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Taiwanese Journal of Obstetrics & Gynecology 50 (2011) 283–291
www.tjog-online.com

Original Article

Analyses of placental gene expression in pregnancy-related hypertensive disorders

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Accepted 25 May 2011

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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1028-4559/$ - see front matter
doi:10.1016/j.tjog.2011.07.005

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

assembly from a random set of nodes (genes) of the same size as the input list [19].

Results

Demographics of the 30 patients included in the hypertensive disorder analysis (Table 1)

Maternal ages in the CS group were greater than those in the preeclampsia group. Ages in mothers with normal cesarean and superimposed preeclampsia were greater than those of the preeclampsia group. Gestational ages in preeclampsia and superimposed preeclampsia groups were significantly lower than those in the CS group, because the most common reasons for preterm deliveries in the preeclampsia and superimposed preeclampsia groups were fetal distress and uncontrollable maternal hypertension. Likewise, fetal body weights in the preeclampsia and superimposed preeclampsia groups were significantly less than those in the CS group.

Blood pressures during early pregnancy (less than 20 gestational weeks) were not statistically different between women with normal pregnancy and those with preeclampsia. Likewise, blood pressures in these two groups were not statistically different during the postpartum period. On the other hand, blood pressures at delivery were significantly elevated in women with preeclampsia and in those with superimposed preeclampsia on chronic hypertension ($p < 0.0001$). During the postpartum period, blood pressures in the preeclampsia group returned to normal, whereas those in the superimposed preeclampsia group remained hypertensive ($p < 0.0001$).

Functional network analyses of differentially expressed genes

To identify differentially expressed genes in preeclampsia and superimposed preeclampsia, we used local weighted regression algorithm to normalize gene expression levels in each microarray, and nonparametric statistics and Mann-Whitney *U* test to compare the expression levels of each gene. Twenty top-ranked differentially expressed genes of each comparison are listed in Table 2. Gene identification number, gene name, *p* values of Mann-Whitney *U* test, and fold changes in the columns titled as Ctr < PE, Ctr < Super, and PE > Super (Table 2) are further summarized in Tables 3–5.

To study the functions of differentially expressed genes in diseases, we analyzed the genes in Tables 3–5 with MetaCore analysis software and database (www.genego.com). Functional network analyses of these lists included the genes that were upregulated in preeclampsia (Fig. 1), those upregulated in superimposed preeclampsia (Fig. 2), and those expressed at a higher level in preeclampsia than in superimposed preeclampsia (Fig. 3). The placental genes that were upregulated in the placentas with preeclampsia (*HSPA1B*, *LIMS1*, *PLAGL1*, *TRIM31*, *PPP2R2C*) (Table 3) and those in superimposed

Table 2
Top-ranked expressed genes in hypertension-related diseases

Ctr > PE	Ctr < PE	Ctr > Super	Ctr < Super	PE > Super	PE < Super
RNF128	*PAF53*	*FOXP2*	*ATBF1*	*HADH2*	*LGALS14*
ADM	*HSPA1B*	*LHPP*	*TGFBI*	*HNRPA1*	*HSD17B2*
ARFIP1	*SLC2A6*	*PRSS16*	*VPREB3*	*C11orf24*	*TPPP3*
SOD1	*LIMS1*	*ACVR1*	*CRIP2*	*MT1A*	*CCAR1*
RNASE1	*FAM53B*	*ZNF397*	*INDO*	*AADAC*	*KCNV1*
CLDN1	*CDC42EP1*	*ABCC2*	*PRAME*	*PAF53*	*SIAH1*
PRPF31	*PLAGL1*	*PEA15*	*KIAA0194*	*HIST1H4J*	*PRDX4*
FIGF	*RNF149*	*AKAP9*	*POGZ*	*CDC42EP1*	*MAN1A2*
BGR4	*RPUSD3*	*DUSP11*	*SIRT4*	*NALP11*	*SGPP1*
PAPPA	*KIF19*	*ISYNA1*	*TEAD3*	*KIAA0141*	*ANK3*
HSPA14	*SLC35D1*	*PROC*	*ABHD2*	*DCAF4L2*	*SELO*
NR2C1	*DAP4*	*MT1G*	*SLC30A5*	*RNPC7*	*ELA3A*
VPS39	*TRIM31*	*SFTPC*	*UBADC1*	*G6PD*	*ZNF430*
MAPK8IP2	*FBXO26*	*BAAT*	*PXMP4*	*KIAA0895*	*UBXD2*
FKBP1A	*PSCD4*	*RPS4X*	*PPM1F*	*TSPAN33*	*FAM63B*
FNDC3A	*UROS*	*TCFL5*	*IST1H2BC*	*RPL24*	*SH3GLB2*
GANAB	*FLJ25006*	*TBL3*	*ZNF22*	*ENG*	*LIME1*
ADAM10	*ANKR*	*TUBB2A*	*CDT1*	*CD48*	*TRH*
NEO1	*PPP2R2C*	*TTC7A*	*PWP1*	*CHI3L1*	*CCNG1*
CHDC1	*MFAP1*	*STAT3*	*MGC2803*	*PLEKHH1*	*CAST*

Ctr = controls; PE = preeclampsia; Super = superimposed preeclampsia.

Table 1
Demographics of the parturient in the hypertensive disorder analysis ($n = 30$)

Clinical information	CS ($n = 10$)	Preeclampsia ($n = 13$)	Superimposed p ($n = 7$)	Overall p[a]
Maternal age (y)	$33.2 \pm 1.6^{1,2}$	$28.1 \pm 1.4^{2,3}$	$33.5 \pm 1.9^{3,4}$	**0.015**
Gravida	2.3 ± 0.5	1.5 ± 0.4	2.8 ± 0.6	0.329
Para	2.0 ± 0.3	1.4 ± 0.3	2.0 ± 0.4	0.261
GA (wk)	$38.8 \pm 1.0^{5,6}$	$33.5 \pm 0.9^{5,7}$	$34.2 \pm 1.2^{6,8}$	**0.0005**
BW (g)	$3270 \pm 262^{9,10}$	$1666 \pm 227^{9,11}$	$2132 \pm 321^{10,12}$	**0.0009**
Systolic BP at GA <20 wk	$109 \pm 4.2^{13,14}$	$121 \pm 3.7^{13,14}$	$155 \pm 5.2^{14,15}$	**< 0.0001**
Diastolic BP at GA <20 wk	62 ± 3.1^{14}	70 ± 2.7^{14}	95 ± 3.8^{14}	**< 0.0001**
Systolic BP at labor	112 ± 4.4^{14}	177 ± 3.8^{14}	167 ± 5.4^{14}	**< 0.0001**
Diastolic BP at labor	61 ± 3.5^{14}	104 ± 3.0^{14}	106 ± 4.3^{14}	**< 0.0001**
Systolic BP, postpartum	114 ± 3.5^{14}	120 ± 2.8^{14}	149 ± 3.3^{14}	**< 0.0001**
Diastolic BP, postpartum	63 ± 3.0^{14}	77 ± 2.6^{14}	101 ± 3.7^{14}	**< 0.0001**

Data are presented as mean ± standard error.

Statistical values were derived from *post hoc* comparisons: $^{1}p = 0.010$; $^{2}p = 0.025$; $^{3}p = 0.033$; $^{4}p = 0.014$; $^{5}p = 0.0006$; $^{6}p = 0.009$; $^{7}p = 0.0005$; $^{8}p = 0.007$; $^{9}p = 0.0002$; $^{10}p = 0.013$; $^{11}p = 0.00004$; $^{12}p = 0.004$; $^{13}p = 0.039$; $^{14}p < 0.0001$; $^{15}p < 0.0001$.

[a] One-way analysis of variance was used for statistics in this table.

BP = blood pressure; BW = fetal body weight; CS = cesarean section; GA = gestational age.

Boldfaced *p* values are those less than 0.05.

Table 3
Top-ranked genes overexpressed in preeclampsia

Gene symbol	Gene ID	Gene name	*U* test	Fold change
PAF53	64425	RNA polymerase I associated factor 53	0.002	1.3
HSPA1B	3304	Heat shock 70 kDa protein 1B	0.018	1.2
SLC2A6	11182	Solute carrier family 2 (facilitated glucose transporter), member 6	0.029	1.2
LIMS1	3987	LIM and senescent cell antigen-like domains 1	0.002	1.2
FAM53B	9679	Family with sequence similarity 53, member B	0.010	1.2
CDC42EP1	11135	CDC42 effector protein (Rho GTPase binding) 1	0.028	1.2
PLAGL1	5325	Pleiomorphic adenoma gene-like 1	0.039	1.2
RNF149	284996	Ring finger protein 149	0.043	1.2
RPUSD3	285367	RNA pseudouridylate synthase domain containing 3	0.045	1.2
KIF19	124602	Kinesin family member 19	0.006	1.1
SLC35D1	23169	Solute carrier family 35, member D1	0.015	1.1
DAP4	22839	Disks large-associated protein 4	0.004	1.1
TRIM31	11074	Tripartite motif-containing 31	0.023	1.1
FBXO26	115290	F-box only protein 26	0.016	1.1
PSCD4	27128	Pleckstrin homology, Sec7 and coiled-coil domains 4	0.037	1.1
UROS	7390	Uroporphyrinogen III synthase (congenital erythropoietic porphyria)	0.009	1.1
FLJ25006	124923	Hypothetical protein FLJ25006	0.035	1.1
ANKR	150709	Ankyrin and armadillo repeat containing	0.032	1.1
PPP2R2C	5522	Protein phosphatase 2, regulatory subunit B (PR 52), gamma isoform	0.037	1.1
MFAP1	4236	Microfibrillar-associated protein 1	0.009	1.1

Gene ID = Gene identification number.

preeclampsia (*ATBF1*, *CRIP2*, *PRAME*, *ABHD2*, *UBADC1*, *HIST1H2BC*, *CDT1*) (Table 4) are mainly involved in the regulation of apoptosis and other types of cell death (Table 6). Of note, the genes that were expressed at a significantly higher level in preeclampsia than in superimposed preeclampsia on chronic hypertension were *HNRPA1*, *G6PD*, and *ENG* (Table 5 and Fig. 3).

Discussion

The placenta plays a central role in fetal and maternal physiology [5]. Not surprisingly, its dysfunctions result in various disorders, such as preeclampsia during pregnancy, coronary heart disease in the later life of the mother, and intrauterine growth restriction and preterm labor of the fetus [1,2,20]. With the advent of genome-wide high-throughput research tools, such as DNA microarrays [13,21], gene expression patterns in the human placenta have been recently explored, demonstrating systemic differences of gene expression profiles among the maternal, fetal, and intermediate layers of the placenta [20].

When DNA microarrays are used to analyze similar tissues, gene expression profiles obtained from different studies have been notoriously varied and even occasionally conflicting. The

Table 4
Top-ranked genes overexpressed in superimposed preeclampsia

Gene symbol	Gene ID	Gene name	*U* test	Fold change
ATBF1	463	AT-binding transcription factor 1	0.027	1.7
TGFBI	7045	Transforming growth factor, beta-induced, 68 kDa	0.027	1.7
VPREB3	29802	Pre-B lymphocyte gene 3	0.014	1.5
CRIP2	1397	Cysteine-rich protein 2	0.029	1.3
INDO	3620	Indoleamine-pyrrole 2,3 dioxygenase	0.008	1.3
PRAME	23532	Preferentially expressed antigen in melanoma	0.033	1.3
KIAA0194	22993	KIAA0194 protein	0.006	1.3
POGZ	23126	Pogo transposable element with ZNF domain	0.025	1.3
SIRT4	23409	Sirtuin (silent mating type information regulation 2 homolog) 4	0.048	1.2
TEAD3	7005	TEA domain family member 3	0.017	1.2
ABHD2	11057	Abhydrolase domain containing 2	0.038	1.2
SLC30A5	64924	Solute carrier family 30 (zinc transporter), member 5	0.041	1.2
UBADC1	10422	Ubiquitin associated domain containing 1	0.017	1.2
PXMP4	11264	Peroxisomal membrane protein 4, 24 kDa	0.036	1.2
PPM1F	9647	Protein phosphatase 1F (PP2C domain containing)	0.034	1.2
HIST1H2BC	8347	Histone 1, H2bc	0.036	1.2
ZNF22	7570	Zinc finger protein 22 (KOX 15)	0.014	1.2
CDT1	81620	DNA replication factor	0.010	1.2
PWP1	11137	Nuclear phosphoprotein similar to *S cerevisiae* PWP1	0.031	1.2
MGC2803	79002	Hypothetical protein MGC2803	0.013	1.2

Gene ID = Gene identification number.

Table 5
Top-ranked genes that expressed at a higher level in preeclampsia than in superimposed preeclampsia

Gene symbol	Gene ID	Gene name	*U* test	Fold change
HADH2	3028	Hydroxyacyl-coenzyme A dehydrogenase, Type II	0.004	2.0
HNRPA1	3178	Heterogeneous nuclear ribonucleoprotein A1	0.012	1.5
C11orf24	53838	Chromosome 11 open reading frame 24	0.033	1.4
MT1A	4489	Metallothionein 1A (functional)	0.029	1.4
AADAC	13	Arylacetamide deacetylase (esterase)	0.028	1.3
PAF53	64425	RNA polymerase I associated factor 53	0.008	1.3
HIST1H4J	8363	Histone 1, H4j	0.036	1.3
CDC42EP1	11135	CDC42 effector protein (Rho GTPase binding) 1	0.009	1.3
NALP11	204801	NACHT, leucine rich repeat and PYD containing 11	0.002	1.3
KIAA0141	9812	KIAA0141 gene product	0.030	1.3
DCAF4L2	138009	DDB1 and CUL4 associated factor 4-like 2	0.017	1.3
RNPC7	58517	RNA-binding region (RNP1, RRM) containing 7	0.046	1.3
G6PD	2539	Glucose-6-phosphate dehydrogenase	0.025	1.3
KIAA0895	23366	KIAA0895 protein	0.014	1.3
TSPAN33	340348	Tetraspanin 33	0.019	1.3
RPL24	6152	Ribosomal protein L24	0.041	1.3
ENG	2022	Endoglin (Osler-Rendu-Weber syndrome 1)	0.017	1.3
CD48	962	CD48 antigen (B-cell membrane protein)	0.013	1.2
CHI3L1	1116	Chitinase 3-like 1 (cartilage glycoprotein-39)	0.003	1.2
PLEKHH1	57475	Pleckstrin homology domain containing, family H member 1	0.010	1.2

Gene ID = Gene identification number.

possible causes for the discrepancy include different assay platforms, nonuniform coverage of gene sets, distinct data-filtering strategies, various statistical stringencies, and data complexity and variability [22–24]. It is, therefore, not surprising that the most differentially expressed genes between normal and preeclamptic placentas in our study were quite different from those of a recent report [25]. In addition to the aforementioned factors, the discrepancy between two studies may also result from the different gestational ages of controls used. Soleymanlou et al [25] compared preeclamptic placentas with those of age-matched controls, and they admitted that the age-matched controls apparently suffered from preterm labor and were not healthy controls. On the other hand, our controls had normal term pregnancies, though not age matched. Given that most cases of preeclampsia end up with preterm delivery, it is impossible to compare gene expression obtained from the

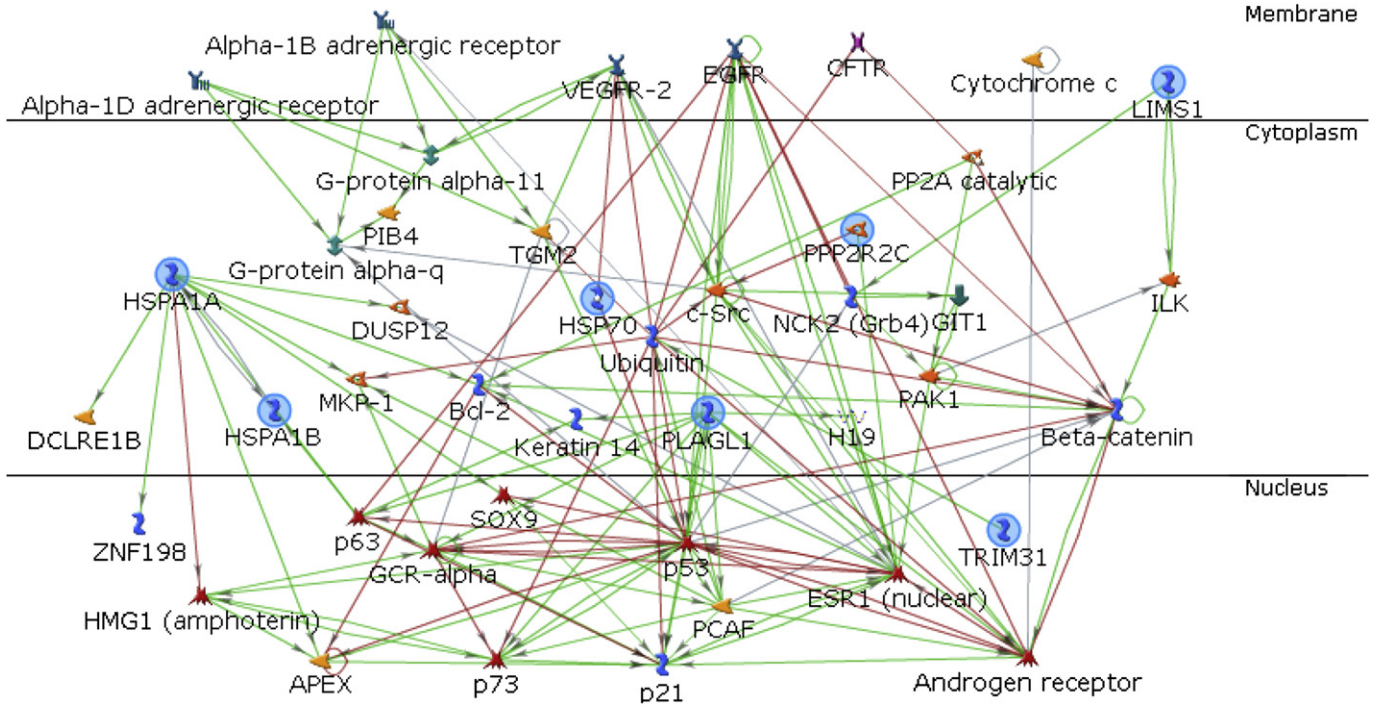

Fig. 1. Network analysis of the genes upregulated (overexpressed) in preeclampsia. Genes in blue circles are the root genes that exhibited upregulation in the placentas with preeclampsia. Green lines indicate stimulation, whereas red lines indicate inhibition.

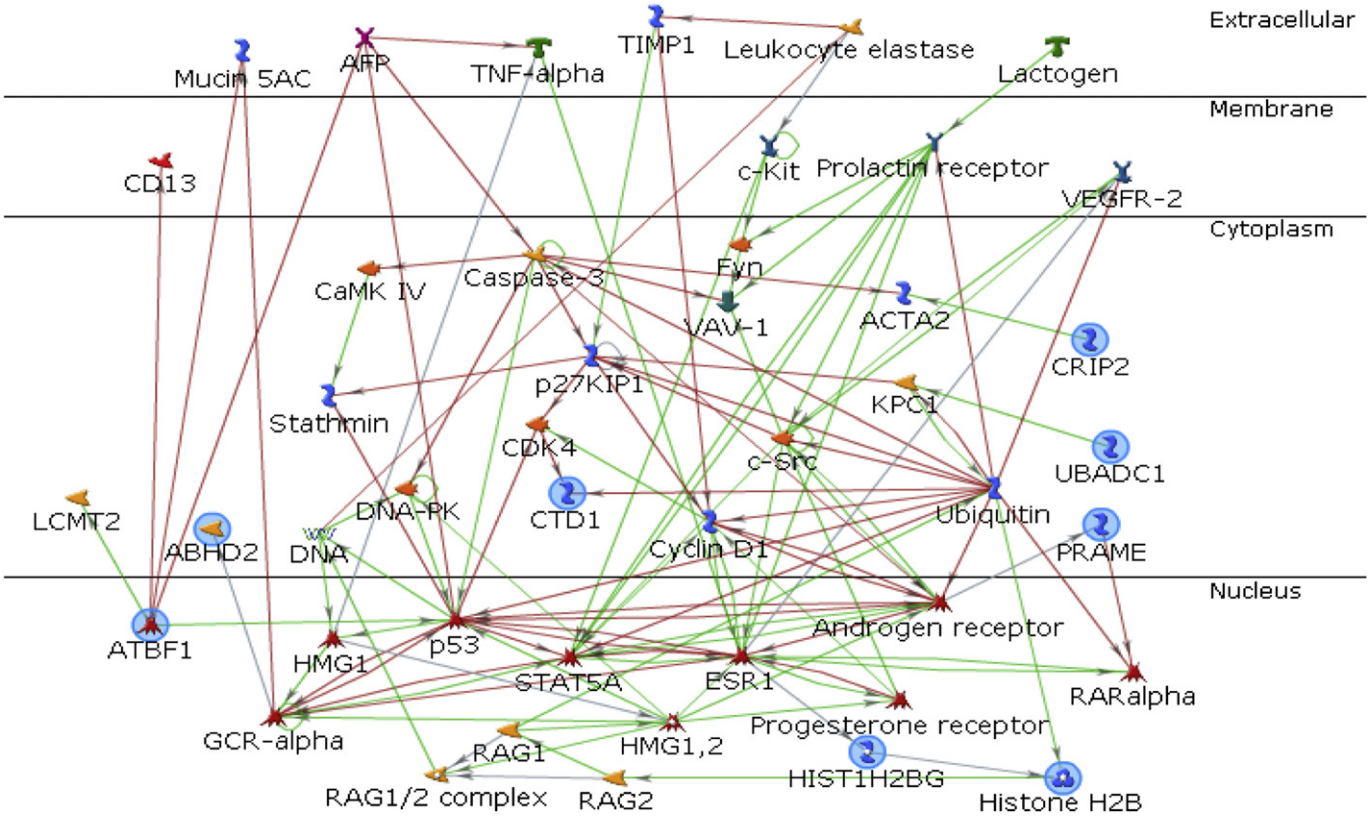

Fig. 2. Network analysis of the genes upregulated (overexpressed) in superimposed preeclampsia. Genes in blue circles are the root genes that exhibited upregulation in the placentas with superimposed preeclampsia. Green lines indicate stimulation, whereas red lines indicate inhibition.

early-onset severe preeclamptic group with that of a perfect age-matched control group [25]. Nevertheless, the consistency of our results on endoglin (ENG) with recent reports validates the quantification of microarray system in this study [26–28].

Among the placental factors that might contribute to the pathogenesis of preeclampsia, ENG has been recently proposed as a placenta-derived soluble transforming growth factor-beta (TGF-β) coreceptor [28]. ENG is elevated in the sera of preeclamptic women; its levels fall after delivery and correlate with disease severity [28]. There was only a mild, though statistically significant, difference in the ENG expression levels between the placentas with preeclampsia and those with chronic hypertension (Table 5). As shown by Reddy et al [26], the ENG levels in both normal and preeclamptic patients decline significantly 24 hours after delivery. The serum levels of ENG in women with preeclampsia dropped rapidly 10 minutes after CS. Our identification of a mild but significantly higher expression level of ENG in the placentas with preeclampsia was consistent with the phenomenon observed [26].

We have been using MetaCore database and software to understand the collaborating functions of groups of genes, which were derived from DNA microarrays [29,30] or proteomics [31,32]. The network generation of MetaCore is based on a frequently updated database, in which each connection between two molecules is established from direct physical interactions between active proteins in human cells using information extracted from experimental literature [19]. In this study, we first selected a list of significantly differentiated expressed genes, which might represent a signature profile for the disease. Then we analyzed those genes based on the database of protein-protein interactions, producing disease-specific functional signature networks consisting of functional pathways organized into a topology tailored for a given condition.

When comparing the functional processes exerted by the upregulated genes in the placentas between preeclampsia and superimposed preeclampsia, most of them are similar—regulation of apoptosis, cell death, and programmed cell death (Table 6). These results suggest that apoptosis and other types of cell death in the placenta are common consequences of both diseases. Because gestational ages were significantly different between normal pregnancy (about 38–39 weeks) and pregnancies complicated with hypertension (about 33–34 weeks) (Table 1), we could not completely rule out the possibility that differential gene expression between normal and preeclamptic pregnancies might partially result from different gestational ages. On the other hand, there was no significant difference in gestational ages between preeclampsia and chronic hypertension with superimposed preeclampsia (Table 1). Therefore, no such confounding factors existed when comparing expression profiles between preeclampsia and superimposed preeclampsia on chronic hypertension.

Based on the results of network analysis (Fig. 3), *HNRPA1*, *G6PD*, and *ENG* were involved in the pathogenesis of preeclampsia. To our knowledge, there is no association

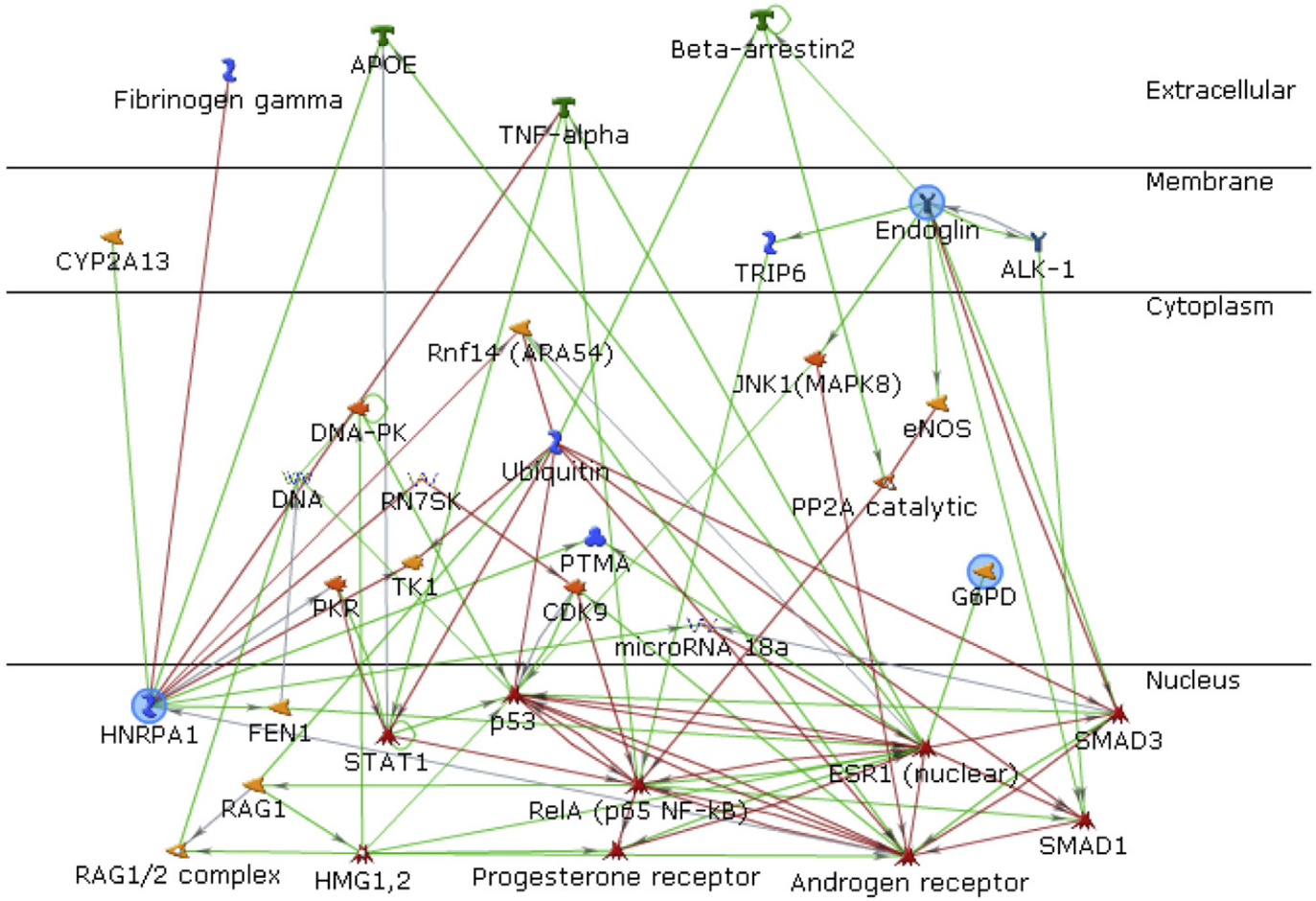

Fig. 3. Network analysis of the genes highly expressed in preeclampsia when compared with superimposed preeclampsia (preeclampsia > superimposed preeclampsia). Genes in blue circles are the root genes that expressed at a significantly higher level in the placentas with preeclampsia than in those with superimposed preeclampsia. Green lines indicate stimulation, whereas red lines indicate inhibition.

between HNRPA1 and preeclampsia, and its role remains unclear. The presence of *G6PD* in both normal and preeclampsia placentas suggests its role in the carbohydrate metabolism of the human placenta [33]. More recently, decreased G6PD activity in preeclampsia was found to be associated with impaired redox regulation in erythrocytes and fetal endothelial cells [34].

On the other hand, ENG activates eNOS [35,36] as well as interacts and modulates Activin receptor-like kinase-1 and -5 signaling [36], resulting in the potentiation of Smad1 and Smad2 and inhibition of Smad3 [36,37], thereby disrupting homeostasis and causing the development of preeclampsia [36]. Venkatesha et al [28] found that ENG decreases the arterial diameter of rat renal microvessels when modulating TGF-β1 and TGF-β3 mediated vasodilation. Subsequently, ENG blocks TGF-β1-mediated activation of eNOS. In human umbilical vein endothelial cells *in vitro*, soluble ENG cooperates with soluble fms-like tyrosine kinase 1 to induce

Table 6
Functional analyses of genes that were upregulated in the placentas with preeclampsia and those with superimposed preeclampsia according to MetaCore database

Upregulated in preeclampsia		Upregulated in superimposed preeclampsia	
Process	*p*	Process	*p*
Regulation of developmental process	3.98E-16	Positive regulation of biological process	6.91E-14
Regulation of apoptosis	5.38E-16	Regulation of developmental process	4.49E-13
Regulation of cell death	7.01E-16	System development	2.81E-12
Regulation of programmed cell death	7.01E-16	Regulation of cell death	3.52E-12
Positive regulation of biological process	4.24E-14	Regulation of programmed cell death	3.52E-12
Regulation of catalytic activity	1.19E-13	Positive regulation of cellular process	3.95E-12
Regulation of molecular function	1.30E-13	Organ development	1.19E-11
Positive regulation of cellular process	4.40E-13	Anatomical structure development	2.12E-11
Negative regulation of apoptosis	5.23E-13	Regulation of apoptosis	2.92E-11
Negative regulation of programmed cell death	6.64E-13	Multicellular organismal development	6.65E-11

E = exponential function.

endothelial dysfunction, and simultaneous administration of both to rats causes a severe preeclampsia-like illness *in vivo* [28,38,39]. Collectively, our results suggest that ENG may play a role in the pathogenesis of preeclampsia through its interference with the eNOS-regulated vasodilation.

Acknowledgments

The authors are grateful to Yu-Chi Lin, Yi-Jun Lin, Yen-Tsun Lin, and Ching-Ling Wang for their technical assistance. This study was supported by grants CMRPG1013-III (S.-D. Chang) and CMRPG361031 (T.-H. Wang) from Chang Gung Memorial Hospital, and NSC-2314-B-182A-156 (T.-H. Wang) from National Science Council, Taiwan.

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