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Research article

Identification of shared gene signatures and biological mechanisms between preeclampsia and polycystic ovary syndrome

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ABSTRACT

Preeclampsia (PE) is one of the most common complications of pregnancy and polycystic ovary syndrome (PCOS) is a prevalent metabolic and endocrinopathy disorder in women of reproductive age. Identifying the shared genetic signatures and molecular mechanisms between PCOS and PE was the objective of this study. The intersections of WGCNA module genes, PPI module genes, and PPI hub genes revealed that 8 immunity-related genes might be shared causative genes of PE and PCOS. Further, qRT-PCR results showed that TSIX/miR-223–3p/DDX58 might play a crucial role in immune dysregulation in PE and PCOS and Spearman rank correlation analysis results illustrated the potential of DDX58 as a novel diagnostic and therapeutic target for PE and PCOS. Our study demonstrated a common disease pathway model TSIX/miR-223–3p/DDX58, illustrating that immune dysregulation may be a possible mechanism of PE and PCOS, and revealed that DDX58 might be a novel predictive target for PE and PCOS.

1. Introduction

Preeclampsia (PE) is a common pregnancy-associated disorder, characterized by hypertension arising at 20 weeks of gestational age or later with proteinuria or other signs of end-organ damage, including thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, new onset headache, and visual symptoms, which might have adverse effects on the mother and fetus [1]. Although progress in unveiling the pathogenesis and pathophysiology of PE have been made, including probably genetic factors, immunological factors, maternal factors or multifactorial, the major mechanism is still controversial in academia [2]. Polycystic ovary syndrome (PCOS), characterized by hyperandrogenism and chronic anovulation, is one of the most common female endocrine disorders [3]. It is a leading cause of female infertility and is associated with a substantially increased lifetime risk of comorbidities while the mechanism underlying the pathophysiology of PCOS also remains to be illustrated [4].

Notably, some studies pointed out that pregnant women with PCOS were at increased risk of PE in pregnancy even when they do

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Abbreviations

PE	preeclampsia
PCOS	polycystic ovary syndrome
GEO	Gene Expression Omnibus
WGCNA	weight gene co-expression network analysis
DEGs	differential expression genes
PPI	protein-protein interaction
TF	transcription factors
lncRNA	long non-coding RNA
miRNA	microRNA
mRNA	messenger RNA
qRT-PCR	Quantitative real-time polymerase chain reaction
GO	gene ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes

not present with other co-existing metabolic conditions [5–9]. Few studies attempted to explain the correlation of these two diseases as the characteristic androgen excess of PCOS might influence the vascular properties of arterial walls of pregnant women, which was a contributor to the development of gestational hypertension [9]. However, the correlation between PE and PCOS has hardly been supported by any molecular biological evidence, despite sufficient clinical research support.

In recent years, with the rapid expansion of gene microarray technology, bioinformatic analysis has been widely used to explore the pathogenesis of diseases from the genetic level more deeply and comprehensively [10,11]. It was the first time using the Gene Expression Integrated Database (GEO) (http://www.ncbi.nlm.nih.gov/geo/) to explore the important differentially expressed genes (DEGs) shared in PE and PCOS at the transcriptome level. Then, we predicted the potential miRNAs, lncRNAs, and transcription factors targeting DEGs and constructed a lncRNA-miRNA-mRNA network. Furthermore, the network was validated by qRT-PCR and the signaling axis was finally identified, which might be the common and promising biomarkers for diagnosis and prognosis of PE and PCOS.

2. Methods

2.1. GEO datasets download and processing

PE and PCOS gene expression profiles were searched in GEO database by using the keywords of "preeclampsia [All Fields]" or "[polycystic ovary syndrome]". The inclusion criteria for analysis datasets and validation datasets were as follows: 1) Including cases and controls in the gene expression profile was essential; 2) Samples for PE or PCOS sequencing must be derived from human placental tissue or granulosa cells, respectively; 3) Datasets must provide processed or raw data that can be reanalyzed. We preprocessed, normalized, and log2-transformed the probe expression matrix and matched the probe to its gene symbol as indicated in the annotation document for the datasets downloaded from the corresponding platform. Gene expression was calculated by averaging probes mapped to the same gene. Batch effect removal were performed on the datasets with the sva package in R and obtained the combined gene expression data for subsequent analysis [12].

2.2. WGCNA (weighted gene Co-expression network analysis) of disease-related modules

Gene networks and diseases can be explored using WGCNA, a genetic network analysis program that clusters genes and constructs modules based on similar gene expression patterns [13]. To determine the association between PE and PCOS, we used the WGCNA package in R. First, the top 50% genes were selected in variance for the WGCNA analysis, and an adjacency matrix was built using soft threshold β (10 for PE, 6 for PCOS). Based on the adjacency matrix, a topological overlap matrix (TOM) was calculated. Gene hierarchical clustering dendrograms were used to identify co-expression modules. The parameters were as follows: minModuleSize = 50 and MEDissThres = 0.3. Further, to assess the potential correlation between modules and traits, the module eigengene (ME) was utilized. The common WGCNA module genes of PE and PCOS were obtained by the intersection operation of their respective key module genes.

3. Detection of shared genes in PE and PCOS

R package limma was used to identify the DEGs between the control and case groups and genes with differential expression threshold $P<0.05$ were selected as DEGs [14]. The DEGs from PE and PCOS were subjected to the intersection operation to obtain the common DEGs. With default settings in Cytoscape, the MCODE algorithm was used to create the protein-protein interaction (PPI) network in PE and PCOS to verify the shared gene signatures [15]. The module of the PPI network with nodes more than 10 was considered a key module and the genes of key modules were further enriched by the biological process (BP) of gene ontology (GO) and

the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis with R clusterProfiler package [16]. In addition, the topological properties of each node, including MCC, MNC, Degree, EPC, Closeness, and Radiality, were analyzed by plugin CytoHubba [17]. The top 30 genes were selected in the above 6 properties respectively, and hub genes of the PPI network were got through the intersection. Finally, the intersection of genes of WGCNA modules, key genes of PPI network modules, and hub genes of the PPI network was identified as the final shared genes of PE and PCOS for further verification. In addition, the Circos diagram was drawn with Circos-0.69-6 software for analyzing the relationship between shared genes.

3.1. Functional annotation and pathway enrichment analysis

We further enriched and analyzed the selected shared genes using the Cytoscape plugin ClueGO and CluePedia for GO and KEGG pathway enrichment analysis [18,19]. *P*-value$<$0.05 was considered significant.

3.2. Validation of shared genes and pathway with validation cohort

With the limma package, we validated the shared genes on PE and PCOS validation datasets. The cutoff value was *P*-value$<$0.05 and $|\log FC|>0.5$, and using the same methods, we analyzed the common DEGs in PCOS and PE for further comparing the results of the enrichment results between the analysis cohort and validation cohort. In addition, the expression levels of the shared genes in the validation datasets were shown in boxplots and the significance of the *P*-value was calculated by Student's t-test.

3.3. The common lncRNA-miRNA-mRNA network construction

MiRNAs and lncRNAs are non-coding RNAs that could regulate gene expression [20,21]. We got PE-related miRNAs and PCOS-related miRNAs from the Human microRNA Disease Database (HMDD) [22], which is a database of diseases-related miRNAs supported by experimental data. Then miRNAs that are associated with both PE and PCOS diseases were selected. Then the common miRNAs were enriched and analyzed by miRPath in DIANA for GO and KEGG pathway [23]. In addition, we predicted the miRNAs and lncRNAs targeting these shared genes with miRanda software (v3.3a) and lncBase respectively, and constructed the lncRNA-miRNA-mRNA regulated network with Cytoscape [24]. Finally, transcription factors (TF) were predicted with TRRUST [25], and the TF-mRNAs network was constructed by Cytoscape.

3.4. Validation samples Collection

Samples with consistent tissue types with the sequencing samples (placental tissues for PE and granulosa cells for PCOS) were conducted for further experimental verification. Preeclamptic (PE group; n=12) and normal (normal group; n=12) placenta samples were collected with informed consent. The diagnosis of PE was: SBP$\geq$140 mmHg or DBP$\geq$90 mmHg after 20 weeks of gestation with previously normal BP and with proteinuria or other end-organ effects, and the blood pressure should be measured at least twice every 4 h according to guide of American College of Obstetricians and Gynecologists (*ACOG*) [26,27]. Patients with cardiovascular diseases, kidney diseases, diabetes, congenital malformations, multiple pregnancies, pregnancies conceived by fertility treatment, and chromosomal anomalies were all excluded. All placental tissue samples were taken from pregnant women receiving cesarean section. Placental tissues were stored at -80 °C until use. The clinical data of the PE patients was present in Supplementary table 1. Placenta samples were collected with informed consent from all the participants.

Human follicular granulosa cells (GCs) were obtained after oocyte retrieval from matched patients (PCOS group, n=10; normal group, n=10) undergoing in vitro fertilization (IVF) during assisted reproductive techniques. PCOS was diagnosed based on the Rotterdam criteria and patients diagnosed met at least 2 of the following 3 features: 1) Abnormal ovulation (oligomenorrhea or amenorrhea); 2) Hyperandrogenism (clinical or biological); 3) Polycystic ovary morphology [28]. Following were the exclusion criteria: thyroid dysfunction, hyperprolactinaemia, non-classical congenital hyperplasia biochemically, and other conditions such as Cushing's syndrome if indicated clinically. The clinical data of the PCOS patients was present in Supplementary table 2. The present study was approved by the International Peace Maternity and Child Health Hospital Ethics Committee, Shanghai, China. Research involving human research participants has been performed in accordance with the Declaration of Helsinki and all research was performed in accordance with relevant guidelines. Shapiro Wilk test was used to evaluate whether the data set was normally distributed. Student's t-test was used to estimate statistically significant differences and all statistical analyses were established by GraphPad Prism 8.3. $P<0.05$ was considered as significant difference.

3.5. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

TRIzol reagent (Invitrogen, United States) was used to extract total RNA from tissue samples and cells, and a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, United States) was used to measure its concentration and purity. Reverse transcription of miRNAs was performed using miRNA First Strand cDNA Synthesis Kit (Accurate Biology, China) and others were performed with TransScript All-inOne First-Strand cDNA Synthesis SuperMix for qPCR (TransGen, China). qRT-PCR was performed by QuantStudio 7 Flex instrument (Thermo Fisher Scientific, United States). SYBR Green Premix Pro Taq HS qPCR Kit (Accurate Biology, China) was used to measure ncRNAs and mRNAs and normalize them to small nuclear RNA U6 or 18S as internal controls. Supplementary table 3 showed the primers for qRT-PCR. Comparative Ct was used to calculate relative expression levels.

3.6. Correlation analysis of the relationship between shared gene expression and clinical characteristics of PE and PCOS

Correlation analyses were performed by Pearson or Spearman rank correlation analysis tests based on the distribution and characteristics of the variables. The main clinical characteristics of patients corresponding to the samples we collected were SBP (the average of two blood pressure measurements), DBP (the average of two blood pressure measurements), mean arterial pressure (MAP) (the average of two blood pressure measurements) and proteinuria in PE and follicle-stimulating hormone (FSH), luteinizing hormone (LH), Oestradiol (E_2), testosterone (T), and anti-Mullerian hormone (AMH) in PCOS. Correlations were evaluated by Pearson correlation coefficient (R_p) or Spearman rank correlation coefficient (R_s). P-value<0.05 was considered as a significant correlation.

4. Results

4.1. GEO datasets information

The PE&PCOS group of the GEO dataset was checked, but no relevant dataset was found. Then, the PE group and PCOS group of the GEO dataset were separately checked. And 2 PE datasets numbered GSE147776, GSE160888, and 3 PCOS datasets numbered GSE95728, GSE114419, and GSE34526 were selected for analysis based on inclusion criteria and the remaining PE dataset numbered GSE148241 and PCOS dataset numbered GSE106724 were used for verification. The details of 7 datasets are shown in Table 1.

4.2. Identification of WGCNA key modules in PE and PCOS

17 modules were identified in PE discovery datasets by WGCNA. Based on Spearman correlation coefficients, a heat map about module-trait relationships was created (Fig. 1 A, C). Compared with other modules, modules “greenyellow”, “magenta”, “salmon” and “white” had a higher association with PE (greenyellow: r=0.45, P=0.03, genes=274; magenta: r=0.52, P=0.01, genes=281; salmon: r=0.54, P=0.01, genes=261; white: r=0.46, P=0.03, genes=76), which were chosen as PE-related modules for further evaluation. Likewise, we identified 6 modules in PCOS and chose the “black” module as a PCOS-related module (Fig. 1 B, D, r=0.72, $P<0.001$, genes=3850). Then the module genes of PE and PCOS were intersected, as shown in Fig. 1 E, and a WGCNA with a total of 346 key module genes was obtained.

4.3. The shared genes in PE and PCOS

According to the threshold of DEGs, 911 up-regulated genes and 476 down-regulated genes in PE, and 1818 up-regulated genes and 1768 down-regulated genes in PCOS were obtained, respectively (Fig. 2 A, B). Among them, as described in Fig. 2 C, 188 genes showed consistent up-regulated, and 86 genes showed consistent down-regulated. We then constructed a PPI network with these genes at the protein levels and filtered 38 key modules with more than 10 nodes (Fig. 2 D, E). To explore the potential functions of these key modules, we performed GO-BP and KEGG pathway enrichment analysis (Fig. 2 F, G). It was found that these genes were enriched in many biological activities, including T cell activation, regulation of cell-cell adhesion, and many signaling pathways, including viral protein interaction with cytokine, cytokine receptor, hematopoietic cell lineage, and chemokine signaling pathway. Next, we analyzed the topological properties of each node by plugin CytoHubba and filtered 20 hub genes of the PPI network, which were the intersection of the top 30 genes of each of the 6 main properties (Fig. 2 H). Finally, we took the intersection of WGCNA key modules, PPI key modules, and PPI hub genes as the shared genes of PE and PCOS, and 8 genes were filtered as follows: CD5, CD22, CD38, CXCL9, DDX58, LAG3, PDCD1LG2 and ZAP70 (Fig. 2 I). In addition, the Circos diagrams for analyzing the relationship between shared genes were present in Supplementary fig. 1.

4.4. The shared genes signatures in PE and PCOS

As mentioned in the previous section, we performed GO-BP and KEGG pathway enrichment analysis of 8 shared genes to explore the potential functions. The results were illustrated in Fig. 3 and immune-related biological activities were mainly enriched again as follows: T cell aggregation, immunoglobulin binding, negative regulation of tissue remodeling, positive regulation of natural killer cell-mediated cytotoxicity, type III interferon production, negative regulation of tissue remodeling and positive regulation of syncytium formation by plasma membrane fusion, indicating great importance of immune-related pathway in PE and PCOS.

Table 1
Characters of the 7 GEO datasets involving PCOS and PE patients.

Disease	Group	Accession ID	Patients	Controls	Platform
Preeclampsia (PE)	Discovery cohort	GSE147776	7	8	GPL20844
		GSE160888	4	4	GPL16956
	Validation cohort	GSE148241	9	32	GPL16791
Polycystic ovary syndrome (PCOS)	Discovery cohort	GSE95728	7	7	GPL16956
		GSE114419	3	3	GPL17586
		GSE34526	7	3	GPL570
	Validation cohort	GSE106724	8	4	GPL21096

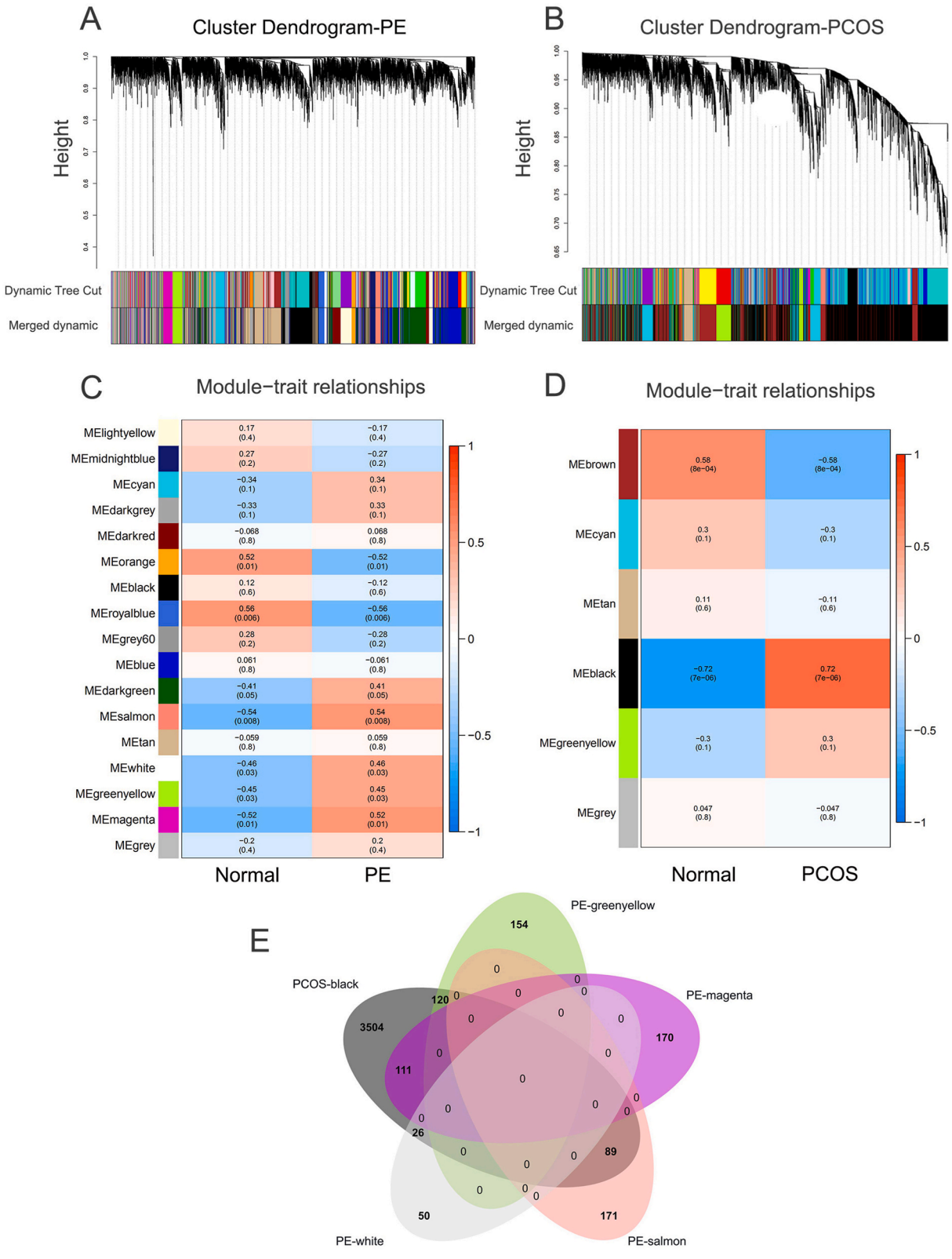

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Fig. 1. WGCNA of PE and PCOS Related Modules. (A) The cluster dendrogram of co-expression genes in PE. **(B)** The cluster dendrogram of co-expression genes in PCOS. **(C)** Module-trait relationships in PCOS. **(D)** Module-trait relationships in PE. Each cell contains the corresponding correlation and *P*-value. Every row represented a Module-trait, and every column represented the group. Each cell contains the corresponding correlation and *P*-value. **(E)** The common WGCNA module genes of PE and PCOS. WGCNA, Weighted Gene Co-expression Network Analysis; PE, preeclampsia; PCOS, polycystic ovary syndrome.

4.5. Validation of shared genes in the validation cohort

To validate above the shared genes, Differential genes analysis in the validation cohort was performed. 20 genes showing consistent up-regulation and 16 genes showing consistent down-regulation were identified (Fig. 4 A). Based on functional enrichment analysis, these DEGs were also enriched for similar biological functions, such as T cell activation, cell-cell adhesion, etc (Fig. 4 B, C). We also validated the expression of shared genes in the validation datasets and the results demonstrated that most shared genes also significantly up-regulated both in PE and PCOS, except ZAP70 in PE and PDCD1LG2 in PCOS. The results in the discovery cohort were highly concordant (Fig. 4 D, E).

4.6. Construction of common lncRNA-miRNA-mRNA network in PE and PCOS

We found that there were 105 miRNAs associated with shared genes and 30 miRNAs in PCOS correspondingly according to the HMDD database. Among these miRNAs, 12 miRNAs were overlapped in PE and PCOS (Fig. 5 A). We performed functional enrichment analysis of these 12 miRNAs and were pleasantly surprised to find that immunity-related biological functions and pathways were enriched again (Fig. 5 B, C). 48 miRNA-target pairs were obtained by the miRanda database of the above 8 shared genes and 12 common miRNAs, including 7 shared genes and 12 miRNAs (Supplementary fig. 2). We further predicted the lncRNAs targeting these miRNAs using lncBase and finally constructed a common lncRNAs-miRNAs-mRNAs network in PE and PCOS (Fig. 5 D). In addition, as shown in Fig. 5 E, a TF-mRNAs network by TRRUST was also constructed.

4.7. Experimental verification of common lncRNA-miRNA-mRNA network in PE and PCOS

To validate the common lncRNA-miRNA-mRNA network of these two diseases, matched human samples were collected, consisting of 12 placental tissues of PE, 10 GCs of PCOS, and a corresponding equal number of normal control samples of these two diseases. First, we detected the mRNA expression levels of 8 shared genes in PE and PCOS samples. These 8 genes were all expressed in both PE and PCOS samples. Among them, compared to the control group, CD5, CD22, CD38, CXCL9, LAG3, PDLD1LG2 had a downward trend in PE, while only DDX58 and ZAP70 had the upward trend. But only the expression of CD5, CD38, and DDX58 showed statistical differences. Compared with the control group, the expression of CD22, CD38, CXCL9, DDX58, LAG3, PDLD1LG2, and ZAP70 in PE showed an upward trend, while only CD5 showed a downward trend. But only the expression of CD22, DDX58, and LAG3 showed statistical differences. Finally, the results showed that DDX58 was the only shared gene with consistent trends in these two diseases (Fig. 6 A, B). Then, as shown in the Sankey diagram, we detected the 9 miRNAs expression targeting DDX58 and filtered miR-223–3p with the same criteria (Fig. 6 C, D). It was worth mentioning that there were 7 ncRNAs targeting miR-223–3p, according to the results predicted by the lncBase, and we further compared them with several other lncRNA databases and found that only 5 ncRNAs (including 1 miRNA miR-6818 and 4 LncRNAs PCAT19, TSIX, KCNQ1OT1 and LOC100190986) excepted RP3-323A16.1 and chr22-38_28785274–29006793.1 were retrieved. Therefore, we detected the expression of these 5 ncRNAs and filtered TSIX (Fig. 6 E, F). Finally, TSIX/miR-223–3p/DDX58 in the network was found had a significant expression change with the same trend by qRT–PCR both in PE and PCOS.

4.8. Correlation analysis of the relationship between shared gene expression and clinical characteristics of PE and PCOS

Spearman rank correlation analysis tests were performed based on the distribution characteristics of the research variables. The expression of DDX58 in PE was significantly correlated with blood pressure, including SBP (Fig. 7 A, R_S=0.4635, P=0.0225), DBP (Fig. 7 B, R_S=0.5016, P=0.0125) and MAP (Fig. 7 C, R_S=0.4873, P=0.0157), and the DDX58 expression in PCOS was significantly correlated with AMH (Fig. 7 D, R_S=0.6363, P=0.0026). The common pathway of PE and PCOS demonstrated that DDX58 might play a crucial role in the pathogenesis of PE and PCOS and immune dysregulation might be the common mechanism of PE and PCOS pathogenesis (Fig. 7 E).

5. Discussion

To date, nearly all previous studies supported that PCOS was a risk factor for PE [5–9]. Considering the strong correlation between PE and PCOS, our goal in this study was to identify genetic signatures, exploring the transcriptional regulatory signatures of these two diseases for further elucidating the pathogenesis and providing targets for the prevention and/or treatment of PE and PCOS. The etiology of PE is not fully understood, in addition to the wildly accepted two-stage theory, immune dysregulation has been purported increasingly to be involved in the pathogenesis of PE in recent years [29,30]. PE develops as a result of improper activation of the innate immune system and subsequent inflammation, leading to dysfunction of the placenta and poor maternal vascular adaptation

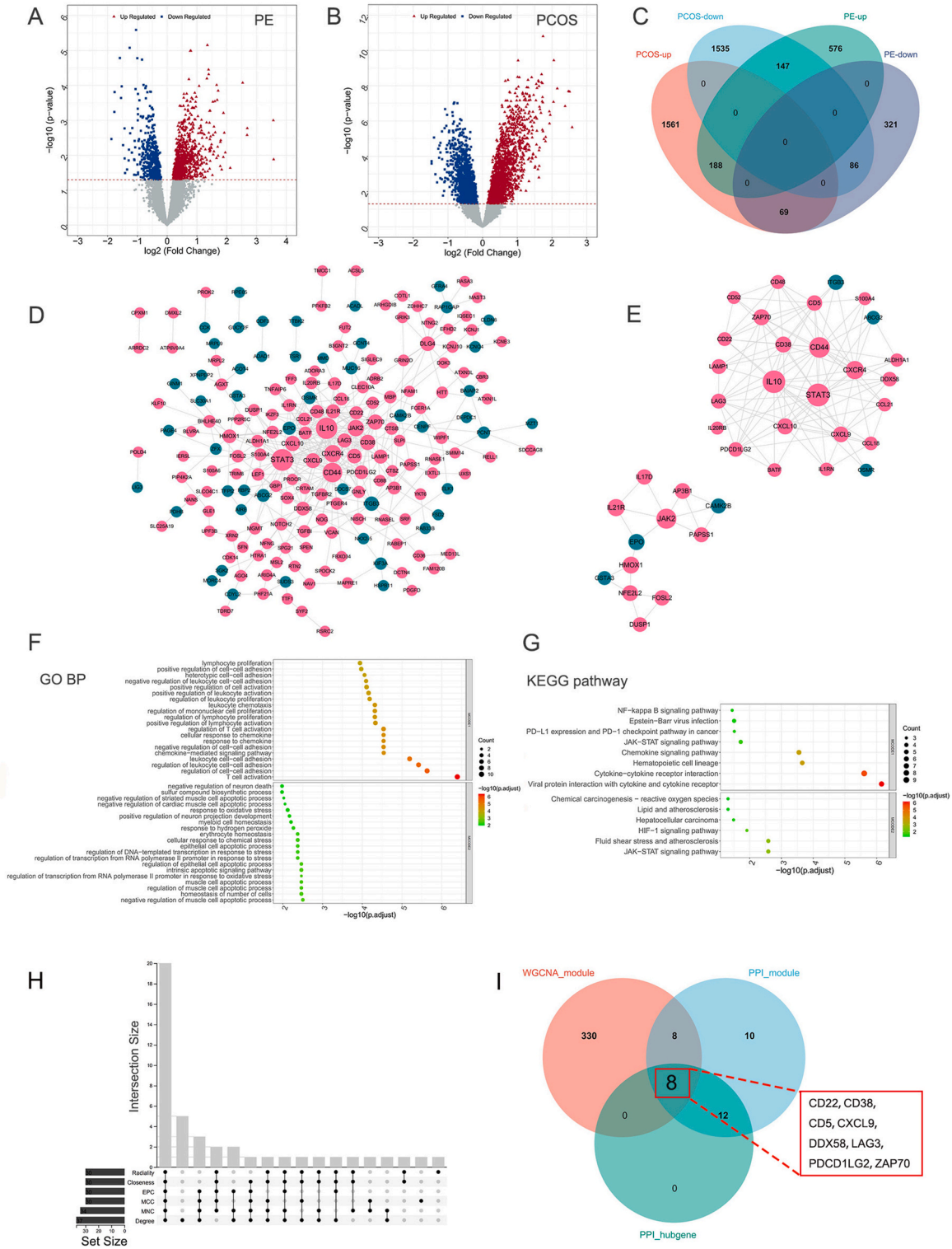

Fig. 2. The shared genes in PE and PCOS. (A) Volcano plot of DEGs in PE (n=1387). **(B)** Volcano plot of DEGs in PCOS (n = 3586). Red dots represent up-regulated DEGs, blue dots represent down-regulated DEGs, and gray dots represent genes that were not significantly different between the two groups. **(C)** Venn diagram of DEGs between PE and PCOS. **(D)** PPI network of common DEGs between PE and PCOS. The pink circles represent the up-regulated genes, the green circles represent the down-regulated genes, and the larger the node, the larger the degree value. **(E)** Key modules of PPI network with more than 10 nodes. **(F)** The GO-BP analyses of key modules of the PPI network. **(G)** The KEGG pathway analyses of key modules of the PPI network. **(H)** The hub genes of PPI network were filtered by plug-in CytoHubba. The bubble size represents the number of

genes associated with each term. The color of each bubble represents the adjusted *P*-value: the redder the color, the higher the enrichment. **(I)** The shared genes were obtained by the intersection operation of genes of WGCNA modules, key genes of PPI network modules, and hub genes of the PPI network. PE, preeclampsia; PCOS, polycystic ovary syndrome; DEGs, differential expression genes; GO, Gene Ontology; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction; WGCNA, Weighted Gene Co-expression Network Analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

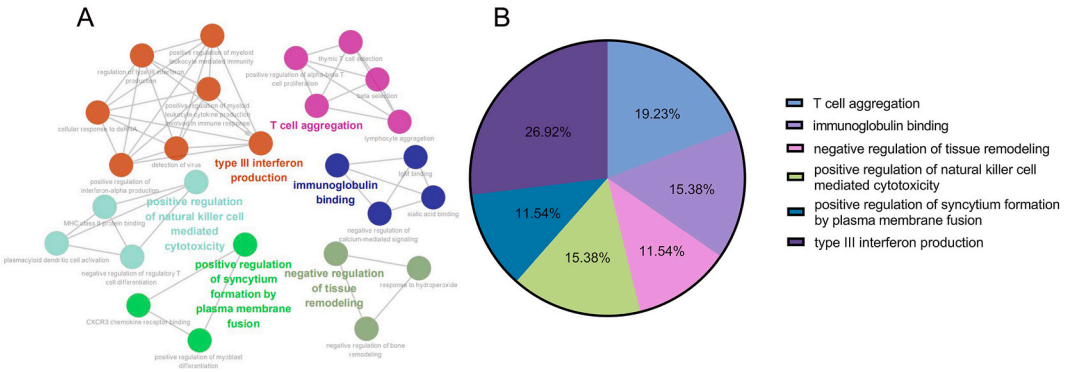

Fig. 3. The shared genes signatures in PE and PCOS. (A) The interaction network of GO terms of shared genes performed by ClueGO and CluePedia enrichment analysis. **(B)** Pie chart of the proportion of GO terms. PE, preeclampsia; PCOS, polycystic ovary syndrome; GO, Gene Ontology.

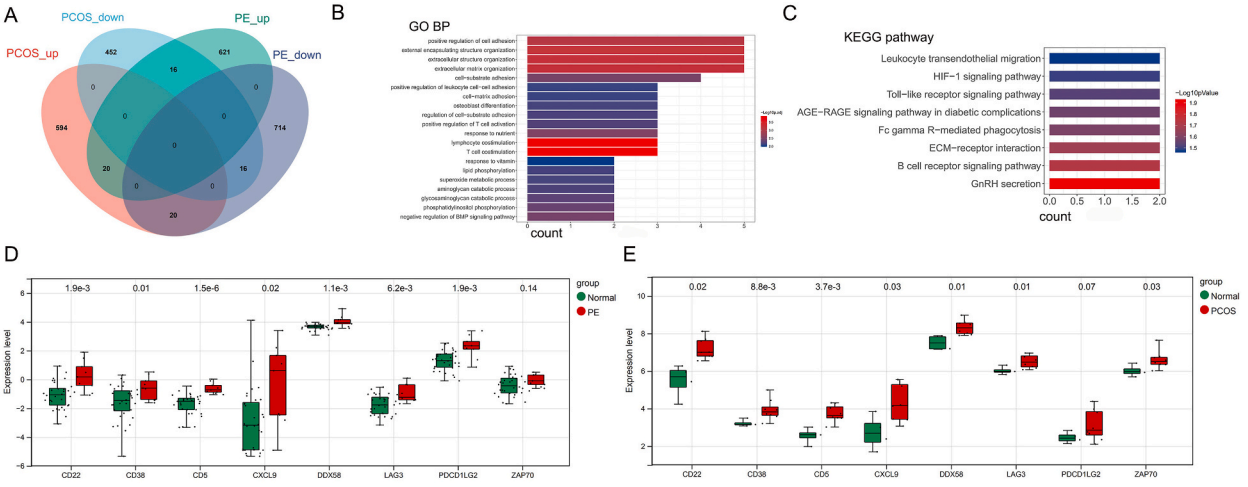

Fig. 4. Validation of shared genes and genes signatures with validation Cohort. (A) Venn diagram of DEGs between PE and PCOS in Validation Cohort. **(B)** The GO biological process analyses of DEGs in Validation Cohort. **(C)** The KEGG pathway analyses of DEGs in Validation Cohort. **(D)** The expression levels of shared genes in PE validation datasets. **(E)** The expression levels of shared genes in PCOS validation datasets. The numbers at the top of the figure represents *P*-values. **(D) (E)**Shapiro Wilk test was used to evaluate whether the data set is normally distributed. Student's t-test was used to estimate statistically significant differences. $P<0.05$ was considered as significant difference. PE, preeclampsia; PCOS, polycystic ovary syndrome; GO, Gene Ontology; DEGs, differential expression genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

[30]. The pathogenesis of PCOS also has yet to be elucidated, the available evidence showed that besides hereditary factors, PCOS may also be affected by environmental factors such as diet and lifestyle choices [3]. Likewise, increasing shreds of evidence suggested that the destruction of immune homeostasis was closely tightly related to the pathological process of PCOS [31,32].

In this study, the top 8 shared genes screened by bioinformatics analysis and their key biological functions suggested that abnormal immune responses were closely related to the etiology of these two diseases. CD5, the marker for identifying several immune cells since its discovery, was recently found to modulate different aspects of immune response and could be used as a target for immune intervention in different pathologies [33]. CD22 is a regulator of B cell responses and autoimmunity and has been widely verified by clinical trials as a target for leukemia treatment in recent years [34]. CD38 is expressed mostly in immune cells and plays an important role during inflammation and autoimmunity [35]. CXCL9 mainly regulates immune cell migration, differentiation, and activation and has gained attention as a target for the management of several types of cancers [36]. LAG3 is an inhibitory coreceptor that serves as a

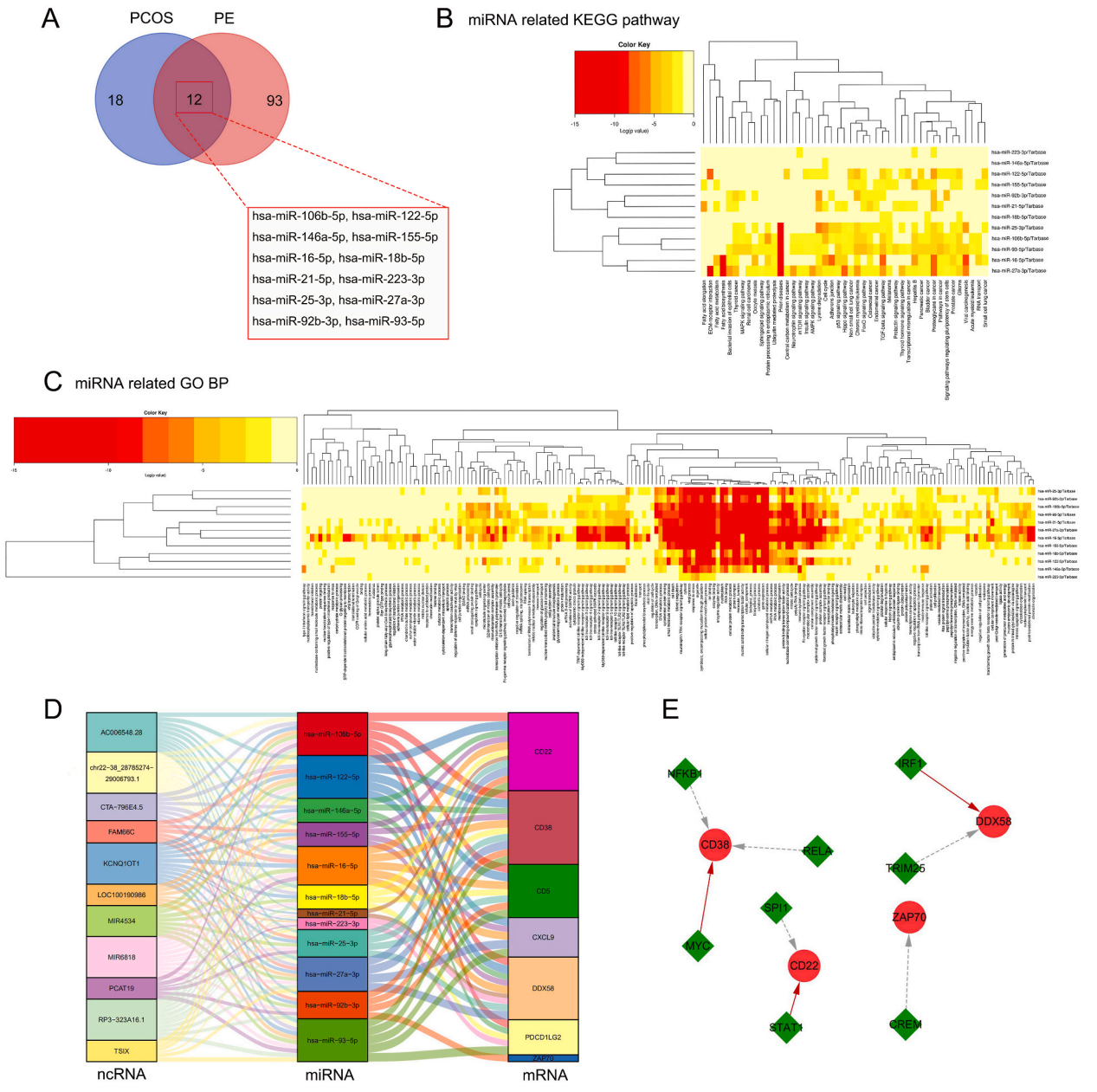

Fig. 5. The common lncRNA-miRNA-mRNA network in PE and PCOS. (A) Venn diagram of common miRNAs associated with shared genes between PE and PCOS. **(B)** The KEGG pathway analyses of common miRNAs. **(C)** The GO biological process analyses of common miRNAs. The redder the color, the more significant the enrichment. **(D)** The Sankey diagram of common lncRNA-miRNA-mRNA network. **(E)** The TF-mRNAs network constructed by TRRUST. The red circles represent the shared genes and the green boxes represent transcription factors. The red solid arrows indicate transcriptional activation and the gray dotted arrows indicate that the regulation modes were obscure. PE, preeclampsia; PCOS, polycystic ovary syndrome; KEGG, Kyoto Encyclopedia of Genes and Genomes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

critical checkpoint in autoimmunity, tumor immunity, and anti-infection immunity [37]. PDCD1LG2, a member of the B7 family, plays a crucial role in the induction and/or maintenance of peripheral tolerance and autoimmune disease [38]. ZAP70 is a master regulator of adaptive immunity, and aberrant ZAP70 expression is a common feature in a broad range of B-cell malignancies [39]. All these genes are well-known in immunomodulatory.

In addition to the shared genes described above, experimental verification showed that DDX58 (DExD/Hbox helicase 58, also known as RIG-1 or RIG-I) was the only shared gene significantly up-regulated both in PE and PCOS, which was consistent with the prediction results of bioinformatics. DDX58, an archetypal member of the cytoplasmic DEAD-box dsRNA helicase family (RIG-I-like

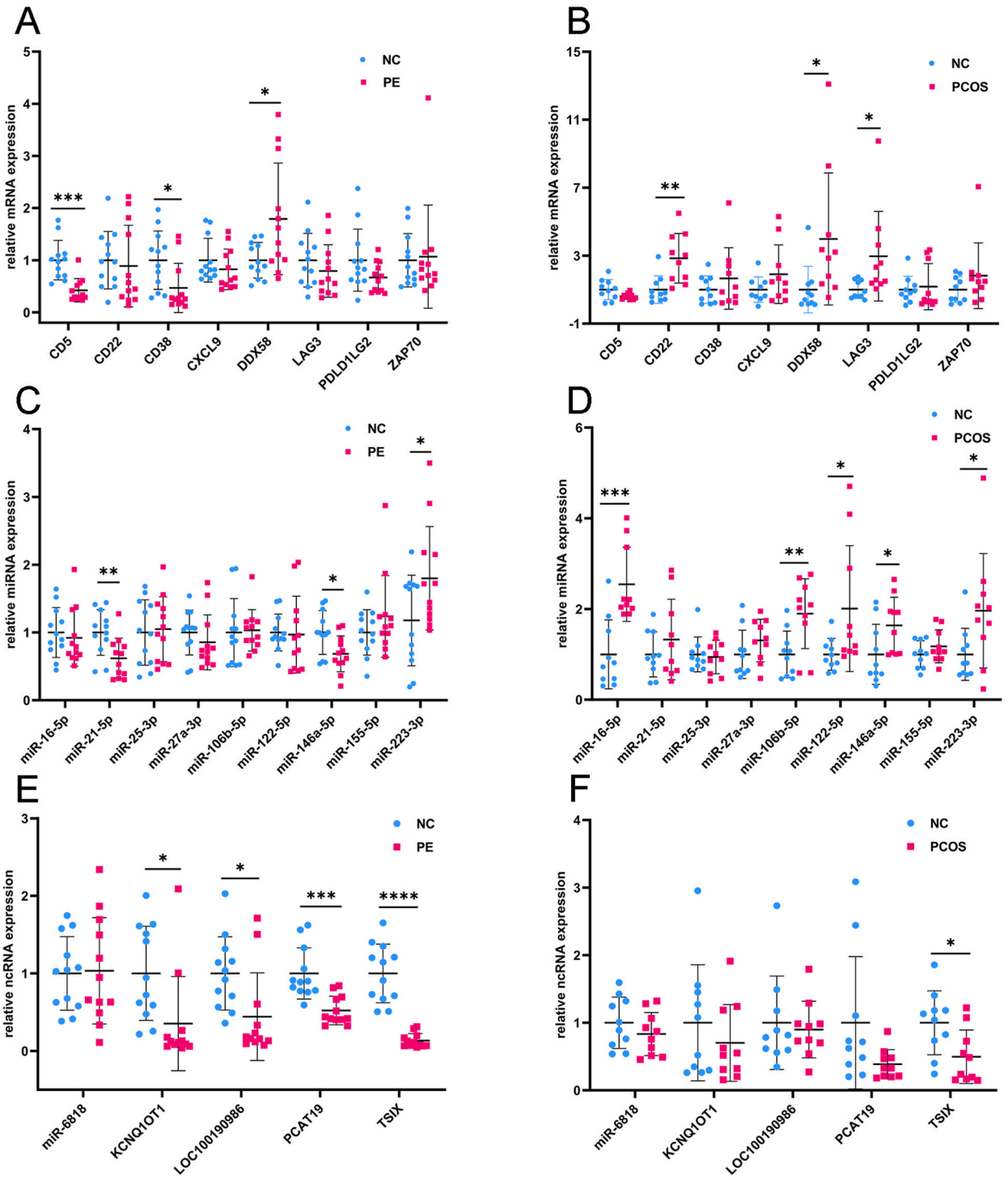

Fig. 6. Verification of common lncRNA-miRNA-mRNA network by qRT-PCR in PE and PCOS. The mRNA expression levels of 8 shared genes in PE **(A)** and PCOS **(B)**. The expression levels of 9 miRNAs targeting DDX58 in PE**(C)** and PCOS **(D)**. The expression levels of 5 ncRNAs targeting miR-223–3p in PE **(E)** and PCOS **(F)**. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ***$P<0.001$. PE, preeclampsia; PCOS, polycystic ovary syndrome; lncRNA, long-coding RNA; miRNA, microRNA.

receptors or RLRs), is wildly known as a pattern recognition receptor (PRR) that detects exogenous viral RNAs as a pathogen-associated molecular pattern (PAMP) [40,41]. However, the long-held paradigm of DDX58 serving as a PRR has been extended due to emerging new findings on the biological functions of DDX58 in other cellular activities, such as cell proliferation and differentiation, and the

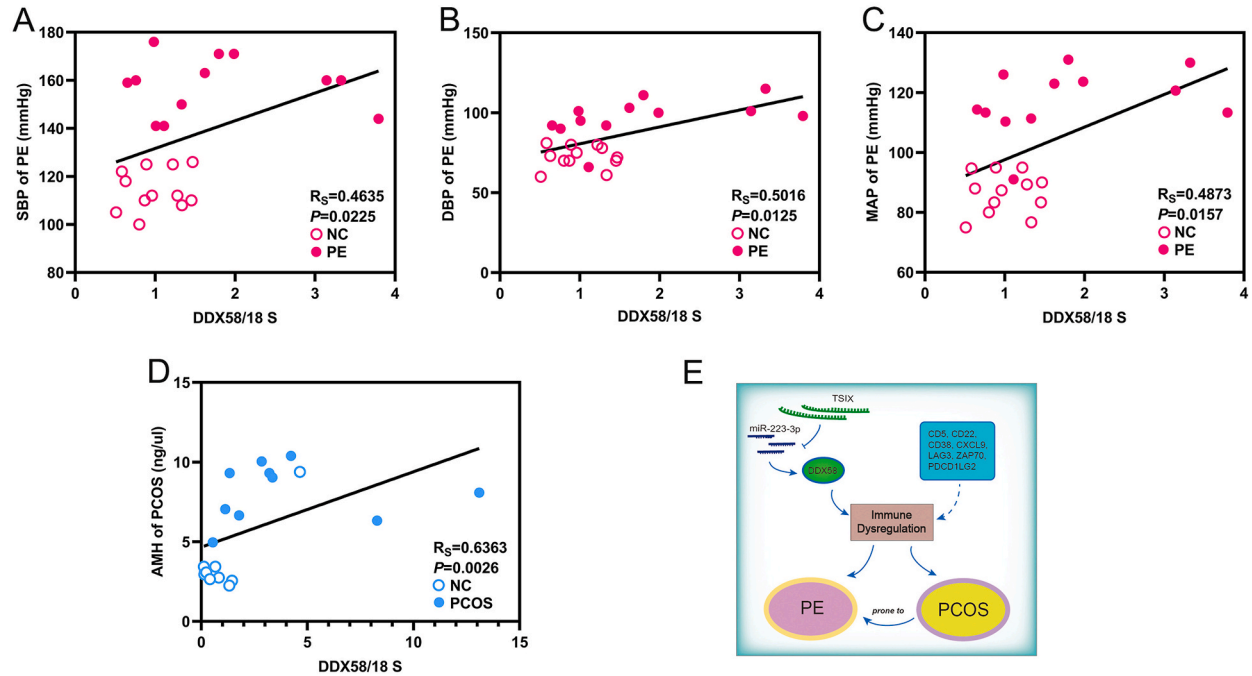

Fig. 7. Correlations between the DDX58 expression and clinical characters of PE and PCOS. Correlations between the expression of DDX58 and SBP **(A)**, DBP **(B)**, and MAP **(C)** in PE. Correlation between the expression of DDX58 and AMH **(D)** in PCOS. **(E)** The common pathway of PE and PCOS demonstrated that DDX58 might play a crucial role in the pathogenesis of PE and PCOS and immune dysregulation might be the common mechanism of PE and PCOS pathogenesis. PE, preeclampsia; PCOS, polycystic ovary syndrome; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; AMH, anti-Mullerian hormone.

activation of T cell-independent B cells [42]. As additional insights into the functions of DDX58, study populations were also not limited to those with immune diseases or cancers. A previous study on PE demonstrated that DDX58/MDA5 mediated induction of IFN-α/β pathway was identified as the seed pathway, in which activity score did the biggest changes occur by the pathway crosstalk network (PCN) related analysis among PE patients and normal controls [43]. Furthermore, there was also another study suggested that dsRNA activating DDX58 contributed to the development of PE by inducing a pro-inflammatory and pro-oxidative stress state [44]. Nevertheless, any similar research has not been reported in PCOS at least at present. To our knowledge, this was the first study to investigate the role of DDX58 both in PE and PCOS.

We demonstrated the predictive potential of DDX58 and related ncRNAs targeting DDX58 for these two diseases by bioinformatics analysis and experimental verification, and we further wondered whether they might become novel therapeutic targets in PE and PCOS. DDX58 possesses two N-terminal caspase-recruitment domains (CARDs), a DExD/H-box RNA helicase domain, and a C-terminal domain (CTD) [45]. Upon ligand recognition and binding, as DDX58 undergoes a conformational change, its CARDs domains become available for homotypic interactions with membrane-associated common adaptor proteins mitochondrial antiviral-signaling (MAVS) [46]. The available studies have held the use of specific DDX58 ligands had great promise as novel pan-antivirals, vaccine adjuvants, or potentiators of anticancer immunotherapies [47], but whether similar interactions also exist in the pathogenesis of PE and PCOS are unclear until now, which needs further experimental exploration. In our study, we assessed the correlations between the DDX58 expression and the main clinical characteristics of these two diseases and found that the increased DDX58 was significantly associated with elevated BP levels in PE and increased secretion of AMH in PCOS. Given the importance of these two indices in the corresponding diseases [48], our findings might partially suggest the potential of DDX58 as a novel diagnostic and therapeutic target for PE and PCOS.

There were also some studies about PE or PCOS by the method of Genome-wide association study (GWAS) and Single cell RNA sequencing (scRNA seq). These findings further confirm the close correlation between cardiovascular health and preeclampsia, and provide new targets for future pathological and physiological research or maternal immune in important in the preeclampsia [49,50]. In addition, a GWAS study of PCOS found that many differentially expressed genes were enriched in immune and inflammation related pathways, confirming the crucial role of inflammation and immunity in the development of PCOS [51,52], which is consistent with our research. However, these two research methods did not investigate the correlation between PE and PCOS. There might be a clue that further study could be done from the data of GWAS and scRNA seq.

In addition, there are several limitations in this study as follows: First, we had only a limited number of paired samples for the experimental validation, the validation results could only partially prove the reliability of DDX58 as a predictive target of PE and PCOS, and the other 7 shared genes could not be completely excluded by the qPCR results alone. Second, samples from those who were simultaneously afflicted with PE and PCOS was difficult to collected, which might not be more convincing to verify the expression levels of shared genes. What's more, so far, progress has been made in revealing the pathogenesis and pathophysiology of

preeclampsia. It may be related to multiple factors, but genetic factors play an important role among them. GWAS found that preeclampsia is a polygenic genetic disease with genetic susceptibility [53]. In addition, conflicts caused by incompatibility between maternal and fetal genomes (maternal-fetal conflicts) result in maternal genetic changes affecting the uterine environment, or fetal genetic changes affecting the mother, ultimately leading to preeclampsia [54]. However, in our study, we were unable to identify the shared genes by which method caused these two diseases. Finally, more evaluations on clinical prediction and treatment prospects for DDX58 and related ncRNAs targeting DDX58 should be performed.

In conclusion, our study demonstrated a common disease pathway model TSIX/miR-223–3p/DDX58, illustrating that immune dysregulation may be a possible mechanism of PE and PCOS, and revealed that DDX58 might be a novel predictive target for PE and PCOS.

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Availability of data and materials

The datasets are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Ethics Committee of the International Peace Maternity and Child Health Hospital, Shanghai Jiao Tong University School of Medicine (Shanghai, China). A written informed consent was obtained from all participants. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Yaoxi Xiong: Writing – original draft, Methodology, Formal analysis. **Chao Chen:** Writing – original draft, Methodology, Formal analysis. **Chengrong He:** Investigation, Data curation. **Xingyu Yang:** Writing – review & editing, Supervision, Funding acquisition. **Weiwei Cheng:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

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