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Original Research

Microarray analysis of preterm preeclampsia

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Abstract: Premature preeclampsia is the second cause of maternal mortalities around the world. To investigate its potential driving mechanism(s), we constructed a multi-regulatory-mediated preeclampsia dysfunction module. Through combining differential expression analysis, co-expression analysis, and enrichment analysis, we obtained 23 sets of preeclampsia expression disorder modules in the disease, which involve the modular aggregations of 3016 genes. The modules were subjected to be analyzed for GO and KEGG paths for enrichment analysis. Based on these pivotal regulators, it is possible to manipulate the essential parts of the modular subnetwork and study their cooperative acts to mediate the driving mechanism of the preeclampsia. Simultaneously, they mainly cause the onset of the disease through the regulation of the apoptotic signaling pathway, down-regulation of an inflammatory response and retinol metabolism. This may present a potential driving mechanism for the disease. The predictor analysis of the regulators showed a series of non-coding RNAs that have potentially significant regulatory effects on the disease, including miR-182-5p, miR-200b-3p, miR-23a-3p, miR -429, miR-590-3p, and transcription factors. These pivotal regulators might mediate the potential driving processes. Based on a comprehensive multivariate analysis, we found a possible driving mechanism in which significant pivotal regulators were used as distinct functional segments in the preterm preeclampsia-driven process.

Key words: Early preeclampsia; Imbalance module; Pivot regulator; Module subnetwork; Drive process.

Introduction

Premature preeclampsia is the second cause of maternal mortalities around the world, and it affects approximately 2-8% of pregnant women, which would be caused by new-onset hypertension and proteinuria during pregnancy (1). Premature birth and preeclampsia would have a common pathophysiological mechanism. If a woman has a premature birth, it might be due to early preeclampsia during labour (2). The preeclampsia may be started by autoantibodies, misfolded proteins, nitric oxide, and the role of oxidative stress, as well as the upstream triggering of the angiogenic pathway, such as the heme oxygenase and hydrogen sulfide pathways (3). During the second half of pregnancy and perinatal period, the blood pressure of more than 140/90 mm Hg and proteinuria might be two main signs of preeclampsia (5, 6).

Regarding the preeclampsia and preventive drugs, low vitamin D level in pregnancy is associated with the increased risk of adverse outcomes (10). For preterm pre-eclamptic pregnant, antiplatelet drugs can help to reduce their spontaneous preterm delivery (11). Besides, in early pregnancy, low-dose aspirin might reduce the prevalence of both preeclampsia and IUGR (12). Statins, as preventive drugs for the disease (13), apparently allow endothelial cells to exert their protective effects by inducing Hmox-1 expression and releasing sFlt-1; as well, the statins cause these cells to produce strong antioxidant products (13). In theory, preeclampsia can reduce the risk of related severe diseases through intensive monitoring and preventive measures (14).

In order to develop new candidates for biochemical markers of preterm preeclampsia severity, the ratio of soluble vascular endothelial growth factor receptor to soluble epidermal growth factor receptor can be determining (15). In addition, Bellos et al. showed that mean platelet volume (MPV) is a valuable biomarker to detect and track the preeclampsia (16).

In the present study, we analyzed the data from the background set of preeclampsia in order to explore the relevant causes of preterm preeclampsia. Our results can provide valuable resources and guidance and offer new insights into effective prevention and treatment of preterm preeclampsia.

Materials and Methods

Difference analysis

From the NCBI Gene Expression Omnibus (GEO) database, we collected an expression microarray dataset for the preterm preeclampsia samples and assigned the code 'GSE66273' to it. Limma package (in R language) performed a differential analysis on the collected samples, i.e., preterm preeclampsia and preterm birth control, to produce differential genes involved in the construction of expression profile matrices (17-21).

Co-expression analysis

Weighted gene co-expression network analysis

(WGCNA) analyzed the associated phenotypes of the expression profiles. Further, the goal is to find gene modules for coordinated expression. The correlation coefficient weighting value is used, that is, the gene correlation coefficient is taken to the Nth power, and the correlation coefficient (Pearson coefficient between any two genes would be calculated. In the network, the genes connections do not have scale-free networks, so it makes the algorithm more biological. Then, a hierarchical clustering tree is constructed by correlation coefficients between genes (22-25).

Enrichment analysis

The gene data were subjected to R Language Cluster profiler package to be analyzed for GO functions (p-value cutoff = 0.01, q-value Cutoff = 0.01) and KEGG pathway (p-value cutoff = 0.05, q-value Cutoff). Based on the functions and pathways involved in the modular genes, it is identified as a key functional pathway that drives preterm preeclampsia. To explore the driving force of co-expression modules, we found data for Pivot analysis as a background set. In the RAID v2.0 database, all ncRNA-mRNAs with P< 0.01 were paired with each other; similarly, in the TRUST v2 database (43), all human transcription factors are targeted to data.

In the target pair, Pivot analysis means that at least two interacting drivers with the module exists. Based on the hypergeometry, we verify the significance of the interaction between the driver and the module. Then, ncRNA and TF were screened. a p-value of less than 0.01 was considered significant. Finally, according to the statistical analysis of Pivot points, in the dysfunction module, the pivot points that have more regulation were identified as the core pivot (26, 27).

Results

Determining expression disorder molecules

Through reviewing different results, 3016 differential genes were obtained.

Identification staging related modules

Based on the WGCNA network, we obtained 23 co-expression modules, in which all their genes were expressed synergistically and formed significant clusters in the sample (Figure 1A, 1B).

Functions and pathways

GO function and KEGG pathway enrichment analyses on 23 modules yielded a collection of GO terms, 824 cell composition entries, 1572 molecular functional words, and 7224 processes (Figure 2A, 2B). The technical analysis indicated relevant functional modular genes that are involved in a variety of functions, such as regulation of protein targeting, positive control of lipoprotein metabolic process, RNA polymerase complex, and regulation of interleukin-4-mediated signaling pathway. The enrichment of 193 KEGG pathways showed the prevalence of the disease. We obtained a relationship between outcomes in both functions and pathways as well as preterm preeclampsia, and we identified these 23 modules as dysfunction modules.

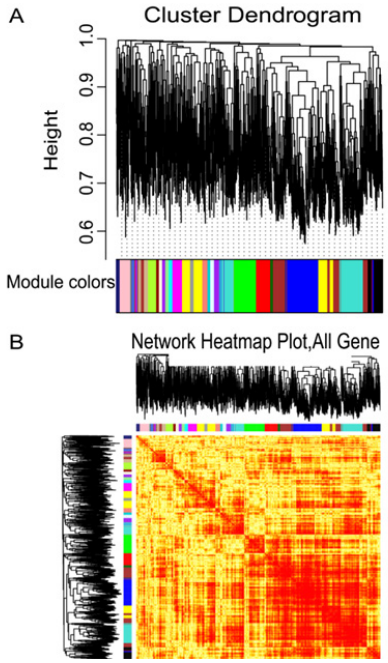

Figure 1. Clustering into a module for the co-expression relationship of preterm preeclampsia-related genes. **A:** According to the synergistic expression relationship of differential genes, the genes are classified into 23 modules; one color represents one module. **B:** Heat map of the modular gene expression in the sample. The expression behavior of 23 modules in 23 samples was significantly consistent.

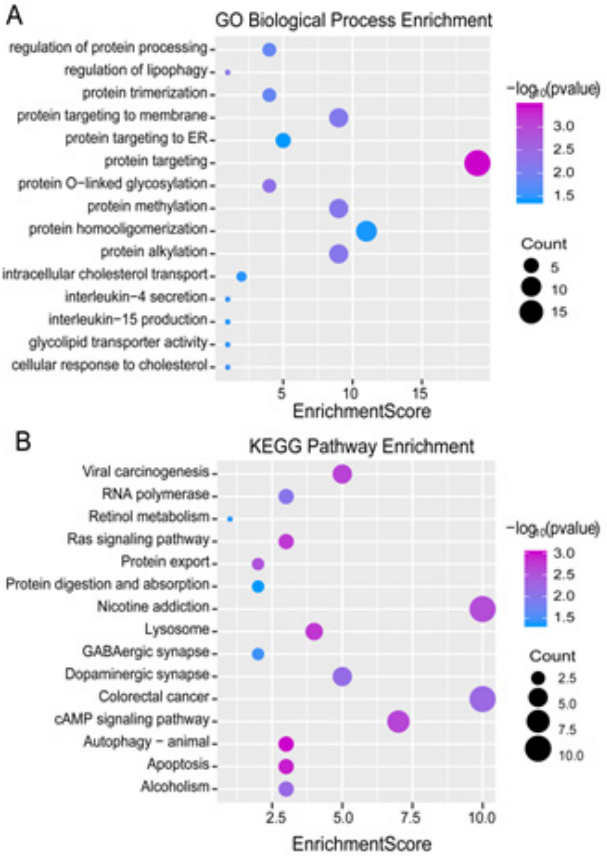

Figure 2. Modules involved in the function and pathway identification of the psoriasis dysfunction module. **A:** GO function enrichment analysis of modular genes. **B:** KEGG pathway enrichment analysis of modular genes. The deeper colors indicate stronger enrichments. The larger circles show more significant proportions of the modular genes accounting for the GO function and in the KEGG pathway entry.

ncRNA that mediates dysfunction modules

The transcription and post-transcriptional regulation in genes have been recognized as crucial factors regulating disease progression, and ncRNA is considered to be an essential regulator. In the disorder module gene, scientific prediction of non-coding RNA with regulatory function is going to help us to explore the transcriptional regulation mechanism for preterm preeclampsia further. So, in order to examine the purpose of the module, we conduct a pivot analysis based on the targeted relationship between ncRNA and genes.

Disordered ncRNA regulator

The prediction showed that 872 ncRNAs have significant regulatory effects on the module, involving 1271 ncRNA-Modular target pairs (Figure 3). These ncRNAs include varying degrees of processes such as mRNAs stability, gene expression, translation, and post-transcriptional regulation. Statistical analysis of the results revealed that miR-182-5p, miR-200b-3p, miR-23a-3p, miR-429, and miR-590-3p have significant regulatory impacts on up to six dysfunction modules, so they contribute to the dysfunction of the module. Other ncRNAs exhibits significant modulation of dysfunction modules, contributing essentially to the onset of the disease.

TF driving premature eclampsia progression

Based on the regulatory relationship of the transcription factors, we performed a pivot analysis (Figure 4). Our analysis showed that 31 transcription factors have significant transcriptional regulation on the dysfunction module for preterm preeclampsia, involving 32 TF-module regulatory pairs. Statistical analysis showed that the transcription factor AR can significantly regulate two dysfunction modules, and it is known that it has a steroid hormone binding function after binding to a hormone ligand, and it can stimulate the transcription of androgen-responsive genes. Furthermore, the transcription factor AR has a potential driving effect on the disease.

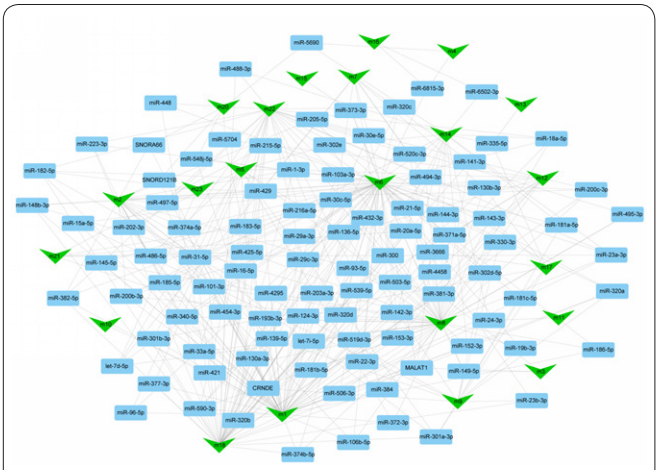

Figure 3. ncRNA regulatory network map of preterm preeclampsia. The green arrow represents the module, and the blue square represents the ncRNA corresponding to the module.

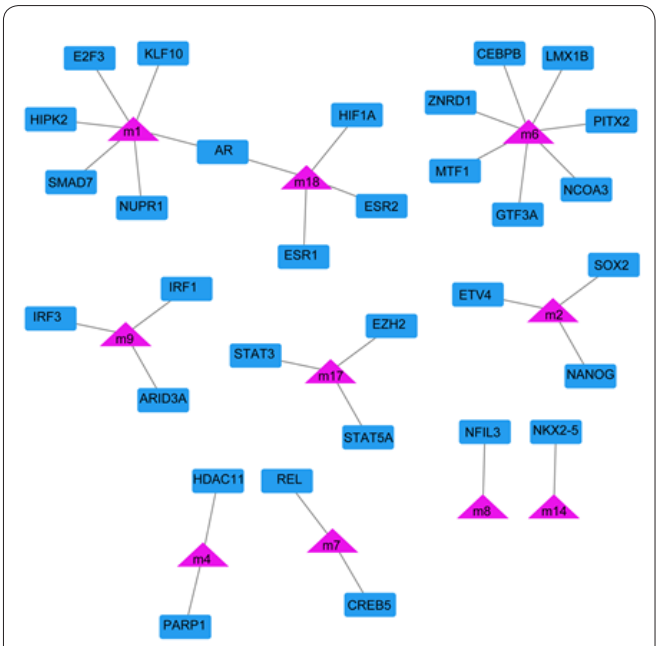

Figure 4. Regulatory network map of transcription factors for preterm preeclampsia. The triangle represents the module, and the light blue rectangle represents the transcription factor.

Discussion

Premature preeclampsia is one of the leading causes of morbidity and mortality in mothers and newborn babies (28). It is unique to a pregnancy and characterized by concurrent hypertension and proteinuria, and it sometimes leads to multiple organ dysfunction syndromes (MODS) with different clinical features (29). It begins with abnormal trophoblast apoptosis and continuously leads to an increase in both inflammation and anti-angiogenic factors, which subsequently affects angiogenesis (30). This syndrome, moreover, is characterized by systemic endothelial dysfunction. Therefore, the drugs improving endothelial functions are expected to alleviate the pre-eclamptic symptoms, delay preterm birth, and improve the prognosis preterm births (4). For example, in maternal blood, angiogenesis and vasculogenesis levels of anti-angiogenic proteins are observed before the diagnosis, and the data have prognostic value to identify women the symptoms during pregnancy, perinatal period (31). Firstly, we combined both enrichment analysis and pivotal regulator analysis; for the pivot regulator, we found that dysfunction modules, which were activated by ncRNA and transcription factors, can mediate a range of functions and pathways. It leads to the development and progression of preterm preeclampsia. As reported by the enrichment analysis results, we found that the modular genes are mainly involved in regulatory responses, including regulation of protein targeting, up-regulation of lipoprotein metabolic process, and RNA polymerase complex. By means of liquid chromatography-mass spectrometry, both the quantitative proteomics methods and targeted proteomics methods could be used to elucidate complications of biological mechanisms associated with protein biomarkers and pregnancy (32).

Retinol-binding protein 4 (RBP4) and lipids may be associated with preeclampsia and preterm birth risk (33). Changes in lipid metabolism are associated with abnormal pregnancy in humans, such as preeclampsia, which may lead to preterm birth (34). Endogenous nitric oxide (NO) damage is associated with preterm preeclampsia so that NO can prevent preeclampsia in high-risk teenage pregnancies. Hence, it reduces the

preeclampsia incidence to improve maternal, fetal, and neonatal outcomes (35). According to McCarthy and Kenny, polymerase chain reaction analysis showed increased expression of the inflammatory markers to-α, tlr-9, and cam-1 in endothelial cells (36). Up to five modules significantly enrich the functions and pathways involved in apoptosis and inflammation, including regulation of apoptotic signaling pathway and negative regulation of inflammatory response. Immune maladaptation might lead to the maternal inflammatory response (mainly hypertension) (37). LXA 4 inhibits certain inflammatory factors such as IL-6, TNF-α, and TNF-γ as well as inflammatory factors such as the upregulation of IL-10. LXA deficiency might lead to preeclampsia (38).

Our findings are consistent with previous studies. Transcriptional and post-transcriptional regulations act as key factors in disease development. In order to elucidate the transcriptional regulators of preterm preeclampsia, we performed a pivotal regulator analysis based on the regulatory relationships between transcriptional and post-transcriptional. MicroRNAs, including miR-182-5p, miR-200b-3p, miR-23a-3p, miR-429, and miR-590-3p as well as AR-based transcription factors have significant regulation in dysfunction modules. MicroRNA is a pathogenesis regulator; it is capable to act as biomarkers and therapeutic targets (39). MicroRNAs, however, may participate in the occurrence of preterm preeclampsia; for example, miR-200b acts as an angiogenesis regulator in the preeclampsia development (40). The transcription factor AR is associated with AR (androgen receptor) and ovarian dysfunction (41).

In preeclampsia, the inflammatory pathway involved in altered α2-AR function is identical to that of other vascular diseases (42). α2 adrenergic receptors in the placenta are involved in the signal transduction of catecholamines through extracellular regulated protein kinases 1 and 2 (ERK 1/2) pathways, which is closely related to the occurrence of preeclampsia in epilepsy (43). These pivotal regulators collectively mediate dysfunction modules and play an overall regulatory role in characterizing the potential driving mechanisms in preterm preeclampsia.

Finally, in the molecular inheritance, the target gene has not only the functions in recognition and binding but also, it also works in function after combination. Our whole module analyzes and observes the target genes that are prominently expressed and mediates the disease development according to the functional pathways. Target genes which have the most obvious effects, including miR-182-5p, miR-200b-3p, miR-23a-3p, miR-429 and miR-590-3p. Studies have shown that miR-23a-3p can regulate the X-linked inhibitor of apoptosis (XIAP) target genes, which are involved in the regulation of apoptosis signals (44). By inhibiting the secretion of MMPs, MiR-200b-3p inhibits apoptosis while promoting cell proliferation and cell growth (45).

In cancer cells, down regulation of miR-182-5p leads to apoptosis (46), down-regulation of miR-429 expression leads to apoptosis (47), further, by targeting TEAD1, miR-590-3p inhibits the growth of cancer cells (48). No further studies have been conducted on the function of miR-200b-3p, miR-429 and miR-590-3p in preterm preeclampsia. Studies have shown that these microRNAs are involved in the development of preterm preeclampsia. In this study, to varying degrees, the series of regulatory factors could have driven the onset of preterm preeclampsia. However, there are other unmentioned ncRNAs and transcription factors that may have a potential drive-in preterm preeclampsia, which requires further exploration. In general, the functional modular-based approach provides not only a comprehensive exploration of the mechanisms for both the occurrence and development of the diseases but also a wealth of resources for TF and pivot ncRNAIt might help clinicians to predict the potential treatments and therapeutic mechanisms.

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Availability of data and materials
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions
HL wrote the manuscript. JY and WY designed the study and performed the experiment. HS and WG were responsible for the analysis and discussion of the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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