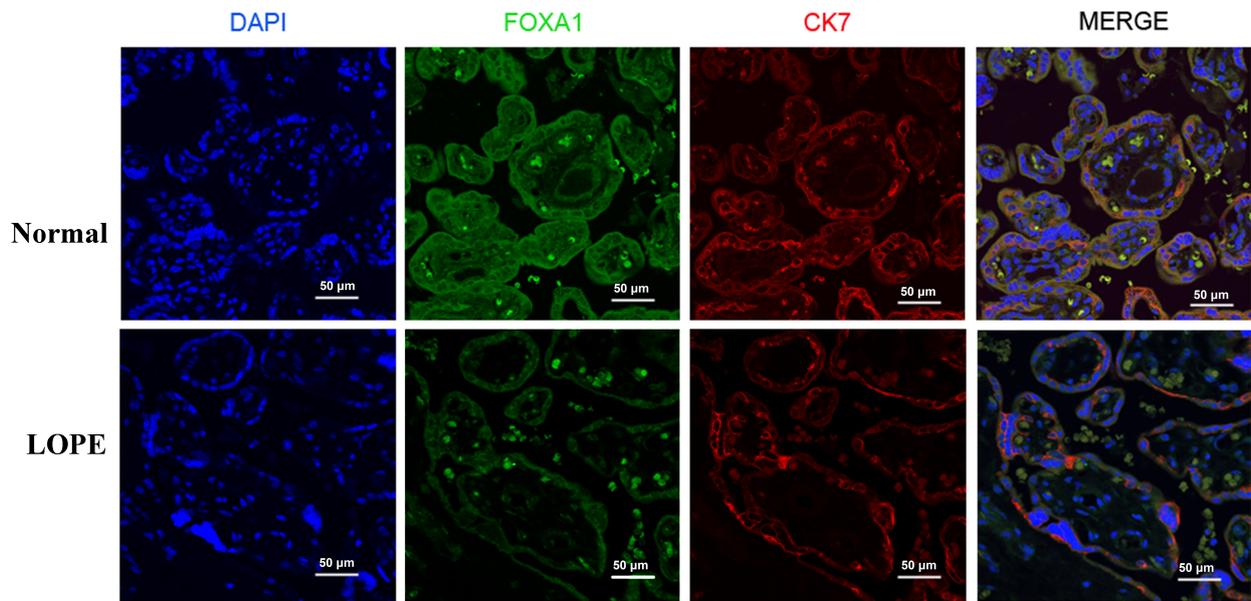


Fig. 1. Immunofluorescent staining of the FOXA1 in the placental tissues from normal pregnant women and women with LOPE. DAPI, diamidino-2-phenylindole; FOXA1, Forkhead Box A1; LOPE, late-onset preeclampsia; CK7, cytokeratin7.

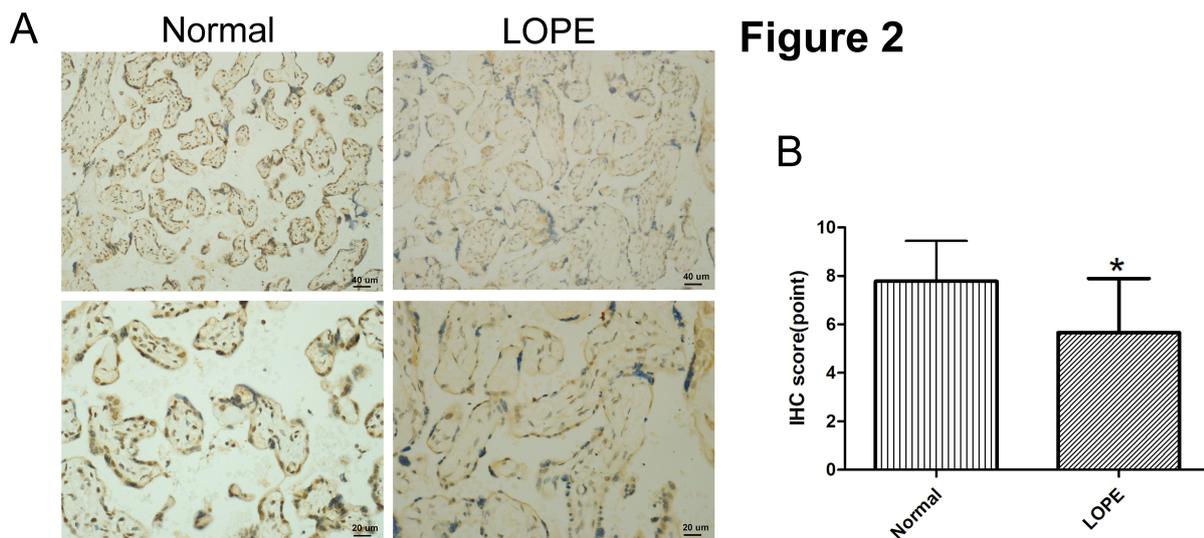


Fig. 2. Immunohistochemical (IHC) staining of FOXA1. (A) IHC staining of FOXA1 in the placental tissues from normal pregnant women and women with LOPE. (B) IHC scores of FOXA1 expression in the placental tissues were compared between the normal group and LOPE group. Bar results represent the mean \pm standard deviation (SD). Student's *t*-test, * $p < 0.05$.

tion, lumen stenosis, and incomplete endothelial cells. The immunofluorescent staining reveals a weaker fluorescence intensity of FOXA1 in LOPE placental tissue compared to the normal group. CK7, a trophoblast-specific marker protein, is expressed in both cytotrophoblast and syncytiotrophoblast cells. Under low magnification, the study reveals FOXA1 expression in both syncytiotrophoblast and cytotrophoblast cells within the villi. When observed at 60 \times magnification, FOXA1 expression is prominently observed in the nuclei and cytoplasm of trophoblast cells.

3.2 FOXA1 Expression in Placental Tissues

Next, in order to confirm if FOXA1 expression was altered in LOPE placental tissues, we performed IHC staining analysis of FOXA1 in placental tissues (Fig. 2). The findings reveal a significantly reduced FOXA1 protein expression in the LOPE group when compared to the normal group.

Table 1. Clinical characteristics and perinatal outcomes of normal group and LOPE pregnancy group.

Characteristics	Normal (n = 18)	LOPE (n = 15)	<i>p</i> -values
Maternal age (year)	32.67 ± 4.058	31.80 ± 5.797	0.618
Pregnancy times	2.72 ± 1.227	2.40 ± 1.404	0.487
Delivery times	0.78 ± 0.548	0.53 ± 0.516	0.200
Gestation weeks at delivery	38.75 ± 0.797	37.89 ± 1.496	0.043
Systolic blood pressure (mmHg)	111.61 ± 9.918	156.67 ± 12.385	<0.001
Diastolic blood pressure (mmHg)	71.28 ± 10.862	91.07 ± 13.430	<0.001
24 h proteinuria (g)	Not test	1.87 ± 2.991	
1-minute Apgar score	9.94 ± 0.236	9.33 ± 1.175	0.066
5-minute Apgar score	10	10	-
Neonate birthweight (g)	3202.78 ± 250.266	2684.60 ± 532.894	0.003
Placental weight (g)	559.00 ± 68.872	496.0 ± 53.559	0.017

Significant differences between groups were determined by Student's *t*-test. LOPE, late-onset preeclampsia.

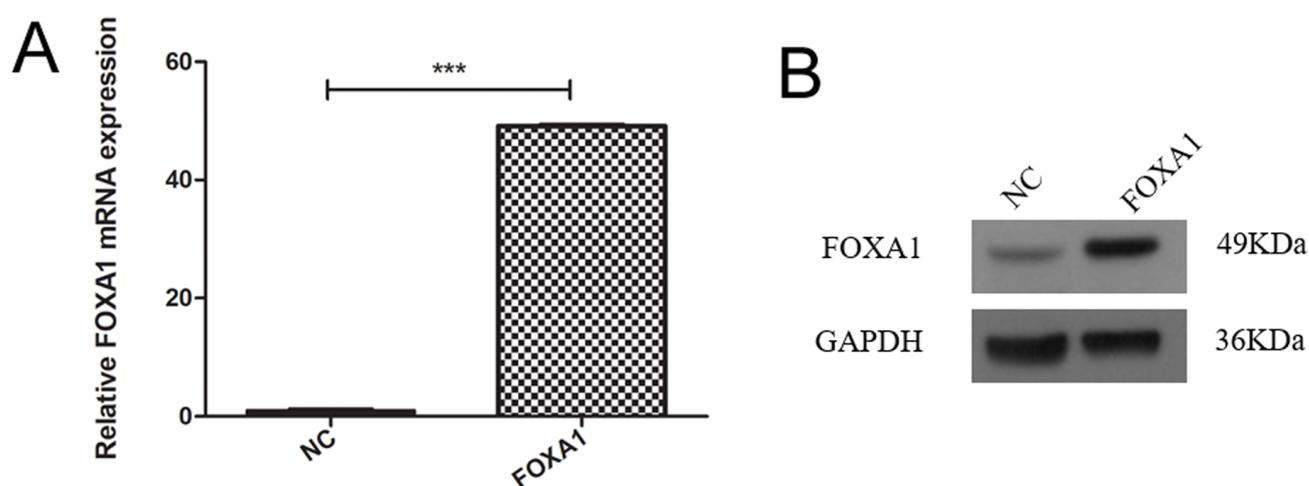


Fig. 3. Overexpression of FOXA1 in the trophoblast cells. (A) The mRNA expression of FOXA1 in the HTR-8/SVneo cells from NC and FOXA1 group was determined by real-time quantitative polymerase chain reaction (qPCR). (B) The expression of FOXA1 protein in the HTR-8/SVneo cells from NC and FOXA1 group was determined by western blot assay. FOXA1, Forkhead Box A1; NC, negative control; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Bar results represent the mean ± SD. n = 3. Student's *t*-test, ****p* < 0.001.

3.3 Clinical Characteristics of Recruited Subjects

No notable differences were detected in delivery times, pregnancy times, maternal age, 1- and 5-minute Apgar scores between the normal and LOPE group. However, the gestation week at delivery was notably shorter in LOPE group compared to normal group. Patients in the LOPE group exhibited higher systolic and diastolic blood pressure in comparison to the normal group. Furthermore, 24-hour proteinuria was detected in the LOPE group, but not in the normal group. Additionally, both placental weight and neonate birth weight were lower in the LOPE group, compared to the normal group (Table 1).

3.4 Actions of FOXA1 Overexpression on the Cell Cycle/Apoptosis of Trophoblasts

To create trophoblasts overexpressing FOXA1, we introduced a retrovirus designed to overexpress FOXA1

into HTR-8/SVneo cells. This resulted in a significant elevation in FOXA1 expression levels compared to the negative control (NC) group (Fig. 3A,B), determined by qPCR and confirmed by western blot assay. Subsequently, we assessed cell apoptosis/cycle through flow cytometry. FOXA1 did not exert any significant effect on the cell apoptotic rate of trophoblasts when compared to the NC group (Fig. 4A). Furthermore, FOXA1 overexpression significantly increased S phase cell population while decreasing the G2/M phase population, without affecting G0/G1 phase population, compared to NC group (Fig. 4B).

3.5 Actions of FOXA1 Overexpression on Trophoblast Migration/Invasion

To further evaluate migration/invasion of trophoblasts, transwell invasion assay deciphered that FOXA1 overexpression enhanced invasive capacity of tro-

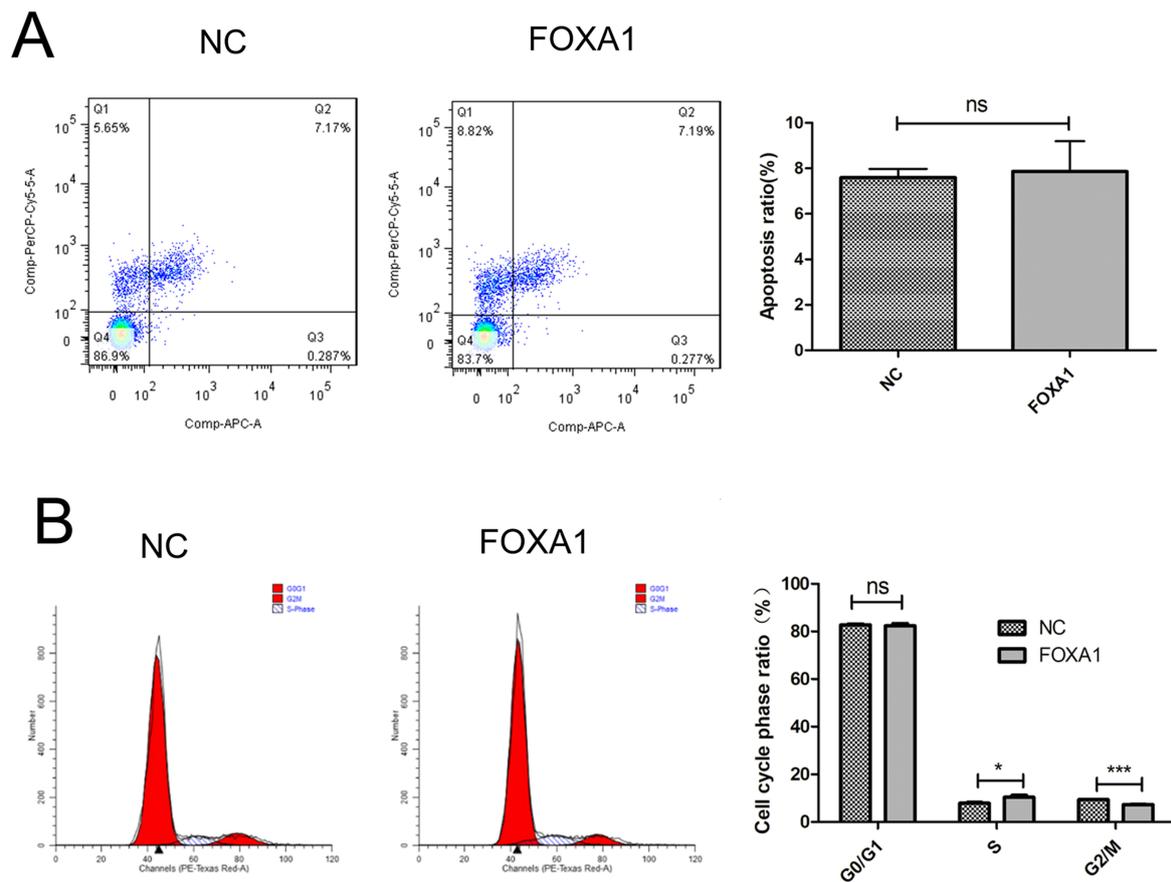


Fig. 4. Effects of FOXA1 overexpression on the cell apoptosis and cell cycle of trophoblast cells. (A) The cell apoptosis of HTR-8/SVneo cells from NC and FOXA1 group was determined by flow cytometry. (B) The cell cycle of HTR-8/SVneo cells from NC and FOXA1 group was determined by flow cytometry. FOXA1, Forkhead Box A1; NC, negative control. Bar results represent the mean \pm SD. $n = 3$. Student's t -test, * $p < 0.05$ and *** $p < 0.001$; ns, not significant.

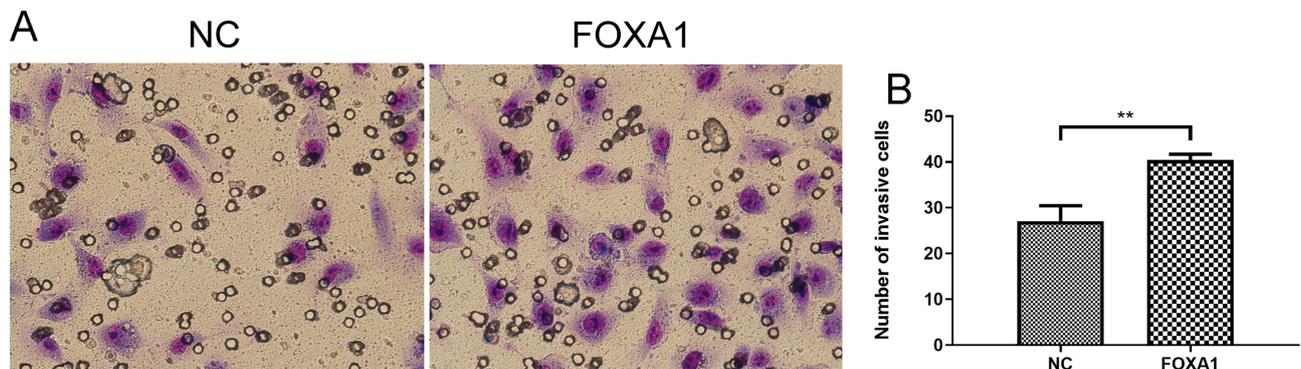


Fig. 5. Effects of FOXA1 overexpression on the cell invasion of trophoblast cells. The cell invasion of HTR-8/SVneo cells from NC and FOXA1 group was determined by Transwell invasion assay. FOXA1, Forkhead Box A1; NC, negative control. Bar results represent the mean \pm SD. $n = 3$. Student's t -test, ** $p < 0.01$.

phoblasts compared to NC group (Fig. 5). Wound healing assay revealed that FOXA1 overexpression significantly accelerated the closure of wounds in trophoblasts when compared to NC group (Fig. 6).

3.6 Effects of FOXA1 Overexpression on the mRNA Expression of N-Cadherin, Vimentin and Fibronectin

To further determine the mechanism responsible for FOXA1-mediated invasion and migration in trophoblast cells, qPCR was performed to determine the mRNA expression levels of N-cadherin, vimentin and fibronectin. As

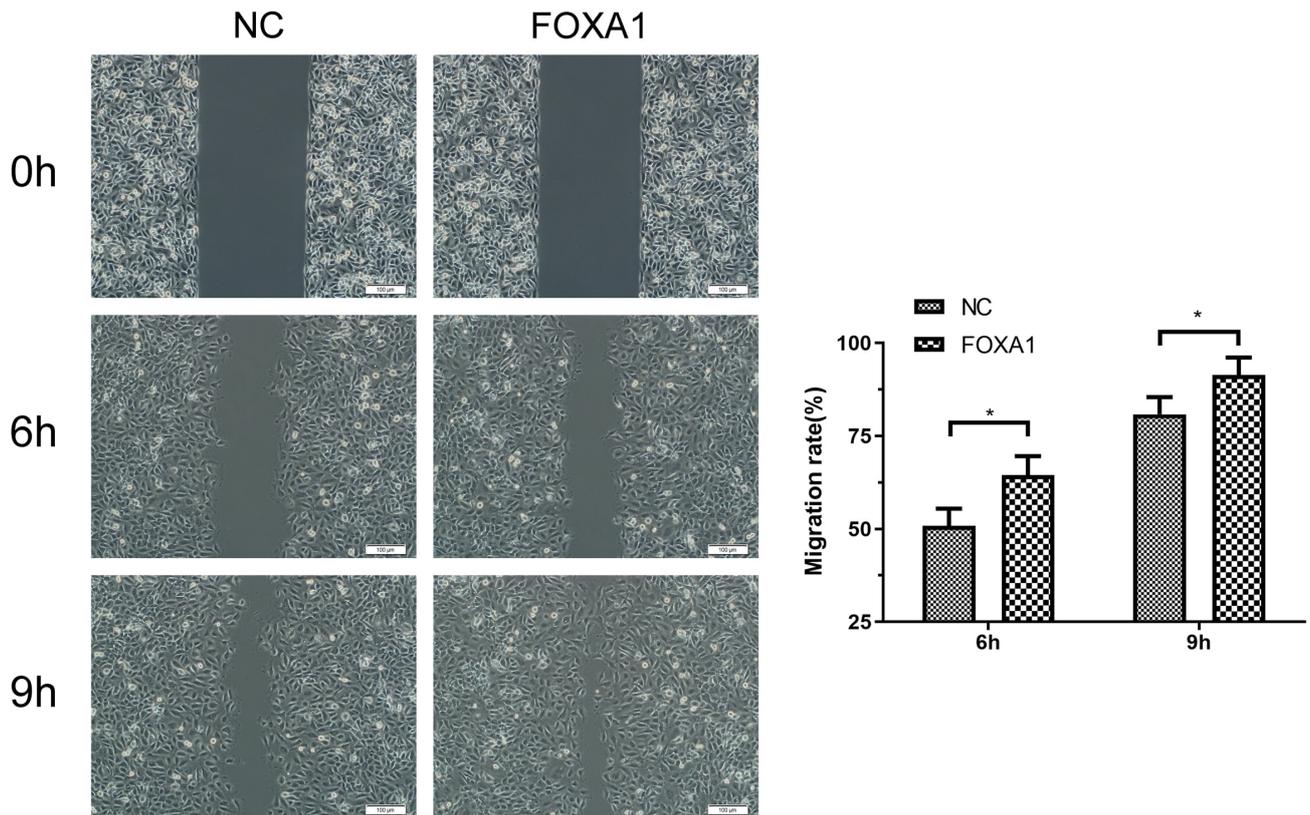


Fig. 6. Effects of FOXA1 overexpression on the cell migration of trophoblast cells. The cell migration of HTR-8/SVneo cells from NC and FOXA1 group was determined by wound healing assay. Scale bar = 100 μ m. FOXA1, Forkhead Box A1; NC, negative control. Bar results represent the mean \pm SD. n = 3. Student's *t*-test, **p* < 0.05.

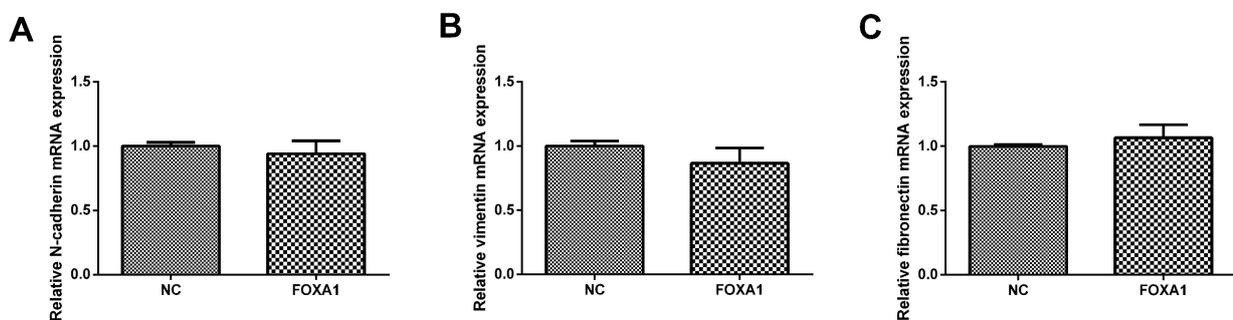


Fig. 7. Effects of FOXA1 overexpression on the mRNA expression of N-cadherin, vimentin and fibronectin in trophoblast cells. The mRNA expression levels of N-cadherin, vimentin and fibronectin in HTR-8/SVneo cells from NC and FOXA1 group was determined by qPCR. GAPDH was used as the internal control. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FOXA1, Forkhead Box A1; NC, negative control. Bar results represent the mean \pm SD. n = 3. Student's *t*-test.

shown in Fig. 7, FOXA1 had no effect on the mRNA expression levels of N-cadherin, vimentin or fibronectin comparing to the NC group.

4. Discussion

PE is a complex disorder observed in pregnant women, yet its underlying pathophysiology remains to be fully understood. Mounting evidence suggests that abnor-

mal trophoblast invasion plays a significant function in PE development [23–25]. Therefore, gaining insights into the molecular mechanisms governing trophoblast invasion is crucial to identify potential treatments for PE.

Our immunostaining results demonstrated a down-regulation of FOXA1 in placental tissues from women with LOPE in comparison to normal controls. *In vitro* studies further revealed that FOXA1 overexpression mitigated

trophoblast apoptosis. Transwell invasion assay indicated that FOXA1 overexpression enhanced trophoblast invasive ability, while the wound healing assay showed that it also potentiated trophoblast migration. Taken together, our findings indicate that FOXA1 is down-regulated in LOPE placental tissues, and its overexpression not only affects cell cycle but also enhances trophoblast invasion and migration.

Numerous studies have explored the role of the forkhead box protein family in the pathophysiology of PE [26–31]. FOXM1 was down-regulated in PE placental tissues [26], and silencing FOXM1 was found to suppress trophoblast proliferation, increase G0/G1 phase cell population, and induce apoptosis [26]. Mechanistically, FOXM1 silencing also reduced the expression of BCL-2 and vascular endothelial growth factor in trophoblasts [26]. Another study revealed that reduced FOXM1 expression limited trophoblast migration and angiogenesis, and was associated with PE [27]. Zhang *et al.* [28] showed that FOXC2 could promote trophoblast invasive ability through the Hedgehog signaling. Chen *et al.* [29] demonstrated that FOXO1 enhanced trophoblast integrin $\beta 3$ expression, thereby mediating cell adhesion and migration. In a recent study, miR-142-3p was identified as a negative regulator of FOXM1 expression, and FOXM1 overexpression was found to promote trophoblast growth and migration [30]. Moreover, Lai *et al.* [31] reported that FOXP1 was observed to promote proliferation/invasion/angiogenesis in chorionic trophoblastic cells. Collectively, these findings indicate that members of the forkhead box protein family are closely associated with the progression of PE, and their dysregulation may contribute to the pathophysiology of the condition.

FOXA1, belonging to forkhead box protein family, has been extensively studied for its role in regulating cellular functions in various cancer types [16,32–37]. However, its specific role in the context of PE has not been fully elucidated. Previous research by Wang *et al.* [21] revealed that miR-20a-mediated repression of FOXA1 could hinder proliferative/invasive capabilities of JEG-3 cells. In our study, we observed that FOXA1 expression was downregulated in LOPE placental tissues. Further functional investigations demonstrated that FOXA1 overexpression could promote trophoblast proliferation, invasion, and migration, while having no significant effect on trophoblast apoptosis. These findings shed light on underlying function of FOXA1 in LOPE pathophysiology, suggesting its involvement in regulating trophoblast behaviors and warranting further exploration.

In early pregnancy, the invasion of EVT is a crucial process for the development of the placenta [38]. The precise mechanism responsible for trophoblast invasion remains not entirely clear. Various factors such as hormones, cytokines, growth factors, and proteinases have been implicated in this process [38]. Notably, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are essential for facilitating trophoblast

invasion by breaking down the extracellular matrix [39]. For instance, MMP9 and MMP2 are two well-known enzymes that play a pivotal role in trophoblast invasion [40]. Moreover, it has been demonstrated that the epithelial-to-mesenchymal transition (EMT) during development and the differentiation of cytotrophoblasts into EVT share similarities [41]. During normal placental formation, EVTs undergo a transformation from an organized epithelial phenotype to a migratory and invasive mesenchymal phenotype, which allows them to infiltrate the maternal decidual stroma and blood vessels [42]. However, the disruption of EMT hinders the trophoblasts' ability to migrate and invade effectively. These findings underscore the importance of regulating EMT during the growth of trophoblast cells [42]. In the present study, we found that the expression of EMT-related markers, including N-cadherin, vimentin and fibronectin was not affected by FOXA1 overexpression in the trophoblast cells. These results indicate that the enhanced trophoblast cell invasion and migration mediated by FOXA1 overexpression could involve other mediators, such as MMPs and TIMPs, which still require further examination.

Our study has several limitations. First, the sample size in the current study is relatively small, and future studies should include larger clinical samples to validate our findings. Second, we only examined the effects of FOXA1 overexpression on trophoblast cell apoptosis, invasion, and migration in the HTR-8/SVneo cell line. As HTR-8/SVneo belongs to a type of first trimester cell line, utilizing primary trophoblast cells, BeWo, or JEG3 cells for similar investigations would bolster our findings. Third, the mechanisms responsible for FOXA1-mediated invasion and migration in trophoblast cells have not been elucidated, and future studies are needed to fully understand these underlying mechanisms.

5. Conclusions

In summary, our findings reveal a reduction in FOXA1 expression in the placentas of patients with LOPE. Furthermore, we observed that the overexpression of FOXA1 enhances the migratory and invasive abilities of trophoblast cells. These results suggest that FOXA1 could potentially be a therapeutic target for managing LOPE.

Availability of Data and Materials

Data are available upon request.

Author Contributions

YM, ZY and JZ conceived/designed the study. JZ, YW, ZW and QJ performed experiments. QJ, PL, HY and HK contributed to analysis. JZ, QJ and ZY analyzed the data. JZ wrote the manuscript. YM and ZY reviewed and edited the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved

the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Ethical approval for study was granted by Ethics Committee of the First Affiliated Hospital of Hainan Medical College (approval number: 2022-50). All subjects provided written informed consent.

Acknowledgment

Not applicable.

Funding

This work was supported by Hainan Province Science and Technology Special Fund (No. ZDYF2020121), Hainan Provincial Natural Science Foundation of China (No. 822RC836, No. 821RC702), National Natural Science Foundation of China (81960283), the specific research fund of The Innovation Platform for Academicians of Hainan Province, Hainan Province Clinical Medical Center.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.ceog5012280>.

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