Pre-eclampsia is a serious multisystem disorder and causes significant increase in both maternal and foetal morbidity and perinatal mortality globally. Due to the limited understanding of the molecular mechanism of pre-eclampsia, the current study conducted bioinformatic analyses to screen key regulators involved in pre-eclampsia. The gene expression profiling dataset GSE44711 containing 8 early-onset pre-eclampsia placentas and 8 gestational-age-matched control placentas was downloaded from the Gene Expression Omnibus database. Differentially expressed genes (DEGs) were screened by llimma software package, which were then subjected to Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis on the Database for Annotation, Visualization, and Integrated Discovery website. Finally, protein–protein interaction network was constructed using the Search Tool for the Retrieval of Interacting Genes database. In total, 192 DEGs including 106 upregulated and 86 downregulated genes were obtained. Proteoglycan 2 and podoplanin were the most significantly up- and downregulated genes, respectively. In addition, three potential pathways and their related DEGs: spermidine/spermine N1-acetyltransferase 1, amiloride-binding protein 1 and adenosynmethionine decarboxylase 1 were associated with arginine and proline metabolism. Vascular endothelial growth factor C; phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit beta; collagen, type I, alpha 1 (COL1A1); and fibronectin 1 (FN1) were associated with focal adhesion. COL6A1 as well as COL1A1 and FN1 were involved in extra-cellular matrix–receptor interaction. The current study identified several potential genes and three pathways which may be considered as candidate targets for diagnosis and therapy of pre-eclampsia.

Keywords: Cell adhesion, differentially expressed genes, nitric oxide, pathway, pre-eclampsia

Introduction

Pre-eclampsia has been classified into early and late onset based on the gestational age at diagnosis or delivery. It is usually referred to an early onset in triploid pregnancies, called ‘early-onset pre-eclampsia’ (Rijhsinghani et al. 1997), which occur during 24–30 weeks’ gestation. Pre-eclampsia is a severe multisystem damage with diverse clinical manifestations, and affects approximately 5%–8% of all pregnancies (Melamed et al. 2012). It can also cause a significant increase in both maternal and foetal morbidity and perinatal mortality (James et al. 2010). Moreover, pre-eclampsia may increase the risk of eclampsia. Until recently, the aetiology and pathophysiology of pre-eclampsia remain largely unknown; however, some factors and mechanisms have been clarified.

It is generally believed that vascular re-modeling throughout gestation is essential for increasing blood flow and nutrient delivery to the foetal-placental unit. The invasion of extra-villous trophoblast cells into the spiral arteries and the replacement of the endothelium mediate spiral arteries re-modeling (Myatt and Webster 2009). Abnormalities in trophoblast invasion and the resulting maternal endothelial dysfunction have important roles in endothelium-dependent vasodilation and could increase thrombus formation (Vanhoutte 2009), ultimately reduce the perfusion of tissues and organs, and cause placental ischaemia and hypoxia (Gilbert et al. 2008), which are symptoms of pre-eclampsia (Ji et al. 2013; Wang et al. 2013). Thus, the failure of cytotrophoblast invasion of pre-eclampsia is the known cause of uterine maternal spiral arteries re-modeling reduction. Many researchers have found that some factors, including angiogenic factors, cell adhesion molecules, hormones and apoptosis-related factors, are important in the invasion process (Fisher 2004; Chen et al. 2012; Louwen et al. 2012; Labarrere et al. 2014). For example, vascular endothelial growth factor (VEGF) mediates the invasion of epithelial and endothelial cells (Fisher 2004). Human chorionic gonadotropin is required for the rapid invasion of extra-villous trophoblast to promote angiogenesis (Chenais and Blanckaert 2012). Moreover, tumour necrosis factor-α was found to induce the impairment of endothelium functions by increasing oxidative stress and decreasing the level of nitric oxide (NO) (Picchi et al. 2006).

ILLUMINATING THE PATHOGENESIS OF PRE-ECLAMPSIA IS VERY IMPORTANT FOR SCREENING PREDICTIVE MARKERS OF PRE-ECLAMPSIA. UNTIL NOW, SOME MARKERS, SUCH AS VEGF AND PREGNANCY-ASSOCIATED PLASMA PROTEIN A (PAPPA), HAVE BEEN VERIFIED TO POSsess SOME PROMISING PREDICTIVE VALUE. HOWEVER, MORE BIOMARKERS ARE STILL NEEDED FOR THE BETTER PREDICTION OF PRE-ECLAMPSIA (GRILL ET AL. 2009). MOREOVER, CURRENT THERAPIES SUCH AS TREDING WATER IMPROVE MATERNAL AND FOETAL HEALTH OUTCOMES ONLY, AND DO NOT ACTUALLY TREAT THE DISEASE (FENTON ET AL. 2013). LACKING TREATMENT MODALITIES, PRE-ECLAMPSIA IS RESOLVED ON PARTURITION, WHICH IS, UP TO NOW,
the only practicable treatment. Overall, treatment modalities that can target the underlying pathophysiological changes and reverse endothelial dysfunction frequently are still blank.

In this study, we introduced bioinformatic analyses to identify differentially expressed genes (DEGs) between normal and pre-eclampsia placentas of pregnancies, which may illuminate the pathogenesis of pre-eclampsia and thus pave the floodgates for possible predictor and targeted drug discovery.

Materials and methods

Affymetrix microarray data

The gene expression profiling data GSE44711 (Blair et al. 2013) employed in this analysis was downloaded from National Center for Biotechnology Information Gene Expression Omnibus database. GSE44711 contains expression data of mRNA from 8 early-onset pre-eclampsia placentas and 8 gestational-age-matched control placentas. Clinical information on the study population is shown in Supplementary Table I to be found online at http://www.informahealthcare.com/doi/abs/10.3109/01443615.2014.990430. Platform of this microarray data is GPL10558 (Illumina Human HT-12 V4.0 expression bead chip).

Pre-processing of microarray data

Normalisation of the microarray data were conducted using quantile normalisation method (Weis 2005) to minimise technical errors in microarray data. The low signal probe data (p > 0.05) were filtered and then the probe IDs were converted into gene symbols using the annotation information in the microarray platform. Finally, mRNA expression values were calculated.

Screening of DEGs

Limma software package (Smyth 2004) was employed to screen DEGs between normal and pre-eclampsia placentas of pregnancies with |log₂ FC (fold change) | > 0.58 and p-value < 0.05 as thresholds. |log₂ FC| > 0.58 equates to fold change > 1.5 or < 0.67 in gene expression.

GO and KEGG pathway analysis of DEGs

Database for Annotation, Visualization, and Integrated Discovery or DAVID is an online accessible software for functional annotation and biochemical pathway analysis (Dennis et al. 2003). For further analysing the potential target gene-related pathways and probing functions of DEGs, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analyses were performed on DAVID website (p-value < 0.05).

Protein–protein interaction network

The database Search Tool for the Retrieval of Interacting Genes (STRING) (Franceschini et al. 2013) is a pre-computed resource providing a global perspective for the exploration and analysis of protein–protein associations. DEGs-associated protein–protein interaction (PPI) network was constructed by the STRING database. Entire list of DEGs was submitted to the STRING database to obtain interaction partners with a threshold of combined score above 0.4. Widely used open-source software Cytoscape network viewer (Kohl et al. 2011) was used to integrate and construct the interaction network. Genes with degree of at least 6 obtained from this network were subsequently subjected to KEGG pathway analysis.

Sub-networks of protein–protein interaction

MalaCards (Rappaport et al. 2013) is an integrated human disease database (http://www.malacards.com/) linked to GeneCards for researchers and clinicians of comprehensive search. DEGs of PPI network were searched in the MalaCards for the pre-eclampsia-related genes.

Clustering with Overlapping Neighbourhood Expansion (ClusterONE) (Nepusz et al. 2012) is a method for detecting possibly overlapping protein complexes in PPI network. Sub-networks containing at least one gene identified in MalaCards of this PPI network were identified with ClusterONE.

Results

Screening of DEGs

After normalisation of the microarray data, DEGs were screened by limma software packages with |log₂ FC| > 0.58 and p < 0.05 as thresholds. In total, 192 DEGs including 106 upregulated and 86 downregulated genes in samples of early-onset pre-eclampsia placentas were obtained. Proteoglycan 2 (PRG2) (Log₂ FC = 3.616) and podoplanin (PDPN) (Log₂ FC = −1.664) were the most significantly up and downregulated genes, respectively (Table I).

KEGG and GO analysis of DEGs

KEGG pathway analysis of DEGs revealed that upregulated genes were enriched in two pathways, namely hsa05200: pathways in cancer and hsa00330: arginine and proline metabolism. Three upregulated DEGs involved in the pathway of arginine and proline metabolism were spermidine/spermine N1-acetyltransferase 1 (SAT1), amiloride-binding protein 1 (ABP1) and adenosylmethionine decarboxylase 1 (AMD1). Downregulated genes were enriched in three pathways. In addition, extra-cellular matrix (ECM)–receptor interaction got the highest enrichment score with p-value = 0.00467 and −log₁₀ (p-value) = 2.331, followed by focal adhesion and nicotinate and nicotinamide metabolism (Figure 1).

GO functional enrichment analysis revealed that upregulated DEGs were enriched in 24 terms of GO-biological process (BP), which mainly focussed on three portions: hormone biosynthetic, cell adhesion and localisation of cell. Meanwhile, downregulated DEGs were intensively associated with 9 terms of BP, which were partly related to cell adhesion and ECM formation (Figure 2).

Protein–protein interaction network of DEGs

There were 26 upregulated and 19 downregulated genes which took part in this PPI network (Figure 3). In total, 14 nodes (proteins) with degrees up to 6 or more were obtained. Among them, fibronectin 1 (FN1); interleukin 2 or IL2; phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta (PI3KB); and VEGFC were upregulated genes, while collagen, type I, alpha 1 (COL1A1) as well as COL6A1 were downregulated genes. Furthermore, KEGG pathway analysis indicated that these 14 genes were enriched in 5 pathways. They were hsa04512: ECM–receptor interaction, hsa04150: mTOR...
signalling pathway, hsa05200: pathways in cancer and hsa04060: cytokine–cytokine receptor interaction. It was interesting to see that COL1A1 and FN1 were both involved in pathways of focal adhesion and ECM–receptor interaction. Additionally, VEGFC and PIK3CB involved in the pathway of focal adhesion. Meanwhile, COL6A1 involved in the pathway of ECM–receptor interaction.

Sub-networks of protein–protein interaction

COL1A1 and FN1 were pre-eclampsia-related genes recorded in MalaCards. In addition to them, three other DEGs in the PPI network including corticotropin releasing hormone (CRH), folliclistatin (FST) and PAPPA were also recorded in MalaCards.

A total of 11 highly interconnected sub-networks were identified from this PPI network using ClusterONE. Five of these sub-networks contained one or more pre-eclampsia-related genes recorded in MalaCards (Figure 4). KEGG analysis showed that genes of sub-network 1 were enriched in focal adhesion and ECM–receptor interaction. Genes of sub-networks 3 and 4 were also partly involved in these two pathways. Besides, mTOR signalling pathway, pathways in cancer and cytokine–cytokine receptor interaction were pathways of genes in enriched sub-network 1.

Discussion

Pre-eclampsia, abnormality of angiogenesis growth and placentation, is a complex pathological process and is closely related with many genes and biological processes (Plaks et al. 2013). In the current study, several bioinformatic analyses were conducted to screen pre-eclampsia-related genes and pathways for a clearer understanding of pre-eclampsia. DEGs including 106 upregulated and 86 downregulated genes were obtained. Further analyses identified 3 key pathways (arginine and proline metabolism, ECM–receptor interaction and focal adhesion) and several relevant genes. These genes and pathways may be responsible for the occurrence and development of pre-eclampsia.

Based on the pathway of arginine and proline metabolism, many molecules were biosynthesized such as proline, ornithine, creatine, polyamine and γ-aminobutyric acid (GABA) (Wu et al. 2009). Arginine is pivotal in this pathway and is utilised as a precursor for the synthesis of these molecules and NO (Mateo et al. 2007; Morris 2007). Vadillo-Ortega et al. (2011) found that L-arginine could prevent preeclampsia. In addition, previous animal studies have shown that placental angiogenesis in mammals is correlated with maternal plasma arginine level (Reynolds et al. 2010) and uteroplacental blood flow (Neri et al. 1995). The L-arginine–NO system is suggested to play important roles in these biological processes. Endothelial cell damage is considered pivotal in the pathogenesis of preeclampsia. Impaired NO bioactivity is strongly linked to endothelial dysfunction and hypertension – symptoms that are common in pre-eclampsia (Ilkka et al. 2011). In addition, SAT1, one of the upregulated DEGs in this pathway, is overexpressed under oxidative stress (Wang et al. 2004) and may help to promote cell survival in poor environment such as oxygen deficiency (Kim et al. 2005). SAT1 may also involve in cell adhesion (Wang et al. 2004). APB1, also known as ‘diamine oxidase’, is a membrane-associated amine oxidase (Ippolito et al. 2005) involved in the pathway of arginine and proline metabolism. Abnormally expressed APB1 is linked with foetal distress and even intra-uterine death (Maintz et al. 2008). The third DEG that participated in arginine and proline metabolism was AMD1. The overexpression of AMD1 in Xenopus embryos could activate maternal apoptosis to protect embryogenesis (Masatake et al. 2003). However, study of AMD1 in human embryo is poor. Taking into account the symptoms of pre-eclampsia, arginine and proline metabolism and these three DEGs may be associated with pre-eclampsia.

Two other notable pathways were ECM–receptor interaction and focal adhesion. Focal adhesion pathway is activated by ECM–receptor interaction, and thus these two pathways would be partly considered as the equivalent system (Birnie et al. 2008). Focal adhesion is a specialised structure of cell adhesion and
controls cell adhesion to ECM. Many researchers have found that ECM–receptor and cell adhesion not only mediate interactions between cells and their environment during early human development (Rozario and DeSimone 2010; Kim et al. 2012), but also play critical roles in differentiation, migration and invasion of trophoblasts (Damsky et al. 1993) at the foetal–maternal interface (Bruchova et al. 2010). The depth of trophoblastic invasion is more shallow in pre-eclampsia patients than in normal women (Kaufmann et al. 2003). Poor differentiation and shallow invasion of trophoblast impede the maternal uterine spiral arteries re-modeling and reduce blood flow, and then lead to placental hypoxia–ischaemia and intra-uterine growth retardation which are symptoms of pre-eclampsia (Ji et al. 2013; Wang et al. 2013). Cell adhesion is also connected with immunity and inflammation. Adhesion molecules mediate the attachment of leucocytes to the endothelium to initiate the endothelial dysfunction (Springer 1990), which is a hallmark of pre-eclampsia (Szarka et al. 2010). Additionally, 5 DEGs (COL1A1, FN1, CRH, FST and PAPPA), the known pre-eclampsia-related genes recorded in MalaCards, were mainly enriched in these two pathways. For example, COL1A1 is associated with ECM–receptor interaction and trophoblastic migration (Goddard et al. 2006); FN1 is involved in cell adhesion and migration processes (Pankov and Yamada 2002). Three other DEGs (VEGFC, PIK3CB and COL6A1) were also engaged in these two pathways. VEGFC is one member of VEGF family. This family is a endothelial-cell-specific growth factor stimulating angiogenesis and vascular permeability (Byrne et al. 2005). VEGFC is associated with pre-eclampsia (Srinivas et al. 2010). PIK3CB is a catalytic subunit beta of phosphoinositide 3-kinases (PI3Ks). PI3Ks are a family of lipid kinase responsible for many cellular responses such as proliferation, vascular trafficking and cell migration (Marone et al. 2008). COL6A1, similar to COL1A1, belongs to the collagens superfamily which are components of ECM (Chung et al. 2005). Therefore, pathways of focal adhesion and ECM–receptor interaction and the above-mentioned three

Figure 2. GO-BPs enrichment analysis of DEGs. Upregulated DEGs (A) and downregulated DEGs (B). The y-axis represents enriched GO terms and gene number. The x-axis represents enrichment score (−log10 0.05 = 1.301).
DEGs as well as the five known genes recorded in MalaCards may play critical roles in pre-eclampsia.

Additionally, PRG2, one of the pregnancy-specific glycoproteins (PSGs) family members, was the most significantly overexpressed gene. PSGs are distributed widely in vessel walls and placenta endothelium (Warda et al. 2008). They may also involve in the development of placenta through regulating the secretion of VEGFC (Lisboa et al. 2011). Composition alternations in PRG of umbilical cord arteries is associated with pre-eclampsia (Gogiel et al. 2002). In addition, the most significantly downregulated gene was PDPN. It encodes a type-I integral membrane glycoprotein distributing in diverse human tissues. Numerous researchers intended to explain the correlation between cell migration and PDPN (Hantusch et al. 2007; Rodrigo et al. 2010). Moreover, Freitag et al. found that PDPN is part of the angiogenesis-associated factors (Freitag et al. 2013). Therefore, PRG2 and PDPN may also be pre-eclampsia-related genes.

In conclusion, our studies implied that arginine and proline metabolism, ECM–receptor interaction and focal adhesion may have vital physiological functions in regulating the development of the placenta with or without pre-eclampsia. Various notable DEGs involved in these pathways including SAT1, ABP1, AMD1, VEGFC, PIK3CB and COL6A1 as well as 5 additional genes recorded in MalaCards may be pre-eclampsia-related genes. In addition, PRG2 and PDPN may also involve in the pathogenesis of pre-eclampsia. This would be a novel illumination for exploring effective diagnose and treatment modalities.

Figure 3. PPI network of DEGs. Red and green nodes represent up- and downregulated DEGs in pre-eclampsia placentas, respectively. Blue nodes represent genes of normal expression. Degree of a node in the network is the number of connections it has with other nodes.
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Figure 4. Sub-networks derived from PPI network. Red and green nodes represent up- and downregulated DEGs in pre-eclampsia placentas, respectively. Blue nodes represent genes of normal expression.
Supplementary material available online

Supplementary Table I.