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Original Article

Expression of markers of endoplasmic reticulum stress-induced apoptosis in the placenta of women with early and late onset severe pre-eclampsia

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ABSTRACT

Objectives: Endoplasmic reticulum (ER) stress-induced apoptosis has been implicated in severe pre-eclampsia (SPE) and is characterized by the activation of three signaling pathways: PKR-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring 1 (Ire1). This study was designed to investigate the role of ER stress in the pathogenesis of SPE.
Materials and methods: Placental tissues were collected from 32 women with normal pregnancies and two cohorts of women with early ($n = 32$) or late onset ($n = 32$) SPE. The expression of glucose-regulated protein 78 (GRP78), PERK, eukaryotic initiation factor 2 subunit a (eIF2α), activating transcription factor 6 (ATF4), ATF6, Ire1, CHOP (CIEBP homologus protein), and caspase 12 mRNA and protein in the placentas was analyzed using real-time reverse transcription-polymerase chain reaction and Western blotting, respectively.
Results: The levels of GRP78, PERK, eIF2α, CHOP, ATF6, and caspase 12 mRNA and protein expression were significantly higher in the placentas of women with early and late SPE than in the control women, whereas there were no differences in ATF6 and Ire1 mRNA and protein.
Conclusion: ER stress-induced apoptosis was important in the development of SPE, especially in early onset SPE and was probably due to the activation of the PERK signaling pathway.

Introduction

Severe pre-eclampsia (SPE) is a pregnancy-specific disease characterized by new onset hypertension, proteinuria, edema, and a series of other systematic disorders after 20 weeks of gestation [1]. SPE is a major cause of clinical morbidity and mortality in pregnant women and prenatal infants, affecting 5–8% of pregnancies worldwide [2,3]. The onset of SPE in most patients occurs before 34 weeks of gestation, a disease defined as early onset SPE. Approximately 10% of women, however, experience late onset SPE, occurring after 34 weeks of gestation [4]. SPE is associated with the abnormal conversion of abnormal implantation and incomplete placental bed vascular remodeling [5]. Early onset SPE that requires preterm delivery has an underlying pathology that may differ from, and be more severe than, that of late onset SPE [4,6,7]. Although the specific pathogenesis of SPE remains unclear, increasing evidence has indicated that endoplasmic reticulum (ER) stress-induced trophoblastic apoptosis may be an important feature of the placental pathophysiology in SPE [3,8–10].

The ER serves multiple functions, including the synthesis, post-translational modification, and trafficking of membrane and secreted proteins. Perturbation of ER homeostasis may result in misfolding or abnormal glycosylation of these proteins, which may reduce their biological activity. The accumulation of unfolded or misfolded proteins within the ER cisternae provokes ER stress and activation of the unfolded protein response (UPR). This UPR attempts to restore ER function by attenuating protein translation, increasing folding capacity, and facilitating degradation of misfolded proteins [11–13]. Normal folding requires that unique conditions be maintained within the ER lumen, and nascent proteins are initially bound to Ca^{2+}-dependent chaperone proteins, such as glucose-regulated protein 78 (GRP78 or BiP) [14,15]. The UPR consists of three principal signaling pathways with overlapping functions (Fig. 1). The three sensor molecules—PKR-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6),

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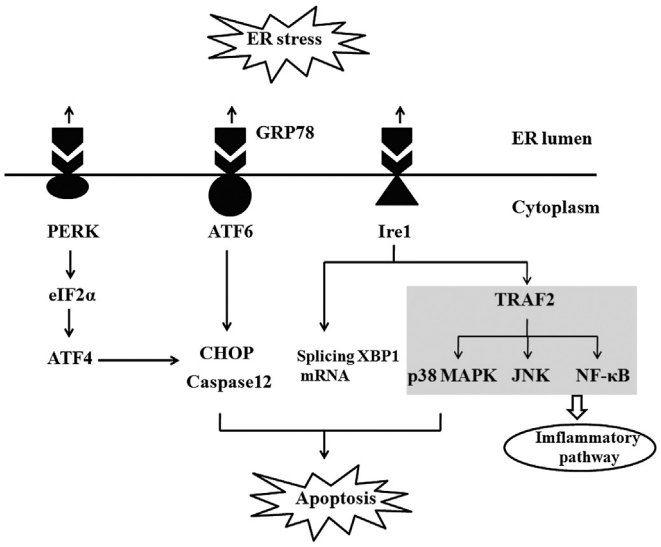

Fig. 1. Diagrammatic representation of the signaling pathways activated in the unfolded protein response (UPR) following endoplasmic reticulum (ER) stress. The sensor molecules PERK, ATF6, and Ire1 are transmembrane proteins normally held inactive by the binding of GRP78, but are released when GRP78 preferentially binds to misfolded proteins accumulating in the ER lumen. The UPR aims to restore homeostasis within the ER, but there are also links to the inflammatory response through the Ire1 pathway. ATF6 = activating transcription factor 6; GRP78 = glucose-regulated protein 78; Ire1 = inositol-requiring 1; PERK = PKR-like endoplasmic reticulum kinase.

and inositol-requiring 1 (Ire1)—are transmembrane proteins with N termini that project into the ER lumen. Normally, these sensors are inactivated through the binding of GRP78 to their N termini, but withdrawal of this chaperone caused by competitive binding to accumulating misfolded proteins results in the dimerization, autophosphorylation, and activation of PERK and Ire1. Activation of PERK results in the phosphorylation of eukaryotic initiation factor 2 subunit a (eIF2α) and translation of activating transcription factor-4 (ATF4), rapidly blocking protein translation and reducing the protein burden within the ER. When ATF6 is released from GRP78, it translocates to the Golgi, where it is cleaved to form a transcription factor that promotes expression of ER chaperone genes. Ire1 contains an endoribonuclease domain; upon activation, Ire1 splices XBP-1 pre-mRNA to produce a variant that activates transcription of genes regulating the breakdown of misfolded proteins, as well as ER biogenesis. Ire1 can also activate proinflammatory pathways through its kinase domain. In severe diseases, PERK and ATF6 can increase the expression of the proapoptotic proteins CHOP (ClEBP homologus protein) and caspase 12, leading to apoptosis [12,16,17].

Although several studies have indicated the importance of ER stress in the development of SPE, the overall expression profiles of these ER stress-related markers in patients with SPE, especially differences between early and late onset SPE, are poorly understood. This study was designed to explore the relationship between ER stress and SPE and to investigate the differences in the expression profile of ER stress-related markers in women with early and late onset SPE. These differences were evaluated by examining the mRNA and protein expression levels of the ER stress markers, GRP78, PERK, eIF2α, ATF4, ATF6, Ire1, CHOP, and caspase 12, in the placentas of normal women and those with early or late onset SPE.

Materials and methods

Patients and sample collection

Patients registered at the Department of Obstetrics and Gynecology, Shandong Weifang People's Hospital affiliated to Shandong University, were enrolled in this study between February 2011 and February 2013. All recruited women were primipara and aged 23–31 years with a body mass index (BMI) ranging from 23 kg/m^2 to 32 kg/m^2. Patients were excluded from the study during the follow-up period if they had a history of hypertension and/or renal disease, infection, smoking, chemical dependency, and multiple pregnancies, and those suffering from confounding pathology, including intrahepatic cholestasis of pregnancy, fetal growth restriction, gestational diabetes mellitus, hyperthyroidism, and hypothyroidism. Common medical and surgical illnesses were excluded according to predelivery examination results from hospital-based electrocardiography, ultrasound, and blood tests. Samples were collected at random from women with normal pregnancies, early onset SPE (<34 weeks of gestation), and late onset SPE (≥34 weeks of gestation) during cesarean sections according to the following diagnostic criteria. SPE was defined as blood pressure >160/110 mmHg on at least two readings within a 4-hour period and proteinuria ≥2 g/d. Sample collection was terminated when the sample size in each group reached 32 cases. The clinical characteristics of the three groups are shown in Table 1.

Placental tissue samples were provided by each pregnant woman following elective cesarean section. The indications for cesarean section in normal pregnancies were breech presentation. The indication for cesarean section in women with early or late onset SPE cases was SPE. Approximately 1 g of tissue was dissected from the central part of the maternal side of each placenta (exclusive of calcified area), rinsed briefly in 0.9% saline, and snap frozen in liquid nitrogen.

The study protocol was approved by the Ethics Committee of Shandong University, and all patients provided written informed consent.

RNA extraction and real-time reverse transcription-polymerase chain reaction

Total RNA was extracted from frozen placentas tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol and reverse transcribed to generate cDNA (PrimeScript RT-PCR kit; Takara Bio; Otsu, Japan). Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed using SYBR green (Bio-Rad, Chicago, USA). The fluorescence emitted by SYBR green was detected using an ABI

Table 1
Clinical characteristics of controls and patients with severe pre-eclampsia (SPE).

	Control (n = 32)	Early onset SPE (n = 32)	Late onset SPE (n = 32)
Maternal age (y)	26.72 ± 2.61	28.06 ± 2.71	26.84 ± 2.84
Gestational weeks	38.37 ± 0.66	32.14 ± 0.52**$^{\#\#}$	36.12 ± 0.79**
BMI (kg/m^2)	26.03 ± 1.90	28.62 ± 3.00**$^{\#}$	27.42 ± 2.41*
Blood pressure (mmHg)			
Systolic	117.21 ± 9.25	158.42 ± 3.22**$^{\#\#}$	165.19 ± 4.87**
Diastolic	74.54 ± 5.58	112.37 ± 3.90**$^{\#\#}$	125.65 ± 2.14**
Proteinuria (g/24 h)	0	3.44 ± 0.68**	3.37 ± 0.64**
Birth weight (g)	3451.13 ± 107.34	1478.25 ± 101.50**$^{\#\#}$	2274.09 ± 78.46**
Placenta weight (g)	561.09 ± 31.22	341.56 ± 27.10**$^{\#\#}$	388.40 ± 33.17**

Data presented as the mean ± standard deviation.
BMI = body mass index.
*p < 0.05, early onset SPE or late onset SPE versus control.
**p < 0.01, early onset SPE or late onset SPE versus control.
$^{\#}p$ < 0.05, early onset SPE versus late onset SPE.
$^{\#\#}p$ < 0.01, early onset SPE versus late onset SPE.