Article

Discovery Medicine 2025; 37(201): 2235–2243
https://doi.org/10.24976/Discov.Med.202537201.190

LASP2 Knockdown Inhibits High Glucose-Induced Suppression of Trophoblast Cell Proliferation and Migration During Gestational Diabetes Mellitus

Yan Huang[1], Jianmei Ling[1,*], Lingyu Zhang[1], Mingjin Zhang[1]

[1]Department of Gynaecology and Obstetrics, Affiliated Matern&Child Health Care Hospital of Nantong University, 226001 Nantong, Jiangsu, China
*Correspondence: jian_ml0513@163.com (Jianmei Ling)
Submitted: 24 April 2025 Revised: 4 July 2025 Accepted: 21 July 2025 Published: 20 October 2025

Background: Gestational diabetes mellitus (GDM), a common complication during pregnancy, is associated with impaired metabolism and endocrine dysfunction, thereby compromising maternal and fetal health. LIM and SH3 protein 2 (LASP2) has been reported to play regulatory roles in several diseases and has been found to be upregulated in the placental tissues of preeclampsia. However, the specific role of LASP2 and underlying signaling pathways in the progression of GDM largely remains uninvestigated. Therefore, this study aims to assess the impact of LASP2 on the high glucose-induced suppression of trophoblast cell proliferation and migration in GDM patients.

Methods: Placental tissue samples were obtained from women with normal pregnancies (n = 15) and those diagnosed with GDM (n = 15). The GDM cell model was successfully established by exposing HTR-8/SVneo cells to high glucose (30 mM). Furthermore, HTR-8/SVneo cells were transfected with siRNAs targeting LASP2 (si-LASP2#1 and si-LASP2#2), along with a negative control (si-NC). The expression levels of LASP2 were evaluated using RT-qPCR and Western blot analysis, and cell proliferation was determined using Cell Counting Kit-8 (CCK-8) and 5-ethynyl-2′-deoxyuridine (EdU) assays. Additionally, cell migratory capability was evaluated using wound healing and Transwell assays, and their angiogenic ability was assessed using the tube formation assay.

Results: LASP2 was upregulated in the placental tissues of patients with GDM ($p < 0.001$). Moreover, the cell viability of HTR-8/SVneo cells was significantly alleviated under high glucose conditions, an effect that was counteracted by LASP2 knockdown ($p < 0.05$). Similarly, cell migration ability was substantially suppressed under high glucose treatment; this impairment was effectively rescued by LASP2 silencing ($p < 0.01$). Additionally, angiogenic ability significantly decreased after high glucose treatment, an effect that was neutralized by LASP2 suppression ($p < 0.001$). LASP2 knockdown effectively provoked the Wingless-related integration site (Wnt)/β-catenin signaling pathway.

Conclusion: LASP2 knockdown effectively reverses high glucose-induced suppression of proliferation and migration in trophoblast cells by modulating the Wnt/β-catenin signaling pathway, providing novel insights into the role of LASP2 in GDM.

Keywords: LASP2; gestational diabetes mellitus; trophoblast cells; cell migration; Wnt/β-catenin pathway

Introduction

Gestational diabetes mellitus (GDM) is an abnormal glucose tolerance that is first recognized during pregnancy, in women who had normal glucose metabolism before conception [1]. It is one of the prevalent pregnancy-related complications, and its incidence has significantly increased due to changes in dietary habits, lifestyles, and the increasing maternal age [2]. The placenta plays a vital role during pregnancy, providing oxygen and essential nutrients to the fetus and facilitating the removal of metabolic waste through maternal circulation [3]. The proliferation and migration of trophoblast cells can offer a biological basis for the development of the placenta [4]. However, high glucose levels can disrupt the viability and migration of trophoblast cells, thereby resulting in adverse pregnancy outcomes [5]. Therefore, identifying critical molecular targets that can modulate the viability and migration of trophoblast cells is crucial for improving GDM outcomes.

LIM and SH3 protein 2 (LASP2), a member of the nebulin family, is regarded as an isoform of the nebulin gene [6]. LASP2 has been reported to play a regulatory role in diverse diseases. For example, its expression is decreased in liver cancer, where it modulates the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway to suppress pernicious phenotypes [7]. In pancreatic cancer, LASP2 restrains transforming growth factor-beta (TGF-β)-triggered epithelial-mesenchymal transition (EMT), thereby alleviating cell migration and invasion [8]. Additionally, LASP2 modulates the c-Jun N-terminal kinase (JNK)/p38 MAPK pathway to delay colorectal cancer progression [9], and has been shown

to prevent tumorigenesis in bladder cancer [10]. Importantly, LASP2 is upregulated in placental tissues from patients with preeclampsia and has been linked to reduced cell migration and invasion [11]. However, the specific function of LASP2 on the progression of GDM remains unexplored.

In this study, we observed that silencing LASP2 reversed high glucose-triggered inhibition of trophoblast cell proliferation and migration in GDM by modulating the Wingless-related integration site (Wnt)/β-catenin pathway. The findings suggest that LASP2 may serve as a potential biomarker and therapeutic target for managing GDM.

Materials and Methods

Tissue Samples

Placental tissues were obtained from women with normal pregnancies (n = 15) and those diagnosed with GDM (n = 15). GDM patients were assessed following the diagnostic criteria established by the International Association of Diabetes and Pregnancy Study Groups (IADPSG). This study was approved by the ethics committee of the Affiliated Matern&Child Care Hospital of Nantong University (Approval No. Y2020037). Informed consent was obtained from all participants in the study.

Human Cell Line Treatment and Establishment of the GDM Model

The human trophoblast cell line (HTR8/SVneo) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) containing 10% FBS (Gibco, Waltham, Massachusetts, USA) in a humidified incubator at 37 °C with 5% CO_2. Cells have been authenticated by short tandem repeat (STR) profiling and tested for mycoplasma contamination. To establish a GDM cell model, HTR8/SVneo cells were treated with high glucose (30 mM) [12], while control cells were exposed to 5.5 mM glucose. Additionally, XAV939 (10 µM), a selective inhibitor of the Wnt/β-catenin pathway, was also used to treat cells [13].

Transfection

The siRNAs targeting LASP2 (si-LASP2#1 and si-LASP2#2), along with a negative control (si-NC), were synthesized. To overexpress LASP2, the full-length of LASP2 was cloned into pcDNA3.1 plasmids (pcDNA3.1/LASP2). All plasmids were purchased from GenePharma (Shanghai, China). HTR8/SVneo cells were transfected using Lipofectamine 2000 (Invitrogen, Waltham, MA, USA). The siRNA sequences used in this study were as follows:

Si-NC sequences: 5′-UUUAUAGGCAGCAUCGCUGAC-3′; 5′-CAAUGCAGCAUUCACCAAATT-3′;

Si-LASP2#1 sequences: 5′-GUCCUAUGCUAAACCAUGUTT-3′; 5′-ACAUGGUUUAGCAAUGGACTT-3′;

Si-LASP2#2 sequences: 5′-CATTCCCAAGGCTATGGCTA-3′; 5′-ATCGTACATGGCTCGGTAGG-3′.

The pcDNA3.1/LASP2 construct was based on the NCBI reference sequence NM_001031692.3, including 1203 bp from the start codon (ATG) to the stop codon (TGA), encoding a protein of 400 amino acids.

RT-qPCR

Total RNA was isolated from placental tissues using the TRIzol reagent (Invitrogen, Waltham, MA, USA) and subsequently converted into cDNA using the PrimeScript™ RT Master Mix kit (Takara, Dalian, China). Quantitative real-time PCR (qRT-PCR) was performed using the SYBR Premix Ex Taq™ (Takara, Dalian, China). The relative mRNA expression levels of LASP2 were assessed using the $2^{-\Delta\Delta Ct}$ method.

The primer sequences used in qRT-PCR were as follows:

LASP2 forward, 5′-CATTCCCAAGGCTATGGCTA-3′, and reverse, 5′-ATCGTACATGGCTCGGTAGG-3′; GAPDH forward, 5′-GACAGTCAGCCGCATCTTCT-3′, and reverse, 5′-GCGCCCAATACGACCAAATC-3′.

Western Blot Analysis

Proteins isolated from placental tissues or HTR8/SVneo cells were resolved using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were then transferred onto PVDF membranes (Beyotime, Shanghai, China). After blocking, the membranes underwent a 12-hour incubation at room temperature with primary antibodies, including LASP2 (1/1000; ab199947; Abcam, Shanghai, China), vascular endothelial growth factor A (VEGFA) (1/1000; ab52917), β-catenin (1/1000; ab16051), c-myc (1/1000; ab32072), and β-actin (1/1000; ab8227). After that, the membranes were incubated with a preadsorbed HRP-conjugated goat anti-rabbit immunoglobulin G (IgG) secondary antibody (1/2000; ab7090) for 2 hours. Finally, protein blots were visualized using a chemiluminescence detection kit (Thermo Fisher Scientific, Inc.).

Cell Counting Kit-8 (CCK-8) Assay

HTR8/SVneo cells were seeded in 96-well plates at a density of 10,000 cells/well. After 24 hours of incubation, 10 µL of CCK-8 solution (Dojindo Laboratories, Kumamoto, Japan) was added to each well, followed by an additional 2-hour incubation. Cell viability was examined at 450 nm using a spectrophotometer (Thermo Fisher Scientific, MA, USA).

5-ethynyl-2′-deoxyuridine (EdU) Assay

The Cell-Light EdU DNA Cell Proliferation Kit (RiboBio, Guangzhou, China) was used to determine cell proliferation. HTR8/SVneo cells were incubated with 50 µM EdU for 2 hours. The cells were then fixed with paraformaldehyde and subsequently permeabilized with

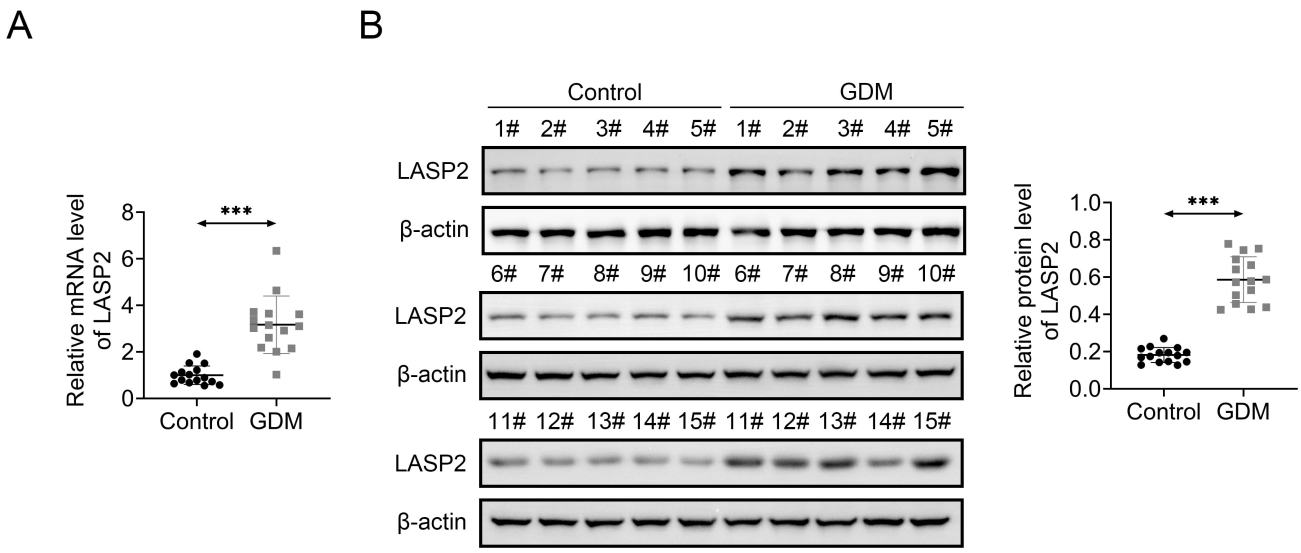

Fig. 1. LIM and SH3 protein 2 (LASP2) expression is upregulated in the placental tissues of gestational diabetes mellitus (GDM). (A) The mRNA expression of LASP2 was compared between the Control (n = 15) and GDM (n =15) groups using RT-qPCR. (B) The protein expression levels of LASP2 were compared between the Control (n = 5) and GDM (n = 5) groups using Western blot analysis. ***$p < 0.001$.

Table 1. Comparison of maternal characteristics between the two study groups.

	Control group (n = 15)	GDM group (n = 15)	*p*-value
Age (years)	31.0 ± 2.83	31.2 ± 2.98	0.8518
Gestational age at delivery (weeks)	38.07 ± 2.94	38.6 ± 4.19	0.6895
BMI (kg/m^2)	28.91 ± 2.71	31.58 ± 2.93	0.0152
OGTT fasting glucose	4.21 ± 0.41	4.91 ± 0.45	<0.0001
OGTT1-h glucose	6.93 ± 0.50	10.61 ± 1.07	<0.0001
OGTT2-h glucose	6.30 ± 0.59	8.18 ± 0.63	<0.0001
HbA1C	4.93 ± 0.54	5.62 ± 0.47	0.0009
Birth weight (g)	3341 ± 261.34	3529 ± 200.81	0.0347

BMI, body mass index; OGTT, oral glucose tolerance test; HbA1C, Hemoglobin A1c.

Triton-X-100. After that, HTR8/SVneo cells were stained with Apollo dye solution and 4′,6-diamidi-diamidino-2-phenylindole (DAPI). Fluorescence microscopy (Leica, Hilden, Germany) was applied to visualize the cells, and the number of EdU and DAPI-positive cells was quantified using Image J software. The percentage of EdU-positive cells was determined using the following formula: EdU (%) = (EdU-positive cells / DAPI-positive cells) × 100%.

Wound Healing Assay

HTR8/SVneo cells were seeded in 6-well plates. After achieving the required confluence, a uniform scratch was made across the cell monolayer using a sterile pipette tip (10 µL) to form a wound. Culture plates were washed to eliminate detached cells and incubated under standard conditions for 24 hours. Images of the scratched area were captured at 0 and 24-hour time points using an inverted microscope. The migration rate (%) was calculated as follows: Migration ratio (%) = (Initial width-final width/Initial width) × 100%.

Transwell Assay

HTR8/SVneo cells suspended in serum-free medium (200 µL) were added to the upper chamber of Transwell inserts (8 µM; Corning, NY, USA). The lower chamber was filled with 600 µL of medium supplemented with 20% FBS. The media in the lower chamber serves as a chemoattractant. After 24 hours of incubation, cells that had migrated to the lower surface were immobilized (fixed) with paraformaldehyde and stained with crystal violet. Finally, migrated cells were visualized and counted under a microscope (Olympus Optical Co., Ltd., Tokyo, Japan) to determine the cell migration capacity.

Tube Formation Assay

Human umbilical vein endothelial cells (HUVEC) (10,000/well) were seeded into 24-well plates pre-coated with 20 µL Matrigel (BD Biosciences, Bedford, MA, USA) and cultured with the conditioned medium for 8 hours. The conditioned medium was collected from HTR8/SVneo cell

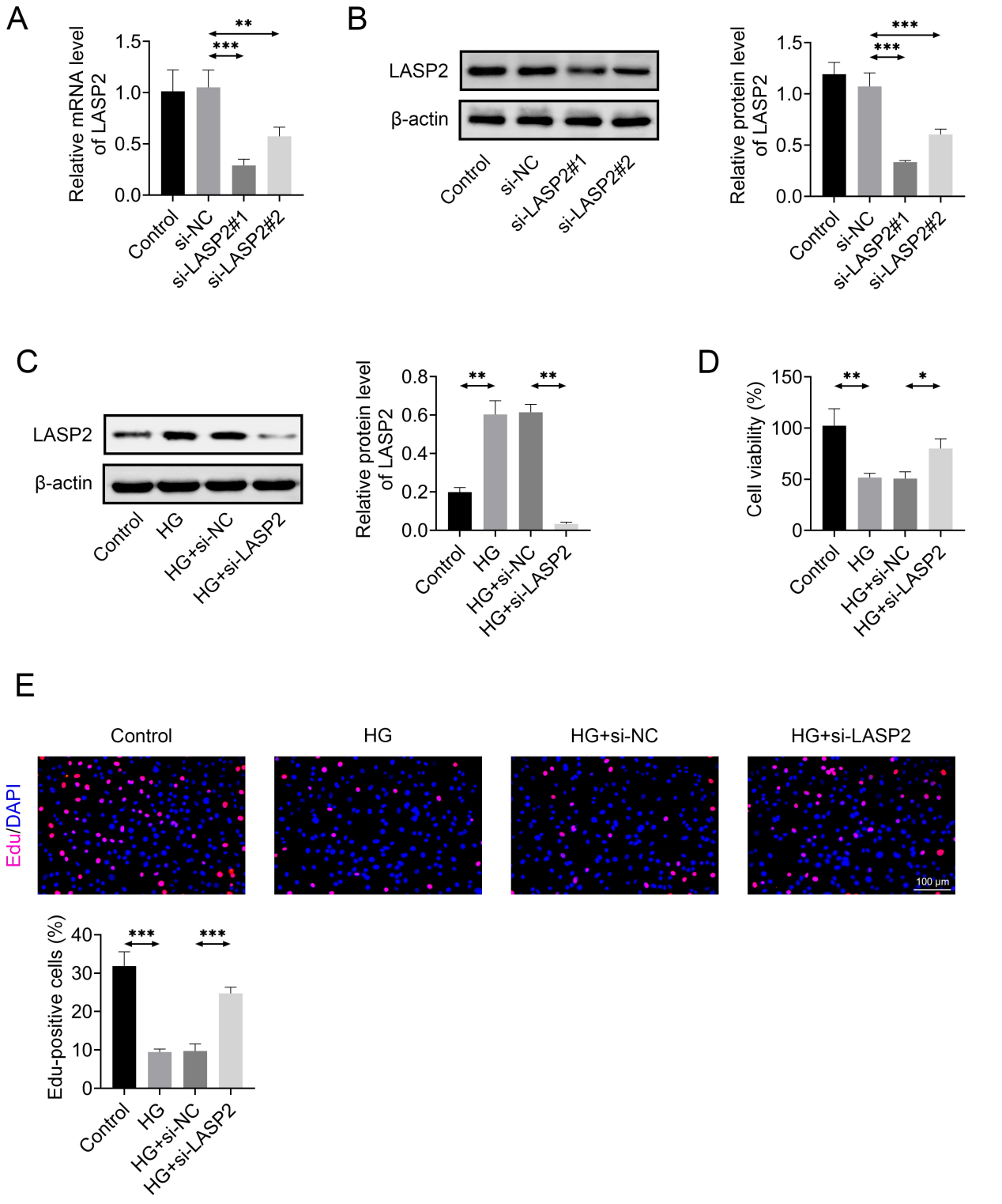

Fig. 2. LASP2 knockdown enhances cell viability in high glucose (HG)-treated HTR-8/SVneo cells. (A) The mRNA expression of LASP2 was confirmed through RT-qPCR in the Control, si-NC, si-LASP2#1 and si-LASP2#2 groups. (B) The protein expression was examined through western blot in the Control, si-NC, si-LASP2#1 and si-LASP2#2 groups. (C) The protein expression of LASP2 was examined through western blot in the Control, HG, HG+si-NC and HG+si-LASP2 groups. (D) The cell viability was tested through Cell Counting Kit-8 (CCK-8) assay in the Control, HG, HG+si-NC and HG+si-LASP2 groups. (E) The cell proliferation was determined through 5-ethynyl-2′-deoxyuridine (EdU) assay in the Control, HG, HG+si-NC and HG+si-LASP2 groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

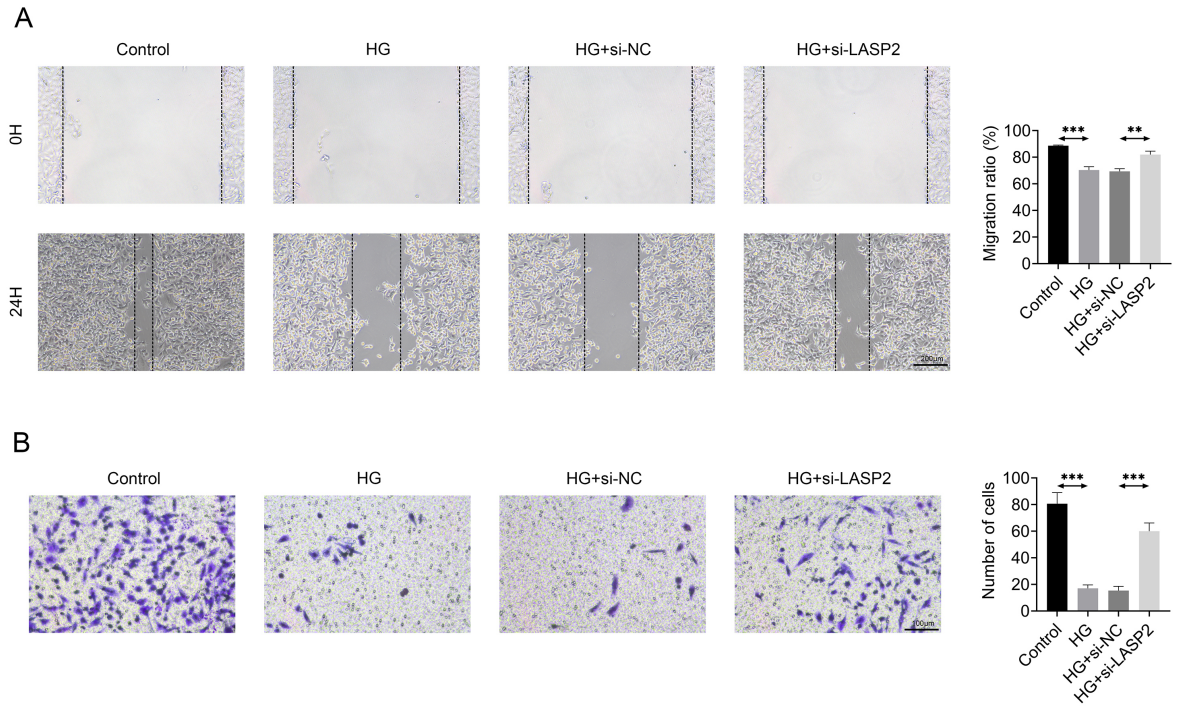

Fig. 3. LASP2 knockdown increases cell migration in HG-stimulated HTR-8/SVneo cells. Cells were designated into the Control, HG, HG+si-NC, and HG+si-LASP2 groups. (A) The migratory ability of the cell was examined using a wound healing assay. (B) The cell migration was assessed using the Transwell assay. $^{**}p < 0.01$, $^{***}p < 0.001$.

cultures corresponding to the Control, HG, HG+si-NC, and HG+si-LASP2 groups, after removing the cells by centrifugation. Eventually, tube formation by HUVECs was determined under an inverted microscope (Olympus Optical Co., Ltd., Tokyo, Japan).

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 9.0 (GraphPad Software, La Jolla, CA, USA). All data were expressed as the mean ± standard deviation (SD). Comparison between two groups was performed using Student's *t*-test, while multiple-group comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A *p*-value < 0.05 was regarded as statistically significant.

Results

LASP2 Expression Is Upregulated in the Placental Tissues of GDM Patients

Initially, LASP2 mRNA expression was significantly elevated in the GDM group compared with the Control group (Fig. 1A, $p < 0.001$). Similarly, LASP2 protein expression levels were substantially higher in the GDM group (Fig. 1B, $p < 0.001$). Maternal characteristics are shown in Table 1. These findings indicate that LASP2 is upregulated in the placental tissues of GDM patients.

Knockdown of LASP2 Enhances Cell Viability in HTR-8/SVneo Cells Under High Glucose Conditions

The mRNA expression of LASP2 was markedly decreased in the si-LASP2#1 or si-LASP2#2 group (Fig. 2A, $p < 0.01$). And, the protein expression of LASP2 was also lessened after LASP2 knockdown (Fig. 2B, $p < 0.001$). The LASP2 protein expression was strengthened after HG treatment, but this impact was attenuated after LASP2 inhibition (Fig. 2C, $p < 0.01$). Additionally, the cell viability was alleviated in HG-evoked HTR-8/SVneo cells, but this change was offset after LASP2 knockdown (Fig. 2D, $p < 0.05$). Besides, cell proliferation was also receded after HG stimulation, but this effect was reversed after LASP2 suppression (Fig. 2E, $p < 0.05$). These observations suggest that LASP2 knock down enhances cell viability in HG-treated HTR-8/SVneo cells.

LASP2 Silencing Elevates Cell Migration in HG-Stimulated HTR-8/SVneo Cells

The migration ratio (%) was substantially decreased after HG treatment; however, this impact was counteracted by LASP2 knockdown (Fig. 3A, $p < 0.01$). Moreover, the Transwell assay revealed that high glucose treatment disrupted the migratory capability of HTR-8/SVneo cells; however, this impact was effectively rescued after LASP2 silencing (Fig. 3B, $p < 0.001$). Overall, these observations suggest that LASP2 knockdown enhances cell migration in HG-stimulated HTR-8/SVneo cells.

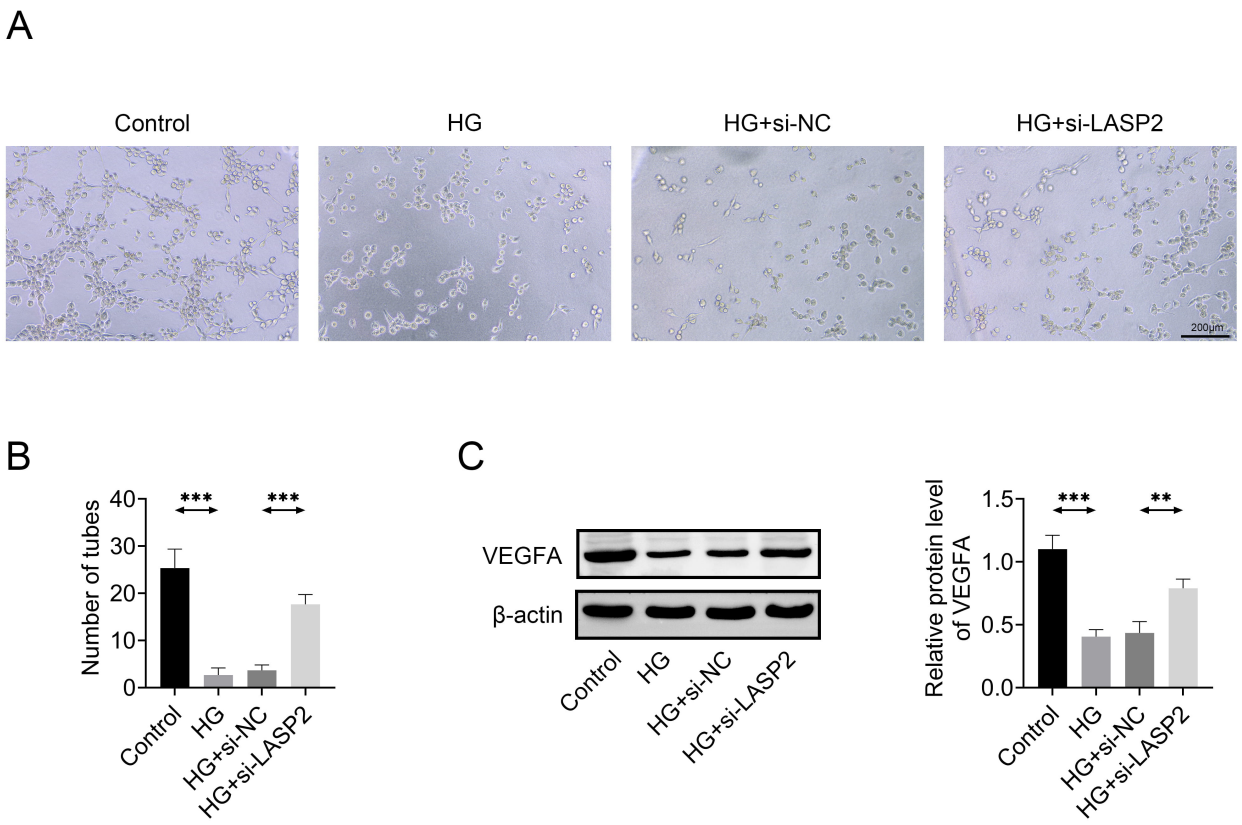

Fig. 4. LASP2 knockdown improves angiogenesis under high glucose conditions. Cells were designated into the Control, HG, HG+si-NC, and HG+si-LASP2 groups. (A,B) The angiogenesis ability of HUVECs was evaluated using the tube formation assay. (C) VEGFA protein expression was determined using Western blot analysis. $^{**}p < 0.01$, $^{***}p < 0.001$.

Suppression of LASP2 Improves Angiogenesis Under High Glucose Conditions

The angiogenic ability of HUVECs was substantially decreased after high glucose stimulation; however, this effect was counteracted by LASP2 silencing (Fig. 4A,B, $p < 0.001$). Additionally, VEGFA protein expression, which was reduced under high glucose conditions, was reversed upon LASP2 downregulation (Fig. 4C, $p < 0.01$). In summary, suppression of LASP2 promotes angiogenesis under high glucose conditions.

LASP2 Knockdown Provokes the Wnt/β-Catenin Signaling Pathway

The protein expression levels of β-catenin and c-myc were significantly downregulated after HG induction, but this effect was reversed upon LASP2 silencing (Fig. 5A, $p < 0.05$). Similarly, Wnt3a and Cyclin D1 protein expression were significantly reduced under high glucose conditions, which were restored after LASP2 suppression (Fig. 5B, $p < 0.01$). The efficiency of LASP2 overexpression was confirmed in Fig. 5C ($p < 0.001$). Furthermore, high glucose-induced decrease in cell viability was further exacerbated by LASP2 overexpression, whereas LASP2 knockdown reversed this effect. Notably, the improvement in cell viability observed in the HG+si-LASP2 group was attenuated upon XAV939 (a Wnt pathway inhibitor) treatment (Fig. 5D, $p < 0.05$). Similarly, the decreased migration ratio mediated by HG treatment was further reduced by LASP2 overexpression and rescued by LASP2 knockdown; however, the migration ratio was attenuated again in the HG+si-LASP2 group upon XAV939 treatment (Fig. 5E, $p < 0.05$). Overall, these findings indicate that LASP2 silencing activates the Wnt/β-catenin pathway.

Discussion

LASP2 has been reported to play regulatory roles in diverse diseases [7–10] and has been found to be upregulated in the placental tissues of patients with preeclampsia [11]. However, its specific role and correlated signaling pathways in the progression of GDM remain unexplored. In this study, LASP2 expression was significantly upregulated in the placental tissues obtained from GDM patients. Furthermore, high glucose treatment substantially alleviated cell viability in HTR-8/SVneo cells, an effect that was counteracted by LASP2 knockdown. Similarly, high glucose-disrupted cell migration was effectively reversed upon LASP2 silencing, indicating its potential regulatory role in GDM progression.

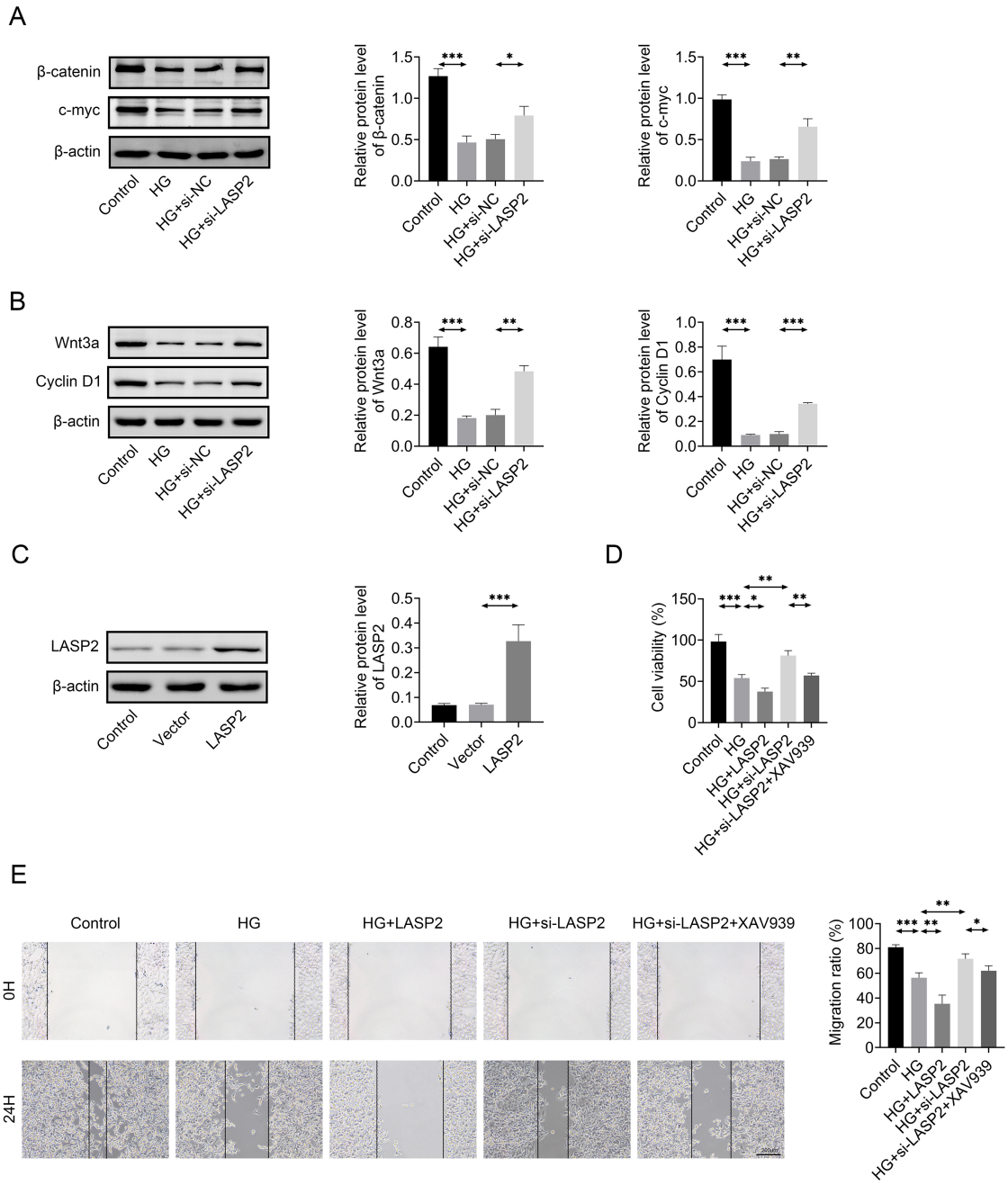

Fig. 5. LASP2 suppression provokes the Wingless-related integration site (Wnt)/β-catenin signaling pathway. Cells were designated into the Control, HG, HG+LASP2, HG+si-LASP2, and HG+si-LASP2+XAV939 groups. (A) β-catenin and c-myc protein expression levels were evaluated using Western blot analysis. (B) Wnt3a and Cyclin D1 protein expression levels were assessed using Western blot analysis. (C) The efficiency of LASP2 overexpression was examined using Western blot analysis. (D) The cell viability was assessed using the CCK-8 assay. (E) The cell migration capability was examined using wound healing assay. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Angiogenesis plays a pivotal role in the progression of GDM and increasing research efforts have focused on modulating the angiogenesis pathway to improve disease outcomes. For instance, lower Unc-5 Netrin Receptor B (UNC5b) expression has been reported to enhance placental angiogenesis during GDM progression [14]. Similarly, miR-195-5p targets VEGFA, aggravating endothelial dysfunction in GDM [15]. Furthermore, homeobox C8 (HOXC8) has been shown to stimulate TGFβ1-mediated Notch1 pathway to mitigate HG-induced

cell damage in GDM [16]. Furthermore, the exosomal circ0074673/miRNA-1200/mesenchyme homeobox 2 (MEOX2) axis has been reported to suppress the proliferation and angiogenesis capacity in HG-mediated HUVECs [17]. Consistent with these results, our study demonstrated that high glucose conditions impaired angiogenesis, an effect that was effectively counteracted by LASP2 suppression.

The Wnt/β-catenin signaling pathway plays a crucial role in embryonic and organ development by modulating key cellular processes, including proliferation, differentiation, polarization, and migration [18]. Furthermore, this pathway regulates trophoblast cell function. For example, secreted frizzled-related protein 2 (SFRP2) modulates the Wnt/β-catenin pathway to inhibit trophoblast cell migration [19], while low-density lipoprotein receptor-related protein 6 (LRP6) activates the Wnt/β-catenin pathway to enhance autophagy, thereby promoting trophoblast cell migration and invasion [20]. In fetal growth restriction, IL-27 knockdown restrains trophoblast cell proliferation and invasion by targeting the Wnt/β-catenin pathway [21]. Additionally, mucin1 has been reported to regulate the Wnt/β-catenin pathway, leading to trophoblast dysfunction in GDM [22]. Interestingly, LASP2 has been implicated in modulating the Wnt/β-catenin pathway in both bladder cancer and preeclampsia [10,11]. However, LASP2's role in regulating the Wnt/β-catenin signaling pathway during GDM progression remains unexplored. In our study, we observed that LASP2 inhibition provokes the Wnt/β-catenin pathway, indicating a potential mechanism by which LASP2 modulates GDM pathogenesis.

Despite promising outcomes, this study has several limitations. Limitations include the lack of dose-response analysis for HG treatment, the lack of validation in animal models, limited investigation of additional associated pathways and cellular phenotypes, and the use of a single cell line without confirmation in other trophoblast models. Additionally, clinical investigations are required to support and validate the *in vitro* findings.

Conclusion

This study is the first to demonstrate that LASP2 knockdown reverses high glucose-triggered suppression of trophoblast cell proliferation and migration in GDM by modulating the Wnt/β-catenin pathway. These observations reveal the previously unrecognized role of LASP2 in GDM pathogenesis, as well as establishing a functional correlation between its expression levels and impaired placental cell characteristics under diabetic conditions. Overall, these findings provide novel insights into the role of LASP2 in GDM and help in devising molecularly targeted approaches to enhance both maternal and fetal health in these patients.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Author Contributions

All authors contributed to the study conception and design. Material preparation and the experiments were performed by YH. Data collection and analysis were performed by JL and LZ. The first draft of the manuscript was written by MZ and all authors critically revised the manuscript for important intellectual content. 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 was obtained from the Ethics Committee of Affiliated Matern&Child Care Hospital of Nantong University (Approval No. Y2020037). Written informed consent was obtained from a legally authorized representative for anonymized patient information to be published in this article. This study adhered to the Declaration of Helsinki.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Szmuilowicz ED, Josefson JL, Metzger BE. Gestational Diabetes Mellitus. Endocrinology and Metabolism Clinics of North America. 2019; 48: 479–493. https://doi.org/10.1016/j.ecl.2019.05.001.

[2] Lende M, Rijhsinghani A. Gestational Diabetes: Overview with Emphasis on Medical Management. International Journal of Environmental Research and Public Health. 2020; 17: 9573. https://doi.org/10.3390/ijerph17249573.

[3] Franzago M, Fraticelli F, Stuppia L, Vitacolonna E. Nutrigenetics, epigenetics and gestational diabetes: consequences in mother and child. Epigenetics. 2019; 14: 215–235. https://doi.org/10.1080/15592294.2019.1582277.

[4] Sheridan MA, Fernando RC, Gardner L, Hollinshead MS, Burton GJ, Moffett A, *et al*. Establishment and differentiation of long-term trophoblast organoid cultures from the human pla-

centa. Nature Protocols. 2020; 15: 3441–3463. https://doi.org/10.1038/s41596-020-0381-x.

[5] Jansson T, Myatt L, Powell TL. The role of trophoblast nutrient and ion transporters in the development of pregnancy complications and adult disease. Current Vascular Pharmacology. 2009; 7: 521–533. https://doi.org/10.2174/157016109789043982.

[6] Pappas CT, Bliss KT, Zieseniss A, Gregorio CC. The Nebulin family: an actin support group. Trends in Cell Biology. 2011; 21: 29–37. https://doi.org/10.1016/j.tcb.2010.09.005.

[7] Li J, Hu S, Zhang Z, Qian L, Xue Q, Qu X. LASP2 is down-regulated in human liver cancer and contributes to hepatoblastoma cell malignant phenotypes through MAPK/ERK pathway. Biomedicine & Pharmacotherapy. 2020; 127: 110154. https://doi.org/10.1016/j.biopha.2020.110154.

[8] Zhang Y, Li JH, Yuan QG, Cao G, Yang WB. Upregulation of LASP2 inhibits pancreatic cancer cell migration and invasion through suppressing TGF-β-induced EMT. Journal of Cellular Biochemistry. 2019; 120: 13651–13657. https://doi.org/10.1002/jcb.28638.

[9] Wang B, Zhang L, Zhao L, Zhou R, Ding Y, Li G, *et al*. LASP2 suppresses colorectal cancer progression through JNK/p38 MAPK pathway meditated epithelial-mesenchymal transition. Cell Communication and Signaling. 2017; 15: 21. https://doi.org/10.1186/s12964-017-0179-9.

[10] Yang R, Liao Z, Cai Y, Kong J. LASP2 suppressed malignancy and Wnt/β-catenin signaling pathway activation in bladder cancer. Experimental and Therapeutic Medicine. 2018; 16: 5215–5223. https://doi.org/10.3892/etm.2018.6836.

[11] Chen L, Wang J, Fan X, Zhang Y, Zhoua M, Li X, *et al*. LASP2 inhibits trophoblast cell migration and invasion in preeclampsia through inactivation of the Wnt/β-catenin signaling pathway. Journal of Receptor and Signal Transduction Research. 2021; 41: 67–73. https://doi.org/10.1080/10799893.2020.1787444.

[12] Zhang J, Bai WP. C1q/tumor necrosis factor related protein 6 (CTRP6) regulates the phenotypes of high glucose-induced gestational trophoblast cells via peroxisome proliferator-activated receptor gamma (PPARγ) signaling. Bioengineered. 2022; 13: 206–216. https://doi.org/10.1080/21655979.2021.2012906.

[13] Yang B, Chen W, Tao T, Zhang J, Kong D, Hao J, *et al*. UBE2N promotes cell viability and glycolysis by promoting Axin1 ubiquitination in prostate cancer cells. Biology Direct. 2024; 19: 35. https://doi.org/10.1186/s13062-024-00469-y.

[14] Prieto CP, Casas BS, Falcón P, Villanueva A, Lois P, Lattus J, *et al*. Downregulation of the Netrin-1 Receptor UNC5b Underlies Increased Placental Angiogenesis in Human Gestational Diabetes Mellitus. International Journal of Molecular Sciences. 2019; 20: 1408. https://doi.org/10.3390/ijms20061408.

[15] Zheng H, Yu Z, Wang H, Liu H, Chen X. MicroRNA-195-5p facilitates endothelial dysfunction by inhibiting vascular endothelial growth factor A in gestational diabetes mellitus. Reproductive Biology. 2022; 22: 100605. https://doi.org/10.1016/j.repbio.2022.100605.

[16] Xu D, Gao C, Cao Y, Xiao B. HOXC8 alleviates high glucose-triggered damage of trophoblast cells during gestational diabetes mellitus via activating TGFβ1-mediated Notch1 pathway. Human Cell. 2023; 36: 195–208. https://doi.org/10.1007/s13577-022-00816-z.

[17] Huang Y, Liang B, Chen X. Exosomal circular RNA circ_0074673 regulates the proliferation, migration, and angiogenesis of human umbilical vein endothelial cells via the microRNA-1200/MEOX2 axis. Bioengineered. 2021; 12: 6782–6792. https://doi.org/10.1080/21655979.2021.1967077.

[18] van der Horst PH, Wang Y, van der Zee M, Burger CW, Blok LJ. Interaction between sex hormones and WNT/β-catenin signal transduction in endometrial physiology and disease. Molecular and Cellular Endocrinology. 2012; 358: 176–184. https://doi.org/10.1016/j.mce.2011.06.010.

[19] Lan R, Yu Y, Song J, Xue M, Gong H. SFRP2 suppresses trophoblast cell migration by inhibiting the Wnt/β catenin pathway. Molecular Medicine Reports. 2024; 29: 66. https://doi.org/10.3892/mmr.2024.13190.

[20] Li L, Peng W, Zhou Q, Wan JP, Wang XT, Qi HB. LRP6 regulates Rab7-mediated autophagy through the Wnt/β-catenin pathway to modulate trophoblast cell migration and invasion. Journal of Cellular Biochemistry. 2020; 121: 1599–1609. https://doi.org/10.1002/jcb.29394.

[21] Zhang XY, Qin XY, Shen HH, Liu KT, Wang CJ, Peng T, *et al*. IL-27 deficiency inhibits proliferation and invasion of trophoblasts via the SFRP2/Wnt/β-catenin pathway in fetal growth restriction. International Journal of Medical Sciences. 2023; 20: 392–405. https://doi.org/10.7150/ijms.80684.

[22] Cui SS, Zhang P, Sun L, Yuan YLL, Wang J, Zhang FX, *et al*. Mucin1 induced trophoblast dysfunction in gestational diabetes mellitus via Wnt/β-catenin pathway. Biological Research. 2023; 56: 48. https://doi.org/10.1186/s40659-023-00460-3.