Original Paper

Comparative Proteomic Profile of the Human Placenta in Normal and Fetal Growth Restriction Subjects

Zhijing Miao Min Chen Hong Wu Hongjuan Ding Zhonghua Shi

State Key Laboratory of Reproductive Medicine, Nanjing Maternity and Child Health Care Hospital
Affiliated to Nanjing Medical University, Nanjing 210004, China

Key Words

TMT • Fetal growth restriction • Placenta • Oxidative stress • Apoptosis

Abstract

Background: Fetal growth restriction (FGR) is the main cause of intrauterine fetal death and the second leading cause of death in the neonatal period. A large body of evidence suggests that FGR may be associated with the placenta, although its etiology and pathogenesis remain to be fully elucidated. **Methods and Results:** To better understand the molecular mechanisms underlying the pathological development of the placenta in FGR, we used tandem mass tags (TMTs) to construct a large-scale comparative proteomic profile of human placentas from normal and FGR pregnancies. A total of 1,198 kinds of proteins were identified in the control and FGR placentas, of which 95 were differentially expressed between two groups. Ingenuity Pathway Analysis (IPA) was used to organize these differentially expressed proteins into networks of interacting proteins and to identify the modules of functionally related proteins. Western blotting was used to verify the expression patterns of several randomly selected proteins. **Conclusion:** The placentas of women with FGR displayed significant proteome differences compared with normal pregnancy. The results indicate that a variety of mechanisms and proteins may contribute to the development of FGR. Further studies and validations are required to elucidate the exact roles of these proteins in FGR pathogenesis.

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Z. Miao and M. Chen contributed equally to this work.

Zhonghua Shi and Hongjuan Ding

123 Tianfei Road, Obstetrics Department, Nanjing Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University, Nanjing 210004 (China)
E-Mail jesse_1982@163.com and E-Mail dinghj_njfy@163.com

Introduction

Fetal growth restriction (FGR) is a major complication of pregnancy and affects up to 10% of all pregnancies [1]. It is the main cause of intrauterine fetal death and the second leading cause of death in the neonatal period [2]. Although the primary mechanism of FGR is still unknown, a considerable body of evidence suggests that FGR could be associated with many factors such as impaired placental function, inadequate trophoblast invasion, deficient spiral arterial remodeling, and increased apoptosis of trophoblastic cells [3-6]. Notably, recent studies have indicated that impaired placental function may hold the key to the etiology of FGR [3, 7].

The placenta is the interface between maternal and fetal circulation, and it plays several significant roles in pregnancy, such as transporting gases, nutrients, and waste products, preventing the rejection of fetal allografts and producing peptides and hormones [3-8]. In short, placental function holds a key to fetal development or its failure. Recent studies have made some progress in better understanding the molecular mechanisms underlying the pathological development of the placenta in patients with FGR. For example, abnormalities of trophoblast invasion and villous vascular development will lead to a failure in establishing adequate uteroplacental blood flow and may increase the risk of oxidative stress and acidosis [9, 10], indicating the imbalance between antioxidants and reactive oxygen species (ROS) production (oxidative stress) is a critical risk factor in FGR [11-13]. Besides, increased apoptosis was significantly observed in placenta trophoblasts, suggesting apoptosis may be one of the pathogenesis of FGR [14, 15].

Dysfunctions of several molecules, such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, alpha-1-antitrypsin (SERPINA1), insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF), might correlate with abnormal trophoblast invasion and vascular development, and oxidative stress in the placenta of FGR subjects [16-22]. The expression levels of apoptosis associated factors, such as ATP-dependent RNA helicase (DDX42), Bcl-2, p53, low-density lipoprotein (LDL), apo B and matrix metalloproteinases, changed during FGR development [6, 23-28]. However, thus far, no large-scale proteomic analyses have been used to investigate the regulatory factors involved in the placenta of pregnancies with FGR. Therefore, here, we aimed to establish a comparative proteomic profile of human placentas in normal and FGR pregnancies using TMTs.

Materials and Methods

Sample preparation

This study was conducted in accordance with the declaration of Helsinki and performed with approval from the Ethics Committee of the Nanjing Maternity and Child Health Care Hospital affiliated to Nanjing Medical University. Written informed consent was obtained from all participants.

Placental tissues were obtained from twenty pregnant women (10 cases for FGR and 10 cases for control) according to the standard operating procedure. All mothers had undergone cesarean section delivery at the Maternal and Child Health Hospital of Nanjing and had the same range of age and gestational age. FGR refers to a fetus that has failed to achieve its genetically determined growth potential and was defined as estimated fetal weight (EFW) less than the tenth percentile with respect to the reference value for current pregnancy age. The details of patient characteristics are presented in Table 1. For each placenta sample, 0.5 g of placental tissue was dissected from the maternal side of the placenta (in the central region excluding the calcified area), rinsed with 0.9% saline, and frozen in liquid nitrogen prior to use.

Protein extraction

Proteinminer (Aurum serum protein mini kit, Bio-Rad) before proteomic analysis was used to remove high abundance proteins to allow the visualization of low abundant proteins as previously described [29].

Table 1. Clinical characteristics of controls and patients with fetal growth restriction. Data are presented as the means $\pm$ SD. * $P < 0.05$, ** $P < 0.01$

	Controls (n = 10)	FGR (n = 10)	P-value
Maternal age (years)	28.20 $\pm$ 3.71	26.30 $\pm$ 2.41	0.193
Gravidity	1.30 $\pm$ 0.48	1.40 $\pm$ 0.70	0.714
BMI (kg/m ²)	25.06 $\pm$ 2.96	25.73 $\pm$ 2.62	0.597
Gestational weeks	38.64 $\pm$ 1.06	37.62 $\pm$ 2.20	0.207
Birth weight (g)	3305.00 $\pm$ 338.67	1949.00 $\pm$ 325.93	<0.001*

The protein was preserved at -20°C . The supernatant was reduced using 10 mM dithiothreitol (DTT) at 56°C for 1 h, and then alkylated by incubation with 55 mM of iodoacetyl moiety biotin (IAM-biotin) in a dark room for 1 h. Precooled acetone was added to the sample liquid until the total volume was five times the original volume, and the mixture was precipitated at -20°C for at least 1 h. The mixture was then centrifuged at rate of 1000 g for 20 min at 4°C . The precipitate was added to 300 μL of buffer with a final concentration of 50% Tetraethylammonium Bromide (TEAB) containing 0.1% SDS. Then, ultrasound was used to enhance homogenates and cell lysis. Finally, Bradford assay was used to quantify the protein.

Protein digestion

Placenta protein (100 μg) from normal and FGR subjects was diluted with TEAB containing 0.1% SDS. Then, 3.3 μg of 1 $\mu\text{g}/\mu\text{L}$ trypsin was added, and the mixture was immersed in a 37°C water bath for 24 h. Then, 1 μg of trypsin was added, and the mixture was incubated in a 37°C water bath for 12 h. Thereafter, the mixture was vacuum freeze-dried, and resuspended in 30 μL of TEAB containing 0.1% SDS (water: TEAB = 1:1). Finally, MALDI TOF/TOF was used to check the digestive efficiency for 1 μL of the lysate.

Peptide labeling and depuration

10 cases of FGR or 10 cases of control were randomly divided into 3 groups respectively, indicating the protein sample of each group was a mixture from 3 or 4 patients. The labeling reagent was equilibrated to room temperature. Then, 70 μL of isopropyl alcohol was added to the labeling reagent and vortexed for 1 min. The homogenized labeling reagent was then added to the peptides, and isotopic labels of different sizes were used for the different samples. The mixture was vortexed again and equilibrated at room temperature for 2 h. The labeled samples were then dried in vacuo and separated by HPLC and C18 reversed phase chromatography and desalted. Finally, the peptides were dissolved by adding 0.1% formic acid.

Mass spectrometry data acquisition and identification

The labeled peptides were analyzed using high-precision LC-MS (Thermo-fisher Q-Exactive Orbitrap). The MS/MS spectra acquired from precursor ions were submitted to Mascot (version 2.3.01) using the following search parameters for quantitative retrieval: the Swissprot Human Library was used for database search, the enzyme used was trypsin, urea methylation was used for fixed modification, methionine oxidation was used for variable modification, the peptide tolerance was set at 15 ppm, MS/MS tolerance was set at 20 ppm, and the maximum number of missed cleavages was 1. Meanwhile, the following search parameters were used for qualitative analysis: the protein ratio was determined using the median value, the minimum peptides was 1, the median normalization method was used, the p value was set at <0.05 , and the fold change was 1.2.

Western blotting

Western blot analysis of all twenty samples was performed as described previously [30]. Lysates from the placentas of normal and FGR subjects were separated on 15% SDS-PAGE gels and the proteins were then transferred on to nitrocellulose membranes (Amersham Biosciences, RPR303D). The membranes were blocked in Tris-Buffered Saline and Tween 20 (TBST) containing 5% nonfat milk powder for 1 h, incubated overnight with primary antibodies against CSNK2B (1:3000 dilution; Abcam Ab133576, Cambridge, UK), GOSR2 (1:2000 dilution; Abcam Ab115642), HNRNP2 (1:2000; Abcam Ab157498), SERPIN A3 (1:3000 dilution; Abcam Ab88527), SERPIN A1 (1:2000 dilution; Novus 2B12) and GAPDH (1:2000 dilution; Abcam

Ab9485), then washed three times (10 minutes each) with TBST. The membranes were then incubated for 1 h with alkaline phosphatase (AP)-conjugated anti-mouse or rabbit IgG (1:1000 dilution; Promega, S372B, WI, USA). The T protein levels were evaluated by detecting alkaline phosphatase activity using a Lumi-Phos kit (Pierce Biotechnology, KJ1243353).

Statistical analysis

For dimethyl labeling, the following criteria were used to consider a protein for further statistical analysis: two or more high-confidence unique peptides could be identified, the p value was <0.1 , and the fold difference was >1.5 or <0.5 . Then, the Student's t test was used to find the significantly altered proteins with the SPSS software (version 18.0).

The visualized bands of the Western blot analysis were quantified with Quantity One software (Bio-Rad). The protein ratio was determined using the median value. The Student's t test was used to generate p values. Differences were considered significant at a p value of <0.05 .

Bioinformatics analysis

Gene ontology (GO) analysis was used to determine the biological significance of the identified proteins by exploring the relationship between biological terms and genes associated with three structured ontologies including the biological process, molecular function, and cellular components. The identified proteins were divided into different categories according to biological function. Ingenuity Pathway Analysis (IPA) (Ingenuity Systems, Redwood City, CA, USA) was used to organize differentially expressed proteins into networks of interacting proteins and to identify modules of functionally related proteins involved during the pathological changes of placenta in FGR. Proteins whose expression was significantly differentially regulated (fold change ≥ 1.2 , p value ≤ 0.05) were selected for the analysis. A detailed description is given in the online repository (<http://www.geneontology.org> and <http://www.ingenuity.com>).

Results

Identification of placental proteins related to pathological development

To identify proteins that were differentially expressed in the placentas of normal and FGR patients, the 1198 identified proteins were analyzed on the Thermo-fisher Q-Exactive Orbitrap. Examination of the mass spectrometry data with Mascot (version 2.3.01) revealed that 95 proteins showed significant ($p < 0.05$) differential expression between the normal and FGR placentas (Table 2).

Bioinformatics analysis of differentially expressed proteins

Ingenuity Pathway Analysis (IPA) was used to elucidate significant molecular networks and pathways associated with FGR. Comparison of the expression of 95 differentially expressed proteins revealed that 36 proteins were downregulated and 59 were upregulated in FGR, with a fold change of 1.2 (Table 2). The networks of interacting proteins and modules of functionally related proteins were shown in Figure 1. These proteins were linked to two major molecular networks that are linked to erythropoiesis and oxidative stress (i.e., hemoglobin (A) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (B) networks).

Western blotting

We validated the TMT results of the identified proteins with Western blotting using GAPDH as an internal reference. For this, we randomly selected the following five proteins (CSNK2B, GOSR2, HNRNP2, SERPINA1 and SERPINA3) from the list of differentially expressed proteins. As shown in Figure 2, the Western blotting results were essentially in agreement with the results of the MS/MS analyses.

Table 2. The list of differentially expressed proteins in human placenta from control and fetal growth restriction pregnancies. Fold change = Controls/fetal growth restriction (FGR). Fold Change < 0: The protein showed higher expression in the FGR placenta. Fold change > 0: The protein showed higher expression in the controls

Protein ID	Protein names	P value	Fold Change
E7EQ34	Golgi SNAP receptor complex member 2 (GOSR2)	1.33E-09	4.475
G3V513	Alpha-1-antichymotrypsin member 3 (SERPINA3)	1.33E-43	1.643
O15347	High mobility group protein B3 (HMGB3) High mobility group protein 2a (HMG-2a) High mobility group protein 4 (HMG-4)	7.86E-26	1.575
P01781	Ig heavy chain V-III region GAL	0.00045	1.245
P01859	Ig gamma-2 chain C region (IGHG2)	5.00E-43	1.734
P02763	Alpha-1-acid glycoprotein 1 (AGP 1)	1.34E-20	1.433
P05120	Plasminogen activator inhibitor 2 (PAI-2) (SERPINB2)	7.88E-21	1.293
P06756	Integrin alpha-V (ITGAV)	9.05E-38	1.268
P09466	Progesterone-associated endometrial protein (PAEP)	7.65E-06	1.804
P22413	Nucleotide pyrophosphatase (NPPS)	1.75E-88	1.33
P24043	Laminin subunit alpha-2 (LAMA2)	0	1.267
P27487	Dipeptidyl peptidase 4 (DPP4) Adenosine deaminase complexing protein 2 (ADCP-2)	1.55E-121	1.227
Q13576	Ras GTPase-activating-like protein (RASAL)	5.16E-147	1.311
Q8TDX7	NimA-related protein kinase 7 (NEK7)	0.00035	1.308
Q92973	Transportin-1 (TRN1)	5.50E-05	1.284
Q9C005	Protein dpy-30 homolog (DPY30)	1.48E-16	1.246
Q9GZM7	Tubulointerstitial nephritis antigen-related protein (TIN-Ag-RP)	3.18E-105	1.22
Q9Y3B3	Transmembrane emp24 domain-containing protein 7 (TMED7)	0.00024	1.253
Q5SRQ6	Casein kinase 2, beta polypeptide (CSNK2B)	6.47E-05	1.265
B4E1Z4	Complement factor B (CFB)	2.69E-08	1.232
J3KR44	Ubiquitin thioesterase (OTUB1)	1.20E-08	1.203
P20936	Ras GTPase-activating protein 1 (RASA1)	1.46E-80	1.222
P10599	Thioredoxin (TXN)	2.98E-11	1.259
P55268	Laminin subunit beta-2 (LAMB2)	0	1.2
O14908	Tax interaction protein 2 (TIP-2)	1.11E-21	1.245
P62330	ADP-ribosylation factor 6 (ARF6)	7.63E-08	1.31
P55795	Heterogeneous nuclear ribonucleoprotein H2 (HNRNP2)	6.75E-35	1.23
Q00577	Transcriptional activator protein Pur-alpha (TAPP)	3.61E-07	1.275
E9PRY8	Elongation factor 1-delta (EEF1D)	4.44E-12	1.315
P02792	Ferritin light chain (FTL)	2.72E-17	1.257
F5H4W9	Serum paraoxonase/arylesterase 1 (PON1)	0.0051527	1.271
P52597	Heterogeneous nuclear ribonucleoprotein F (HNRNPF)	1.71E-42	1.217
Q6P1A2	Lysophospholipid acyltransferase 5 (LPLAT 5)	7.51E-06	1.233
P31040	Succinate dehydrogenase (SD)	1.66E-23	1.255
P23786	Carnitine palmitoyltransferase II (CPT II)	2.13E-15	1.506
B7ZKJ8	Inter-alpha-trypsin inhibitor heavy chain H4 (ITI4H)	6.01E-35	1.439
P35555	Fibrillin-1 (FBN1)	3.83E-94	-1.2
F8W031	Uncharacterized protein (Fragment)	2.02E-19	-1.202
P05997	Collagen alpha-2(V) (COL5A2)	8.21E-05	-1.206
P04040	Catalase (CAT)	5.09E-56	-1.21
Q14019	Coactosin-like protein (CLP)	1.43E-14	-1.213
P05164	Myeloperoxidase (MPO)	1.64E-39	-1.223
P60903	S100 calcium-binding protein A10 (S100A10)	3.75E-39	-1.225
Q9HA64	Fructosamine-3-kinase-related protein (FN3KRP)	0.001366	-1.228
Q4KWH8	Phospholipase C-like protein 3 (PLC-L3)	3.08E-06	-1.234
P14625	Heat shock protein 90 kDa beta member 1 (HSP90B1) Tumor rejection antigen 1 (TRA1)	5.44E-270	-1.235
P49721	Proteasome subunit beta type-2 (PSMB2)	1.75E-06	-1.239
Q12805	EGF-containing fibulin-like extracellular matrix protein 1 (EFEP1)	7.27E-12	-1.245
P68871	Hemoglobin subunit beta (HBB)	0	-1.246
O75306	NADH dehydrogenase iron-sulfur protein 2 (NDUFS2)	2.35E-15	-1.254
P01860	Ig gamma-3 chain C region (IGHG3)	5.23E-48	-1.256
P16452	Erythrocyte membrane protein band 4.2 (EPB42)	0.0057523	-1.26
P69892	Hemoglobin subunit gamma-2 (HBG2)	1.54E-263	-1.265
Q96BM9	ADP-ribosylation factor-like protein 8A (ARL8A)	2.36E-25	-1.266
P07237	Protein disulfide-isomerase (PDI)	3.06E-189	-1.269
MOQYN0	UPF0556 protein (C19orf10)	2.79E-16	-1.272
P02751	Fibronectin (FN)	0	-1.272
O43396	Thioredoxin-like protein 1 (TXNL1)	0.00043767	-1.275
P21128	Placental protein 11 (PP11)	2.42E-24	-1.277
P16157	Ankyrin-1 (ANK-1)	1.02E-36	-1.279
Q14956	Transmembrane glycoprotein NMB (GPNMB)	1.17E-37	-1.286
P11277	Spectrin beta chain (SPTB)	1.60E-179	-1.299
Q9UNN8	Endothelial protein C receptor (EPCR)	0.00042307	-1.308
Q9NRJ4	Tubby-related protein 4 (TUBL4)	0.014235	-1.31
P46781	40S ribosomal protein S9 (RPS9)	1.25E-06	-1.311
Q04837	Single-stranded DNA-binding protein (SSBP)	3.02E-41	-1.315
MOQYS1	60S ribosomal protein L13a (RPL13A)	1.48E-05	-1.332
K7ER00	Phenylalanine--tRNA ligase alpha subunit t(FARSA)	1.90E-07	-1.339
Q14554	Protein disulfide-isomerase A5 (PDIA5)	5.15E-06	-1.353
J3QQ67	60S ribosomal protein L18 (RPL18)	5.93E-06	-1.359
P32119	Natural killer cell-enhancing factor B Thioredoxin-dependent peroxide reductase 1 (TDPX1)	3.55E-43	-1.364
Q92820	Gamma-glutamyl hydrolase (GGH)	1.89E-11	-1.365
P02549	Spectrin alpha chain, erythrocytic 1 (SPTA1)	2.30E-152	-1.372
P61225	Ras-related protein Rap-2b (RAP2B)	2.31E-09	-1.374
P11498	Pyruvic carboxylase (PCB)	1.75E-07	-1.377
P01009	Alpha-1-antitrypsin (SERPINA1)	2.30E-202	-1.387
P02730	Anion exchange protein 1(AE1) Solute carrier family 4 member 1(SLC4A1)	5.77E-181	-1.398
P07093	Glia-derived nexin (GDN) Peptidase inhibitor 7 (PI-7) (SERPINE2)	3.60E-06	-1.4
P69891	Hemoglobin subunit gamma-1 (HBG1)	1.96E-257	-1.434
P01215	Chorionic gonadotrophin subunit alpha (CGA)	0.00053799	-1.44
J3KN66	Torsin-1A-interacting protein 1 (TOR1AIP1)	1.03E-09	-1.493
O15554	Intermediate conductance calcium-activated potassium channel protein 4 (SK4)	5.12E-13	-1.561
Q3ZCQ8	Mitochondrial import inner membrane translocase subunit TIM50 (TIM50)	1.15E-07	-1.586
P60842	Eukaryotic initiation factor 4A-1 (EIF4A1)	4.47E-90	-1.597
P37108	Signal recognition particle 14 kDa protein (SRP14)	5.58E-10	-1.607
Q14839	Chromodomain-helicase-DNA-binding protein 4 (CHD-4)	8.35E-08	-1.651
C9JV77	Alpha-2-HS-glycoprotein (A2HS)	4.07E-11	-1.657
P69905	Hemoglobin subunit alpha (HBA)	2.14E-136	-1.691
Q9BU23	Lipase maturation factor 2 (LMF2)	1.75E-15	-1.732
Q02543	60S ribosomal protein L18a (RPL18A)	0.00027036	-2.045
Q13361	Microfibrillar-associated protein 5 (MFAP-5) Microfibril-associated glycoprotein 2(MAGP-2)	0.00018138	-2.322
O00442	RNA terminal phosphate cyclase domain-containing protein 1 (RTC1)	9.39E-06	-2.518
G3V1K3	Paraoxonase 2(PON2)	8.93E-22	-3.034
B4DR87	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1 (PLOD1)	1.06E-07	-3.592
Q86XP3	ATP-dependent RNA helicase DDX42(DDX42)	3.25E-05	-4.459

Fig. 1. Schematic representation of network using Ingenuity pathway analysis (IPA) of differentially expressed genes. 35 of 95 total differentially expressed proteins are linked to two major molecular networks: erythropoiesis and oxidative stress which had the highest significant scores (A: hemoglobin network; B: nicotinamide adenine dinucleotide phosphate (NADPH) oxidase network). Relationships are defined in the legends. Green is indicative of genes going up and red depicts genes suppressed in the FGR placenta.

Discussion

FGR is a major complication of pregnancy and affects up to 10% of all pregnancies [1]. It is the main cause of intrauterine fetal death and the second leading cause of death in the neonatal period [2]. Placenta is known as a critical determinant of fetal growth. Many previous studies have shown that structural and functional alterations in the placenta from FGR pregnancies might affect fetal growth and development. However, the fundamental mechanisms underlying these defects are poorly understood [3-6]. Therefore, we attempted to gain a comprehensive map of the proteins that are differentially expressed in FGR placentas.

Here, we carried out a comparative proteome study to determine the proteins that are differentially expressed in human placentas of normal and FGR pregnancies. A total of 1198 unique proteins expressed in the human placenta were identified with high confidence. Of these, 95 were found to be differentially expressed between these two groups. Ingenuity Pathway Analysis (IPA) was used to elucidate the significant molecular networks and pathways associated with FGR. Thirty five of these 95 total differentially expressed proteins

Fig. 2. Western blotting analyses and proteome profiles of five proteins from fetal growth restriction and normal placentas. (A) Western blotting analyses of the five proteins. (B) Corresponding contrast analyses of the blots in A. (C) Proteome profiles of the five proteins in total protein extracts with LC-MS/MS analyses. Student's T-test was used to generate p values. Significant difference was recognized as a p value less than 0.05.

are linked to two major molecular networks that are linked to erythropoiesis and oxidative stress.

Several proteins such as NADPH oxidase, low-density lipoprotein (LDL), and SERPINA1 play critical roles in the pathological development of FGR. NADPH oxidase is an enzyme complex that can generate large amounts of reactive oxygen species (ROS). They are generated at the maternal-fetal interface at the decidual, trophoblast, and mesenchymal levels. Physiological ROS levels play an important regulatory role through various signaling transduction pathways in uterine function, embryogenesis, embryonic implantation, and

feto-placental development [16, 31, 32]. However, the imbalance between antioxidants and ROS production (oxidative stress) is a causative agent in FGR [11-13]. Nutritional and environmental factors are the sources of oxidative stress [33, 34]. There is considerable evidence that FGR is associated with oxidative stress [35-37]. SERPINA1, also known as alpha-1 antitrypsin (AAT), is an acute phase protein and one member of the serpin superfamily of serine protease inhibitors. SERPINA1 has been shown to be a broad-spectrum anti-inflammatory, immunomodulatory, anti-infective and tissue-repair molecule. Moreover, SERPINA1 can increase LDL binding and uptake, and upregulate the levels of LDL receptors [38, 39]. However, decreased SERPINA1 activity, which is associated with oxidative stress, has been reported in FGR placentas [17-19]. SERPINA1 has been shown to be oxidized by peroxide lipids and various oxidants, and oxidized SERPINA1 has multiple effects on cytokine expression, generation of ROS, and intracellular lipid metabolism. Furthermore, oxidized SERPINA1 can release elastase, which may induce vascular abnormalities and stimulate NADPH oxidase, catalyzing the production of superoxides, which in turn causes direct oxidative damage to biomolecules, including SERPINA1 itself [40-42]. All in all, NADPH oxidase, LDL, and SERPINA1 are all associated with oxidative stress, which plays an important role in the pathological changes observed in FGR placentas. Several studies have suggest that using antioxidants (e.g., vitamins C and E) to treat pregnancies that are at high risk of FGR have shown positive effects of reducing the incidence of maternal disease and improving the prognosis of fetuses with FGR [43-45].

Studies have shown that hypoxia can enhance erythropoiesis to improve the oxygen carrying and buffering capacities by increasing the erythrocyte mass and hemoglobin concentration, and high hemoglobin concentrations have been associated with FGR [46-48]. In this work, HBA1, HBA2, HBG1, HBG2, and HBB were all found upregulated in the placenta of FGR. Therefore, abnormal erythropoiesis may be one cause of FGR.

Apoptosis is an essential feature of normal placental development but is notably exaggerated in association with some pregnancy complications [49], such as FGR [14]. DDX42 is a putative ATP-dependent RNA helicase implicated in cellular growth and apoptosis. It is a major apoptosis inducer known to enhance p53 transactivation of proapoptotic genes [23]. In this study, DDX42 was approximately 4.5-fold upregulated. At present, the pathological mechanism underlying increased cell apoptosis in FGR is still not understood. Crocker et al. found an increased susceptibility to apoptosis of primary trophoblasts isolated from patients with FGR [15]. In any case, the end result would be placental dysfunction and suboptimal pregnancy outcomes.

In summary, through comparative proteome analysis of the placentas of normal and FGR pregnancies, we constructed a protein expression profile; identified modules of functionally related proteins; and outlined important proteins that play key roles in the incidence and development of FGR, such as NADPH oxidase, SERPINA1, and DDX42. Further functional analyses of these differentially expressed proteins will help in understanding the critical biological processes and discovery of potential biomarkers and therapeutic targets, and eventually improve the prognosis of fetuses with FGR.

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Disclosure Statement

All authors have no conflicts of interest to declare.

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