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The relationship between nuclear factor-erythrocyte-related factor 2 and antioxidant enzymes in the placenta of patients with gestational diabetes and the metabolism of umbilical cord endothelial cells

Xiaoxiao Yin, Juan Nie, Xiaoqian Nie, Yumei Nie*

Obstetric department, Tai 'an Central Hospital Affiliated to Qingdao University, Tai 'an 271099, China

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ABSTRACT

The paper aimed to explore the relationship between nuclear factor erythroid 2 related factor 2 (Nrf2), antioxidant enzymes and the metabolism of umbilical cord endothelial cells in the placenta of patients with gestational diabetes (GDM). A total of 200 pregnant women who underwent an obstetric examination at the Municipal Maternity and Child Health Hospital from March 2018 to December 2019 were selected, with an average age of 30.91±3.24. According to the plasma glucose level and oral glucose tolerance test (OGTT), pregnant women were divided into a control group and an observation group. Blood samples were collected from these pregnant women, serum was removed, put into a centrifuge tube and stored in the refrigerator of the laboratory at - 80 °C. Placental tissue was collected for biochemical analysis. GSH level was detected by absorbance kit, and serum MDA content was detected by spectrophotometry. The expression levels of Heme oxygenase-1(HO-1), Nrf2, and NQO1 protein in placental tissue were analyzed by gel electrophoresis. Western blot analysis was used to detect the protein expression levels of Bach1 and Keap1 in endothelial cells. PCR real-time analysis was used to detect the expression of GSH and NQO1 mRNA. Results showed that the SOD and GSH levels in the serum of the observation group were lower than those of the control group ($P<0.05$). The protein expression levels of HO-1, Nrf2 and NQO1 in the observation group were higher than those in the control group ($P<0.05$). The GSH level of the HNE+ observation group was lower than that of the HNE+ control group before stimulation ($P<0.05$). 1.5 hours after the stimulation, the GSH levels of the two groups of cells were decreased. After 6 hours, the GSH levels of the two groups began to increase. The GSH level of HUVEC in the HNE+ observation group was lower than that of the HNE+ control group after 48 hours. The expression level of Bach1 protein in the HNE+ observation group was lower than that in the HNE+ control group ($P<0.05$). The expression level of Keap1 protein in the HNE+ observation group and HNE+ control group did not change ($P>0.05$). The expression levels of GSH and NQO1 mRNA in the HNE+Nrf2 silence group were lower than that in the Nrf2 silence group ($P<0.05$). The expression levels of GSH and NQO1 mRNA in the HNE+Nrf2 overexpression observation group were higher than those of the HNE+Nrf2 silence group ($P<0.05$). The apoptosis of trophoblast in the placenta of GDM patients was significantly decreased. The continuous lack of redox signals in fetal endothelial cells in patients with gestational diabetes can destroy the defense ability of cells in the uterus against oxidative stress. Nrf2 antioxidant defense pathway can provide therapeutic targets for reducing oxidative stress associated with diabetes and aging.

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Introduction

GDM is defined as type 1 or type 2 diabetes mellitus diagnosed after the first three months of pregnancy. The global prevalence of GDM ranges from 2% to 14%, while that of the Chinese population ranges from 2.4% to 6.8%, and this rate continues to increase every year (1). Pregnancy and delivery complications, including preeclampsia, cesarean section, premature delivery and macrosomia, are more common in GDM women (2). GDM was also associated with abortion, premature rupture of membranes and other abnormal rates, which were higher than those of non-diabetic pregnancies (3). In addition, children born to GDM mothers are at high risk of obesity and type 2 diabetes as they grow up (4). For example, it is reported that women with GDM are older and report higher weight 2 years after delivery (5). It has been reported that pregnant women with GDM have increased circulating oxidative stress and decreased antioxidant enzymes caused by hyperglycemia (6). Increased oxidative stress may have adverse effects on both the mother and fetus. GSH has a reducible GSH form and an oxidized GSH form. Reduced GSH is the main form and one of the most common antioxidants (7). HO-1 is one of the most easily induced proteins, and various factors (such as cytokines, oxidative stress and inflammatory factors) can induce HO-1 expression (8). Up-regulation of HO-1 gene expression and increase of HO-1 enzyme activity are considered to play a protective role in the development of diabetic complications (9). Nrf2 is activated after oxidative stress is induced. This

* Corresponding author. Email: Nieyumei2023@126.com

protein has been proven to be involved in the regulation of cell-protective genes mediated by oxidative stress. Nrf2 is considered to be an important upstream transcription factor regulating HO-1 (10). Endothelial cells are the main target of oxidative damage caused by hyperglycemia, and elevated intracellular glucose levels increase the production of mitochondrial superoxide, which leads to the activation of polyol and hexosamine pathways, and ultimately leads to endothelial dysfunction. Interestingly, endothelial dysfunction is associated with insulin resistance in young offspring of first-degree relatives of type 2 diabetes mellitus (11). It is attempted to determine the effects of GDM on redox status and Nrf2 antioxidant defense in fetal endothelial cells treated with 4-hydroxynonenoic acid (HNE) (12). Therefore, the purpose of this study was to study the changes in oxidative stress indexes and to evaluate the relationship between Nrf2 and antioxidant enzymes in the placenta of GDM patients and the metabolism of umbilical cord endothelial cells.

Materials and Methods

General information

A total of 200 pregnant women, aged 23 to 45, with an average age of 30.91 ± 3.24, were selected from March 2018 to December 2019 in the municipal maternal and child health care hospital. Blood samples were collected from these pregnant women. The serum was removed and placed in a centrifuge tube and stored in the refrigerator of laboratory at - 80 ℃ until further processing.

Inclusion criteria

Pregnant women aged 18-45 years; those who met one of the following criteria were considered to have GDM: fasting, ≥ 5.1 mmol / L; 1 hour, ≥ 10 mmol / L; 2 hours, ≥ 8.5 mmol / L.

Exclusion criteria

History of hypertension; cardiovascular and cerebrovascular diseases; preeclampsia; placenta previa; incomplete data; patients with infectious diseases; patients who did not agree to sign the informed participation letter.

Medical ethics

All patients or their guardians signed informed consent or oral consent.

Method

Endothelial cell culture

As mentioned previously, HUVECs were cultured in M199 containing 5 mmol/L D-glucose. Prior to treatment, the absence of 3-generation HUVEC serum in 1% FCS M199 was performed for 4 h. endothelial phenotype was confirmed by characteristic cobble morphology and von Willebrand factor immunostaining.

Adenovirus overexpression of Nrf2 siRNA and Nrf2

Normal HUVECs were transfected with 40 pmol/24 well Nrf2 siRNA for 24 hours and then treated with HNE (20 μ mol/ L) or solvent for 20 hours. The levels of Nrf2 and NQO1 proteins or intracellular GSH were measured. GDM HUVEC was transfected with an adenovirus vector (50 mol/L) for 12 hours to coordinate the expression of AD containing green fluorescent protein or active AdNrf2. The expression of the NQO1 protein was determined.

GSH level detection

GSH was evaluated by absorbance-based assay. The kit was purchased from Shanghai cable bridge Biotechnology Co., Ltd. Thermo Scientific varioskan flash was preheated for 30 minutes and the wavelength was adjusted to 412 nm. Reagent 2 was heated in water at 37 ° C for 30 minutes, and 20 μ l of distilled water, 140 μ l of reagent 2 and 40 μ l of reagent 3 were put into 96 well plates. After mixing for 2 minutes, the method was simple, rapid and accurate, the absorbance A1 at 412 nm was measured by varioskan flash as a blank tube. The above steps were repeated by using serum instead of distilled water in the test tube. The absorbance A2 at 412 nm was read. Finally, the density of GSH (μmol/ mL) was calculated using the formula 6.67 × (A2-A1).

Detection of SOD and MDA levels

Plasma SOD was determined by the microdilution method (the kit of Shanghai cable bridge Biotechnology Co., Ltd.). The blood sample was replaced by distilled water in the control tube. All mixtures were kept at room temperature for 30 minutes. The absorbance of the mixture was measured with a Thermo Scientific Varioskan Flash at 560 nm. The SOD content was calculated according to the instructions. The value was expressed as U/mLof SOD. The amount of MDA was evaluated by spectrophotometry (the kit of Shanghai cable bridge Biotechnology Co., Ltd.). 0.3 mL of reagent 1 was transferred into a 1.5 ml centrifuge tube, 0.1 ml serum was added, and fully mixed. The mixture was heated in water at 95 ° C for 30 minutes, the test tube was placed in an ice bath. The absorbance of 532 nm (= A532) and 600 nm (= A600) was determined by Thermo Scientific varioskan flash, ΔA = A532-a600. The unit of MDA used in the laboratory is nmol / mL.

Expression of HO-1, Nrf2 and NQO1 in placenta

Differential gel electrophoresis (DIGE) and nanophase liquid chromatography-tandem mass spectrometry (UPLC-MS) were used for proteomic comparison between normal and GDM-stimulated HUVECs. The identified proteins were analyzed using the ingenuity system (mountain view, CA) to determine the most relevant interaction networks and biological functions. Pairs of samples from different normal and GDM HUVEC cultures were paired for two-dimensional separation, and Ettan DIGE Imager (GE Healthcare) was used to capture the fluorescent gel images, and DeCyder software was used to analyze the differentially expressed proteins between normal and GDM samples.

Intracellular GSH measurement

HUVEC was treated with solvent (0.08% v / hexane) or HNE (20 μ Moll L) for 0-24 hours. GSH was extracted on ice with 6.5% trichloroacetic acid for 10 minutes. The total GSH level of HUVEC was measured by fluorescence method.

Western blot

Whole-cell and nuclear proteins were extracted by lysis buffer or nuclear extraction kit (Active Motif). Denatured samples were separated by gel electrophoresis. α - tubulin or laminin C was used as a reference protein and detec-

ted with a monoclonal antibody. The protein expression of ovalbumin was determined by enhanced chemiluminescence, and the images were captured in Ingenius, Syngenee bioimaging, and the optical density was measured by ImageJ software (National Institutes of Health, Baltimore, Maryland).

RT-PCR

RNA was extracted and separated using the nucleon spin RNA Kit (Macherey Nagel). The integrity of RNA was evaluated by capillary electrophoresis using RNA 6000 nano assay, and analyzed by Agilent 2100 Bioanalyzer system (Agilent Technologies, Santa Clara, California). Quantitative reverse transcription Kit (Qiagen) was used to reverse the total RNA. A quantitative probe PCR kit (Qiagen) was used to evaluate Nrf2 linkage and NOX4 (data not shown) gene expression in a real-time PCR system (Corbet Rotor-Gene). The mRNA levels were normalized to geometric mean values of three housekeeping genes: β - actin, ribosomal protein L13a and succinate dehydrogenase unit complex A.

In situ nick end labeling (TUNEL)

Paraffin sections were dewaxed to water, placed in 3% hydrogen peroxide (H2O2) at room temperature for 10 minutes, and washed with distilled water for 3 times. The samples were added with protease K (0.01 M TBS 1:200 dilution) and digested at 37 ℃ for 15 minutes. 1 μ l of terminal deoxyribonucleic acid transferase (TD T) and digoxin labeled digoxigenin triphosphate deoxyuridine (dig-d-utp) were taken from each section, added to 18 μ l labeled buffer, mixed, and placed in the wet box, labeled at 37 ℃. The biotinylated anti-digoxin antibody was diluted with antibody diluent 1:100, then 50 μ L / tablet was added to the specimen, and the sample was placed in the wet box. The antibody dilution 1:100 was used to dilute the streptavidin peroxidase (SABC). After DAB color development and hematoxylin re-dyeing, the film was sealed. Under a high power microscope, brown granules in the nucleus were positive apoptotic cells. The positive cells in 8 consecutive non-overlapping visual fields were counted. Apoptosis index (AI) = (number of apoptotic cells / total number of cells) × 100%.

Statistical analysis

The results were analyzed by SAS 9.1 and GraphPad Prism5 software. Chi-square test, t-test and Pearson correlation coefficient analysis were used. The results were expressed as arithmetic mean with standard error (mean ± SEM) and mean with standard deviation (mean ± SD). $P < 0.05$ was considered to be statistically significant. The detailed statistical analysis method of each experiment was described in the corresponding legend.

Results

Comparison of general information

In the control group, the average age was 31.09 ± 3.42 years, the average BMI was 22.16 ± 1.15, the average gestational age was 38.29 ± 0.46 weeks, the proportion of hereditary disease history was 4 (4.00%), the average systolic blood pressure was 113.42 ± 15.08, the diastolic blood pressure was 71.34 ± 8.24. In the observation group, the average age was 30.64±6.24 years, the average BMI was 22.37±1.63, the average gestational age was 38.81±0.39 weeks, and the proportion of hereditary disease history was 3 (3.00%). There was no difference in general data between the two groups ($P > 0.05$). (Table 1).

Detection of serum oxidative stress level

The detection of serum-related oxidative stress levels showed that the level of MDA in the observation group was higher than that in the control group ($P < 0.05$), while SOD and GSH levels were decreased ($P < 0.05$). It was indicated that the level of oxidative stress in GDM patients was increased (Table 2).

The expression of oxidative stress molecules was analyzed by Western blot

Since the oxidative stress protein showed strong changes in healthy pregnant women and GDM patients, whether there were any changes in placental tissue was discussed. The expression of HO-1, Nrf2 and NQO1 in the placenta of the control group and observation group was analyzed by Western blot, and the protein expression of HO-1, Nrf2 and NQO1 in the placenta of the observation

Table 1. General Statistics.

Project	Control group	Observation group	*T* value χ^2value	*P value*
Age (years)	31.09±3.42	30.64±6.24	6.381	0.366
BMI	22.16±1.15	22.37±1.63	5.228	0.175
Gestational weeks of delivery	38.29±0.46	38.81±0.39	4.162	0.528
Other genetic histories (%)	4 (4.00%)	3 (3.00%)	6.537	0.822
Systolic blood pressure (MMG)	113.42±15.08	115.37±16.29	5.088	0.296
Diastolic blood pressure (MMG)	71.34±8.24	68.25±10.37	6.735	0.422

Table 2. Serum oxidative stress levels.

Group	MDA (μmol/mL)	SOD (U/ml)	GSH (μmol/mL)
control group	0.64±0.08	16.64±3.88	2.42±0.38
Observation group	1.23±0.14	12.25±2.58	2.07±0.26
T value	6.337	5.558	4.725
P value	0.012	0.003	0.0234

Table 3. Protein expression of HO-1, Nrf2 and NQO1.

Group	HO-1	Nrf2	NQO1
Control group	1.21±0.15	1.08±0.16	1.15±0.16
Observation group	1.84±0.22	1.95±0.28	1.83±0.27
T value	4.117	6.082	5.417
P value	0.011	0.006	0.037

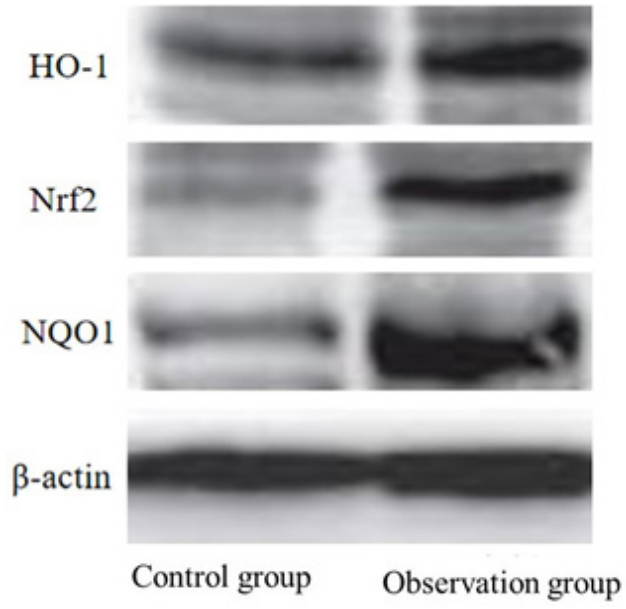

Figure 1. Western blot analysis.

group was higher than that of the control group (P < 0.05). (Figure 1, Table 3).

Trophoblast apoptosis in the placenta

TUNEL staining was used to detect the apoptosis of the trophoblast. As shown in Figure 2, the apoptosis of trophoblast in the observation group was significantly lower than that in the control group.

GDM impaired HNE induced GSH synthesis

The GSH levels in normal and GDM cells stimulated by HNE were compared. Before stimulation, the GSH level in HNE + observation group was lower than that in HNE + control group (P < 0.05). At 1.5 hours after stimulation, GSH levels in both groups decreased, and GSH levels began to increase after 6 hours. The GSH level of HUVEC in the HNE+ observation group was lower than that of the HNE+ control group after 48 hours. These results indicated that the adaptation of GSH induced by HNE in the HUVEC of the observation group was decreased (P < 0.05), and the antioxidant defense ability was damaged. (Figure 3, Table 4).

Western blot analysis of Bach1 and Keap1 levels

Whether the activation of damaged Nrf2 in GDM was

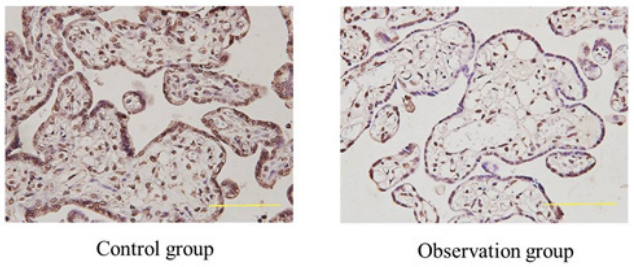

Figure 2. Trophoblast apoptosis in the placenta.

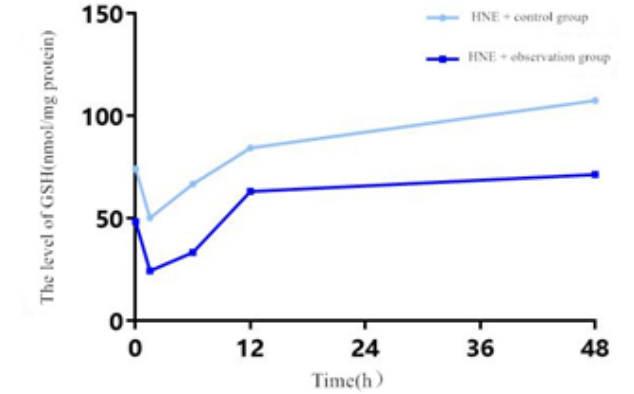

Figure 3. GSH level analysis (nmol/mg protein).

Table 5. The protein content of Bach1 and Keap1.

Group	Bach1	Keap1
HNE + control group	1.74±0.21	1.08±0.12
HNE + observation group	1.13±0.25	1.14±0.10
T value /χ^2 value	5.668	6.082
P value	0.017	0.006

affected by Keap1 or Bach1 was examined. The expression level of Bach1 protein in the HNE+ observation group was lower than that in the HNE+ control group (P<0.05). The expression level of Keap1 protein in the HNE+ observation group and HNE+ control group did not change (P>0.05).

Antioxidant effect of Nrf2

In order to confirm that the adaptive increase of GSH and NQO1 induced by HNE was mediated by Nrf2. Nrf2 siRNA was used to knock down the transcriptional activity in normal cells. The expression levels of GSH and NQO1 mRNA in the HNE+Nrf2 silence group were lower than that in the Nrf2 silence group (P<0.05). The expression levels of GSH and NQO1 mRNA in the HNE+Nrf2 overexpression observation group were higher than those of the HNE+Nrf2 silence group (P<0.05). These results indicated that Nrf2 overexpression can partially restore the damaged Nrf2 redox signal in the HUVEC of the observation group (Figure 4 and Table 6).

Table 4. GSH level analysis (nmol/mg protein).

Group	0h	1.5h	6h	12h	48h
HNE + control group	74.11±5.09	50.16±4.82	66.74±5.26	84.31±7.05	107.46±10.24
HNE + observation group	48.27±3.08	24.37±2.67	33.26±2.91	63.11±5.19	71.28±7.14
T value	5.038	6.417	6.882	5.037	4.119
P value	0.251	0.034	0.015	0.011	0.005

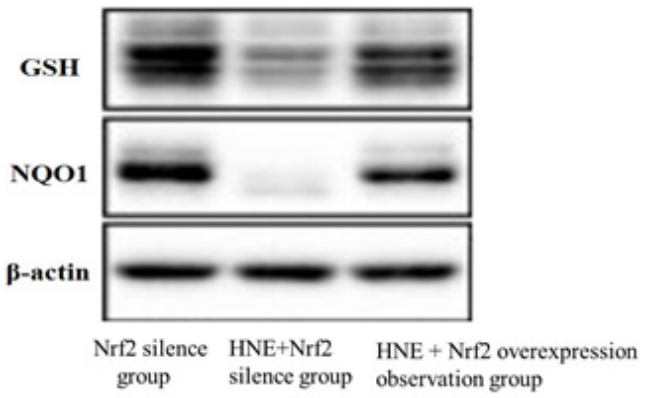

Figure 4. Real-time RT-PCR analysis.

Table 6. Nqo6 and GSH mRNA expression scale.

Project	GSH	NQO1
Nrf2 silence group	1.82±0.27	1.79±0.20
HNE + Nrf2 silencing group	1.03±0.11	1.11±0.12
HNE + Nrf2 overexpression observation group	1.67±0.13	1.54±0.14
F	9.038	11.551
P value	0.027	0.015

Discussion

Oxidative stress is an imbalance between ROS and antioxidant defense, which may lead to tissue damage (13). Although the exact mechanism of GDM is not clear, previous studies have solved the problem of oxidative stress in GDM. In current studies, it was found that some oxidative stress proteins are overexpressed in GDM patients. In addition, abnormal serum levels of these molecules were associated with poor clinical prognosis.

Studies have shown that the decrease of trophoblast apoptosis is one of the important mechanisms for the increase of placental mass and fetal birth weight. Placenta is a temporary but extremely important organ produced by women during pregnancy. It is the only nutrient transport channel between the mother and the fetus. Only when the fetal disc grows and develops normally can it perform its normal metabolic function, gestational trophoblast is the main component of the placenta, which plays an important role in the formation and function of the placenta. Foreign scholars have shown that the decrease of trophoblast apoptosis in the placenta of GDM patients can lead to larger placental tissue development. In this study, TUNEL staining was used to detect the apoptosis of trophoblast in the GDM group.

MDA is the end product of ROS-mediated lipid peroxidation (14). In this study, serum levels of MDA in GDM patients were higher than those in healthy pregnant women. Our results are consistent with previous studies. SOD and GSH are ubiquitous antioxidants in the human body, and keep the balance of redox reaction (15). The dysfunction of these molecules will lead to various diseases. Whether they also have an impact on the development of GDM is still uncertain. Our results showed that the levels of SOD and GSH in GDM patients were decreased. The expression patterns of SOD and GSH in the placental diseases of GDM patients were similar to the serum levels of the two molecules. As an inducible stress protein, HO-1 is widely considered a highly sensitive and reliable marker of oxidative stress. Nrf2, known as the upstream regulator of HO-1, is a new regulator of cell resistance to oxidants. It has been reported that Nrf2 overexpression increases the expression of basic Nrf2 and NQO1 in GDM cells (16). Nrf2 antioxidant defense pathway may provide therapeutic targets for reducing oxidative stress associated with diabetes mellitus (17). Our study also showed that Nrf2 and NQO1 proteins in the GDM group were higher than those in the control group. This was consistent with the expression of HO-1. Previous studies found that the concentration of MDA in amniotic fluid was higher than that in healthy pregnancy during pregnancy with intrauterine growth restriction, and the determination of MDA can be used as an endometrial biochemical test (18). It was found that there was a close relationship between SOD content in the GDM group and preterm delivery. Studies have found that the level of superoxide dismutase (SOD) in the placenta of women with full-term delivery is higher than that of women with full-term delivery, which is consistent with our findings.

The study provided the first whole-cell proteomics analysis of the effect of GDM on fetal endothelial cells, characterizing the phenotypic changes of proteins involved in redox signaling. It was further demonstrated that GDM HUVEC abolished Nrf2 nuclear accumulation, Nrf2 mediated GSH adaptive increase, and lipid peroxidation product HNE induced NQO1 and Bach1 expression. These findings are consistent with increased protein oxidation levels, increased sensitivity to HNE-induced DNA damage, and increased ROS production in GDM cells.

Previous studies have reported that in fetal plasma triglycerides, GSH levels in cord blood and newborns of GDM pregnancies are reduced. Associated inflammatory markers, such as C-reactive protein, adhere to molecule 1 and interleukin-6 in cells and neonatal fat mass (19,20). In addition, childhood obesity is closely related to the degree of methylation of DNA encoding endothelial nitric oxide synthase and vitamin A X receptor - α at birth (21). These results suggest that our relatively normal DNA methylation and redox and the regulation of GDM may provide valuable insights into the epigenetic role of GDM in the uterus. Increased glucose load leads to mitochondrial dysfunction and increased superoxide production, which has been widely involved in the development of glucose intolerance and vascular dysfunction in diabetic patients (22). Studies using a rodent intrauterine growth retardation model have shown that elevated intrauterine stress and mitochondrial dysfunction are associated with type 2 diabetes and metabolic diseases in offspring (23). In our study, fetal endothelial cells from GDM pregnancies showed increased carbonylation of basic proteins and enhanced DNA damage due to the fact that physiologically related concentrations of HNE rarely exceeded 100 μ mol / L in cells and tissues.

Western blot analysis showed that the GSH and phase II detoxification pathways in GSH cells were changed, which suggested that Nrf2 activation might be impaired in GDM. Due to the increased adaptability in GSH and XCT mediated by HNE and the decrease of GCLM mRNA and protein levels in GDM cells, these findings highlight the importance of Nrf2 and GSH synthesis-related enzymes in promoting cell survival and oxidative stress adaptation. Although lower basal GSH levels in GDM HUVECs may contribute to increased sensitivity to HNE-mediated inju-

ry, the adaptive increase in Nrf2-linked gene expression in normal HUVECs seems to be unrelated to mitochondrial ROS production. The initial consumption of GSH (1.5 hours) is parallel to the maximum of Nrf2 nuclear translocation within 1-2 hours, which indicates that HNE-induced Nrf2 activation is the result of redox environment changes, rather than the direct production of ROS itself. Without an adaptive increase of GSH, cardiomyocytes exposed to HNE show a higher level of apoptosis, this finding is consistent with our observation of the increase of DNA damage induced by HNE in GDM HUVEC. The induction of NQO1 and Bach1 mediated by HNE is abolished in GDM cells, therefore, it seems unlikely that the down-regulation of Bach1 weakens Nrf2 activation in GDM cells. The activity of GSK-3 β in fibroblasts of type 2 diabetic rats is increased. Therefore, GSK may negatively regulate the nuclear accumulation of Nrf2 stimulated by HNE and the induction of ndm01, XCT, GCLM and Bach1 in GDM endothelial cells.

The induction of NQO1 by HNE was mainly mediated by Nrf2. In this context, it was demonstrated that knockdown of Nrf2 in normal HUVECs eliminated HNE-induced increase in GSH level and NQO1 expression, while Nrf2 overexpression partially restored NQO1 in GDM cells. There are conflicting reports on Nrf2 activation in endothelial cells exposed to hyperglycemia. When human skin microvascular endothelial cells are exposed to high glucose (30 mmol / L), the nuclear accumulation changes of Nrf2 and NQO1 expression are negligible, while the increased glucose enhances Nrf2 / ARE driven luciferase activity and NQO1 mRNA level in human coronary artery (24,25). In both studies, inhibition of Nrf2 exacerbates glucose-induced ROS production. It was found that elevated glucose only caused a small increase in ROS production and Nrf2-mediated antioxidant response in HUVEC, and the difference between normal cells and GDM cells was negligible. Epigenetic modification is associated with prenatal procedure changes and may regulate Nrf2-linked antioxidant response in GDM endothelial cells. Although CpG island methylation in the Nrf2 promoter seems to inhibit transgenic adenocarcinoma. Vascular Nrf2 levels and adaptive antioxidant defense have been shown to decrease with aging. Since we have demonstrated that Nrf2 signaling in endothelial cells of GDM fetuses is impaired, while others have reported vascular cells in older rodents (altered Nrf2 signaling), it is speculated that the redox phenotype of GDM may be due to persistent intrauterine aging. Spontaneous hypertensive rats are involved in the premature aging of the uterus, in which the intrauterine environment of diabetes is related to the decrease of the life span of offspring. We speculate that intrauterine exposure to maternal diabetes may alter the redox proteome of fetal endothelial cells, resulting in the impairment of Nrf2-mediated antioxidant defense function. Nrf2 plays an important role in maintaining mitochondrial function and protecting cells from endoplasmic reticulum stress.

In conclusion, studies have shown that oxidative stress exists in GDM. GSH, MDA, SOD, HO-1, Nrf2 and NQO1 can be regarded as useful markers to evaluate oxidative stress in GDM. Persistent deficiency of redox signal in endothelial cells of GDM will damage the cellular defense ability of cells in the uterus against oxidative stress and make offspring more susceptible to type 2 diabetes mellitus and cardiovascular disease. Therefore, the Nrf2 antioxidant defense pathway can provide therapeutic targets for reducing diabetes, aging and related oxidative stress.

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