Article

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ATF2 Promotes Preeclampsia Accompanied by Gestational Hypertension via Transcriptionally Activating *ARRDC3* to Suppress Trophoblast Functions

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Background: Growing evidence suggests that arrestin domain-containing protein 3 (*ARRDC3*) plays a pivotal role in enhancing preeclampsia (PE) progression, and activating transcription factor 2 (*ATF2*) serves as a key regulator of trophoblast cell functions. Therefore, this study aims to investigate the role of the *ATF2*/*ARRDC3* axis in mediating trophoblast cell viability, migration, invasion, and epithelial-mesenchymal transition (EMT), providing mechanistic insights into PE pathogenesis.
Methods: The expression of ARRDC3, ATF2, EMT-related markers, both at mRNA and protein levels, was assessed using quantitative reverse transcription polymerase chain reaction (qRT-PCR) and western blot analyses. Trophoblast cell viability, apoptosis rate, invasion, and migration were evaluated using the Cell counting kit-8 (CCK-8) assay, flow cytometry, Transwell assay, and wound healing assay. Additionally, a correlation between ATF2 and the ARRDC3 promoter region was determined using the dual-luciferase reporter and ChIP assays.
Results: *ARRDC3* overexpression inhibited trophoblast cell viability, invasion, migration, and EMT, while promoting apoptosis ($p < 0.05$). *ATF2* promoted *ARRDC3* expression by binding to its promoter region ($p < 0.05$). Upregulated *ATF2* suppressed trophoblast cell viability, invasion, migration, and EMT; however, these effects were reversed by *ARRDC3* knockdown ($p < 0.05$).
Conclusion: *ATF2* binds to the *ARRDC3* promoter region, upregulating its expression, thereby inhibiting trophoblast cell functions which in turn accelerate PE progression.

Keywords: preeclampsia; *ARRDC3*; *ATF2*; trophoblast cells

Introduction

Preeclampsia (PE) is an obstetric disorder characterized by hypertension and multi-organ dysfunction [1]. Hypertension is a major risk factor for PE, and evidence indicates that cardiovascular dysfunction occurs several days before the development of PE [2,3]. Trophoblast cells, the primary structure of the placenta, are essential for invading the uterine wall and forming placental blood vessels to ensure adequate oxygen and nutrient supply to the fetus, playing a vital role in PE development [4]. Furthermore, reduced proliferation and invasion of trophoblast cells have been correlated with the development of PE [5,6]. Abnormal trophoblast invasion and impaired spiral artery remodeling lead to decreased placental perfusion, ischemia, and hypoxia, which are recognized as crucial pathological mechanisms underlying PE [7,8]. Therefore, elucidating the pathogenesis of PE holds immense clinical implications for improving patient management and treatment.

Arrestin domain-containing protein 3 (*ARRDC3*) is a newly reported member of mammalian inhibitory proteins (α-arrestins) and has been identified as a potential tumor suppressor [9,10]. Liu *et al*. [11] found elevated *ARRDC3* expression in the gastric mucosa of *H. pylori*-infected patients, and confirmed that its expression levels was positively correlated with the severity of gastritis. Importantly, higher *ARRDC3* expression has also been reported in both PE patients and human placental trophoblast cells, suggesting *ARRDC3* as a potential therapeutic target for PE [12]. Therefore, investigating the precise molecular mechanisms involving *ARRDC3* in PE progression may provide new perspectives for developing effective interventions.

Activating transcription factor 2 (*ATF2*), a member of the leucine zipper family, plays a pivotal role in regulating many cellular biological processes [13,14]. *ATF2* regulates gene expression by forming homodimers or heterodimers with proteins, including cAMP-responsive element binding protein (CREB) and c-Jun N-terminal kinase (c-Jun) [15, 16]. Elevated *ATF2* expression has been reported in gestational diabetes mellitus, where it suppresses trophoblast cell proliferation and promotes ferroptosis, thereby impacting trophoblast function [17]. This suggests that *ATF2* may

be a crucial contributor to trophoblast cell dysfunction. Furthermore, previous research has revealed that *ATF2* is overexpressed in PE patients, indicating its potential role in PE progression [18]. In this study, the binding sites between *ATF2* and the *ARRDC3* promoter region were identified using the Jaspar software (https://jaspar.genereg.net/analysis). However, whether *ATF2* affects the progression of PE by regulating *ARRDC3* remain unexplored.

Therefore, we investigated the involvement of the *ATF2*/*ARRDC3* axis in regulating trophoblast cell viability, migration, invasion, and epithelial-mesenchymal transition (EMT), providing new insights into PE pathogenesis.

Materials and Methods

Tissue Sample Collection

Placental tissues were obtained from 33 PE patients and 29 healthy pregnant women recruited from The No. 4 People's Hospital of Hengshui, China. Each participant signed a written informed consent form, and the study was approved by the Hospital's Ethics Committee (2022 No. 20).

Cell Culture

The human choriocarcinoma-derived JEG-3 cell line (CL-0127; Procell, Wuhan, China) and HTR-8/SVneo trophoblast cell line (CL-0765; Procell) were cultured in MEM (PM150410; Procell) or RPMI-1640 medium (PM150110; Procell) containing 1% penicillin/streptomycin (PB180120, Procell) and 10% fetal bovine serum (FBS) (164210-50, Procell). The cell lines were authenticated via STR and tested negative for mycoplasma contamination.

Cell Transfection

The pcDNA3.1-*ARRDC3* and pcDNA3.1-*ATF2* overexpression vectors were prepared by inserting the full-length *ARRDC3* (GenBank™ NM_020801.4) or *ATF2* (GenBank™ NM_001880.4) sequences into the pcDNA3.1 vector (V79020; Invitrogen, Carlsbad, CA, USA). The siRNAs targeting *ATF2* (si-ATF2: F 5′-ACUAGUUCAUGAUCCUUUCGG-3′, R 5′-GAAAGGAUCAUGAACUAGUGA-3′) and *ARRDC3* (si-ARRDC3: F 5′-AGAUUUCUUAAAAAGUCAGGG-3′, R 5′-CUGACUUUUUAAGAAAUCUCA-3′), along with a negative control siRNA (si-NC: F 5′-GGAGUAGGGAGCAAACCUAUAGGAA-3′, R 5′-UUCCUAUAGGUUUGCUCCCUACUCC-3′), were synthesized by RiboBio (Guangzhou, China). JEG-3 and HTR-8/SVneo cells were seeded into 24-well plates (1×10^5/well) and cultured until achieving 60% confluence. Then, the cells were transfected with pcDNA vector and siRNAs using Lipofectamine 3000 (L3000150; Invitrogen). After 48 hours of incubation at 37 °C, the cells were collected for subsequent functional analyses.

Western Blot (WB) Analysis

Total protein was extracted using RIPA buffer, resolved on 10% SDS-PAGE (P0690; Beyotime, Shanghai, China), and subsequently transferred onto PVDF membranes (FFP22; Beyotime). After that, the membranes were blocked and incubated overnight with primary antibodies (Abcam, Cambridge, CA, USA), including anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, ab9485, 1:2500), anti-ARRDC3 (ab64817, 1:500), anti-E-cadherin (ab40772, 1:1000), anti-N-cadherin (ab76011, 1:5000), anti-ATF2 (ab32160, 1:10,000), anti-Vimentin (ab45939, 1:1000), anti-Snail (ab216347, 1:1000), anti-PI3K (ab191606, 1:1000), anti-p-PI3K (ab182651, 1:500), and anti-AKT (ab8805, 1:500), anti-p-AKT (ab18206, 1:1000). The next day, the membranes were thoroughly washed and incubated with secondary antibody (ab205718, 1:50,000) for 2 hours. Protein bands were visualized using an ECL reagent (P0018AS; Beyotime) and examined on an ImageQuant LAS 4000 (GE Healthcare, Little Chalfont, Buckinghamshire, UK) imaging system. The gray values of the protein bands were quantified using ImageJ software (v1.8.0; NIH, Bethesda, MD, USA). GAPDH was employed as the loading control during blot analysis.

Cell Counting Kit-8 (CCK-8) Assay

The CCK-8 assay was used to assess cell viability. Transfected cells were inoculated into 96-well plates and cultured for 48 hours. CCK-8 reagent (10 µL; C0037; Beyotime) was added to each well, and absorbance was measured at 450 nm using a microplate reader (SpectraMax i3x, Molecular Devices, Sunnyvale, CA, USA).

Flow Cytometry

Cells were resuspended with binding buffer and stained with Annexin V-FITC and PI solution (C1062S; Beyotime). The apoptosis rate was then assessed using a flow cytometer (FACScalibur, BD Biosciences, San Jose, CA, USA).

Transwell Assay

Cells were seeded into the upper chambers of the Transwell insert, which was pre-coated with Matrigel (354230; Corning Inc., Corning, NY, USA), while the lower chamber was filled with complete medium as a chemoattractant. After 24 hours, the cells were fixed and stained, and the number of cells that had invaded the lower membrane surface was counted using a microscope (IX71, Olympus, Tokyo, Japan).

Wound Healing Assay

Cells were seeded into 6-well plates, and a wound was created by scratching the cell layer using a sterilized pipette tip. The cells were then cultured in serum-free medium for 24 hours. After that, the wound area was photographed using a microscope (IX71, Olympus), and the migration distance was calculated.

Bioinformatics Prediction

The Genecards database predicted *ATF2* as an upstream transcription factor of the *ARRDC3* gene promoter, while the potential binding sites between *ATF2* and the *ARRDC3* promoter region were determined using the Jaspar website.

Dual-luciferase Reporter Assay

Wild-type and mutant-type fragments of the *ARRDC3* promoter region were cloned into the pGL3-basic vector to develop the WT-ARRDC3 and MUT-ARRDC3 vectors. Then, the cells were transfected with si-NC or si-ATF2 vectors along with the WT-ARRDC3 and MUT-ARRDC3 vectors using Lipofectamine 3000. Finally, luciferase activity was evaluated using the appropriate assay kit (E1910; Promega, Madison, WI, USA).

ChIP Assay

Using the Magna ChIP A/G Kit (17-10086; Millipore, Billerica, MA, USA), cells were cross-linked with 1% formaldehyde, and the resulting cell lysates were sonicated to obtain DNA fragments. The DNA fragments were incubated with anti-IgG or anti-ATF2 antibodies, and the precipitated DNA was used as a template for quantitative reverse transcription polymerase chain reaction (qRT-PCR) to evaluate the enrichment of the ARRDC3 promoter region.

qRT-PCR

Total RNA was isolated and reverse-transcribed into cDNA using the cDNA Synthesis Kit (RR037A; Takara, Tokyo, Japan). qRT-PCR was then performed using SYBR Green (RR820A; Takara) on a CFX96 PCR system (Bio-Rad, Hercules, CA, USA). Specific primers used in qRT-PCR are listed in Table 1. The relative mRNA expression of the ATF2 and ARRDC3 genes was determined using the $2^{-\Delta\Delta Ct}$ method.

Table 1. Primer sequences used in qRT-PCR.

Name		Sequences for PCR (5′-3′)
ARRDC3	Forward	CCTCTGCTGCCCTGACTTTT
	Reverse	GTGTGGAAGCCTTCTTCGGA
ATF2	Forward	GTAGTCTGATTGGCTTAACTCGTAT
	Reverse	TCTTGTTGGTGTTGGGGTCT
GAPDH	Forward	GACCACAGTCCATGCCATCAC
	Reverse	ACGCCTGCTTCACCACCTT

ARRDC3, arrestin domain-containing protein 3; ATF2, activating transcription factor 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

Statistical Analysis

Statistical analyses were performed using GraphPad 7.0 software. Data were expressed as mean ± standard deviation (SD). Comparison between two groups was conducted using Student's *t*-test, while multiple group comparisons were performed using one-way ANOVA followed by the Tukey post-hoc test. All experiments were performed in triplicate to ensure the reliability of the findings. A *p*-value of <0.05 was considered statistically significant.

Results

ARRDC3 Overexpression Inhibits Trophoblast Cell Proliferation, Invasion, Migration, and EMT

To investigate the role of *ARRDC3* in PE, JEG-3 and HTR-8/SVneo cells were transfected with an *ARRDC3* overexpression vector. After transfection, both ARRDC3 mRNA and protein expression levels were substantially elevated ($p < 0.05$, Fig. 1A,B). Furthermore, *ARRDC3* overexpression suppressed cell viability and promoted the apoptosis rate ($p < 0.05$, Fig. 1C–E). Moreover, the upregulation of *ARRDC3* suppressed cell invasion and reduced migration distance ($p < 0.05$, Fig. 1F–H). Additionally, *ARRDC3* overexpression increased the E-cadherin protein expression levels while decreasing N-cadherin expression ($p < 0.05$, Fig. 1I,J). Overall, these results suggest that ARRDC3 inhibits trophoblast cell functions.

ATF2 Regulates ARRDC3 Transcription

The Genecards database indicated that transcription factor *ATF2* could bind to the *ARRDC3* promoter region to mediate its transcription (Fig. 2A). Furthermore, the Jaspar database identified specific binding sites between the *ATF2* and *ARRDC3* promoter regions (Fig. 2B). To validate these observations, we constructed si-ATF2 to downregulate ATF2 mRNA and protein expression in JEG-3 and HTR-8/SVneo cells ($p < 0.05$, Fig. 2C,D). A dual-luciferase reporter assay revealed that si-ATF2 substantially reduced the luciferase activity of the WT-ARRDC3 vector, while it had no effect on the MUT-ARRDC3 vector ($p < 0.05$, Fig. 2E,F). Additionally, the *ARRDC3* promoter region was found to be enriched for anti-ATF2 ($p < 0.05$, Fig. 2G). Similarly, si-ATF2 significantly inhibited *ARRDC3* mRNA expression ($p < 0.05$, Fig. 2H). Moreover, both *ATF2* and *ARRDC3* were found to be overexpressed in the placental tissue obtained from PE patients ($p < 0.05$) (**Supplementary Fig. 1A,B**), and Pearson correlation analysis revealed a positive correlation between *ATF2* and *ARRDC3* expression in the tissue samples ($p < 0.05$) (**Supplementary Fig. 1C**). These results demonstrate that *ATF2* enhances *ARRDC3* expression by binding to its promoter region.

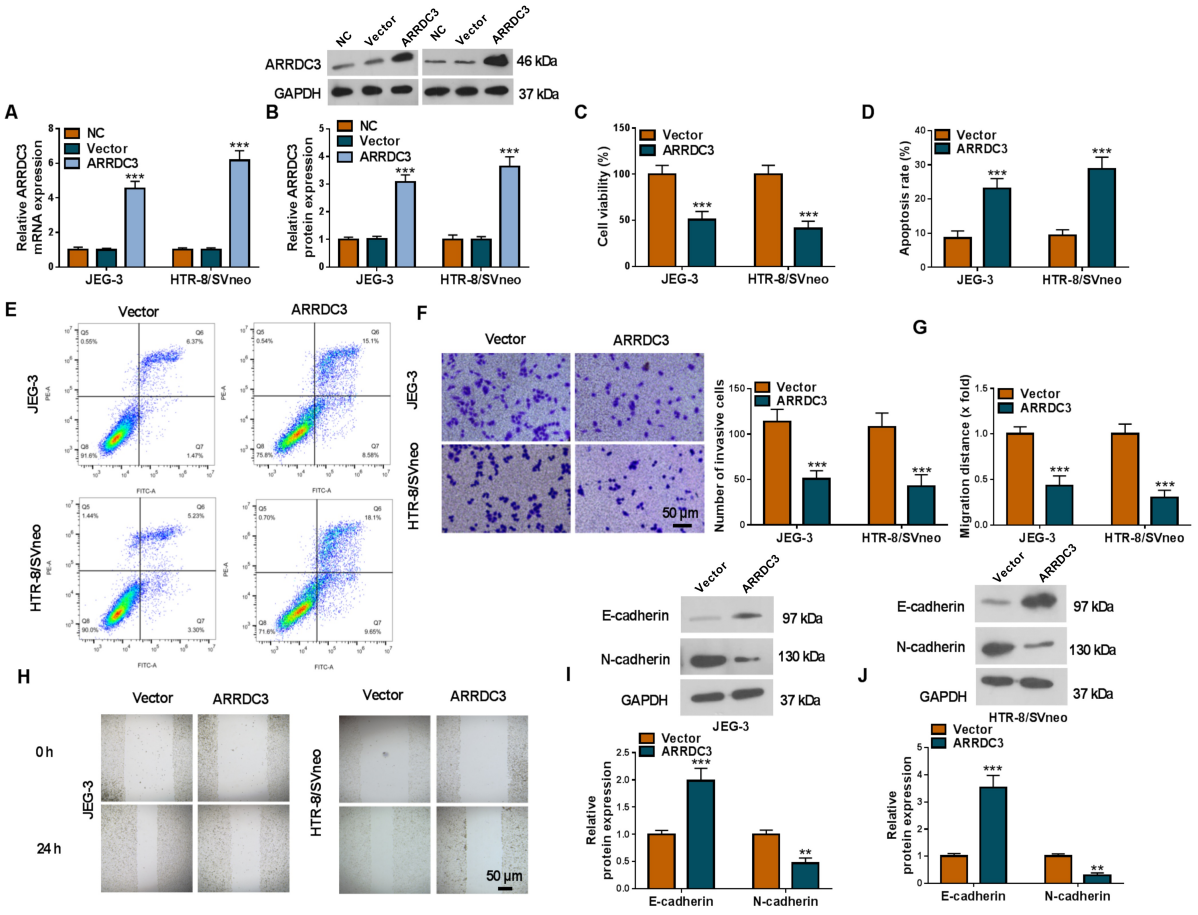

Fig. 1. Effect of ARRDC3 upregulation on trophoblast cell functions. JEG-3 and HTR-8/SVneo cells were transfected with a vector or an ARRDC3 overexpression vector. (A,B) ARRDC3 mRNA and protein expression in the NC group, the vector group, and the ARRDC3 overexpression group (qRT-PCR and WB analysis). Cell viability and the apoptosis rate in the vector group and the ARRDC3 overexpression group were assessed using CCK-8 assay (C) and flow cytometry (D,E). Transwell assay (F) and wound healing assay (G,H) were used to assess cell invasion and migration in the vector group and the ARRDC3 overexpression group. (I,J) WB analysis was performed to determine the expression levels of E-cadherin and N-cadherin proteins in the vector group and the ARRDC3 overexpression group. $^{**}p < 0.01$ and $^{***}p < 0.001$. ARRDC3, arrestin domain-containing protein 3; JEG-3, human choriocarcinoma cell line JEG-3; HTR-8, human extravillous trophoblast cell line HTR-8/SVneo; NC, negative control; qRT-PCR, quantitative reverse transcription polymerase chain reaction; WB, Western blot; CCK-8, cell counting kit-8.

ATF2 Overexpression Suppresses Trophoblast Cell Functions

Next, we explored the role of *ATF2* in PE progression. *ATF2* overexpression substantially elevated ATF2 mRNA and protein expression levels in JEG-3 and HTR-8/SVneo cells ($p < 0.05$, Fig. 3A,B). Furthermore, *ATF2* upregulation reduced cell viability, decreased the number invasive cells and migration distance, and promoted apoptosis ($p < 0.05$, Fig. 3C–H). Additionally, *ATF2* upregulation increased E-cadherin protein levels while reducing N-cadherin protein levels ($p < 0.05$, Fig. 3I,J). These results suggest that *ATF2* suppresses trophoblast cell viability, invasion, migration, and EMT.

ATF2 Modulates Trophoblast Cell Functions by Regulating ARRDC3

To examine whether *ATF2* regulates trophoblast cell functions via *ARRDC3*, JEG-3 and HTR-8/SVneo cells were co-transfected with an *ATF2* overexpression vector and si-ARRDC3. Analysis of ARRDC3 mRNA and protein expression levels confirmed that si-ARRDC3 reverted the upregulation of ARRDC3 induced by *ATF2* overexpression ($p < 0.05$, Fig. 4A,B). Functional assays revealed that si-ARRDC3 counteracted the inhibitory effects of the *ATF2* overexpression on cell viability, invasive cell numbers, and migration distance, as well as the pro-apoptotic effects ($p < 0.05$, Fig. 4C–G). Similarly, the *ATF2*-mediated increase in

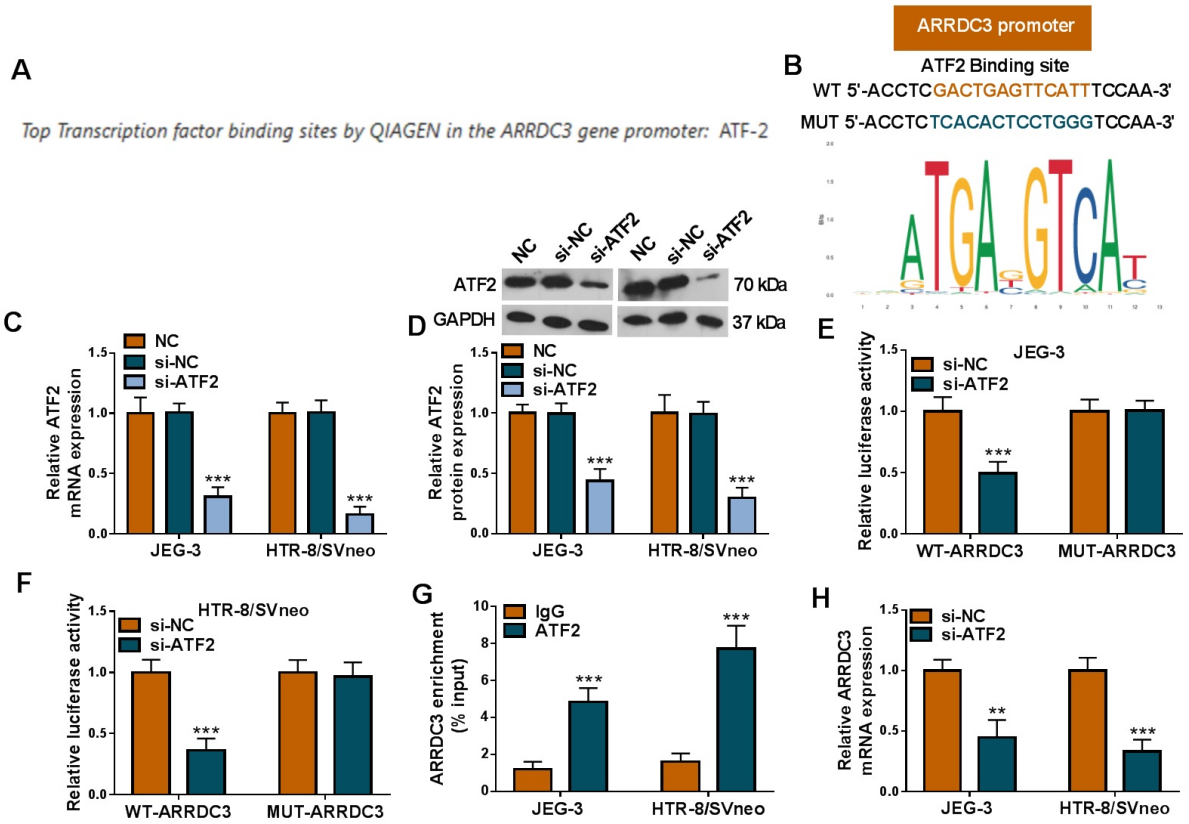

Fig. 2. Effect of ATF2 on ARRDC3 transcription. The Genecards database (A) and the Jaspar website (B) identified the binding sites between ATF2 and the ARRDC3 promoter region. (C,D) ATF2 mRNA and protein levels were detected using qRT-PCR and WB analysis in si-NC or si-ATF2-transfected JEG-3 and HTR-8/SVneo cells. (E,F) A dual-luciferase reporter assay was used to examine the luciferase activity of the WT-ARRDC3 and MUT-ARRDC3 vectors in JEG-3 and HTR-8/SVneo cells transfected with si-NC/si-ATF2. (G) The enrichment of the ARRDC3 promoter in the IgG and ATF2 antibodies was determined using a ChIP assay. (H) qRT-PCR was used to evaluate ARRDC3 mRNA expression in JEG-3 and HTR-8/SVneo cells transfected with si-NC/si-ATF. $^{**}p < 0.01$ and $^{***}p < 0.001$. ATF2, activating transcription factor 2; WT, wild type; IgG, immunoglobulin G.

E-cadherin levels, along with the decrease in N-cadherin, Vimentin, and Snail levels, was reversed by si-ARRDC3 ($p < 0.05$, Fig. 4H,I and **Supplementary Fig. 2A,B**). A previous study have reported that *ARRDC3* inhibits the PI3K/AKT signaling pathway to regulate liver fibrosis [19]. Thus, we further evaluated the effect of the *ATF2*/*ARRDC3* axis on the activity of the PI3K/AKT pathway. The results indicated that *ATF2* overexpression reduced the protein expression of p-PI3K/PI3K and p-AKT/AKT, while *ARRDC3* knockdown effectively reversed these effects ($p < 0.05$) (**Supplementary Fig. 3A,B**). Overall, these findings indicate that *ATF2* promotes *ARRDC3* expression to repress trophoblast cell functions by inhibiting the PI3K/AKT signaling axis.

Discussion

Globally, PE accounts for about 60,000 maternal deaths and 500,000 preterm births annually [20]. Despite its significant clinical outcomes, the underlying mechanisms of PE remains unclear. Abnormal regulation of trophoblast cell function has been associated as a major contributor with PE development [21]. In this study, we explored the role of *ATF2*-mediated regulation of *ARRDC3* in trophoblast cell functions to provide valuable insights into PE pathogenesis.

Evidence suggests that *ARRDC3* plays a pivotal in regulating various diseases [22,23]. For instance, *ARRDC3* reduces liver fibrosis by alleviating liver injury and inhibiting the EMT process [19], whereas *ARRDC3* knockdown has been found to enhance the proliferation of lung cancer cells [24]. Lei *et al.* [12] reported that *ARRDC3* overexpression inhibits trophoblast cell invasion and tube for-

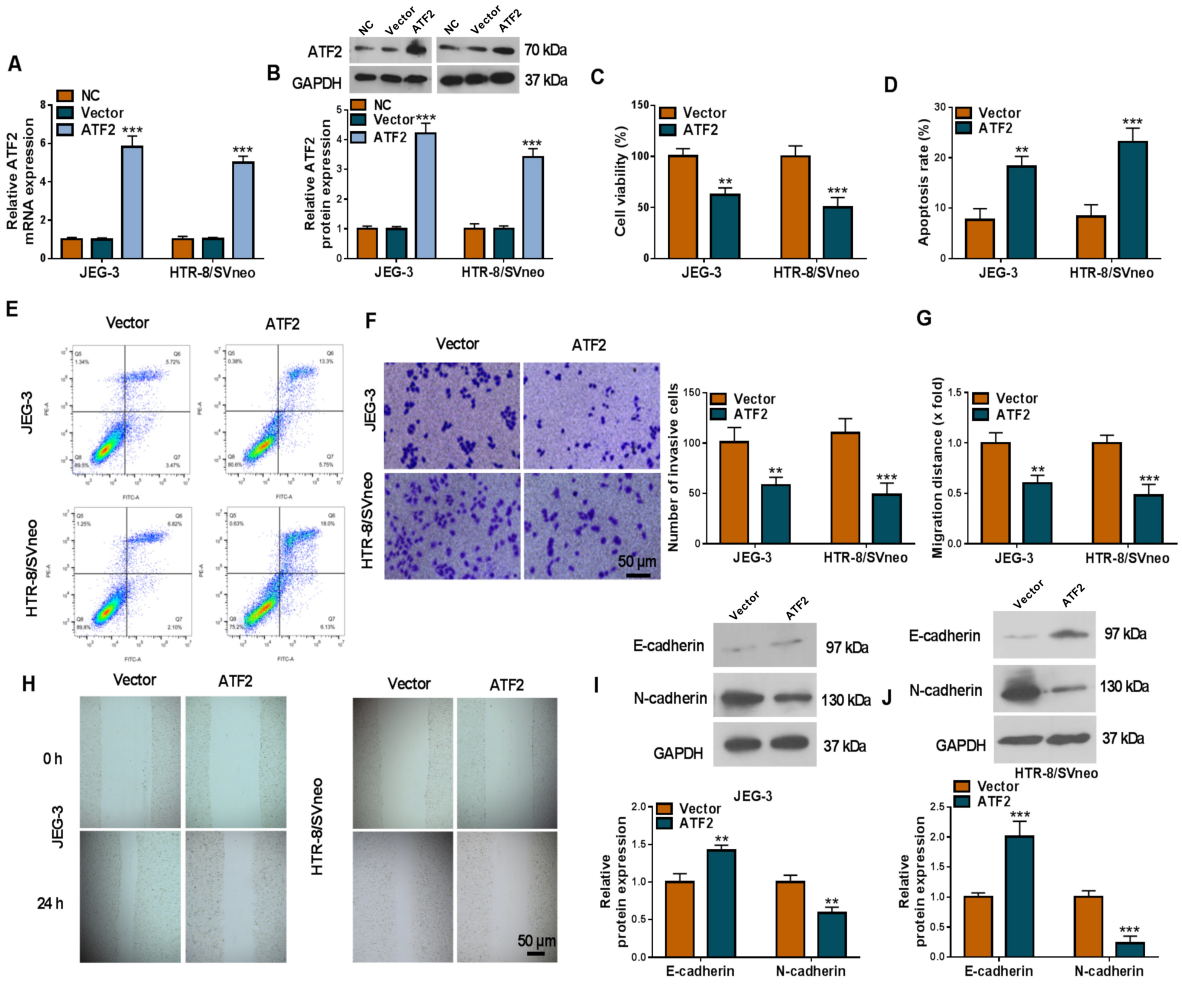

Fig. 3. Impact of ATF2 upregulation on trophoblast cell functions. JEG-3 and HTR-8/SVneo cells were transfected with a vector or an ATF2 overexpression vector. (A,B) ARRDC3 mRNA and protein expression levels were assessed using qRT-PCR and WB analysis in the NC group, the vector group, and the ATF2 overexpression group. CCK-8 assay (C) and flow cytometry (D,E) were used to determine cell viability and the apoptosis rate in the vector and ATF2 overexpression groups. Cell invasion and migration in the vector and the ATF2 overexpression groups were examined using the Transwell assay (F) and wound healing assay (G,H). (I,J) WB analysis assessed E-cadherin and N-cadherin protein levels in the vector and the ATF2 overexpression groups. $^{**}p < 0.01$ and $^{***}p < 0.001$.

mation. Moreover, *ARRDC3* has been observed to be upregulated in PE patients, whereas circ_0004904 suppresses trophoblast cell proliferation and invasion by increasing *ARRDC3* expression [25]. Additionally, the upregulation of hsa_circ_0001740 restrains trophoblast cell proliferation and invasion by sponging miR-188-3p, thereby promoting ARRDC3 levels [26]. Consistent with these findings [12,25,26], our results showed that *ARRDC3* upregulation substantially suppressed the viability, invasion, migration and EMT in trophoblast cells, suggesting its potential role in trophoblast cell dysfunctions and might be a promising therapeutic target for PE.

ATF2 is a transcription factor that is involved in disease onset and progression by regulating gene transcription. In hepatocellular carcinoma patients, *ATF2* is upregulated, enhancing cell proliferation and metastasis by mediating miR-548p expression [27]. In contrast, Qin *et al.* [28] reported that *ATF2* is downregulated in thyroid cancer, where it binds to the *GAS8-AS1* promoter to activate its expression, thereby accelerating autophagy in thyroid cancer cells. These observations suggest that ATF2 is differentially expressed in different tissues. In the context of PE progression, the role of *ATF2* has been explored in a previous study. It has been reported that low doses of aspirin may alleviate PE progression by reducing the expression of *ATF2* [18]. Similarly, Xia *et al.* [17] confirmed that *ATF2* contributes to trophoblast cell dysfunction. Our study revealed that *ATF2* increases *ARRDC3* levels by di-

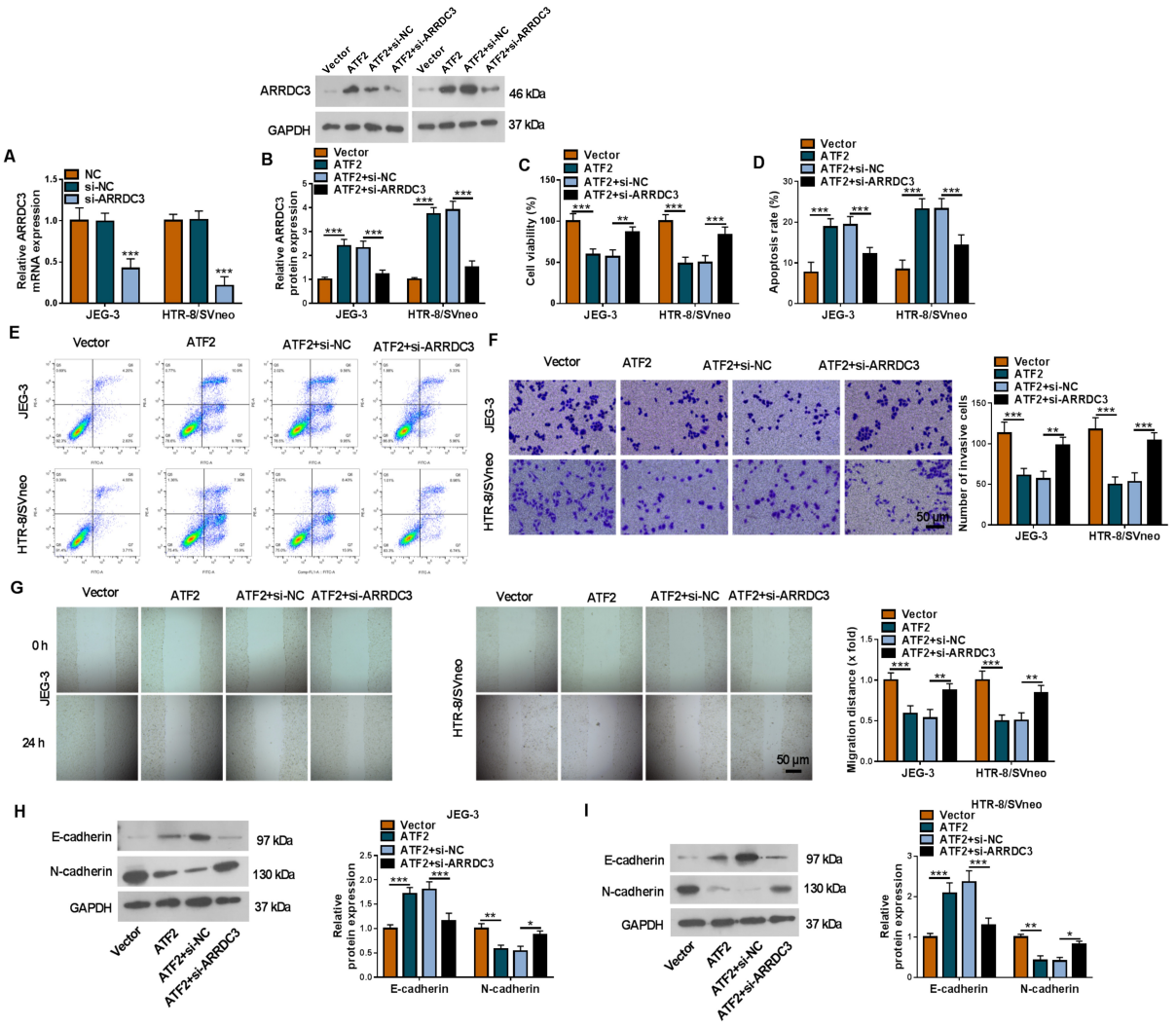

Fig. 4. Impact of the ATF2/ARRDC3 axis on trophoblast cell functions. JEG-3 and HTR-8/SVneo cells were co-transfected with vector, ATF2 overexpression vector, si-NC, and si-ARRDC3. (A,B) qRT-PCR and WB analysis were performed to test ARRDC3 mRNA and protein expression levels in the vector, ATF2 overexpression, ATF2+si-NC, and ATF2+si-ARRDC3 groups. Cell viability and the apoptosis rate in the vector, ATF2 overexpression, ATF2+si-NC, and ATF2+si-ARRDC3 groups were assessed using CCK-8 assay (C) and flow cytometry (D,E). Cell invasion and migration were examined in the vector, ATF2 overexpression, ATF2+si-NC, and ATF2+si-ARRDC3 groups using the Transwell assay (F) and wound healing assay (G). (H,I) WB analysis was used to determine E-cadherin and N-cadherin protein levels in the vector, ATF2 overexpression, ATF2+si-NC, and ATF2+si-ARRDC3 groups. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

rectly binding to its promoter. Furthermore, functional experiments demonstrated that *ATF2* suppresses trophoblast cell viability, invasion, migration, and EMT by upregulating *ARRDC3*. Consistent with previous studies [17,18], our results confirm the positive role of *ATF2* in promoting trophoblast dysfunction and propose novel mechanistic insights, indicating that *ATF2* accelerates PE progression through the ATF2/ARRDC3 axis.

Despite several valuable insights, we acknowledge some limitations to this study. Our results confirm that the *ATF2*/*ARRDC3* axis regulates the expression of p-PI3K/PI3K and p-AKT/AKT proteins, suggesting that the PI3K/AKT pathway is a critical downstream signaling of the *ATF2*/*ARRDC3* axis. However, future studies involving activators or inhibitors of the PI3K pathway are needed to confirm whether the *ATF2*/*ARRDC3* axis mediates PE progression by regulating the PI3K/AKT pathway. Furthermore, this study has only explored the effect of the *ATF2*/*ARRDC3* axis on trophoblast cells at the *in vitro* cellular level, without validating the findings in *in vivo* disease model. Future research should include the development of PE mouse models to determine the *in vivo* effects of the

ATF2/*ARRDC3* axis on PE progression. Additionally, although the human HTR-8/SVneo trophoblast cell lines and the human placental choriocarcinoma cell line (JEG-3) are commonly employed as model cells to reflect trophoblast function [29,30], these cells have inherent limitations. To address these limitations, future research should include primary trophoblast cells to more precisely determine the regulatory effects of the *ATF2*/*ARRDC3* axis on PE progression.

Conclusion

In conclusion, our findings revealed that *ATF2* binds to the promoter region of *ARRDC3*, upregulating its level, thereby inhibiting trophoblast cell viability, invasion, migration, and EMT. These findings provide new insights into the pathogenesis of PE accompanied by gestational hypertension.

Availability of Data and Materials

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

Author Contributions

Conceptualization, SXW, CZ and SSL; Data Curation, SXW, AWM, CZ, SSL and GXH; Formal Analysis, AWM, SSL and GXH; Investigation, SXW, AWM, and SSL; Methodology, SXW, AWM, CZ, SSL and GXH; Validation, SXW, CZ and SSL; Visualization, SXW, AWM, CZ, SSL and GXH. All authors were involved in the drafting and critical revision of the manuscript. All authors have 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

Each participant signed written informed consent, and this study was approved by the Ethics Committee of The No.4 People's Hospital of Hengshui (2022 No. 20). All procedures performed were in accordance with the Declaration of Helsinki.

Acknowledgment

Not applicable.

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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/10.24976/Discov.Med.202537200.157.

References

[1] ACOG Practice Bulletin No. 202: Gestational Hypertension and Preeclampsia. Obstetrics and Gynecology. 2019; 133: 1. https://doi.org/10.1097/AOG.0000000000003018.

[2] Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222. Obstetrics and Gynecology. 2020; 135: e237–e260. https://doi.org/10.1097/AOG.0000000000003891.

[3] Melchiorre K, Giorgione V, Thilaganathan B. The placenta and preeclampsia: villain or victim? American Journal of Obstetrics and Gynecology. 2022; 226: S954–S962. https://doi.org/10.1016/j.ajog.2020.10.024.

[4] Yang J, Wang Y, Chen X, Zhang H, Xue Y, Chen H. Trophoblast-derived proteins and their effects on the pathogenesis of preeclampsia. Histology and Histopathology. 2025; 18930. https://doi.org/10.14670/HH-18-930.

[5] Liu Y, Huang J, Yu N, Wei S, Liu Z. Involvement of WNT2 in trophoblast cell behavior in preeclampsia development. Cell Cycle (Georgetown, Tex.). 2020; 19: 2207–2215. https://doi.org/10.1080/15384101.2020.1802913.

[6] Xu Y, Sui L, Qiu B, Yin X, Liu J, Zhang X. ANXA4 promotes trophoblast invasion via the PI3K/Akt/eNOS pathway in preeclampsia. American Journal of Physiology. Cell Physiology. 2019; 316: C481–C491. https://doi.org/10.1152/ajpcell.00404.2018.

[7] Li Y, Wu H, Pei X, Liu S, Yan Q. Alpha 1,3 N-Acetylgalactosaminyl Transferase (GTA) Impairs Invasion Potential of Trophoblast Cells in Preeclampsia. International Journal of Molecular Sciences. 2024; 25: 7287. https://doi.org/10.3390/ijms25137287.

[8] Sun L, Shi M, Wang J, Han X, Wei J, Huang Z, *et al*. Overexpressed Trophoblast Glycoprotein Contributes to Preeclampsia Development by Inducing Abnormal Trophoblast Migration and Invasion Toward the Uterine Spiral Artery. Hypertension (Dallas, Tex.: 1979). 2024; 81: 1524–1536. https://doi.org/10.1161/HYPERTENSIONAHA.124.22923.

[9] Wedegaertner H, Pan WA, Gonzalez CC, Gonzalez DJ, Trejo J. The α-Arrestin ARRDC3 Is an Emerging Multifunctional Adaptor Protein in Cancer. Antioxidants & Redox Signaling. 2022; 36: 1066–1079. https://doi.org/10.1089/ars.2021.0193.

[10] Luo J, Tao H, Chen L, Hu H, Mao L, Guan H. LncRNA MEG3 suppresses prostate cancer progression by mediating macrophage polarization via the miR-148a-3p/ARRDC3 signaling axis. Carcinogenesis. 2025; 46: bgaf009. https://doi.org/10.1093/carcin/bgaf009.

[11] Liu YG, Teng YS, Shan ZG, Cheng P, Hao CJ, Lv YP, *et al*. Arrestin domain containing 3 promotes Helicobacter pylori-associated gastritis by regulating protease-activated receptor 1. JCI Insight. 2020; 5: e135849. https://doi.org/10.1172/jci.insight.135849.

[12] Lei D, Deng N, Wang S, Huang J, Fan C. Upregulated ARRDC3 limits trophoblast cell invasion and tube formation and is associated with preeclampsia. Placenta. 2020; 89: 10–19. https://doi.org/10.1016/j.placenta.2019.10.009.

[13] Giannoudis A, Malki MI, Rudraraju B, Mohhamed H, Menon S, Liloglou T, *et al*. Activating transcription factor-2 (ATF2) is a key determinant of resistance to endocrine treatment in an in vitro model of breast cancer. Breast Cancer Research: BCR. 2020; 22: 126. https://doi.org/10.1186/s13058-020-01359-7.

[14] Kirsch K, Zeke A, Tőke O, Sok P, Sethi A, Sebő A, *et al*. Co-regulation of the transcription controlling ATF2 phosphoswitch by JNK and p38. Nature Communications. 2020; 11: 5769. https://doi.org/10.1038/s41467-020-19582-3.

[15] Li JKH, Lai PF, Tribe RM, Johnson MR. Transcription factors regulated by cAMP in smooth muscle of the myometrium at human parturition. Biochemical Society Transactions. 2021; 49: 997–1011. https://doi.org/10.1042/BST20201173.

[16] Huebner K, Procházka J, Monteiro AC, Mahadevan V, Schneider-Stock R. The activating transcription factor 2: an influencer of cancer progression. Mutagenesis. 2019; 34: 375–389. https://doi.org/10.1093/mutage/gez041.

[17] Xia D, Zhang Y, Zhang C, Wang H. Regulatory role of ATF2 in trophoblast ferroptosis via the PI3K/Akt/Nrf2 pathway in gestational diabetes mellitus. Journal of Diabetes Investigation. 2025; 10.1111/jdi.70115. https://doi.org/10.1111/jdi.70115.

[18] Xiao S, Guo L, Zhang M, Hu R, Liu R. Low-dose Aspirin may Prevent Preeclampsia by Inhibiting the Expression of ATF2. Endocrine, Metabolic & Immune Disorders Drug Targets. 2023; 23: 702–710. https://doi.org/10.2174/1871530323666221103105349.

[19] Zhang B, Wu F, Li P, Li H. ARRDC3 inhibits liver fibrosis and epithelial-to-mesenchymal transition via the ITGB4/PI3K/Akt signaling pathway. Immunopharmacology and Immunotoxicology. 2023; 45: 160–171. https://doi.org/10.1080/08923973.2022.2128369.

[20] Ma'ayeh M, Costantine MM. Prevention of preeclampsia. Seminars in Fetal & Neonatal Medicine. 2020; 25: 101123. https://doi.org/10.1016/j.siny.2020.101123.

[21] Liu Z, Yu Y, Zhang X, Wang C, Pei J, Gu W. Transcriptomic profiling in hypoxia-induced trophoblast cells for preeclampsia. Placenta. 2023; 136: 8–17. https://doi.org/10.1016/j.placenta.2023.03.005.

[22] Chen M, Yin B, Liu Y, Li M, Shen S, Wu J, *et al*. ARRDC3 regulates the targeted therapy sensitivity of clear cell renal cell carcinoma by promoting AXL degradation. Cell Cycle (Georgetown, Tex.). 2024; 23: 56–69. https://doi.org/10.1080/15384101.2024.2308411.

[23] Zheng Y, Lin ZY, Xie JJ, Jiang FN, Chen CJ, Li JX, *et al*. ARRDC3 Inhibits the Progression of Human Prostate Cancer Through ARRDC3-ITGβ4 Pathway. Current Molecular Medicine. 2017; 17: 221–229. https://doi.org/10.2174/1566524017666170807144711.

[24] Tang Q, Wang X, Zhou Q, Li Q, Yang X, Xu M, *et al*. Fuzheng Kang-Ai inhibits NSCLC cell proliferation via regulating hsa_circ_0048091/hsa-miR-378g/ARRDC3 pathway. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology. 2023; 114: 154819. https://doi.org/10.1016/j.phymed.2023.154819.

[25] Cao C, Cui J, Liu G. circ_0004904 regulates the trophoblast cell in preeclampsia via miR-19b-3p/ARRDC3 axis. Open Medicine (Warsaw, Poland). 2023; 18: 20220546. https://doi.org/10.1515/med-2022-0546.

[26] Long M, Wang S. Hsa_circ_0001740 mediates trophoblast cell function via regulating miR-188-3p/ARRDC3. Molecular Reproduction and Development. 2023; 90: 406–416. https://doi.org/10.1002/mrd.23695.

[27] Li ZJ, Zhang JP, Li DY, Yang HY, Liu BR. ATF2 accelerates the invasion and metastasis of hepatocellular carcinoma through targeting the miR-548p/TUFT1 axis. Hepatology Research: the Official Journal of the Japan Society of Hepatology. 2022; 52: 281–297. https://doi.org/10.1111/hepr.13740.

[28] Qin Y, Sun W, Wang Z, Dong W, He L, Zhang T, *et al*. ATF2-Induced lncRNA GAS8-AS1 Promotes Autophagy of Thyroid Cancer Cells by Targeting the miR-187-3p/ATG5 and miR-1343-3p/ATG7 Axes. Molecular Therapy. Nucleic Acids. 2020; 22: 584–600. https://doi.org/10.1016/j.omtn.2020.09.022.

[29] Shen J, Hu N, Wang Z, Yang L, Chen R, Zhang L, *et al*. Ghrelin alleviates placental dysfunction by down-regulating NF-κB phosphorylation in LPS-induced rat model of preeclampsia. European Journal of Pharmacology. 2024; 972: 176569. https://doi.org/10.1016/j.ejphar.2024.176569.

[30] Lin J, Huang X, Zhang J, Yang W, Sun F, Huang B, *et al*. Amniotic fluid-derived exosomal miR-146a-5p ameliorates preeclampsia phenotypes by inhibiting HIF-1α/FLT-1 expression. Placenta. 2025; 162: 35–44. https://doi.org/10.1016/j.placenta.2025.02.013.