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Increased expression of fatty acid binding protein 4 in preeclamptic Placenta and its relevance to preeclampsia

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ABSTRACT

The aim of this investigation was to determine the expression of fatty acid binding protein 4 (FABP4) in the placenta from women with preeclampsia and normal pregnancy, and to delineate the regulatory effects on thophoblast cell by FABP4. We determined the expression of FABP4 by real-time polymerase chain reaction (PCR) for messenger ribonucleic acid (mRNA) or enzyme-linked immunesorbent assay (ELISA) and Western blotting for protein. Small interference of ribonucleic acid (siRNA) and specific FABP4 inhibitor were used to inhibit FABP4. The proliferation, migration and invasion of trophoblastic cells (Swan-71 and Jar) were evaluated with cell counting kit-8, wound-healing test and transwell analysis respectively. We found the expression of FABP4 was significantly higher in the placenta of preeclamptic women than that of women with normal pregnancy ($t = 4.244$, $P < 0.001$ for mRNA; $t = 4.536$, $P < 0.001$ for protein). FABP4 siRNA significantly reduced the proliferation of trophoblasts ($P < 0.001$). The specific inhibition of FABP4 inhibited the proliferation of trophoblasts in a dose-dependent manner ($P < 0.001$) and the inhibitory effect increased as the concentration of inhibitor increased. FABP4 siRNA and specific inhibitor significantly decreased the migration ($P < 0.001$) and invasion ($P < 0.001$) of trophoblasts. We concluded the increase in placental FABP4 expression in preeclampsia may affect the function of trophoblast, and this increase may have a role in the pathogenesis of preeclampsia.

1. Introduction

Preeclampsia affects 2–8% of all pregnancies, and is one of the major causes for maternal mortality and morbidity, preterm birth, perinatal death and intrauterine growth restriction [1–3]. The condition is characterized by hypertension and proteinuria in the second half of pregnancy [1,2]. Preeclampsia is a multisystem disorder that affects the liver, kidneys and cardiovascular and clotting systems. These symptoms are alleviated after the delivery of the fetus and the placenta. The etiology of the disorder is not completely understood [1,2].

Abnormal placentation due to the abnormal function of trophoblastic cell and inadequate maternal response to trophoblast invasion has been regarded as the pivotal and initial pathology of preeclampsia [1,2,4,5]. During the normal development of the placenta, extravillous trophoblasts invade the muscular layer of spiral arteries, and this leads to the remodeling of maternal spiral artery [1,2,6,7]. In preeclampsia, incomplete invasion of trophoblasts into the maternal spiral arteries was observed [1,2,8]. On the other hand, excessive proliferation of placental trophoblast has been found in the placenta of preeclampsia [8–10]. However, the mechanisms behind the abnormal function of trophoblast remain unclear.

Previously, we described that maternal serum level of FABP4 was significantly increased in preeclamptic women compared with normal pregnant women [11]. Scifres et al. further observed that maternal serum FABP4 levels were elevated before the clinical onset of preeclampsia, and this increase occurs independently of maternal body mass index [12]. These findings implicate that FABP4

Abbreviations: FABP4, fatty acid binding protein 4; PCR, poly-chain reaction; siRNA, small interfering RNA.

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may play a role in the development of preeclampsia. FABP4 was expressed in placental trophoblast, and hypoxia, a characteristic pathology of preeclampsia, induced the expression of FABP4 via hypoxia inducible factors 1α and 2α [5,13,14]. However, it remains unknown if placental expression of FABP4 is elevated in preeclampsia and if the elevation of circulating FABP4 is associated with altered expression of FABP4, and also little is known if FABP4 regulates the biological function of trophoblast cell.

The aims of the current investigation were to determine the expression of FABP4 in placenta of women with preeclampsia and normal pregnancy, and to delineate the regulatory effects of FABP4 on trophoblastic cells.

2. Materials and methods

2.1. Subjects

Twenty-nine normal pregnant women and 29 women of preeclampsia (18 of mild and 11 of severe) were recruited in Women's Hospital, School of Medicine, Zhejiang University. Pregnancy was diagnosed upon positive human chorionic gonadotropin test after missed menstruation. Gestational age was calculated by menstrual dating. Ultrasound was performed to confirm pregnancy and gestational age. Preeclampsia were diagnosed and classified according to the criteria recommended by American College of Obstetrics and Gynecologist (ACOG): a systolic blood pressure of 140 mm Hg or higher or a diastolic blood pressure of 90 mm Hg or higher on two occasions at least 6 h apart occurring after 20 weeks of gestation in a pregnant woman with previously normal blood pressure and detectable urinary protein ($\geq$1 + by dipstick or 0.3 g/24 h and more) [15]. Severe preeclampsia was defined as a blood pressure greater than or equal to 160/110 mm Hg with either a urine dipstick showing 3 + or 4 + in a random urine sample or greater than 5.0 g of proteinuria over 24 h [15]. Other evidence of severe disease included elevated serum creatinine, eclampsia, pulmonary edema, oliguria (less than 500 ml per 24 h), fetal growth restriction, oligohydramnios and symptoms suggesting significant end-organ involvement (headache, visual disturbance, or epigastric or right upper quadrant pain). Women who met criteria of preeclampsia but not severe preeclampsia were diagnosed mild preeclampsia.

Exclusion criteria were multiple gestation, diabetes mellitus, chronic hypertension, infectious diseases recognized in pregnancy, premature rupture of membrane, active labor, polyhydramnios and signs of other concurrent medical complication. The control women had no sign of gestational complications and fetal distress and gave birth to healthy neonates of appropriate size for gestational age.

Clinical data and demographic data were collected according to the medical records. The approval of the current study was obtained from Institutional Ethical committee of Women's Hospital, School of Medicine, Zhejiang University, and all the participants provided their informed consents.

2.2. Real-time polymerase-chain reaction (PCR)

Ribonucleic acid (RNA) was isolated using the Trizol RNA reagent according to manufacturer's instructions (Invitrogen Life Technologies, CA, USA). Reverse transcription was performed with random primers using PrimeScript RT reagent kit (Takara Bio Inc, Tokyo, Japan). The sequences of primers for FABP4 were as following:

F: GAATGCGTCATGAAAGGCG;
R: CAATGCGAACTTCAGTCCAGG.

Beta-actin was used as the internal control, and the primers for beta-actin were as following:

F: CAGTCGGTTGGAGCGAGCAT;
R: GGATGGCAAGGGACTTCCTGTA.

Primers were synthesized by Shanghai Sangong Biological Engineering Technology & Services Co. Ltd (Shanghai, China). Real-time PCR was performed on the ABI7900 HT Fast real-time PCR System (Applied Biosystems, USA) using the SYBR Premix EXTaq (Takara Bio Inc, Tokyo, Japan) according to the manufacturer's instructions. cDNA template of 2 μl was used for each reaction in a system of 25 μl. All PCR reactions were performed in triplicate. Thermal cycling conditions included pre-incubation at 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 10 s. LightCycler collected data automatically and analyzed the value of Threshold Cycle (Ct). The fold changes of mRNA expression were detected by using $2^{-\Delta\Delta Ct}$ method.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Placental tissue was minced and homogenized. The resulting suspension was sonicated and centrifuged, and then FABP4 level in the supernatants was measured with ELISA following the instructions of manufacturer (Cloud-Clone Corp., Houston, TX, USA). The protein level was determined with bicinchoninic acid (BCA) kit (Jierui Biotech., Shanghai, China). The placental FABP4 level was normalized against protein level through divided by protein level for each sample and expressed in pg/mg.

2.4. Cell culture, silence and inhibition of FABP4

Trophoblastic cells, Swan-71 and JAR, were cultured in RPMI-1640 medium (phenol red free) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. Cells were transfected with small interference ribonucleic acid (siRNA) (Santa Cruz Inc., CA, USA; catalog number: sc-43592; concentration: 10 μmol/mL) or scrambled siRNA (Santa Cruz; sc37007) using Lipofectamine 2000 (Life Technologies Co., Taipei, Taiwan) according to the manufacturer's protocol. The transfection medium was replaced with complete medium 6 h after transfection, and the cells were incubated for further experiment. To inhibit the function of FABP4, a selective inhibitor of FABP4 (EMD Millipore Inc., Darmstadt, Germany; catalog number: 341310), ((2′-(5-Ethyl-3,4-diphenyl-1H-pyrazol-1-yl) (1,1′-biphenyl) -3-yl) oxy)-acetic acid, which targets fatty acid binding pocket, was added to the medium at the indicated concentrations for 12 h and then the medium was replaced.

2.5. Western blotting

Cells were collected and lyzed in 50 μl cell lysis buffer containing protease inhibitors. The protein concentration was quantified using the BCA Protein Kit (Applygen Inc. Ltd, Beijing, China). The cell lysates were separated on 10% sodium dodecyl sulfate-polyacrylamide gelelectrophoresis (SDS-PAGE) and the proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Massachusetts, USA), blocked with Tris-buffered saline (TBS) and 0.1% Tween 20 (TBS/T) containing 5% bovine serum albumin, and then incubated with anti-human FABP4 antibody (Cell Signaling Technology, USA; diluted at 1:1000 in TBS/T) or anti-human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Cell Signaling Technology Co.; diluted at 1:2000 in TBS/T)) at 4 °C overnight. The membranes were washed three times with TBS/T and then incubated with the appropriate Horseradish-