ORIGINAL RESEARCH

Effect of PLGF and EZH2 Expression in Placenta Tissue on GDM Placental Structure and Pregnancy Outcome

Jianfen Hong, BM; Yifeng Chen, BM

ABSTRACT

Objective • To explore the relationship between the expression of placental growth factor (PLGF) and Zeste homolog enhancer 2 (EZH2) in placental tissues of women with gestational diabetes (GDM), placental function, and pregnancy outcome.

Methods • Select 100 women with GDM diagnosed in our hospital from January 2019 to May 2020 as the GDM group and 100 women with normal pregnancy at the same time as the control group. Detection and analysis of the expression levels of PLGF and EZH2 proteins in placental tissue after delivery of the two components. Observation of the expression of different PLGF and EZH2 proteins using an electron microscope, and analyze the ultrastructural changes in placental tissue of women with GDM. Finally, assess the differences in pregnancy outcomes.

Results • The expression intensity of PLGF protein in the GDM group was higher than that in the control group ($P < .001$), and the expression intensity of EZH2 protein in the GDM group was lower than that in the control group ($P < .001$); the positive rate of PLGF protein in the GDM group was 67.00% higher than that of the control group 35.00% ($P < .001$), the positive rate of EZH2 protein in the GDM group was 15.00% lower than 33.00% in the control group ($P = .003$); the placental ultrastructural change rate of PLGF-positive GDM women was 71.64% higher than that in the negative expression group 45.45 % ($P = .011$); the placental ultrastructural change rate of EZH2 protein-positive GDM mothers was 33.33% lower than that of negative expression of A mixed 68.24% ($P = .01$); the incidence of premature infants (26.87%) and fetal respiratory distress (13.43%) in the PLGF-positive GDM group, the rate was higher than that in the negative expression group (9.09%, 0%) ($P = .027, .04$); the incidence of preterm infants in the EZH2 protein-positive GDM group (0.00%) was lower than that in the negative expression group (24.71%) ($P = .03$).

Conclusion • The expression of PLGF is up-regulated and the expression of EZH2 is down-regulated in the placental tissue of GDM women, which causes ultrastructural changes in the placental tissue and increases the incidence of preterm birth and fetal respiratory distress to a certain extent. (*Altern Ther Health Med.* 2024;30(10):206-211).

Jianfen Hong, BM, Associate chief physician**; Yifeng Chen, BM,** Attending doctor; Department of Obstetrics and Gynecology, Yueqing Second People's Hospital, Yueqing, China.

Corresponding author: Yifeng Chen, BM
E-mail: 13867718796@163.com

INTRODUCTION

Gestational diabetes mellitus (GDM) is a condition in which varying degrees of impaired glucose tolerance or diabetes mellitus occur during pregnancy, which in severe cases leads to fetal malformations and other unfavorable outcomes for mother and child.[1] The development of the disease may be related to factors such as decreased pancreatic islet cell function and elevated glucocorticoids during pregnancy. Risk factors for GDM include a family history of diabetes mellitus, obesity, and hypertension.[2] The placenta is an important organ connecting mother and baby, and trophoblast cells are the main component of the placenta and play an important role in its formation and maintenance.[3] Studies have shown [4] that apoptosis of trophoblast cells is reduced in the placenta of patients with GDM, leading to overgrowth of placental tissue. Still, it is not clear what mechanism mediates the reduction in apoptosis of placental trophoblast cells in patients with GDM. Placental growth factor (PLGF), found in patients with preeclampsia, inhibits trophoblast apoptosis,[5] but there are few reports on whether PLGF is involved in trophoblast apoptosis under hyperglycaemic conditions. Zeste homolog enhancer 2 (EZH2) is the human homolog of the Drosophila zeste gene enhancer, located in the 7q35-7q36 region of the human chromosome, and is an important member of the polycomb group (PcG), which promotes cancer cell growth, differentiation, and proliferation as well as It can promote the growth, differentiation, and

proliferation of cancer cells as well as control the local infiltration and metastasis of tumor cells.[6]

To investigate the relationship between the expression of PLGF and EZH2 in placental tissues of women with GDM, placental function, and pregnancy outcome, the present study was conducted in women with GDM diagnosed in Yueqing Second People's Hospital.

MATERIALS AND METHODS

Materials

100 women with GDM diagnosed in Yueqing Second People's Hospital were selected as the GDM group and 100 women with normal pregnancy in the same period as the control group, and the time range of patient inclusion was from January 2019 to May 2020. Inclusion criteria: (1) the diagnostic criteria of women with GDM refer to the diagnostic criteria set by the International Association for the Study of Diabetes in Pregnancy and Complicated Gestation Study Group (IADPSG) in 2010;[7] (2) all of them underwent prenatal checkups and completed delivery in our hospital, and placental tissues were obtained immediately after the delivery of the placenta with the placenta; (3) all of them had a singleton, cephalic pregnancy; and the study complied with the relevant provisions of medical ethics. Exclusion criteria: (1) with other types of pregnancy complications or comorbidities (gestational hypertension, intrahepatic cholestasis syndrome in pregnancy); (2) tumors of the reproductive system; (3) with hyperthyroidism or hypothyroidism; (4) with a history of diabetes mellitus before pregnancy; and (5) long-term use of antipsychotics, glucocorticoid hormones and so on.

In the GDM group, the pregnant women were 22-37 years old, with an average of 27.6±2.9 years old; the gestational weeks of delivery ranged from 36 to 41 weeks, with an average of 38.3±1.1 weeks; the systolic blood pressure (SBP) was 126.8±8.4 mmHg and the diastolic blood pressure (DBP) was 73.9±6.2 mmHg; there were 81 cases of primigravida and 19 cases of menstruation; and there were 11 cases with a history of miscarriage. In the control group, the pregnant women's age ranged from 22 to 36 years old, with an average of 27.7±2.5 years old; gestational weeks of delivery ranged from 36 to 41 weeks, with an average of 38.5±1.3 weeks; systolic blood pressure (SBP) was 124.6±7.1 mmHg and diastolic blood pressure (DBP) was 74.0±6.8 mmHg; there were 77 primigravida and 23 menstruating women; and there were 6 cases of miscarriage history. A comparison of the above indicators between the two groups of pregnant women showed no statistically significant difference ($P > .05$).

Western-blot detection method

Placenta tissue from patients was taken, and crushed in a glass homogenizer, lysate was added for 30min under an ice bath, transferred to a centrifuge tube, centrifuged at 10 000 rpm for 5 min at 4°C, the supernatant was taken, and he protein concentration was determined by bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). Proteins were separated by SDS-PAGE electrophoresis, then transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA), rinsed with tris buffered saline-tween (TBST), closed with 5% skimmed milk powder for 1 h at room temperature, rabbit anti-human primary antibody working solution (anti-PLGF, EZH2) was added and incubated overnight at 4°C, horseradish peroxidase-labelled goat anti-rabbit secondary antibody working solution was added and incubated for 1 h at 37°C, rinsed with TBST, for 15 min each time, ECL chemiluminescence was performed and grayscale analysis was performed using Image J.

Immunohistochemical staining detection method

The surgically resected specimens were fixed in 10% formaldehyde for 24 to 48 hours and then embedded in paraffin. The paraffin specimens were cut into 4 consecutive tissue sections of about 4 mm thickness, baked at 60°C for 3 hours, deparaffinized routinely, incubated in 3% H_2O_2 for 10 minutes at room temperature, washed 3 times with PBS for 3 minutes each, fixed with a citric acid solution, washed 3 times with PBS for 3 minutes each time, incubated with primary antibody overnight, washed with PBS for 3 times for 3 minutes each, incubated with polymer booster for 20 min at room temperature, washed 3 times with PBS for 3 minutes each, Polymer enhancer was added, and the tissue was incubated for 20 minutes then washed with PBS for 3 minutes each time. Incubate for 20 min, wash with PBS 3 times, 3 min each, add enzyme-labeled anti-mouse polymer (secondary antibody, incubate for 30 min at room temperature) at room temperature, wash with PBS 3 times, 3 min each, rinse with water for DAB chromatography, re-stain with hematoxylin, and seal with conventional dehydration. The kit was purchased from Beijing Zhongshan Biological Products Co. (Beijing, China).

Immunohistochemical results were determined: PLGF protein, EZH2 protein positive staining expressed in the cytoplasm, positive staining expressed in the cytoplasm, positive staining of the two proteins was yellow, brown, brown expression, (1) according to the degree of staining is divided into: no staining (0 points), only yellowish staining (1 point), brown staining (2 points), black (3 points); (2) according to the staining (2) According to the percentage of stained cells: 1 point for ≤10%, 2 points for 11%-50%, 3 points for 51%-75%, 4 points for >75%, and <3 points for negative and ≥3 points for positive for the product of staining degree and positive cell count.

Ultrastructural observation of placenta by projection electron microscopy

Immediately after delivery of the placenta, 2-3 pieces of tissue measuring about 0.5 cm×0.5 cm×0.5 cm were taken from the center of the placenta. After short-term fixation with 2.5% glutaraldehyde, the tissue blocks were cut into 1 mm × 1 mm × 1 mm blocks, double-fixed with 4% glutaraldehyde, 1% osmiic acid, eluted with 30%, 50%, 70%, 80%, 95%, and anhydrous ethanol gradient, embedded in epoxy resin, positioned in semi-thin sections, and ultrathin sections were stained with lead citrate and uranyl acetate, and

Table 1. Comparison of the results of PLGF protein and EZH2 protein detection in placental tissues of the two groups ($\bar{x} \pm s$, /β-actin)

Group	n	PLGF	EZH2
GDM group	100	0.772±0.150	0.207±0.065
Control group	100	0.516±0.100	0.496±0.081
t		14.200	-27.827
P value		.000	.000

Table 2. Comparison of positive expression rates of PLGF protein and EZH2 protein [n (%)]

		PLGF		EZH2	
Group	n	positive	negative	positive	negative
GDM group	100	67(67.00)	33(33.00)	15(15.00)	85(85.00)
Control group	100	35(35.00)	65(65.00)	33(33.00)	67(67.00)
χ^2		20.488		8.882	
P value		.000		.003	

photographed by transmission electron microscopy (JEM-1230, JEL) to take photographs.

Statistical analysis

Statistical analysis was performed using Statistic Package for Social Science (SPSS) version 21.0 software (IBM, Armonk, NY, USA), and the measurement data such as PLGF protein and EZH2 protein detection and fixation values of the two groups were expressed as ($\bar{x} \pm s$), and the comparison between the two groups was performed using *t* test; meanwhile, for comparing the positive expression rates of PLGF and EZH2 proteins in placental tissues, as well as the ultrastructural changes in the placenta, and the delivery outcomes of gestational diabetes mellitus (GDM) patients, the χ^2 test was employed; the difference was considered to be statistically significant at $P < .05$.

RESULTS

Comparison of the results of PLGF protein and EZH2 protein detection in placental tissues of the two groups

Table 1 presents a comparison of PLGF protein and EZH2 protein detection results in the placental tissues of the GDM (Gestational Diabetes Mellitus) group and the control group. The values are expressed as mean ± standard deviation (v) and normalized to β-actin. In the GDM group (n = 100), PLGF protein expression was significantly higher (0.772±0.150) compared to the control group (0.516±0.100), indicated by a t-value of 14.200 ($P < .001$). Conversely, EZH2 protein expression in the GDM group (0.207±0.065) was significantly lower than in the control group (0.496±0.081), with a t-value of -27.827 ($P < .001$). These findings underscore significant differences in PLGF and EZH2 protein levels between the GDM and control groups, suggesting potential implications in the context of gestational diabetes. see Table 1 and Figure 1.

Comparison of the positive expression rate of PLGF protein and EZH2 protein in placental tissues of the two groups

Table 2 presents the comparison of positive expression rates of PLGF protein and EZH2 protein in the GDM (Gestational Diabetes Mellitus) group and the control group, represented as counts and percentages (n (%)). In the GDM group (n = 100), 67 individuals (67.00%) exhibited positive PLGF protein expression, whereas 15 individuals (15.00%) showed positive EZH2 protein expression. In contrast, the control group (n = 100) had 35 individuals (35.00%) with positive PLGF protein expression and 33 individuals (33.00%) with positive EZH2 protein expression. The differences in positive expression rates were statistically significant, as indicated by the χ^2 test. For PLGF protein, $\chi^2 = 20.488$, $P < .001$, and for EZH2 protein, $\chi^2 = 8.882$, $P = .003$. These results highlight significant disparities in the positive expression rates of PLGF and EZH2 proteins between the GDM and control groups, suggesting their potential relevance in the context of gestational diabetes. see Table 2, Figures 2 and 3.

Comparison of ultrastructural changes of the placenta in GDM patients with different PLGF protein and EZH2 protein expression

Table 3 illustrates the relationship between PLGF protein expression and ultrastructural changes in the placenta,

Figure 1. Western-blot results of PLGF protein and EZH2 protein

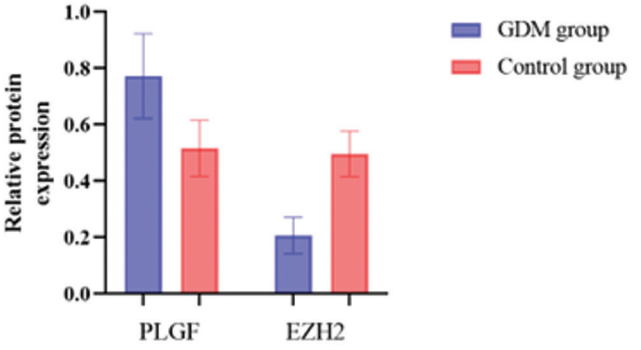

Figure 2. Immunohistochemical results of PLGF proteins, A is the GDM group, B is the control group, (×200)

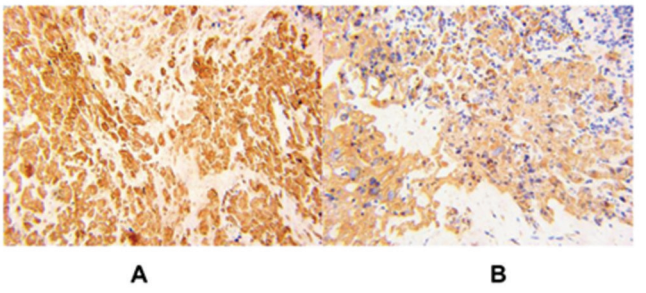

Figure 3. Immunohistochemical results of EZH2 protein, A is the GDM group and B is the control group, (×200)

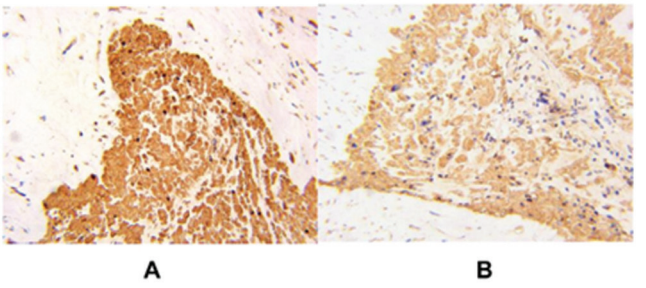

Table 3. Relationship between PLGF protein expression and ultrastructural changes in the placenta [n (%)]

PLGF	n	Ultrastructural changes	No ultrastructural changes
positive	67	48(71.64)	19(28.36)
negative	33	15(45.45)	18(54.55)
χ^2		6.505	
P value		.011	

Table 4. Relationship between EZH2 protein expression and ultrastructural changes in the placenta [n (%)]

EZH2	n	Ultrastructural changes	No ultrastructural changes
positive	15	5(33.33)	10(66.67)
negative	85	58(68.24)	27(31.76)
χ^2		6.663	
P value		.010	

Table 5. Association of PLGF protein expression with adverse pregnancy outcomes

PLGF	n	respiratory distress	premature	giant baby
positive	67	9(13.43)	18(26.87)	11(16.42)
negative	33	0(0.00)	3(9.09)	3(9.09)
χ^2		4.871	4.211	0.986
P value		.027	.04	.321

Table 6. Association of EZH2 protein expression with adverse pregnancy outcomes

EZH2	n	respiratory distress	premature	giant baby
positive	15	1(6.67)	0(0.00)	1(6.67)
negative	85	8(9.41)	21(24.71)	13(15.29)
χ^2		0.117	4.691	0.780
P value		.732	.030	.375

represented as counts and percentages (n (%)). Among the PLGF-positive cases (n = 67), 48 individuals (71.64%) exhibited ultrastructural changes, while 19 individuals (28.36%) showed no ultrastructural changes. In the PLGF-negative group (n = 33), 15 individuals (45.45%) had ultrastructural alterations, and 18 individuals (54.55%) did not. The differences in ultrastructural changes between PLGF-positive and PLGF-negative cases were statistically significant, demonstrated by the χ^2 test ($\chi^2 = 6.505$, $P = .011$). These findings suggest a significant association between PLGF protein expression and placental ultrastructural alterations, providing valuable insights into the potential implications of PLGF in gestational diabetes mellitus. Table 4 displays the relationship between EZH2 protein expression and ultrastructural changes in the placenta, presented as counts and percentages (n (%)). Among the EZH2-positive cases (n = 15), 5 individuals (33.33%) exhibited ultrastructural changes, while 10 individuals (66.67%) showed no ultrastructural alterations. In the EZH2-negative group (n = 85), 58 individuals (68.24%) had ultrastructural changes, and 27 individuals (31.76%) did not. The differences in ultrastructural changes between EZH2-positive and EZH2-negative cases were found to be statistically significant, as indicated by the χ^2 test ($\chi^2 = 6.663$, $P = .010$). These results highlight a significant association between EZH2 protein expression and placental ultrastructural alterations, underscoring the potential role of EZH2 in gestational diabetes mellitus pathogenesis. Table 3 and Table 4.

Delivery outcomes of GDM pregnant women with different PLGF protein and EZH2 protein expressions

Table 5 shows the association of PLGF protein expression with adverse pregnancy outcomes. Among PLGF-positive cases (n = 67), 13.43% experienced respiratory distress, 26.87% had premature births, and 16.42% had giant babies. In PLGF-negative cases (n = 33), no cases reported respiratory distress, 9.09% experienced premature births, and 9.09% had giant babies. Significant associations were found for respiratory distress ($\chi^2 = 4.871$, $P = .027$) and premature births ($\chi^2 = 4.211$, $P = .040$), indicating a higher incidence in PLGF-positive pregnancies.

Table 6 displays the association of EZH2 protein expression with adverse pregnancy outcomes. Among EZH2-positive cases (n = 15), 6.67% experienced respiratory distress, none had premature births, and 6.67% had giant babies. In EZH2-negative cases (n=85), 9.41% reported respiratory distress, 24.71% experienced premature births, and 15.29% had giant babies. A significant association was found for premature births ($\chi2 = 4.691$, P =0.030), indicating a higher incidence in EZH2-negative pregnancies. No significant associations were observed for respiratory distress ($\chi2 = 0.117$, P =0.732) and giant babies ($\chi2 = 0.780$, P =0.375). These results suggest a potential link between PLGF and adverse pregnancy outcomes, particularly respiratory distress and premature births, in gestational diabetes mellitus.; Table 5 and Table 6.

Electron microscope observation results

Electron microscopy of the placental tissue of the control group showed that the syncytiotrophoblasts, microvilli, and organelles were well arranged. Electron microscopy of the placental tissue of pregnant women in the GDM group showed that the microvilli were disorganized and sparse, some of which were missing, and the nuclei of the cells were irregular, with abnormally distributed chromatin, tortuous nuclear membranes, and a curved and inhomogeneous thickening of the basement membrane. The results of this study show that the placental tissue of pregnant women in the GDM group was not well arranged.

DISCUSSION

GDM is defined as abnormal glucose metabolism that is first detected or occurs during pregnancy and may, be influenced by genetics, intrauterine environment, and endocrine and placental transport functions. GDM is usually considered a pregnancy complication that severely damages the fetus, and may be associated with the risk of neonatal asphyxia, birth injuries, neonatal hypoglycemia, and death.[7] The placenta is an important organ that maintains the development of the fetus in the uterus during pregnancy, mainly performing functions such as material exchange, metabolism, hormone secretion, and defense.[9,10] The integrity of the placenta plays an important role in maintaining pregnancy and has been studied as a special discipline by many scholars. In recent years, light microscopy, electron

microscopy, immunohistochemistry, and ultrasound have been widely used for placental studies.[7] It has been found that alterations in the ultrastructure of the placenta can lead to adverse pregnancy outcomes, and the study of placental ultrastructure can help guide clinical diagnosis and treatment.[11] In recent years, studies have begun to focus on placental ultrastructure and pregnancy outcomes. It has been found that changes in placental ultrastructure can affect the blood and oxygen supply between the placenta and the fetus, leading to fetal growth restriction, distress, and neonatal asphyxia, which is one of the major causes of adverse pregnancy outcomes in patients with GDM.[12,13]

PLGF was first isolated and purified from a human placental cDNA library, which is mainly synthesized by placental trophoblast cells, inhibits the apoptosis of trophoblast cells in placental tissues in preeclampsia, and plays an important role in the growth and development of the placenta.[14] Currently, domestic and international studies have confirmed that changes in PLGF levels are associated with episodes of eclampsia, and preeclampsia is one of the complications of GDM, and similar pathological changes exist in the placenta, so we hypothesized that PLGF may also be involved in the genesis and development of the placenta in GDM.[14,15] EZH2 is located on human chromosome 7q35, and it is one of the core members of the PCG gene family, which can regulate Wnt, Notch, and other signaling pathway genes expression, inhibit cellular senescence and promote epithelial-mesenchymal transition, which is closely related to the development of malignant tumors.[16] Since the discovery of EZH2, scholars in many fields have analyzed its correlation with malignant tumors, and its high expression is associated with poor patient prognosis.[17] The results of this study showed that the intensity of PLGF protein expression and protein positivity in the GDM group were significantly higher than those in the control group, and the intensity of EZH2 protein expression and protein positivity in the GDM group were significantly lower than those in the control group. It is suggested that PLGF and EZH2 may be involved in the occurrence and development of GDM.

In this study, we observed the ultrastructure of the placenta of normal pregnant women, pregnant women with gestational diabetes mellitus (GDM) with satisfactory glycaemic control, and pregnant women with GDM with unsatisfactory glycaemic control by electron microscopy, and monitored the corresponding glucose levels to investigate the effect of the management of glucose during pregnancy on the ultrastructure of the placenta of GDM. The rate of ultrastructural alteration of the placenta of GDM women who were PLGF-positive was higher than that of those who were negatively expressed, and the rate of ultrastructural alteration of the placenta of GDM women who were EZH2 protein The rate of placental ultrastructural alteration was significantly lower in PLGF-positive GDM women than in those with negative expression. PLGF and EZH2 could effectively inhibit high glucose-induced apoptosis of trophoblast cells, suggesting that PLGF and EZH2 may be an important mechanism leading to the overdevelopment of placenta in GDM. It can be seen that hyperglycemia is a risk factor for ultrastructural alterations of the placenta. When pregnant women with gestational diabetes mellitus have suboptimal glycaemic control, it leads to high blood glucose levels during pregnancy. Continued stimulation of placental syncytiotrophoblast cells by hyperglycemia beyond their adaptive capacity leads to damage such as microvillous swelling, mitochondrial swelling, and endoplasmic reticulum swelling. The ultrastructure of the placenta in patients with GDM affects placental function and may further lead to fetal distress and other adverse pregnancy outcomes. This study also confirms the importance of blood glucose management during pregnancy in pregnant women with GDM, and strict monitoring of blood glucose during pregnancy may improve the ultrastructure of the placenta in gestational diabetes mellitus and contribute to the improvement of pregnancy outcomes. The incidence of preterm birth and fetal respiratory distress was significantly higher in the PLGF protein-positive GDM group than in the negative-expression group, and the incidence of preterm birth was significantly lower in the EZH2 protein-positive GDM group than in the negative-expression group. expression group. This suggests that the expression of PLGF and EZH2 is related to the pregnancy outcome of the patients, but the mechanism needs to be further studied.

Current clinical research has found that EZH2 is a cancer-related gene, which is closely related to the proliferation and differentiation of tumor cells, and is mainly used in the study of malignant tumors,[18,19] and this study chose to investigate its effect on placental structure and pregnancy outcome in GDM patients, and found that there is a certain correlation, which provides a better basis and foundation for later research, but the mechanism of its action is still unclear, and it is worth further clinical research. However, its mechanism is still unclear and deserves further clinical research.

Future research in gestational diabetes mellitus (GDM) should focus on several key areas. Firstly, mechanistic studies employing advanced molecular techniques can unravel the intricate pathways through which PLGF and EZH2 influence placental development. Functional experiments, including gene manipulation studies, can further elucidate their roles. Longitudinal studies tracking PLGF and EZH2 levels throughout pregnancy and their correlation with adverse outcomes offer valuable insights. Additionally, biomarker discovery efforts may yield diagnostic tests for early pregnancy screening. Interventional studies, exploring pharmacological or lifestyle interventions, could modulate PLGF and EZH2 levels to observe subsequent effects on placental structure and pregnancy outcomes. Large-scale clinical cohort studies should validate findings across diverse populations, considering genetic and environmental factors. Utilizing advanced imaging techniques and integrating multi-omics approaches can provide a comprehensive understanding of GDM-related placental alterations. Patient stratification based on PLGF and EZH2 expression levels can uncover distinct GDM phenotypes. Animal models may

confirm causative relationships. Addressing these aspects will deepen our understanding of GDM's molecular basis, facilitating targeted therapies and improved management strategies for this condition.

In conclusion, the up-regulation of PLGF expression and the down-regulation of EZH2 expression in the placental tissue of GDM women caused ultrastructural changes in the placental tissue, which increased the risk of adverse pregnancy outcomes to a certain extent.

ETHICAL COMPLIANCE

This study was approved by the ethics committee of Yueqing Second People's Hospital. Signed written informed consent was obtained from the patients and/or guardians. Informed consent was obtained from all study participants, ensuring their voluntary participation, understanding of the study procedures, and awareness of their rights by ethical standards. Providing these specific details ensures transparency and demonstrates the ethical conduct of the study.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to report relevant to this article.

AUTHOR CONTRIBUTIONS

JH and YC designed the study and performed the experiments, JH collected the data, YC analyzed the data, and JH and YC prepared the manuscript. All authors read and approved the final manuscript.

FUNDING

This study did not receive any funding in any form.

REFERENCE

1. Fiskin G, Sahin N; Nonpharmacological Management of Gestational Diabetes Mellitus: Diaphragmatic Breathing Exercise. *Altern Ther Health M*. 2021;27(90-96.
2. Chen X, Huang J, Peng Y, Han Y, Wang X, Tu C. The role of circRNA polyribonucleotide nucleoside transferase 1 on Gestational Diabetes Mellitus. *Cell Mol Biol (Noisy-le-grand)*. 2022;68(6):148-154. doi:10.14715/cmb/2022.68.6.24
3. Santander Ballestín S, Giménez Campos MI, Ballestín Ballestín J, Luesma Bartolomé MJ. Is Supplementation with Micronutrients Still Necessary during Pregnancy? A Review. *Nutrients*. 2021;13(9):3134. doi:10.3390/nu13093134
4. Gou BH, Guan HM, Bi YX, Ding BJ. Gestational diabetes: weight gain during pregnancy and its relationship to pregnancy outcomes. *Chin Med J (Engl)*. 2019;132(2):154-160. doi:10.1097/CM9.0000000000000036
5. Azadbakht Z, Fanaei H, Karajibani M, Montazerifar F. Effect of type 1 diabetes during pregnancy and lactation on neonatal hypoxia-ischemia injury and apoptotic gene expression. *Int J Dev Neurosci*. 2023;83(4):346-356. doi:10.1002/jdn.10262
6. Rais R, Starikov R, Robert W, Has P, He M. Clinicopathological correlation of large-for-gestational age placenta in pregnancies with pregestational diabetes. *Pathol Res Pract*. 2019;215(3):405-409. doi:10.1016/j.prp.2018.12.029
7. Metzger BE, Gabbe SG, Persson B, et al; International Association of Diabetes and Pregnancy Study Groups Consensus Panel. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care*. 2010;33(3):676-682. doi:10.2337/dc09-1848
8. Li Y, Cooper A, Odibo IN, et al. Expression of Concern: discrepancy in insulin regulation between gestational diabetes mellitus (GDM) platelets and placenta. *J Biol Chem*. 2019;294(13):5208. doi:10.1074/jbc.EC119.008295
9. Heidari Z, Mahmoudzadeh-Sagheb H, Narouei M, Sheibak N. Effects of gestational diabetes mellitus on stereological parameters and extravillous trophoblast cells of placenta compared to the control group. *J Obstet Gynaecol*. 2019;39(7):928-933. doi:10.1080/01443615.2019.1578948
10. Jayabalan N, Lai A, Ormazabal V, et al. Adipose Tissue Exosomal Proteomic Profile Reveals a Role on Placenta Glucose Metabolism in Gestational Diabetes Mellitus. *J Clin Endocrinol Metab*. 2019;104(5):1735-1752. doi:10.1210/jc.2018-01599
11. Pérez-Pérez A, Vilariño-García T, Guadix P, Dueñas JL, Sánchez-Margalet V. Leptin and Nutrition in Gestational Diabetes. *Nutrients*. 2020;12(7):1970. doi:10.3390/nu12071970
12. Ding R, Liu XM, Xiang YQ, et al. Altered Matrix Metalloproteinases Expression in Placenta from Patients with Gestational Diabetes Mellitus. *Chin Med J (Engl)*. 2018;131(10):1255-1258. doi:10.4103/0366-6999.231530
13. Li Y, Cooper A, Odibo IN, et al. Withdrawal: discrepancy in insulin regulation between gestational diabetes mellitus (GDM) platelets and placenta. *J Biol Chem*. 2019;294(24):9656. doi:10.1074/jbc.W119.009465
14. Chau K, Hennessy A, Makris A. Placental growth factor and pre-eclampsia. *J Hum Hypertens*. 2017;31(12):782-786. doi:10.1038/jhh.2017.61
15. Wang X, Yip KC, He A, et al. Plasma Olink Proteomics Identifies CCL20 as a Novel Predictive and Diagnostic Inflammatory Marker for Preeclampsia. *J Proteome Res*. 2022;21(12):2998-3006. doi:10.1021/acs.jproteome.2c00544
16. Bugide S, Green MR, Wajapeyee N. Inhibition of Enhancer of zeste homolog 2 (EZH2) induces natural killer cell-mediated eradication of hepatocellular carcinoma cells. *Proc Natl Acad Sci USA*. 2018;115(15):E3509-E3518. doi:10.1073/pnas.1802691115
17. Zhou T, Sun Y, Li M, et al. Enhancer of zeste homolog 2-catalysed H3K27 trimethylation plays a key role in acute-on-chronic liver failure via TNF-mediated pathway. *Cell Death Dis*. 2018;9(6):590. doi:10.1038/s41419-018-0670-2
18. Lu F, Xu H, Wang Q, Li M, Meng J, Kuang Y. Inhibition of enhancer of zeste homolog 2 increases the expression of p16 and suppresses the proliferation and migration of ovarian carcinoma cells *in vitro* and *in vivo*. *Oncol Lett*. 2018;15(3):3233-3239. doi:10.3892/ol.2017.7712
19. Homko CJ, Sivan E, Reece AE. Is there a role for oral antihyperglycemics in gestational diabetes and type 2 diabetes during pregnancy? *Treat Endocrinol*. 2004;3(3):133-139. doi:10.2165/00024677-200403030-00001