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CLINICAL ARTICLE

Placental proteome alterations in women with intrahepatic cholestasis of pregnancy

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ABSTRACT

Objective: To investigate differences in the placental proteomes of women with intrahepatic cholestasis of pregnancy (ICP) and those with a normal pregnancy. *Methods:* Ten pregnant women diagnosed with ICP were recruited at the First People's Hospital of Yuhang District from October 2011 to September 2012; 10 age-matched healthy pregnant women acted as controls. Total placental proteins were extracted and subjected to two-dimensional polyacrylamide gel electrophoresis followed by mass spectrometry to identify proteins that were differentially expressed in the two groups. *Results:* In total, 37 protein spots with differentially expressed proteins were found. These comprised proteins involved in cytoskeleton activity, blood coagulation, and platelet activation as well as chaperones, heat shock proteins, RNA-binding and calcium-binding proteins, and various enzymes. *Conclusion:* The placentas of women with ICP displayed significant proteome differences compared with women with a normal pregnancy. The results indicate that a variety of mechanisms and proteins may contribute to the development of ICP. Further verification and research are required to elucidate the exact roles of these proteins in ICP pathogenesis.

1. Introduction

Intrahepatic cholestasis of pregnancy (ICP) is a cholestatic disorder that develops in the second or third trimester of pregnancy. It is characterized by elevated bile acid and serum aminotransferase levels, pruritus, and spontaneous resolution of signs and symptoms within several weeks after delivery [1]. However, ICP has considerable effects on the fetus. It increases the risk of spontaneous pre-term labor, fetal distress, and sudden intrauterine death [2]. The disorder can occur in women of all racial backgrounds, but there is considerable geographic variation in the incidence of ICP [3,4]. The pathologic mechanisms underlying ICP-related fetal distress, pre-term labor, and intrauterine death are not fully understood. It seems to be a multifactorial disease. The pathogenesis of ICP involves many factors, including genetic, hormonal, and environmental factors [3]. As yet, the pathogenesis and etiology of ICP remain elusive and incompletely understood.

The placenta has a key role in the regulation of fetal growth and development during pregnancy. Although the pathogenesis and etiology of ICP have not been clarified, increasing evidence demonstrates that the placenta has a vital role in the development of ICP. The normalization of liver tests and the disappearance of pruritus after the delivery of the placenta support this hypothesis [5].

A previous study [5] identified a number of genes that are differentially expressed in ICP placentas. These genes were believed to play an important role in the pathogenesis of ICP. However, changes in RNA levels do not provide direct information about the link between placental abnormalities and metabolic dysfunction in ICP, and the level of RNA expression may also not be representative of the level of protein expression. Therefore, it would be interesting to investigate whether the alterations in RNA expression lead to changes in the functional proteome of the ICP placenta.

Proteomics is a key area of research in the postgenomic era. Advances in the use of two-dimensional electrophoresis (2-DE) and mass spectrometry have led to a rapid expansion of this field in biomedical research [6]. The availability of two-dimensional proteomic maps of placenta and the identification of the placental proteome should facilitate the identification of proteins that are over- or underexpressed in association with ICP.

The present study was conducted to compare the proteomes of ICP and normal placentas to clarify the pathogenesis of ICP.

2. Materials and methods

Demographic and pregnancy-related data from 10 women with ICP and 10 women with a normal pregnancy were collected from the

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medical records at First People's Hospital of Yuhang District, Hangzhou, China from October 1, 2011, to September 1, 2012. Written informed consent was obtained from all participants and the research protocol was approved by the Ethics Committee of the Affiliated Women's Hospital of Zhejiang University in Hangzhou, China. All of the women were Han Chinese. Women with diabetes and high blood pressure were excluded. To prevent potential interference from labor, all placentas were obtained after combined spinal anesthesia and cesarean delivery.

Immediately after cesarean delivery, placental samples were obtained and thoroughly washed in ice-cold phosphate-buffered saline to remove any blood. The samples (size 1 cm^3) were taken from the central area of the placenta between the basal and chorionic plates; the amniotic membranes and decidua were removed. The tissue samples were snap-frozen in liquid nitrogen and immediately stored at −80 °C.

To extract proteins from the placental samples, the frozen tissues were mixed and disrupted in liquid nitrogen and homogenized in lysis buffer containing 7 M urea, 2 M thiourea, 4% w/v 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), 65 mM dithiothreitol (DTT), 0.5% v/v immobilized pH gradient (IPG) buffer (pH 3–10), and 1% v/v protease inhibitor cocktail. For each 100 mg of tissue, 1 mL of lysis buffer was used. After incubation on ice for 30 minutes, the samples were centrifuged at 16 000 g and 4 °C for 30 minutes. The supernatant was collected and the protein concentration was detected using the 2-D Quant Kit (GE Healthcare; Piscataway, NJ, USA). The purified protein samples were stored at −80 °C until 2-DE was performed.

The protein samples from each group were run on three gels to generate a total of six 2-DE maps for analysis. For each gel, the protein sample was mixed with rehydration buffer (7 M urea, 2 M thiourea, 65 mM DTT, 4% CHAPS, 0.5% IPG buffer at pH 3–10, and 0.001% trace bromophenol blue), and the total volume was adjusted to 250 μL (80 μg of protein). The protein samples were applied to nonlinear IPG strips (pH 3–10; Bio-Rad, Hercules, CA, USA) and the strips were then actively rehydrated at 30 V for 12 hours. Isoelectric focusing was carried out using the Ettan IPGphor 3 (GE Healthcare; Piscataway, NJ, USA) system at 200 V for 2 hours, 500 V for 1 hour, 1000 V for 1 hour, 8000 V for 8 hours, and 500 V for 4 hours. Following the first dimension of the 2-DE, the IPG strips were equilibrated for 15 minutes in equilibration buffer 1 (6 M urea, 20% glycerol, 2% sodium dodecyl sulfate [SDS], 0.375 M Tris–HCl at pH 8.8, 1% DTT) before being transferred to equilibration buffer 2 (6 M urea, 20% glycerol, 2% SDS, 0.375 M Tris–HCl at pH 8.8, 2.5% iodoacetamide) for 15 minutes. After equilibration, second-dimension SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 12.5% polyacrylamide gels using the Mini-Protean 3 (Bio-Rad; Hercules, CA, USA) system at 5 mA per gel for 1 hour followed by 15 mA per gel until the bromophenol-blue dye front had run off the bottom of the gels.

The gels were stained with silver nitrate or coomassie brilliant blue (CBB) as previously described [7,8]. After staining, the gels were scanned (ImageScanner III; GE Healthcare) and the protein spots were analyzed using ImageMaster 2D Platinum version 6.0 (GE Healthcare). All gel images were edited and the spots were matched manually. A unique identification number was assigned to matching spots on different gels. The spot volumes were normalized and the volume ratios were calculated for each matching pair. A 1.5-fold change in volume was considered to indicate significantly different protein expression levels.

Spots where the protein levels differed between the normal and ICP groups were excised from the CBB-stained gels and subjected to in-gel digestion with trypsin as previously prescribed [9]. The extracted samples were analyzed by matrix-assisted laser-desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) mass spectrometry, which was carried out by Sangon Biotech (Shanghai, China) using the 4800 Plus MALDI TOF/TOF Analyzer (AB Sciex, Framingham, MA, USA).

Peptide mass fingerprinting was used for protein identification. The mass spectral data were processed using GPS Explorer software version 3.5 (Applied Biosystems; Foster City, CA, USA) and searched

Table 1
Demographic and pregnancy characteristics of the women whose placentas were sampled.[a]

Parameter	ICP (n = 10)	Controls (n = 10)	*P* value
Gravidity	2.7 (1–6)	2.8 (1–4)	>0.2
Parity	1.4 (1–3)	1.5 (1–2)	>0.2
Gestational age, wk	38.1 ± 1.2	38.6 ± 1.1	>0.2
Maternal age, y	28.3 ± 4.4	28.7 ± 3.1	>0.2
Birth weight, kg	3.1 ± 0.1	3.2 ± 0.2	>0.2
Placental weight, g	435.5 ± 32.5	448.0 ± 38.8	>0.2
Sex of neonate			
Female	5	4	
Male	5	6	

Abbreviation: ICP, intrahepatic cholestasis of pregnancy.
[a] Values are given as mean (range) or mean ± SD.

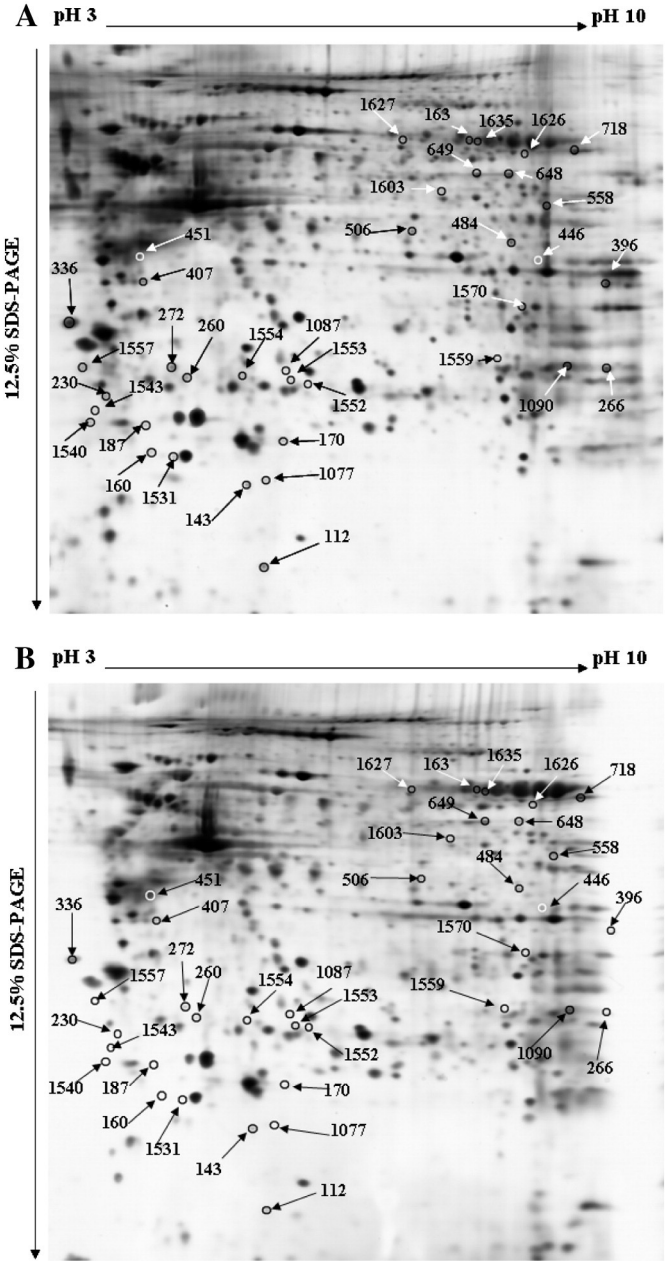

Fig. 1. Representative two-dimensional gel electrophoresis images of placenta samples from women with intrahepatic cholestasis of pregnancy (A) and with a normal pregnancy (B). Spots representing differentially expressed proteins are encircled and labeled with unique spot numbers, which correspond to the numbers in Table 2. White circles indicate downregulated proteins, whereas black circles indicate upregulated proteins.

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