Original Article

Methylation-based epigenetic studies and gene integration analysis of preeclampsia

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Background: Preeclampsia (PE) is a multi-factor and multi-mechanism disease, which may jeopardize the life safety of affected pregnant women and fetuses. Our study aimed to detect the potential molecular indicators of PE that might be helpful for its diagnosis and treatment.

Methods: Methylation assay of PE and normal pregnancies placental biopsies was analyzed using the Illumina Human Methylation-27 Assay. Differentially expressed genes (DEGs) were analyzed using R-DESeq2 software. Subsequently, the relationship between DNA methylation genes and DEGs were evaluated. Furthermore, immunohistochemical (IHC) and quantitative reverse transcription polymerase chain reaction (qRT-PCR) validation analyses were conducted for the hub genes.

Results: These hub genes (including *PLXNB1*, *PMCH*, *PPARG*, *GOPC*, *CD79A*, and *MME*) were found to be differentially methylated genes and DEGs. Further analysis revealed that *PPARG*, *CD79A*, and *PLXNB1* may be diagnostic gene markers for PE; down-regulation of PPARG expression was closely correlated with the development of PE. The IHC analysis demonstrated that the expression levels of *PLXNB1*, *PMCH*, *GOPC*, *CD79A*, and *MME* genes were increased, whereas that of PPARG was decreased in PE tissues. The PCR results showed that *PLXNB1*, *PMCH*, *GOPC*, *CD79a*, and *MME* were upregulated, whereas *PPARG* was downregulated. The results of the 2 experiments were consistent with those of bioinformatics analysis.

Conclusions: The molecular indicators identified in this study could facilitate the development of potential biomarkers and therapeutic targets for PE.

Keywords: Preeclampsia (PE); DNA methylation; epigenetics; placenta; CpG sites

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Introduction

Preeclampsia (PE) is a common and serious complication of pregnancy and the Leading cause of maternal and infant mortality worldwide (1). The pathogenesis of PE is unclear, the important factors are immune imbalance, placental superficial implantation, oxidative stress, thrombosis, and genetic factors (2). Clinical symptoms include hypertension and obvious proteinuria, vascular endothelial dysfunction, and systemic inflammatory response (3). In addition, endocrine, genetic, environmental elements, and immune

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response are intimately associated with the formation and progression of PE (4). Furthermore, environmental factors may cause epigenetic changes, which are strongly associated with PE (5).

Epigenetics describes conditions where the DNA sequence remains unchanged, but changes in gene expression result in phenotypic changes, including histone modification, chromatin remodeling, genomic imprinting, random chromosome inactivation, and chemical modification of DNA and RNA (6). Genetic methylation is an important molecular genetic mechanism, involved in regulating the function of trophoblasts and closely linked to the pathogenesis of PE. Genome-wide abnormal methylation content, methylation of PE related genes, and so forth, may promote the onset of PE (7). A study has confirmed that aberrant methylation of CpG islands in the human genome is associated with related gene silencing. During trophoblast differentiation, methylation and unmethylation of CpG islands in the promoter region of some genes can change the state of gene expression, which in turn affects the physiological function of trophoblasts to participate in the pathogenesis of PE (8).

Placental abnormalities are a prominent trigger for the development and progression of PE (9). Extensive studies have indicated that the pathogenesis of PE is related to DNA methylation of genes. DNA methylation is an epigenetic modification of the genome, which is linked to the regulation of various cellular activities (10) and acts as a regulator in the regulation of the genetic phenotype (11). The release of vasoactive factors, pro-inflammatory cytokines, and syncytial debris from the placenta into the maternal circulation can cause systemic endothelial dysfunction and ultimately induce PE (12). Epigenetic modulation has recently been indicated as having a critical role in the physiological regulation of the placenta (13). Therefore, the evaluation of the association between gene transcription and DNA methylation modifications is vital to reveal the epigenetic regulatory mechanisms of PE. Hence, in this study, an integrated analysis of DNA methylation and gene expression data of PE was performed, and then key modules and hub genes were screened out using the weighted gene co-expression network analysis (WGCNA) approach. Finally, 6 candidate genes (*PLXNB1*, *PMCH*, *PPARG*, *GOPC*, *CD79A*, *MME*) were identified as potential diagnostic and therapeutic targets for PE. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5556/rc).

Highlight box

Key findings

- This study identified novel gene-specific differences in DNA methylation in PE placentas.

What is known and what is new?

- This study reveals that *PLXNB1*, *PMCH*, *GOPC*, *CD79A*, and *MME* gene expression levels were elevated in PE, whereas the *PPARG* was reduced. Immunohisto- chemistry and qRT-PCR experiments have verified the results of the bioinformatics analysis.
- This study revealed differentially expressed novel genes may serve as molecular markers in the future.

What is the implication, and what should change now?

- This study will contribute the development of potential biomarkers or therapeutic targets for PE, the function of the hub gene needs to be studied later.

Methods

Sample collection

During March 2021–December 2021, 20 cases with PE and 20 normal pregnant women who had undergone cesarean section were recruited from the Department of Obstetrics, Second Hospital of Hebei Medical University (Shijiazhuang, Hebei, China). The diagnosis of PE was according to the relevant guidelines of the American College of Obstetricians and Gynecologists (ACOG) Practice Bulletin 2019. *Table 1* shows the baseline characteristics of all participants. All pregnant women enrolled in the study were excluded from multiple pregnancies, primary hypertension, congenital malformations, chromosomal abnormalities, and suspected signs of perinatal infection. Placental tissue was obtained within 10 minutes after cesarean sections. We collected placentas located near the root of the umbilical cord to avoid infection, hemorrhage, and calcification. The tissues obtained were cut into 1 cm^3 size, after which they were washed with phosphate-buffered saline (PBS) and wrapped with gauze to absorb the surface fluid. Then, we cut them into pieces and immediately after adding 1 mL of trizol reagent, they were frozen at –80 ℃. We then selected 3 cases and 3 paired controls as samples and tested them according to the quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay. The other part was fixed with 4% paraformaldehyde for immunohistochemistry (IHC). The study was conducted in accordance with the Declaration

Table 1 Characteristics of samples used in the study

Characteristics	Preeclampsia (n=20)	Control (n=20)	P value
Maternal age, years	29.75±2.57	28.65±2.46	0.175
Gestational age, weeks	35.50±1.62	37.04±0.97	0.030
Prenatal maternal BMI, kg/m^2	25.48±2.23	25.60±2.25	0.870
Systolic pressure, mmHg	155.8±9.56	113.8±10.21	5.92E-16
Diastolic pressure, mmHg	106.45±8.58	67.7±7.7	1.37E-17
Proteinuria, g/24 h	2.86±1.32	0±0	7.74E-12
Birth weight, kg	2.52±0.37	3.06±0.27	4.90E-06

All the data were expressed as mean ± SEM. BMI, body mass index; SEM, standard error of the mean.

of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Second Hospital of Hebei Medical University (No. 2018-R270) and informed consent was taken from all the patients.

Methylation assay

The data for each sample was run repeatedly to determine the random location of each sample on the plate by establishing a plate maps. All samples were routed in a particular bunch. Illumina Human Methylation-27 Assay (Illumina, San Diego, CA, USA) was used to assess the methylation of CpG sites.

DNA methylation analysis

Differential methylation sites were screened using R-COHCAP packages. Δβ is the value of differentially methylated sites. β-free methylation sites with >20% distribution were first screened in the samples. Next, differentially methylated sites and regions were obtained with Δβ >0.2, FDR <0.05, and then genomic functional enrichment of differentially methylated sites was analyzed using Fisher's precision assays (dominance ratio ≠1, P<0.05).

Identification of differentially expressed genes (DEGs) between PE and normal

DEGs were screened using the R-DESeq2 package. The degree of methylation of each gene was inferred by analyzing changes in its expression levels at different times and locations. Genes with >20% distribution in the sample were first screened (count =0). These genes are involved in transcriptional regulation, signaling, and so on. Secondly, DEGs were identified using |log2FC|≥1 and P<0.05.

Construction of WGCNA

First, the WGCNA program in R (http://www.r-project.org/) was used to screen important modules and genes in PE at a threshold of β=5. The correlation between the modules and PE were explored by |correlation coefficients|>0.2 and P<0.05. The enrichment analysis was followed by Fisher's Exact test (P<0.05) to analyze the correlation between the module and PE/normal, and the relationship between the modules and DEGs. Finally, visualization using Cytoscape software (https://cytoscape.org/), the threshold >0.1. The criteria for selecting HUB genes from the optimal modules was |correlation. module module| (|Cor.MM|) >0.8.

Identification of hub genes in the turquoise module

To explore key genes associated with PE, we introduced DEGs from the top 100 in the modules most associated with PE traits into the STRING online database (http://string-db.org) for protein-protein interaction (PPI) analysis. Important candidate subsites were identified in this dataset by predicting PPIs and function, and these potential mechanisms of action were speculated in combination with other experimental evidence. The visualization network was created by Cytoscape (version 3.5; https://cytoscape.org/).

Correlation analysis of DNA methylation regions and DEGs

Correlation analysis of DNA methylation genes and gene expression profiles was performed, focusing on

Table 2 Primer sequences in the qRT-PCR

Primer name	Primer sequence (5' to 3')
ACTB-F (internal reference)	TGGCACCCAGCACAATGAA
ACTB-R (internal reference)	CTAAGTCATAGTCCGCCTAGAAGCA
GAPDH-F (internal reference)	CAGGAGGCATTGCTGATGAT
GAPDH-R (internal reference)	GAAGGCTGGGGCTCATTT
PPARG-F	GCAGGTGATCAAGAAGACGGAGAC
PPARG-R	AAATGTTGGCAGTGGCTCAGGAC
PMCH-F	AGTCTACCGACCTTGTTGGCA
PMCH-R	GGCTTCTCCTCCCATTGGCA
PLXNB1-F	GGATTCGGAGAGGAAGGAGGAGAG
PLXNB1-R	AGGATGTGGCAGCAGCAACAAG
MME-F	ATGGGCAAGTCAGAAAGTCAGATGG
MME-R	TGGTGAGGAGCAGGACAAGGAC
GOPC-F	TGGAGGTGCTGGAGAAGGAGTTC
GOPC-R	CATAAGTGATGTCCGCTTGGTCTGG
CD79A-F	TCTTCCTCCTCTTCCTGCTGTCTG
CD79A-R	CGTTGGCGTTGTTGCTGCTATTG

qRT-PCR, quantitative reverse transcription polymerase chain reaction.

the association between different methylation regions and differences in corresponding gene expression. First, CpG islands of differentially methylated genes were integrated with DEGs in an up-regulation/downregulation relationship. Next, single nucleotide polymorphisms were detected at each differentially methylated site. Thirdly, correlation between differentially methylated regions (DMRs) and DEGs was analyzed by $P<0.05$ and |correlation| (|COR|) >0.5. Finally, DEGs with significant negative correlation to DMRs were identified by $P<0.05$.

qRT-PCR confirmation

Twenty cases of PE and normal pregnant women were selected according to the diagnostic criteria of PE. Retrospective qRT-PCR was performed on PE and normal placental tissue samples. We used RNA-easy™ Isolation Reagent (Vazyme, Beijing, China) to isolate total RNA from cells. The RNA samples were reliably amplified and labeled to generate complementary DNA (cDNA) with Hiscript® III RT SuperMix (Vazyme, China) based on CFX96™ Real-Time System (Bio-Rad, Hercules, CA, USA). ChamQ Universal SYBR qPCR Master Mix (Vazyme, China) was used for qRT-PCR following the instructions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and actin beta (ACTB) were used as internal references. Gene expression levels were measured using the $2^{-\Delta\Delta Ct}$ method. All experiments were performed in triplicate with three technical replicates. All primers designed by Sangon Biotech Co., Ltd. (Shanghai, China) are shown in *Table 2*.

Immunohistochemical (IHC) analysis

The paraffin embedded sections were stained with IHC. Placenta samples were treated with 3% hydrogen peroxide for 30 minutes after dewaxing, hydration, and antigen recovery to block endogenous peroxidase activity. Afterwards, the slides were incubated with primary antibody overnight at 4 ℃ (PLXNB1, 1:500 dilution, Rabbit pAb, 23795-1-AP, Proteintech®; PMCH, 1:500 dilution, Rabbit pAb, 18725R, Bioss; PPARG, 1:300 dilution, Rabbit pAb, 340844, ZEN BIO; GOPC, 1:200 dilution, Rabbit pAb, 383119, ZEN BIO; CD79A, 1:50 dilution, Rabbit mAb, R23860, ZEN BIO; MME 1:200 dilution, Mouse mAb, 250203, ZEN BIO). The slides were washed by PBS and reheated 30min for the next day. Following the addition of secondary antibody, slices were incubated with diaminobenzidine (DAB) color developer for 5 minutes, washed in PBS, counterstained in hematoxylin, continuously dehydrated, and the images were recorded by two senior pathologists in a double blind manner. All sections were observed in 5 visual fields under an inverted fluorescence microscope. Brown showed positive expression. All experiments were performed in triplicate with three technical replicates.

Statistical analysis

The average beta significance between 0 and 1 was created for each CpG site, indicating the ratio of methylated cytosine residues to the number of cytosine residues. Student's *t*-test was applied to compare the mean methylation between the PE group and the control group. The significant difference in methylation was defined as

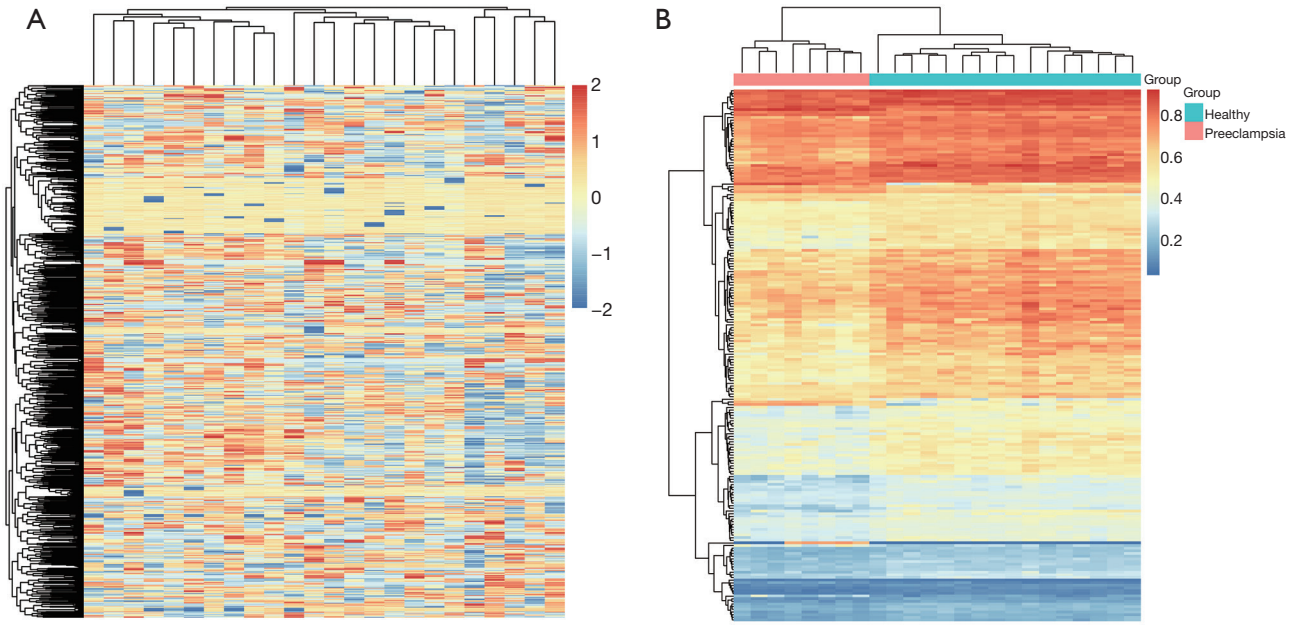

Figure 1 DNA methylation analysis. (A) The heat map of the differentially methylated sites. (B) The heatmap of the top 200 differentially methylated sites in terms of histological diagnosis.

P<0.05 and beta values ≥0.02.

Results

A total of 830 DEGs and 1,828 differentially methylated sites were identified. The results of the WGCNA analysis showed that the royal blue module was the optimal module. Besides, the hub genes (*PLXNB1*, *PMCH*, *PPARG*, *GOPC*, *CD79A*, *MME*) in this module were also differentially methylated genes and DEGs. Studies revealed that *PPARG*, *CD79A*, and *PLXNB1* may be diagnostic gene markers for PE. Furthermore, low expression of *PPARG* was closely connected with the development of PE. The results of the IHC analysis demonstrated that the expression level of *PLXNB1*, *PMCH*, *GOPC*, *CD79A*, and *MME* were increased in PE tissues. The PCR results showed that *PLXNB1*, *PMCH*, *GOPC*, *CD79a*, and *MME* were upregulated, and *PPARG* was downregulated. The results of both experiments were in line with the bioinformatics results.

DNA methylation analysis

For the analysis of differential methylation in PE, 1,828 differentially methylated sites were identified, including 1,035 hypermethylated sites and 793 hypomethylated sites, the heatmaps of which are shown in *Figure 1A*. *Figure 1B* demonstrates the heatmap of the top 200 differentially methylated sites in the histological diagnosis. A total of 1,340 differentially methylated CpG islands were identified, including 1,037 hypermethylated regions and 303 hypomethylated regions, using Δβ>0.2 and FDR <0.05 as the criteria.

Identification of DEGs between PE and normal samples

According to the screening criteria of |log2FC|≥1 and P<0.05, we gleaned 830 DEGs, including 409 up-regulated genes and 421 down-regulated genes. The top 100 DEGs were screened out with a heatmap (*Figure 2*).

Construction of WGCNA

Based on the WGCNA co-expression network analysis, The total number of modules we obtained was 49 (*Figure 3*). To investigate the characterization of those modules, we carried out correlation analysis between modules and PE/normal condition cases. We found that 8 modules were markedly associated with PE/normal status, and turquoise was the most connected with PE/normal condition.

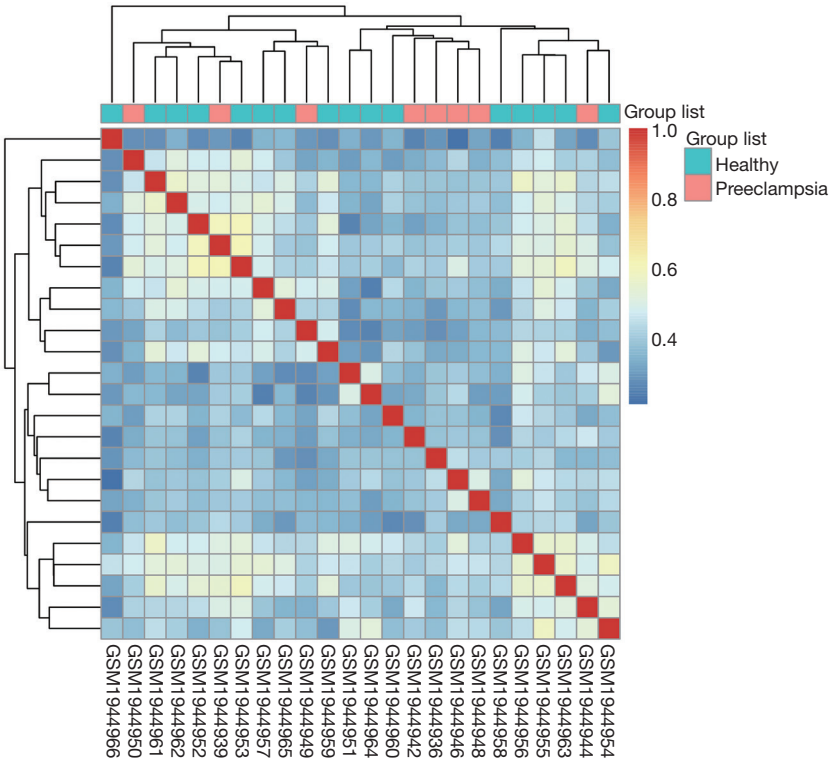

Figure 2 Identification of DEGs between preeclampsia and normal. The top 100 DEGs were screened out with a heat map. DEGs, differentially expressed genes.

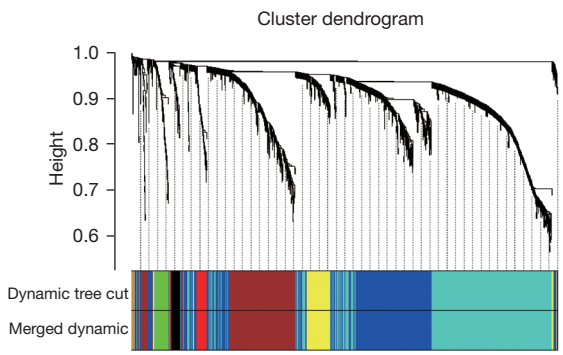

Figure 3 Construction of WGCNA. WGCNA co-expression network identified key modules. WGCNA, weighted gene co-expression network analysis.

Identification of hub genes in the turquoise module

To investigate the hub genes involved in PE, we introduced the top 100 DEGs into the Search Tool for the Retrieval of Interacting Genes/proteins (STRING) online database (http://string-db.org) for PPI analysis, and networks were created by Cytoscape (version 3.5; https://cytoscape.org/). As shown in *Figure 4A-4F*, the top 6 hub DEGs were identified in the turquoise module, namely *PLXNB1*, *PMCH*, *PPARG*, *GOPC*, *CD79A*, and *MME*.

Correlation analysis of DNA methylation regions and DEGs

A total of 117 DMRs-DEGS association combinations were obtained, including 67 combinations of hypermethylated and down-regulated DEGs pairs and 35 combinations of hypomethylated and up-regulated genes. Among them, *PLXNB1*, *PMCH*, *PPARG*, *GOPC*, *CD79A*, and *MME* were both DMRs and DEGs.

qRT-PCR confirmation

We used qRT-PCR to explore whether *PLXNB1*, *PMCH*, *PPARG*, *GOPC*, *CD79A*, and *MME* were involved in the progression of PE. The messenger RNA (mRNA) expression of *PLXNB1*, *PMCH*, *PPARG*, *GOPC*, *CD79A*,

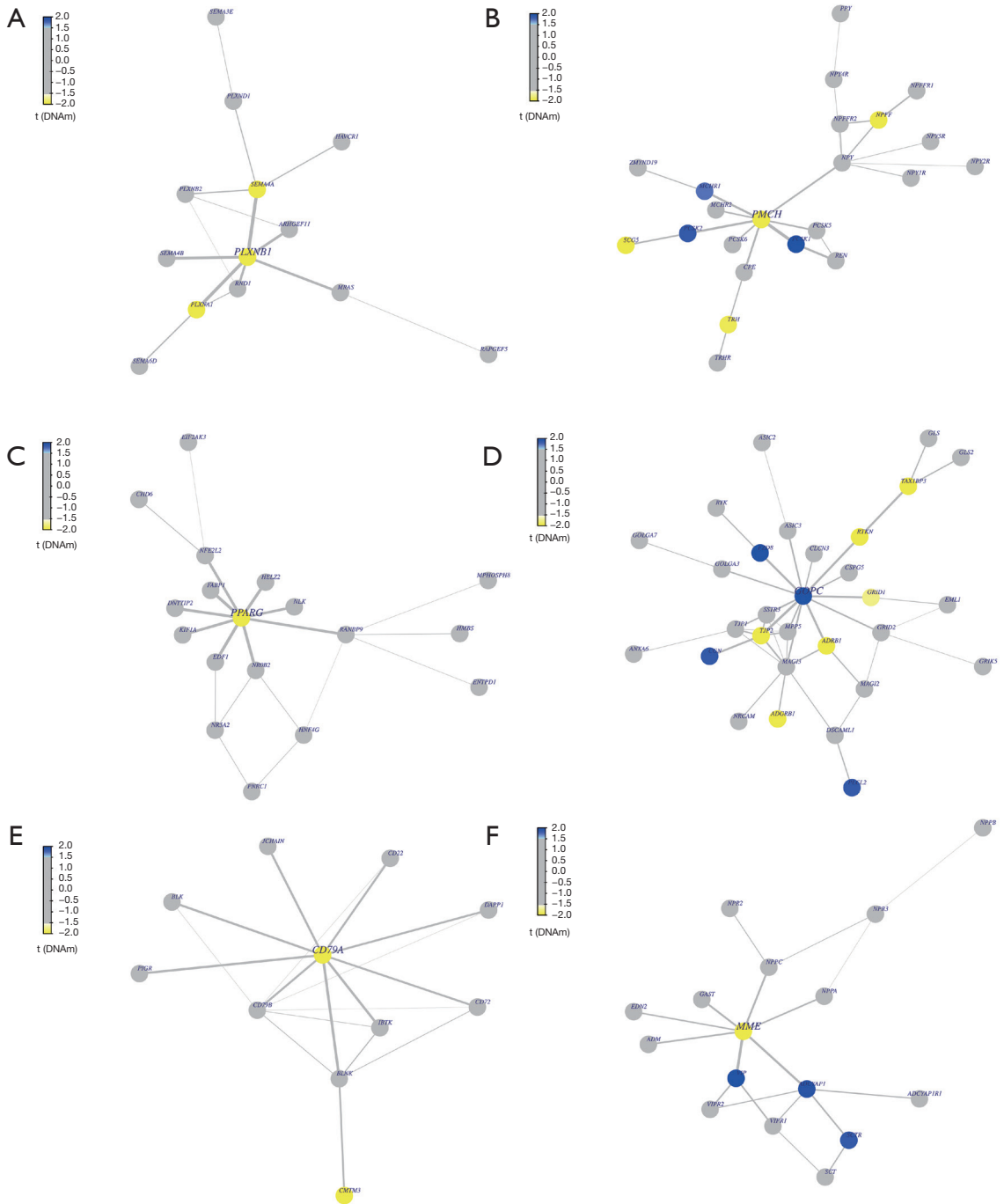

Figure 4 Identification of hub genes in the turquoise module. (A-F) The top 6 hub DEGs were identified in the turquoise module. DEGs, differentially expressed genes.

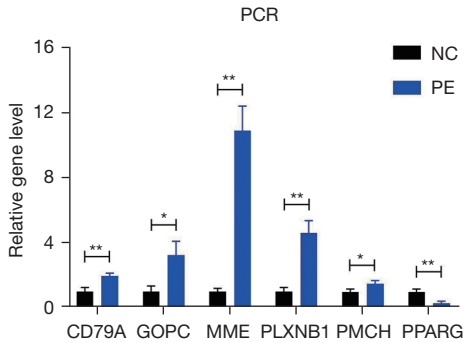

Figure 5 qRT-PCR Confirmation. qRT-PCR was used to evaluate the mRNA expression of *PLXNB1*, *PMCH*, *PPARG*, *GOPC*, *CD79A*, and *MME*. *, P<0.05, **, P<0.01. qRT-PCR, quantitative reverse transcription polymerase chain reaction; mRNA, messenger RNA; NC, normal contrast; PE, preeclampsia.

and *MME* was examined using qRT-PCR verification. The results exhibited that *PLXNB1*, *PMCH*, *GOPC*, *CD79A*, and *MME* were up-regulated and *PPARG* down-regulated (*Figure 5*), which was consistent with our findings in bioinformatics.

IHC analysis for PLXNB1, PMCH, PPARG, GOPC, CD79A, and MME

We performed IHC to validate the roles of *PLXNB1*, *PMCH*, *PPARG*, *GOPC*, *CD79A*, and *MME* in the placental tissues obtained from patients with PE. In PE placenta tissues, the number and degree of positive cells staining for the *PLXNB1*, *PMCH*, *GOPC*, *CD79A*, and *MME* genes were appreciably enhanced in PE tissues compared with normal tissues (*Figure 6*). Furthermore, the expression of *PLXNB1*, *PMCH*, *GOPC*, *CD79A*, and *MME* genes was positively correlated with the occurrence and development of PE. The results of IHC were consistent with qRT-PCR.

Discussion

PE is a complication of pregnancy that can lead to the death of both the fetus and mother (14). It is often accompanied by renal insufficiency, abnormal liver function, neurological symptoms, and thrombocytopenia (15). PE can cause serious fetal growth restriction, and long-term cardiovascular diseases such as hypertension in pregnant women, resulting in maternal and neonatal mortality, which has become one of the leading causes of death worldwide (16).

In the current study, we identified differential methylation in 6 genes: *PLXNB1*, *PMCH*, *PPARG*, *GOPC*, *CD79A*, and *MME* were closely related to the development of PE. In addition, *PLXNB1*, *PMCH*, *GOPC*, *CD79A*, and *MME* were enhanced whereas *PPARG* was reduced and hypomethylated in PE tissues.

PPARG belongs to the nuclear receptor superfamily of ligand-activated transcription factors, which exert their function by regulating the binding of gene fragments to response elements (17). *PPARG* regulates transcription by forming a heterodimer with *RXR*, a member of the secondary nuclear receptor family (18,19). The binding of *PPARG* to natural ligands (fatty acids and prostaglandins) may activate *PPARG/RXR* heterodimers, and while the latter is coupled with peroxisome proliferator response components, they will remain inactive (20). Furthermore, ligand binding induces a conformational alteration of the *PPARG/RXR* heterodimer, triggering the release of co-repressors and the recruitment of co-activators (21).

CD79A is a membrane protein that is conserved in a wide range of species. It is expressed at the primary stage of B-cell growth and its continued expression is observed until the final stage of B-cell maturation (22). Generally, *CD79A* consists of a disulfide-linked heterodimer that polymerizes with *CD79B* and assembles non-covalently with membrane-bound IgM to form the B-cell signaling complex receptor (BCR) (23). The role of dimeric CD79a/b is to transfer the signal generated by the binding of antigen to the BCR into the cell and initiate B cell activation (24).

Plexin-B1 (*PLXNB1*) is a transmembrane receptor for semaphoring 4D (25). Earlier findings have shown that *PLXNB1* is implicated in various cellular processes (26). Moreover, several studies have shown that *PLXNB1* plays a crucial role in the development of cancer (27,28). Nevertheless, to date, the biological role of *PLXNB1* in PE remains unknown.

At present, the research on DNA methylation of PE is mainly focused on the following aspects: genome-wide methylation, methylation of PE-related genes, methylation related to stress response, and methylation of imprinted genes. Blair *et al.* use genome-wide expression profiling revealed a negative correlation between abnormal gene expression, such as angiogenic factor endothelial PAS protein 1 (EPAS1), and Fms related tyrosine kinase 1 (FLT1), and methylation level (29). Syncytin-1, a PE-related gene, is a functional fusion protein that mediates the fusion of trophoblasts to syncytiotrophoblast cells. In the placenta of PE, the mean methylation level of the HERVW promoter region of the Syncytin-1 gene

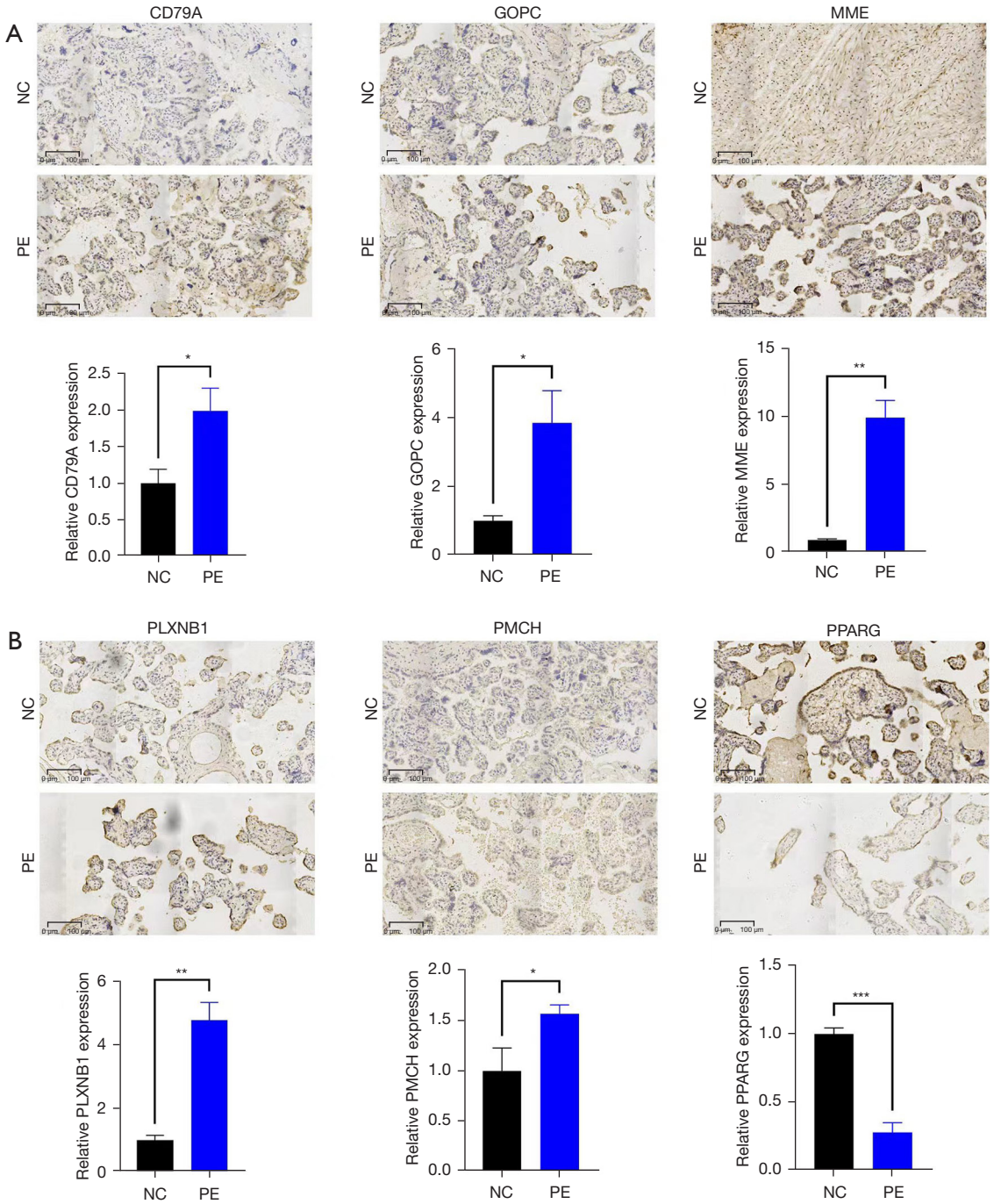

Figure 6 (A) Immunohistochemistry was used to validate the expression of *CD79A*, *GOPC*, and *MME* in the placental tissues. (B) Immunohistochemistry was used to validate the expression of *PLXNB1*, *PMCH*, and *PPARG* in the placental tissues. *, P<0.05, **, P<0.01, ***, P<0.001. NC, normal contrast; PE, preeclampsia.

was higher than that of the normal pregnancy group (30). Hogg *et al*. found the hypermethylation at CpG sites of stress-related genes such as the glucocorticoid receptor (NR31C1) and corticotropin-releasing hormone-binding protein (CRHBP) in PE may be regulated by DNA methylation (31).

As the detection of DNA methylation continues to advance, we can screen from methylation changes of specific genes in PE for novel indicators of biological diagnostics, and enrich the pathogenesis in the future.

Conclusions

In the present study, *PLXNB1*, *PMCH*, *PPARG*, *GOPC*, *CD79A*, and *MME* were revealed as genes that were both differentially methylated and differentially expressed. The IHC analysis revealed that *PLXNB1*, *PMCH*, *GOPC*, *CD79A*, and *MME* gene expression levels were elevated in PE tissues, whereas the gene expression of *PPARG* was reduced, which was consistent with the bioinformatics results. Hence, these identified molecular indicators could be invaluable in diagnosing and curing PE.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5556/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5556/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5556/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Second Hospital of Hebei Medical University (No. 2018-R270). Informed consent was taken from all the patients.

References

1. Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin Summary, Number 222. Obstet Gynecol 2020;135:1492-5.
2. Portelli M, Baron B. Clinical Presentation of Preeclampsia and the Diagnostic Value of Proteins and Their Methylation Products as Biomarkers in Pregnant Women with Preeclampsia and Their Newborns. J Pregnancy 2018;2018:2632637.
3. Nakamura E, Mikami Y, Era S, et al. Differences in the prognosis of preeclampsia according to the initial symptoms: A single-center retrospective report. Pregnancy Hypertens 2019;16:126-30.
4. Esteve-Valverde E, Ferrer-Oliveras R, Gil-Aliberas N, et al. Pravastatin for Preventing and Treating Preeclampsia: A Systematic Review. Obstet Gynecol Surv 2018;73:40-55.
5. Attini R, Kooij I, Montersino B, et al. Reflux nephropathy and the risk of preeclampsia and of other adverse pregnancy-related outcomes: a systematic review and meta-analysis of case series and reports in the new millennium. J Nephrol 2018;31:833-46.
6. Kaliman P. Epigenetics and meditation. Curr Opin Psychol 2019;28:76-80.
7. Almomani SN, Alsaleh AA, Weeks RJ, et al. Identification and validation of DNA methylation changes in pre-eclampsia. Placenta 2021;110:16-23.
8. Kamrani A, Alipourfard I, Ahmadi-Khiavi H, et al. The role of epigenetic changes in preeclampsia. Biofactors 2019;45:712-24.
9. Velicky P, Windsperger K, Petroczi K, et al. Pregnancy-associated diamine oxidase originates from extravillous trophoblasts and is decreased in early-onset preeclampsia. Sci Rep 2018;8:6342.
10. Xu Y, Wang N, Liu R, et al. Epigenetic Study of

Esophageal Carcinoma Based on Methylation, Gene Integration and Weighted Correlation Network Analysis. Onco Targets Ther 2021;14:3133-49.
11. Walsh SW, Nugent WH, Al Dulaimi M, et al. Proteases Activate Pregnancy Neutrophils by a Protease-Activated Receptor 1 Pathway: Epigenetic Implications for Preeclampsia. Reprod Sci 2020;27:2115-27.
12. Ralser DJ, Klümper N, Gevensleben H, et al. Molecular and Immune Correlates of PDCD1 (PD-1), PD-L1 (CD274), and PD-L2 (PDCD1LG2) DNA Methylation in Triple Negative Breast Cancer. J Immunother 2021;44:319-24.
13. Kobayashi H, Tsunemi T, Akasaka J, et al. Preeclampsia as a parental epigenetic disease. Clin Exp Obstet Gynecol 2018;45:493-502.
14. Leavey K, Wilson SL, Bainbridge SA, et al. Epigenetic regulation of placental gene expression in transcriptional subtypes of preeclampsia. Clin Epigenetics 2018;10:28.
15. Kattah A, Milic N, White W, et al. Spot urine protein measurements in normotensive pregnancies, pregnancies with isolated proteinuria and preeclampsia. Am J Physiol Regul Integr Comp Physiol 2017;313:R418-24.
16. Hamza RZ, Diab AAA, Zahra MH, et al. A Potential Role of Apelin-13 against Hepatic Injury and Metabolic Disorders in Preeclampsia Induced by L-NAME. Coatings 2021;11:391.
17. Auwerx J. PPARgamma, the ultimate thrifty gene. Diabetologia 1999;42:1033-49.
18. Lian M, Tao Y, Chen J, et al. Variation of PPARG Expression in Chemotherapy-Sensitive Patients of Hypopharyngeal Squamous Cell Carcinoma. PPAR Res 2021;2021:5525091.
19. Javanrouh Givi N, Najd Hassan Bonab L, Barzin M, et al. The joint effect of PPARG upstream genetic variation in association with long-term persistent obesity: Tehran cardio-metabolic genetic study (TCGS). Eat Weight Disord 2021;26:2325-32.
20. Wilson HE, Stanton DA, Rellick S, et al. Breast cancer-associated skeletal muscle mitochondrial dysfunction and lipid accumulation is reversed by PPARG. Am J Physiol Cell Physiol 2021;320:C577-C590.
21. Cataldi S, Aprile M, Melillo D, et al. TNFα Mediates Inflammation-Induced Effects on PPARG Splicing in Adipose Tissue and Mesenchymal Precursor Cells. Cells 2021;11:42.
22. Bell PB, Rooney N, Bosanquet AG. CD79a detected by ZL7.4 separates chronic lymphocytic leukemia from mantle cell lymphoma in the leukemic phase. Cytometry 1999;38:102-5.
23. Cruse JM, Lewis RE, Pierce S, et al. Aberrant expression of CD7, CD56, and CD79a antigens in acute myeloid leukemias. Exp Mol Pathol 2005;79:39-41.
24. Hoeller S, Zihler D, Zlobec I, et al. BOB.1, CD79a and cyclin E are the most appropriate markers to discriminate classical Hodgkin's lymphoma from primary mediastinal large B-cell lymphoma. Histopathology 2010;56:217-28.
25. Perälä NM, Immonen T, Sariola H. The expression of plexins during mouse embryogenesis. Gene Expr Patterns 2005;5:355-62.
26. Li G, Ma L, Lu H, et al. Transactivation of Met signalling by semaphorin4D in human placenta: implications for the pathogenesis of preeclampsia. J Hypertens 2018;36:2215-25.
27. Yu L, Petyuk VA, Gaiteri C, et al. Targeted brain proteomics uncover multiple pathways to Alzheimer's dementia. Ann Neurol 2018;84:78-88.
28. Cong X, Li X, Li S. Crystal structure of the aromatic-amino-acid aminotransferase from Streptococcus mutans. Acta Crystallogr F Struct Biol Commun 2019;75:141-6.
29. Blair JD, Yuen RK, Lim BK, et al. Widespread DNA hypomethylation at gene enhancer regions in placentas associated with early-onset pre-eclampsia. Mol Hum Reprod 2013;19:697-708.
30. Ruebner M, Strissel PL, Ekici AB, et al. Reduced syncytin-1 expression levels in placental syndromes correlates with epigenetic hypermethylation of the ERVW-1 promoter region. PLoS One 2013;8:e56145.
31. Hogg K, Blair JD, McFadden DE, et al. Early onset pre-eclampsia is associated with altered DNA methylation of cortisol-signalling and steroidogenic genes in the placenta. PLoS One 2013;8:e62969.

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