Physiological Changes in the Pattern of Placental Gene Expression Early in the First Trimester

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Abstract

Objective: To assess the physiological changes in the placental expression pattern of a panel of genes related to angiogenesis and oxidative stress during the early part of the first trimester of pregnancy. **Methods and Results:** The expression of a selected panel of genes was quantified by reverse transcriptase–polymerase chain reaction in samples of villous trophoblasts obtained from women between 6 and 11 weeks of gestation undergoing elective artificial abortion. We found that the levels of messenger RNA (mRNA) expression of placental growth factor (PlGF), heme oxygenase 1 (HO-1), and superoxide dismutase (SOD) increased significantly with gestational age ($r = .37$, $P = .001$; $r = .24$, $P = .04$; and $r = .52$, $P < .001$, respectively). Conversely, the mRNA expression level of fms-like tyrosine kinase 1 (FLT-1) decreased significantly ($r = -.30$, $P = .009$). **Conclusion:** During the early part of the first trimester of pregnancy, the placental gene expression levels of PlGF, HO-1, and SOD increase with gestational age, whereas the expression of FLT-1 decreases. The alteration in this pattern of gene expression in early pregnancy may therefore play an important role in placenta-related disorders such as preeclampsia.

Keywords

placenta, villous trophoblasts, fms-like tyrosine kinase 1, vascular endothelial growth factor-A, placental growth factor, heme oxygenase 1, superoxide dismutase

Introduction

Preeclampsia is one of the most common complications of pregnancy, and worldwide about 5% of the pregnant women have this complication.¹ It is associated with serious adverse events, including maternal liver dysfunction, acute renal failure, seizures, cerebral hemorrhage, fetal growth restriction, and death of the mother and fetus.² The pathophysiological mechanisms underlying preeclampsia are not fully understood. However, increasing evidence supports the idea that failure of normal placentation, involving a series of events such as defective remodeling of the maternal spiral arteries,³ a placental hypoxic environment,⁴ oxidative stress,⁵ inflammatory responses,⁶ and endothelial dysfunction⁷ are potential factors contributing to preeclampsia. The search for circulating biomarkers of preeclampsia has been the subject of many ongoing studies. Therefore, increased levels of soluble fms-like tyrosine kinase 1 (FLT-1) and soluble endoglin (ENG) as well as decreased levels of placental growth factor (PlGF) have been reported in the maternal circulation at the time of diagnosis of preeclampsia and even several weeks before the clinical onset.^{7, 8}

In a previous study using tissue obtained by chorionic villous sampling, we showed that the expression levels of genes

related to angiogenesis and oxidative stress were already altered at 11 weeks of gestation in women with subsequent preeclampsia.⁹ These findings suggest that aberrant expression of these genes in the first trimester may contribute to the pathogenesis of preeclampsia. Furthermore, we surmised that the expression pattern of these genes in the trophoblastic villi has physiological variations during pregnancy, particularly in the early stages of placentation, and any alteration in this pattern of expression could potentially contribute to the pathogenesis of preeclampsia. To our knowledge, no previous research assessing the pattern of expression of placental genes in normal pregnancies in the early stage of placentation has been published. In this preliminary study, we aimed to assess the physiological changes in the placental expression pattern of a

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selected panel of genes related to angiogenesis and oxidative stress in the early stage of placentation.

Methods

Participants

Totally, 74 pregnant women between 6 and 11 weeks of gestation undergoing artificial abortion, in private clinics in Tokyo, were prospectively enrolled for this study. All pregnancies were singleton and normally progressing. Fetal heart movement was detected just prior to the operation. Gestational age was calculated by the last menstrual period or the crown-rump length measured by ultrasonography when appropriate (ie, when there was a difference of more than 4 days between these 2 approaches). Informed consent was obtained from all the patients before the surgical procedure. The study was approved by the Ethics Committee of Showa University School of Medicine, Tokyo, Japan.

Samples

Samples of villous trophoblasts were immediately collected and washed using saline solution to remove maternal blood. After removing the vessels and decidual tissues, villous trophoblast samples were stabilized in RNeasy Lysis Buffer (QIAGEN, Hilden, Germany) solution and stored at -80°C until the further analysis.

RNA Preparation and Real-Time Quantitative Reverse Transcription PCR

RNA was extracted from 20 mg of villous trophoblast samples using an RNeasy Mini Kit and an RNase-Free DNase Set (QIAGEN) according to the manufacturer's protocol. A total of 15 μg of RNA in 30 μL of the solution was obtained. In all, 2 μL of this solution was reverse transcribed with an Omniscript RT kit (QIAGEN). Complementary DNA (cDNA) products were amplified by real-time quantitative polymerase chain reaction (PCR), according to the manufacturer's instructions (QuantiTect Probe PCR kit; Qiagen), using 2 μL aliquots of cDNA and the kit components in a reaction volume of 20 μL . We quantified the expression of the messenger RNAs (mRNAs) of the following genes: (1) vascular endothelial growth factor-A (VEGFA), (2) (FLT-1), (3) (ENG), (4) (PIGF), (5) transforming growth factor- β 1 (TGF- β 1), (6) heme oxygenase 1 (HO-1), and (7) superoxide dismutase (SOD). The TaqMan PCR analyses for VEGFA, FLT-1, ENG, PIGF, TGF- β 1, HO-1, and SOD were performed using predeveloped and commercially available primers and probe sets (Cat # Hs00900054_m1 for VEGFA, Cat # Hs01052936_m1 for FLT-1, Cat # Hs00923997_g1 for ENG, Cat # Hs00182176_m1 for PIGF, Cat # Hs0000171257_m1 for TGF- β 1, Cat # Hs00157965_m1 for HO-1, and Cat# Hs00166575_m1 for SOD; Applied Biosystems, Foster City, California). As an initial step, we verified that each PCR assay was specific to mRNA and not to genomic DNA. Amplification data were collected and analyzed with an ABI Prism 7900 T Sequence Detector (Applied Biosystems). Each sample was

analyzed in duplicate, and multiple negative water blanks were included in every analysis. The PCR thermal conditions were as follows: 15 minutes of denaturation at 95°C , followed by 15 seconds of annealing at 94°C , and 1 minute of extension at 60°C . The amounts of mRNA in samples were expressed in terms of copies per milliliter. The expression levels were normalized to those of ribosomal protein large P1 (RPLP1) mRNA and reported as a ratio (ie, relative expression). Previous to this study, a panel of 15 candidate genes in early pregnancy was explored using Human Housekeeping Gene Primer Set (Takara, Tokyo, Japan, Code#3790) and the RPLP1 appeared as the most suitable housekeeping gene (A. Sekizawa, oral communication, September 2011). Reported values are the means of the experiments that were performed in duplicate.

Statistical Analysis

The Spearman correlation analyses were performed to assess the differences in the relative expression of every gene for each gestational age. The statistical analyses were performed by using the JMP software program, version 10.0 (SAS Institute, Cary, North Carolina). A value of $P < .05$ was considered to be statistically significant.

Results

Figure 1 shows the distribution of the samples according to the gestational age in weeks. Graphs depicting the correlation between the placental expression of each gene and the gestational age are shown in Figure 2(A-G). The respective correlation coefficients are shown in Table 1. The relative expression levels of PIGF mRNA increased significantly as the pregnancy progressed from 6 to 11 weeks (Spearman $\rho = .37$, $P = .001$), and conversely, the expression levels of FLT-1 decreased significantly (Spearman $\rho = -.3$, $P = 0.009$). The relative expression levels of ENG and VEGFA showed a tendency to increase with gestational age, although the differences in expression did not reach statistical significance. A study of the expression of genes related to oxidative stress, HO-1 mRNA and SOD mRNA, also showed a significant tendency to increase as the pregnancy progressed (Spearman $\rho = .241$, $P = .04$; Spearman $\rho = .52$, $P < .001$, respectively).

Discussion

In this preliminary report, we focused on the physiological changes in the expression pattern of a panel of genes related to angiogenesis and oxidative stress during the early part of the first trimester. We herein demonstrated that the relative expression level of PIGF mRNA increased significantly with the gestational age, while the relative expression of FLT-1 mRNA decreases with gestational age. Additionally, the expression levels of HO-1 mRNA and SOD mRNA, which are genes related to oxidative stress, also increased significantly with gestational age. Although the relative expression levels of other angiogenic factors, such as TGF- β 1 and VEGFA, showed a

Figure 1. Distribution of cases according to gestational age.

tendency to increase as pregnancy progressed, this tendency did not reach statistical significance.

All of these results showed that physiological variations in the trophoblastic villi are evident even during early placentation. These findings may indicate that the mechanism of angiogenesis in the villi starts at a very early gestational age and is upregulated as the pregnancy progresses. It has previously been reported that the protein levels of soluble FLT-1 (sFLT-1) and PlGF in the maternal circulation increase with the gestational age during pregnancy,¹⁰ but no work has reported the physiological variations in the placental expression of these genes at the very early stages of placentation. Furthermore, previous studies have reported that increased levels of sFLT-1 and reduced levels of PlGF in maternal circulation are detected in preeclampsia at the time of diagnosis, and even several weeks before the clinical onset.^{7,8} In a previous study using tissues obtained during chorionic villous sampling (CVS), we showed that the mRNA expression levels of FLT-1 are increased and the levels of PlGF are decreased as early as 11 weeks of gestation in women who subsequently developed preeclampsia.⁹ In the present study, we showed that there are physiological variations in the pattern of expression of these genes depending on the gestational age, and, interestingly, these variations start during the early stages of placentation. Taken together, these data led us to hypothesize that any of the alterations in this gestational age-dependent physiological pattern of expression may be associated with the pathogenesis of preeclampsia, and this could be evident even earlier than 11 weeks of gestation.

The HO-1, an enzyme that catalyzes the degradation of heme, is known to have antioxidant, anti-inflammatory, and cytoprotective functions.¹¹ It hinders vascular endothelial damage due to oxidative stress, and its upregulation has been proposed as a marker of increased oxidative stress in cultured trophoblast cells.¹² On the other hand, SOD is an enzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. The expression of the HO-1 and SOD genes is stimulated by oxidative stress.¹¹ We have previously shown that the mRNA concentrations of HO-1 and SOD are decreased in the cellular component of the maternal blood of pregnant women with preeclampsia,¹³ and that these mRNA levels are decreased in the cellular component during the first and early second trimester of women who subsequently developed preeclampsia.^{14,15}

In the present study, the expression levels of both genes increased significantly with gestational age from 6 to 11 weeks. Remodeling of the spiral arteries probably begins in the early first trimester and is completed by 18 to 20 weeks of gestation, although the exact gestational age at which trophoblast invasion of these arteries ceases is unclear. This progressive remodeling of the spiral arteries leads to increasing oxygen tension in the intervillous spaces. This event may increase the oxidative stress of villous trophoblasts and correlate with the level of placental expression of HO-1 and SOD. Additionally, a previous study reported that villous trophoblasts cultured under hypoxic conditions (1% O₂) expressed higher levels of VEGFA, FLT-1, and sFLT-1 mRNA. In fact, these cells also produced higher levels

Figure 2. Correlations between the gestational age and the gene expression levels. A, placental growth factor; B, vascular endothelial growth factor-A; C, transforming growth factor- β 1; D, fms-like tyrosine kinase 1; E, endoglin; F, heme oxygenase 1; G, superoxide dismutase.

Table 1. Correlation Coefficients Between the Placental Expression of Genes and Gestational Age.

mRNA	Spearman ρ	Significance
FLT-1/RPLP-1	-.304	0.009
PIGF/RPLP-1	.367	0.001
ENG/RPLP-1	.029	0.806
VEGF-A/RPLP-1	.193	0.099
TGFB-1/RPLP-1	-.058	0.624
SOD-1/RPLP-1	.522	0.000
HO-1/RPLP-1	.241	0.039

Abbreviations: ENG, endoglin; FLT-1, fms-like tyrosine kinase 1; HO-1, heme oxygenase 1; mRNA, messenger RNA; PIGF, placental growth factor; RPLP-1, ribosomal protein large P1; SOD-1, superoxide dismutase 1; TGFB-1, transforming growth factor- β 1; VEGF-A, vascular endothelial growth factor-A.

of the VEGFA and sFLT-1 proteins than cells cultured under normoxic conditions.¹⁶ In contrast, both the PIGF mRNA and the protein levels were decreased by hypoxia, and the ENG mRNA level was not significantly altered.¹⁶ These in vitro results are consistent with our present in vivo work. These findings support the idea that the oxygen tension is crucial for the production of angiogenic and antiangiogenic factors, and that a decreased oxygen tension may disturb the physiological development of the placenta. Furthermore, the hypoxic condition may be reflected by altered placental levels of FLT-1 mRNA and PIGF mRNA, which may be detectable early in the first trimester.

Conclusion

In this study, a number of genes related to angiogenesis (PIGF and FLT-1) and oxidative stress (HO-1 and SOD) were found

to have a distinct pattern of expression that was dependent on the gestational age, the timing of which correlated with the early stages of placentation. Our findings suggest that these physiological variations are crucial for normal placentation, and any early disturbance in the expression of these key genes may play a role in the pathogenesis of placenta-related disorders such as preeclampsia.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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