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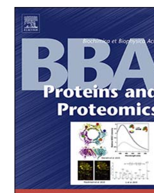

Proteomics analysis of human placenta reveals glutathione metabolism dysfunction as the underlying pathogenesis for preeclampsia☆

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ABSTRACT

Hypertensive disorder in pregnancy (HDP) refers to a series of diseases that cause the hypertension during pregnancy, including HDP, preeclampsia (PE) and eclampsia. This study screens differentially expressed proteins of placenta tissues in PE cases using 2D LC-MS/MS quantitative proteomics strategy. A total of 2281 proteins are quantified, of these, 145 altering expression proteins are successfully screened between PE and control cases ($p < 0.05$). Bioinformatics analysis suggests that these proteins are mainly involved in many biological processes, such as oxidation reduction, mitochondrion organization, and acute inflammatory response. Especially, the glutamine metabolic process related molecules, GPX1, GPX3, SMS, GGCT, GSTK1, NFκB, GSTT2, SOD1 and GCLM, are involved in the switching process from oxidized glutathione (GSSG) conversion to the reduced glutathione (GSH) by glutathione, mercapturic acid and arginine metabolism process. Results of this study revealed that glutathione metabolism disorder of placenta tissues may contribute to the occurrence of PE disease.

1. Introduction

Preeclampsia (PE) was a unique and multisystem syndrome with unknown etiology, and belonged to human hypertension disorder pregnancy (HDP). Nowadays, the incidence of HDP in China arrived at 5.6% to 9.4%, which could increase the risks of the systemic vascular resistance, platelet aggregation, activate the coagulation system and induce endothelial cells function disorder [1]. Clinical manifestations are presented as maternal symptoms of hypertension, proteinuria and multiple system function disorder, the fetal syndrome of growth restriction, amniotic fluid and hypoxia [2]. Especially, the epidemiological investigation presented that risk of death from cardiovascular diseases in women with PE before 34 weeks of pregnancy was 4-8 folds than that of healthy maternal group [3].

Many studies confirmed that the occurrence of PE was closely related to the immune rejection, excessive apoptosis of trophoblastic cells and uterine spiral artery remodeling defect [4]. Immune disorders activated mononuclear phagocyte system to perform the antigen presenting process, which promoted lymphocyte to differentiate into plasma cells, and then produced the excessive antibodies [5]. Abnormal invasion ability of trophoblastic cells in PE patients resulted in the abnormal remodeling of uterine spiral artery and induced the hypoxia in the placenta tissues [6]. The abnormal trophoblastic cells also can secrete pathogenic factors into the peripheral circulation and activate the endothelial cells, and then induced dysfunction of multiple organs [7]. Many studies also showed that continuous hypoxia stimulation

Abbreviations: HDP, hypertensive disorder in pregnancy; PE, preeclampsia; GO, Gene Ontology; iTRAQ, isobaric tags for relative and absolute quantitation; DTT, dithiothreitol; IAA, iodoacetamide; 2D LC-MS/MS, two-dimensional liquid chromatography-tandem mass spectrometry; OD, optical density; IHC, immunohistochemistry; SD, standard deviation; S.E.M., standard error of means; ROC, receiver operating-characteristic curves; AUC, area under the curve; PPI, protein-protein interaction; FDR, false discovery rate; BP, biological process; CC, cellular component

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decreased the invasion ability of trophoblastic cells and increased the TNF-α synthetic, which excessively activated the VEGF signaling and injured the vascular endothelial cells in placenta tissues [8].

Although the mechanism research of PE had made great progresses, it still failed to reduce its incidence. However, many new clues suggested that the occurrence of blood perfusion reduction in placenta tissues was usually accompanied with symptoms of PE. Oxidative stress might be a primary factor connecting this two courses [9]. Many studies showed that microvillus membrane particles originated from apoptotic trophoblastic cells bonded monocytes and neutrophils, increasing the production of inflammatory factors, such as TNF, IL-12 and superoxide radicals. These factors directly disrupt the function of vascular endothelial cells in placenta [10]. Meanwhile, reactive oxygen species (ROS) was also regarded as a critical molecular in activation of inflammation and immune response, furthermore, the degree of oxidative stress in PE patients was significantly higher than normal pregnancy by detecting the biomarker of oxidative stress in their exhaled air [11]. However, the underlying mechanism of excessive oxidative stress-induced the occurrence of PE remained obscure. Otherwise, the classic studies also screened many critical molecules as biomarker of PE, including of glycogen phosphorylase isoenzyme BB (GCBB), CD73, placental growth factor (PlGF), and angiotensin II type I receptor (AT1R) agonistic autoantibodies (AT_1-AA) [12–15]. An increasing number of studies have already focused on seeking for biomarkers with favorable economic, specificity and sensitivity of PE. Here, we provided evidences that glutathione metabolism disorder of placenta tissues contributes to the pathogenesis of preeclampsia using proteomics strategy. Mechanistically, we found that decreased GSH level and the elevated GSSG level of placenta tissues are key roles for excessive oxidative stress and thereby contribute to the development of preeclampsia.

2. Materials and methods

2.1. Reagents

Formic acid (56302) and Acetonitrile (14261) were from Sigma-Aldrich. Isobaric tags for relative and absolute quantitation (iTRAQ) Reagent Multi-Plex Kit (4352135) were from AB Sciex (Foster City, CA). Anti-SOD1 (ab20926), anti-NFκB (ab32360), anti-GCLM (ab124827), anti-SMS (ab156879), anti-GPX1 (ab22604), anti-GPX3 (ab27325), anti-GSTK1 (ab134173), and anti-GGCT (ab198503), anti-GSTT2 (ab176336) were from Abcam. Diaminobenzidine (DAB) Kit (ZLI-9018, China) and Biotin-Streptavidin HRP Detection systems were from ZSGB-Bio Company (Beijing, China). Oxidized Glutathione (GSSG) assay kit and glutathione (GSH) assay kit were from Jiancheng Institute (Nanjing, China).

2.2. Ethics statements

The study was approved by the Medical Ethics and Human Clinical Trial Committee of Pingjing Hospital and First Central Hospital of Tianjin (China) and it was carried out in compliance with the Helsinki Declaration. In the study, a written informed consent to participate was acquired from each individual prior to recruitment. The used materials in this study were collected at the department of obstetrics and gynecology of Pingjing and First Central Hospital (Tianjin, China) in 2014-2016. All methods were carried out in accordance with approved guidelines.

2.3. Clinical samples

The diagnostic criteria of PE was defines as classic studies, which is systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg, and proteinuria (protein/creatinine ratio ≥ 0.3, protein ≥ 1 g/L, or one dipstick measurement ≥ 2 +) appearing after 20 weeks' gestation [16]. The placenta tissues provided by Pingjing Hospital (Tianjin, China) and the First Central Hospital (Tianjin, China) were obtained from 20 health maternal and 20 PE cases, and serum samples were provided by mentioned hospitals. The quartiles of systolic and diastolic pressure in PE and health maternal of development cohort were 143 mm Hg, 127 mm Hg and 95 mm Hg, 78 mm Hg. The amount of 24 h-urinary protein for in PE and health maternal were 6.3 g and 0.1 g. And the baseline characteristics of participate were shown in Table. 1.

Table 1
Baseline characteristics of patients.

Characteristic	PE	Control
Median age (IQR) — year	32 (29–33)	32 (28–34)
Median week of gestation (IQR)	31.5 (28.2–33.4)	32.1 (28.1–35.2)
Median BMI before pregnancy (IQR)[a]	27.3 (25.6–31.3)	24.6 (22.3–30.1)
Median blood pressure (IQR) (mm Hg)		
Systolic	143 (138–148)	127 (113–135)
Diastolic	95 (83–101)	78 (62–88)
24 h-urinary protein	6.3 (5.7–7.8)	0.1 (0–0.2)

[a] The bodymass index (BMI) is the weight in kilograms divided by the square of the height in meters.

2.4. Quantitative proteomics

The potential changes between PE and health maternal groups were analyzed using iTRAQ coupled to two-dimensional liquid chromatography-tandem mass spectrometry (2D LC-MS/MS) strategy (Fig. 1.). To ensure the accuracy of quantitative proteomics, the placenta tissues were divided into the four groups, including two normal groups and two PE groups. To reduce the individual differences and homogenates, 10 independent placenta tissues in each group were pooled together and then were lysed with the lysis buffer (8 M Urea, 5 mM IAA, 50 mM NH_4HCO_3 and protein inhibitor cocktail) followed by sonication and centrifugation. The protein concentration was determined by BCA assay. The equal amount of lysates from each of pooled tissue sample (~100 μg) were reduced with dithiothreitol (DTT) and alkylated with iodoacetamide (IAA). The method of Trypsin/Lys-C Mix digestion in gel was performed according to the previous description [17]. Briefly, 100 μg peptide samples from normal_1 and 2, PE_1 and 2 were labeled by iTRAQ4-113, 114, 115 and 116. Differentially labeled peptides were equally mixed, dried with a speed-vac and then desalted with Sep-Pak C18 Vac cartridges. The labeled samples were separated into 20 fractions with off line Agilent 1100 HPLC system with Xbridge® Peptide BEH C18 column(3.5 μm, 4.6 × 250 mm, Waters, Milford, MA). The fractionated peptides were measured by using LC-MS/MS on an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), which was equipped with an EASY-nLC 1000 ultra-high-pressure liquid chromatography (HPLC) system (Thermo Fisher Scientific, Waltham, MA, USA). The peptides were dissolved in buffer A (0.1% formic acid, 2% acetonitrile in water) and loaded on an home-made C_{18} capillary column (75 μm ID × 15 cm). Then peptides were eluted with a linear 60 min gradient of 8–38% of buffer B (0.1% formic acid in 90% acetonitrile), followed by 38–80% buffer B for 6 min and 80% buffer B for 4 min at a flow rate of 300 nL/min. The eluted peptides were ionized under 2.2 kV voltage by using nano-spray ion source (NSI). For the full MS scan, peptides were measured by Orbitrap analyzer, the mass range was set to 450–1, 500 m/z and the resolution was 60,000 at m/z 200. The automatic gain control (AGC) target was set to 5×10^5, and maximum injection time was set to 50 ms. The 15 most intense ions were subjected to fragmentation via high energy collision induced dissociation (HCD) with 40% normalized collision energy (NCE). The fixed first mass of 100 m/z was used for MS/MS scans in Orbitrap analyzer(resolution was 15,000 at m/z 200). The AGC target was set to 5×10^5, the isolation width was 1 m/z, the dynamic exclusion range was 60 s, and maximum ion injection time was set as 80 ms. All raw

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