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Original Article

Analyses of placental gene expression in pregnancy-related hypertensive disorders

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Abstract

Objective: To explore the changes in placental gene expression between women with preeclampsia and those with superimposed preeclampsia on chronic hypertension.
Materials and Methods: In Taiwanese population, we compared gene expression between the placentas from preeclamptic patients and those with superimposed preeclampsia on chronic hypertension.
Results: Although top-ranked activated genes between preeclampsia and superimposed preeclampsia on chronic hypertension were different, functional network analyses indicate that these genes are mainly involved in the regulation of cell death and apoptosis. These results suggest that apoptosis and other types of cell death in the placenta are common consequences of both diseases. However, placental endoglin (ENG) was expressed at a significantly higher level in preeclampsia than in superimposed preeclampsia. Results of functional network analysis indicated that ENG may play a role in the pathogenesis of preeclampsia through its interference with the endothelial nitric oxide synthase-regulated vasodilation.
Conclusion: Our results support the fact that ENG is the culprit for the development of preeclampsia. In addition, this study identifies several other genes in the placenta, which are transcriptionally regulated in pregnancy-related hypertensions.

Keywords: Gene expression; Hypertensive disorders; Microarray; Preeclampsia; Pregnancy

Introduction

Preeclampsia is a multisystem disorder unique to human pregnancy, and it occurs in 2–7% of nulliparous women [1]. It is a major cause of maternal and neonatal death and morbidity worldwide [2]. This condition, diagnosed by sustained *de novo* hypertension and proteinuria after 20 weeks of gestation, typically occurs in the third trimester of gestation. The affected mother demonstrates increased blood pressure, edema, proteinuria, abnormal clotting, and liver and renal dysfunction, whereas fetal preeclampsia syndrome can manifest as preterm delivery; growth restriction; placental abruption; fetal distress; and, in some cases, fetal death [2].

Preeclampsia is caused by an adverse maternal response to placentation. To date, however, no single theory could fully explain the pathogenesis of preeclampsia [1]. Historically, two opposing schools of thought have been the immunologists, who consider preeclampsia as a maternal-embryonic immune maladaptation [3–5], and the vascularists, who propose that ischemia-reperfusion leads to oxidative stress and vascular disease [6,7]. Both of these perspectives may be equally important in a recent convergent model for preeclampsia pathogenesis [8].

Pregnant women with chronic hypertension have an increased risk of developing superimposed preeclampsia,

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estimated at 25% versus 2–7% of the general population [1]. Although mixed presentations exist, two broad categories of preeclampsia have been proposed: placental and maternal [8]. In placental preeclampsia, to which pure preeclampsia belongs, the syndrome arises from placenta under oxidative stress. Maternal preeclampsia arises from the interaction between a normal placenta and a maternal constitution that is susceptible to microvascular diseases, such as hypertension or diabetes. Chronic hypertension with superimposed preeclampsia most likely belongs to maternal preeclampsia. With reference to the aforementioned two-stage process of preeclampsia, superimposed preeclampsia might skip the first stage of the immune-related faulty placentation process to start off at the second stage of the vasoconstriction-causing placental ischemia process. However, it remains unknown whether placental preeclampsia and maternal preeclampsia operate from distinct molecular mechanisms.

Given that preeclampsia is of placental origin, it is sensible to compare the gene expression profiles of placentas among pregnant patients with preeclampsia, those with chronic hypertension with superimposed preeclampsia, and normal controls, to understand the pathogenesis of different types of preeclampsia [9]. In this study of Taiwanese women, we analyzed distinct groups of differentially expressed genes and their corresponding functional networks among the placentas collected from women with normal pregnancies, preeclampsia, and superimposed preeclampsia on chronic hypertension.

Methods

Experimental designs and inclusion criteria of the parturient

In this study, 30 cases were included: 10 women in the scheduled cesarean section (CS) group, 13 in the preeclampsia group, and seven in the superimposed preeclampsia on hypertension group. We obtained written informed consent from all participating women. This study has been approved by Institute Review Board of Chang Gung Memorial Hospital (No. 96-0630B).

Preeclampsia was diagnosed in the presence of hypertension and proteinuria [1]. Hypertension is defined as a blood pressure higher than 140 mmHg (systolic) or 90 mmHg (diastolic) on at least two occasions at least 4–6 hours apart. Proteinuria is defined as the excretion of greater than 300 mg protein within 24 hours or a protein concentration of 300 mg/L or more (>1+ on dipstick) in at least two random urine samples taken at least 4–6 hours apart [1]. When a woman was documented as hypertensive before 20th week of gestation, she was considered to have chronic hypertension. Preeclampsia superimposed on chronic hypertension is defined by worsening hypertension and proteinuria occurring in women with documented chronic hypertension [10]. Additional criteria for superimposed preeclampsia include proteinuria development; neurological symptoms, such as severe headaches and visual disturbances; generalized pathological edema; oliguria; pulmonary edema; increased serum creatinine; thrombocytopenia; and appreciable elevations of serum hepatic transaminase.

Specimen collection and processing

Immediately after delivery of placentas, four pieces (about 0.5 cm × 0.5 cm × 0.5 cm each) of placental tissue were cut from the maternal side of placenta, snap frozen in liquid nitrogen, and stored at −80°C.

RNA isolation and DNA microarray experiments

We used Trizol and RNA Easy kit (Qiagen, Valencia, CA, USA) to isolate RNA from placental tissues. The quality and quantity of total RNA were evaluated with Agilent Bioanalyzer 2100 (Agilent Technologies Inc., Palo Alto, CA, USA). Detailed information in Minimal Information About a Microarray Experiment format [11,12] from the Genomic Medicine Research Core Laboratory Human 15K chips can be accessed at the Website http://www.cgmh.org.tw/intr/intr2/c32a0/chinese/corelab_intro/genetics/index_1.htm. We used 2-μg total RNA for labeling and hybridization using the 3DNA Array 350RP Detection kit (Genisphere, PA, USA); scanned slides with a microarray scanner (Axon Instruments, Inc., Foster City, CA, USA); and acquired spot and background intensities with GenePix Pro 4.1 software (Axon Instruments, Inc.).

Microarray data analysis

To carry out within-slide normalization, we used a local weighted regression (www.stat.berkeley.edu/users/terry/zarray/Html/soft.htm) method in which changes of intensity were assumed to be symmetrical for all spots. Thus, normalization was performed in each bin of spots, as previously described [13–16].

Using the matrix transformation [17], the expression level of each gene in individual placental samples was represented as the fold change relative to that of a virtual common reference. For statistical analyses of each gene expression level, Mann-Whitney *U* tests were used with Statistica version 6.1 (Statsoft Inc, Tulsa, OK, USA). *Chi*-square analysis was used for the study of fetal sex in various groups of placental origins. A p value less than 0.05 was used to determine statistical significance.

Network visualization and analysis

Network analyses of differentially expressed genes were performed using MetaCore Analytical Suite (GeneGo Inc, St Joseph, MI, USA) [18,19]. MetaCore is a Web-based computational platform designed for systems biology and drug discovery. It includes a curated database of human protein interactions and metabolism; hence, it is useful for analyzing a cluster of genes in the context of regulatory networks and signaling pathways. For the network analysis of a group of genes, MetaCore can be used to calculate the statistical significance (p value) based on the probability of