International Journal of General Medicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Screening Preeclampsia Genes and the Effects of CITED2 on Trophoblastic Function

Xiujing Lu*, Xi Lan*, Xiaoqian Fu, Jing Li, Min Wu, Lu Xiao, Yachang Zeng

Department of Obstetrics and Gynecology, The First Affiliated Hospital of Guangxi Medical University, Nanning, 530021, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yachang Zeng, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Guangxi Medical University, Nanning, 530021, People's Republic of China, Email zengyachang@163.com

Purpose: Preeclampsia (PE) is a serious complication of obstetrics and represents a significant challenge in terms of understanding its underlying mechanism. It has been shown that a number of disorders involve dysregulation of the CBP/p300-interacting transactivator with glutamic acid/aspartic acid-rich carboxyl-terminal domain 2 (CITED2). However, the relationship between PE and CITED2 is still mostly unclear. This work aimed to confirm the hub genes linked to PE and explore the roles of CITED2 in trophoblast using experimental and bioinformatic methods.

Methods: To determine the hub genes, bioinformatics research was performed on two datasets from the Gene Expression Omnibus (GEO) public database. Immune infiltration analysis and enrichment analysis were also used to identify the related pathways and immune cells. PCR and WB were then used to validate the mRNA and protein levels of CITED2 in the PE samples. Finally, the expression of CITED2 was knocked down using siRNA to investigate the function of CITED2 in trophoblast development in vitro.

Results: The study's findings showed that the NOTCH signaling pathways, glycolysis, and hypoxia were the main areas of enrichment for the six PE-related genes that were tested. The results of immune infiltration suggest that activated NK cells and regulatory T cells may play an important role in this process. CITED2 was significantly upregulated in the PE placenta. In functional tests, the knockdown of CITED2 may enhance apoptosis while suppressing migration, invasion, and proliferation of cells.

Conclusion: This study offers important proof that CITED2 influences trophoblast cell function and may one day be a therapeutic target for PE.

Keywords: preeclampsia, CITED2, placenta, cell function, bioinformatics

Introduction

Preeclampsia (PE) is a pregnancy-specific condition that is typically characterized by proteinuria and new-onset hypertension after 20 weeks of gestation.[1] PE can affect several organ systems throughout the body, and in extreme circumstances, it can result in mortality, convulsions, coma, and disseminated intravascular coagulation.[2] Intrauterine growth retardation, oligohydramnios, intrauterine discomfort, early birth, etc. are the main impacts on the fetus.[1] PE affects 2–5% of pregnant women globally and results in about 70,000 prenatal fatalities and 500,000 neonatal deaths annually.[3,4] The placenta is thought to play a central role in the pathogenesis of PE,[5] and inflammation, oxidative stress, and angiogenesis disorders are recognized as the underlying mechanisms involved.[6] Nonetheless, the pathogenesis of PE remains unclear.

Although delivery is currently the most effective treatment, it does not mitigate the long-term consequences of PE for the mother or the fetus. These long-term consequences include an increased risk of cardiovascular disease in mothers and various fetal diseases.[7] Consequently, the etiology and management of PE have long been popular subjects in obstetrics. Currently, no precise markers exist that can reliably predict PE or provide a genetic explanation for its occurrence.[8] For the sake of the health of the mother and fetus, it is crucial to thoroughly investigate the connection between the presence of additional new genes and PE.

Received: 14 June 2024
Accepted: 7 August 2024
Published: 14 August 2024

CBP/p300-interacting transactivator with glutamic acid/aspartic acid-rich carboxyl-terminal domain 2 (CITED2) is a widely expressed transcriptional cofactor protein. As the most studied member of the CITED family, it plays a key role in many biological processes.[9] Numerous cell processes and organelles are impacted by this factor, including the inhibition of endothelial cell development and proliferation,[10,11] the induction of mitochondrial death, the induction of nerve cell apoptosis and DNA damage.[12,13] CITED2 plays a key role in placenta formation, which is a significant process by which the regulatory network controls trophoblast cell development.[14] It contributes to the typical development of the mouse placenta and controls the differentiation of trophoblast cells.[15,16] The absence of CITED2 limits placental and fetal development in mice.[17]

Moreover, CITED2 is a conserved regulator of the uterine-placental interface. Decreased expression of CITED2 limits the differentiation and cell invasion of extravillous trophoblast cells, and in rats, CITED2 reduction results in placental and fetal growth restriction.[14] Under hypoxic conditions, trophoblast cells express more CITED2, which competitively inhibits p300/CBP's recruitment to the PlGF HRE-HIF-1 complex in trophoblast.[18] CITED2 protein expression declines and extracellular vesicle release rises in an endoplasmic reticulum stress model of BeWo cells, which could explain the heightened maternal systemic inflammatory response in PE.[19] To summarize, while many reports have indicated a correlation between CITED2 and placental tissue, its significance for PE remains uncertain, as does its impact on trophoblast cells migration, proliferation, and other related processes. We first investigated the biological function of CITED2 in trophoblast cells in order to better understand its involvement in the development of PE.

Six hub genes, CITED2, BAF nuclear assembly factor 1 (BANF1), pleckstrin homology and RhoGEF domain containing G2 (PLEKHG2), plectin (PLEC), SAM and SH3 domain containing 1 (SASH1), and notch receptor 3 (NOTCH3) were found to be important for PE in this study after bioinformatics techniques were applied to analyze two public datasets. The hub genes and PE may be intimately associated with regulatory T cells and activated NK cells, and glycolysis, hypoxia, and the NOTCH signaling pathway may be crucial in these processes. In addition, we confirmed CITED2's function in in vitro studies. This research may contribute to the development of CITED2 as a viable therapeutic target for PE by shedding light on the genes linked to PE and the biological role of CITED2.

Materials and Methods

Bioinformatics

Datasets

Downloading a single-cell RNA sequencing (scRNA-seq) dataset (GSE173193) and a bulk RNA sequencing dataset (GSE10588) from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/). The quality of GSE173193 raw data was assessed by Seurat v.5.1.0 and obtain a preliminary single-cell document. To generate the gene expression matrice for GSE10588, probe IDs were converted to gene symbols using probe annotation information, and unique gene was retained by averaging expression. The basic information of two datasets was shown in Table 1.

Single-Cell Data Analysis

The DropletUtils v.1.24.0, Scater v.1.24.0 and Seurat v.5.1.0 were used for quality control of the raw data of the GSE173193 dataset, removing barcodes without gene expression and filtering out genes with less than 200 unique molecular identifiers (UMI), more than 10% mitochondrial gene expression, and less than 10% ribosome gene expression. We performed linear scaling and dimensionality reduction analysis of the expression data using ScaleData and RunPCA functions. The principal components (PCs) with large standard deviation were used for cell clustering. Nonlinear dimensionality reduction analysis was performed using the reduceDimension function. Differentially

Table 1 Characteristics of the Datasets

Datasets	Type	Control	PE	Source
GSE173193	High throughput sequencing	2	2	GPL24676
GSE10588	Array	26	17	GPL2986

expressed genes between each cluster and all other cells were identified using the FindMarkers function, with minimum cell population expression≥25%, log2FC≥0.1, and p<0.05. We used the top logFC markers to label the cells.

Differentially Expressed Genes (DEGs) Analysis

To identify DEGs in the GSE10588 dataset, we used the normalizeBetweenArrays function to standardize the samples. Next, DEGs based on the criterion "| logFC | > 1 and adj. P.Value < 0.05" were obtained using the limma v.3.60.3. The ggplot2 v.3.5.1 was used for visualization and marked genes corresponding to the criteria "| logFC | > 2, adj. P.Value < 0.01".

Weighted Gene Co-Expression Network Analysis (WGCNA)

The GSE10588 dataset was analyzed using the WGCNA v.1.72.5. The filter standard MeanFPKM was set to 0.5, and anomalous data were excluded. The bare minimum of genes in each module (minModuleSize=100) and the optimal soft threshold (soft power=9) were set to divide the gene modules. Modules with similarities greater than 0.75 were aggregated based on a MEDissThres of 0.25. Then modules that showed a strong positive correlation with PE were identified.

Enrichment Analysis and Machine Learning Algorithm

Venn v.1.12 and randomcoloR v.1.1.0.1 were used to obtain intersection genes associated with PE from scRNA-seq, DEGs, and WGCNA analysis, and plot the diagram. Next, LASSO regression and random forest, two machine learning methods, were used to examine and filter the intersection genes in more detail. Glmnet v.4.1.8 was used to get the genes corresponding to the minimum lambda value, setting "nlambda=100, alpha=1, nfolds = 10". Then RandomForest v.4.7.1.1 was used to obtain genes corresponding to the minimum error point. And the remaining genes from two algorithms were intersected using venn v.1.12 to obtain hub genes. In addition, in order to understand the possible gene enrichment pathway, we performed gene set enrichment analysis (GSEA) enrichment analysis. It was performed for genes of GSE10588 dataset using org.Hs.eg.db v.3.19.1 and clusterProfiler v.4.12.0, setting "pvalueCutoff = 0.1".

Immune Infiltration and Gene Set Enrichment Analysis

Immune infiltration analysis was performed on the GSE10588 dataset and hub genes to investigate the association between PE and immune cells. The CIBERSORT v.1.03 was used to calculate immune scores between each sample and different immune cells. R 4.4.1 was used to generate relevant graphs of immune cell infiltration in the control group and PE group, as well as the correlation between various immune cells and hub genes expression. The scores of different sample gene sets were calculated by single-sample gene set enrichment analysis (ssGSEA) in GSVA v.1.52.3. The differences between the control group and PE group in GSE10588 were compared with *t* test and using the limma v.3.60.3 and GSEABase v.1.66.0. The correlation between the expression of hub genes was also analyzed.

Gene Expression and CITED2 Single Gene Analysis

Firstly, hub genes expression between different groups in GSE10588 were compared by *t* test, and expression boxplots were drawn using the ggpubr v.0.6.0 and ggsci v.3.2.0. We then used clinical samples to validate their expression. Secondly, the signal pathways associated with CITED2 were analyzed by single gene GSEA using the org.Hs.eg.db v.3.19.1. PvalueCutoff set to 0.05 and the top 3 enriched pathways were plotted. And then receiver operating characteristic (ROC) curves were used to assess the ability of CITED2 to discriminate PE in GSE10588. ROC objects were established and AUC values were calculated according to the data set and samples. Confidence intervals (CIs) were determined by 2000 repeated calculations. The ROC curve was drawn, and further information was added using the pROC v.1.18.5. Finally, drugs associated with CITED2 were predicted by Enrichr (https:// maayanlab.cloud/Enrichr/). The DSigDB database, which is rich in gene-targeted drugs, was used in conjunction with the Enrichr website. The conditions were set as p value <0.01 and adj.p value <0.05 for drug prediction.

Experiment

Clinical Samples

This study included 24 clinical samples, and their clinical characteristics were shown in Table 2. Fresh samples, including control and PE placenta tissues, were collected during cesarean section at the First Affiliated Hospital of Guangxi

Table 2 Characteristics of Clinical Samples

Parameters	Control (n=10)	PE (n=14)	P value
Maternal age (year)	32.70±3.59	32.00±5.63	0.733
BMI (kg/m2)	27.60±3.14	28.50±3.58	0.531
Termination of gestational age (day)	266.30±2.67	262.43±9.63	0.084
Gravida (time)	3.20±1.48	1.79±1.05	0.012
SBP (mmHg)	109.80±9.11	154.64±16.44	<0.0001
DBP (mmHg)	69.70±10.33	98.29±15.76	< 0.0001
Fetal birth weight (g)	3266.00±368.79	2358.57±383.56	< 0.0001
Urine protein	- ~ ±	- ~+++	NA
Proteinuria (mg/24h)	NA	158.10~8803.10	NA

Notes: Urine protein was presented by degree symbol (-~+++), "-" represents negative, "+" represents positive, and the number of "+" is positively correlated with the degree. Proteinuria (mg/24h) in the disease group is represented by a range and the remaining indicators were described by mean ± SD. *t* test was used in all parameters except Mann–Whitney test for the termination of gestational age. P value <0.05 was considered as significant difference. Proteinuria (quantity of 24 h urine protein excretion) higher or equal to 120 mg/24 h was proteinuria positive and lower than 120 mg/24 h was proteinuria negative. NA indicates that information was missing or the result cannot be calculated.
Abbreviations: BMI, body mass index; SBP, systolic blood pressure on admission; DBP, diastolic blood pressure on admission; SD, standard deviation.

Medical University. All collected samples were stored at −80°C until future tests. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, Guangxi, China. Informed consent was obtained from the patients upon hospitalization.

Cell Culture and Transient Transfection

HTR-8-Svneo (CL-0765) cell line were purchased from Wuhan Procell Life Science&Technology Co., Ltd. The cells were cultured with 10% fetal bovine serum (FBS, OriCell), 1% penicillin-streptomycin, and 5% carbon dioxide at 37°C. After HTR-8-Svneo cell line were inoculated for 12h, three siRNA and si-NC were transiently transfected into cells using the Lipofectamine™ 3000 (Thermo Fisher Scientific, US), respectively. Three siRNA sequences were in Table 3 (RiboBio, Guangzhou, China).

Real-Time Polymerase Chain Reaction (RT-PCR)

MagZol Reagent (Magen, China) and chloroform were added to the sample in a 5:1 ratio to extract total RNA. After high-speed centrifugation, the supernatant containing RNA was retained, and equal volume of isopropyl alcohol was added and left for rest at −20°C. Then the RNA precipitation was obtained by centrifugation again, and washed 2 to 3 times with 75% alcohol. Finally, the RNA was preserved in enzyme-free water after drying. Afterwards, the cDNA template was obtained by RevertAid™First Strand cDNA Synthesis Kit. And the PowerUp™SYBR™Green Master Mix and StepOnePlus real-time PCR systems for RT-PCR (Thermo Fisher Scientific, US). With β-actin as the internal parameter, and $2^{-\Delta\Delta Ct}$ method was used to analyze the relative expression of all factors. The information of primers was shown in Table 4.

Western Blotting (WB)

Firstly, the total protein was extracted by RIPA lysis buffer and the concentration was determined with BCA kit. Next, the total protein was separated by 12% SDS-PAGE gel and transferred to PVDF membrane (Millipore). Then,

Table 3 Three siRNA Sequences Used to Silence CITED2

Number	Name	Target sequence
stB0001598A	genOFFTM st-h-CITED2_001(si-1)	TCGACGAGGAAGTTCTTAT
stB0001598B	genOFFTM st-h-CITED2_002(si-2)	TAATGGGCGAGCACATACA
stB0001598C	genOFFTM st-h-CITED2_003(si-3)	AACCACTACATGCCGGATT

Table 4 Primer Sequence Used in This Study

Primer Name	Sequence (5' to 3')	Primer Length(bp)
PLEC-F	CGGAATACTTCACGGCAGAGCAG	23
PLEC-R	GTCGGATGGCAGCAGGTCTTTC	22
BANF1-F	CAACCTCCCAAAAGCACCGAGAC	23
BANF1-R	GCCACAAGTGTCTTTCAGCCATTC	24
PLEKHG2-F	GAAGAGGAGGAGGAGGAAGAGGAAG	25
PLEKHG2-R	GGAGCCAGGAGTCACAGGAGAG	22
CITED2-F	AAAGGGAACGGCTCCGAATC	20
CITED2-R	TGCCATTTCCAGTCCTTCCG	20
β-actin-F	CCTGGCACCCAGCACAAT	18
β-actin-R	GGGCCGGACTCGTCATAC	18

membranes were blocked under indoor environment with 5% slim milk for 1 hour, and incubated with primary antibodies against CITED2(28KDa, 1:600, Abmart) and internal reference β-actin (42 kDa, 1:10000, Servicebio) overnight at 4°C. Second day, Rabbit IgG (H+L) secondary antibody (1:200000, Cell Signaling Technology) was incubated in a shaker for 2 hours at room temperature and away from light. Finally, Odyssey (LI-COR, US) was used to visualize the immunoblots.

Cell Proliferation

The cell proliferation level was detected by Cell Counting Kit-8 (Uelandy, Suzhou, China). The cells (3.5×10^3 per well) were first cultured in 96-well plates (100 μL/well) in medium containing 10% FBS, and each group was set with six replicates. After 12 hours, the cells in each group were treated accordingly. Next, the liquid in each well was replaced with 90ul fresh medium and 10ul CCK-8 reagent at the appointed time point, and incubated at 37°C for 1 hour. Finally, OD values at 450nm were detected by enzyme marker. Three independent experiments were performed.

Wound Healing Assay

6-well plate were cultured 5×10^5 cells per well. After overnight, the cells of each group were treated accordingly. Then they were scratched straight line with a 200 μL pipette tip when they were 90% confluence. And the cells were cultured in serum-free medium under 5% CO2 atmosphere at 37 °C for 24 hours. Subsequently, taking pictures with the Leica microscope (Leica, Germany). Three independent experiments were performed.

Transwell Invasion Assay

The membrane was prepared in Transwell chamber (BIOFIL, Guangzhou, China) with 100μL Matrigel solution (Corning, CA, USA). 600μL DMEM containing 20% FBS was added to the lower chamber, and 200 μL serum-free medium containing 3.5×104 HTR8-SVneo cells were added to the upper chamber. After incubation for 36 h, the cells in the upper chamber were cleaned with cotton swabs, while the broken membrane cells were fixed with paraformaldehyde, and then stained with 0.1% crystal violet. Finally, photographs of the cells were taken using a Leica microscope (Leica, Germany). Three separate experiments were conducted.

Detection of Cell Apoptosis Rate

Annexin V-FITC/PI apoptosis Kit (Multisciences, Hangzhou, China) for the detection of apoptosis in each group was carried out according to the corresponding instructions. Trypsin was used to harvest cells that had been treated with siRNA and cultured for 24 hours. The cells were then washed twice with pre-cooled PBS, and next re-suspended with 500ul 1 × binding buffer and transferred into the flow tube. 10μL PI and 5μL Annexin V-FITC were added to each tube, mix well and incubate at room temperature for 5 minutes away from light. Finally, the apoptosis rate was detected by a C5 Plus flow cytometer (BD Biosciences, USA). Three independent experiments were performed.

Statistical Analysis

R 4.4.1 completed the bioinformatics analysis. GraphPad Prism 8.0.2 was used for statistical analysis of the experimental part. Data were indicated by mean ± standard deviation (SD). And the significance of the differences between the two groups was determined using a two-sided unpaired *t*-test when variance was homogeneous, otherwise, non-parametric test was used. $P < 0.05$ was considered as the standard of statistical significance. The symbols *p < 0.05, **p < 0.01, ***p < 0.001, ****p<0.0001, ns, were not statistically significant.

Results

The hypothesis diagram of our study is shown in Figure 1.

Gene Screening and Enrichment Analysis

There were 267 genes with significantly different expression between the control group and PE group in GSE10588, with met the criteria "|logFC| > 1, adj. P.Val < 0.05" (Figure 2A). In WGCNA, the modules with the strong correlations were identified (Figure 2B). We found strong positive associations between salmon and grey60 modules and PE, and they contained 884 genes. At the same time, a total of 7708 DEGs were identified based on "expression ratio of each cell > 0.25, adj.P.Val < 0.05" in single cell data analysis. They were used to annotate 18 clusters to 12 cell types (Figure 2C) and screen genes associated with PE. After performing intersection analysis on scRNA, WGCNA, and DEGs, there were 74 overlapping genes (Figure 2D). The results of GSEA enrichment analysis indicated that the genes were mainly enriched in Galactose metabolism, Adipocytokine signaling pathway, AMPK signaling pathway, Homologous recombination, Taste transduction (Figure 2E).

Hub Genes Were Obtained by a Machine Learning Algorithm

To further narrow the list of genes that could have a greater correlation with PE, we used LASSO regression and random forest algorithms. LASSO regression identified 11 genes (Figure 3A), and random forest analysis identified 35 genes (Figure 3B and C). Six genes (BANF1, CITED2, PLEC, PLEKHG2, NOTCH3, and SASH1) that overlapped between the two algorithms were identified via a venn diagram (Figure 3D).

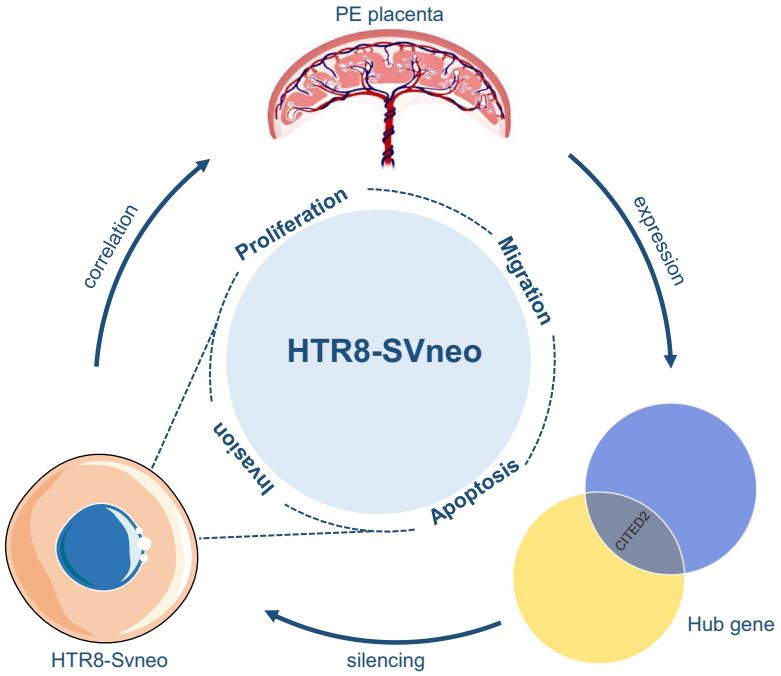

Figure 1 Research hypothesis diagram.

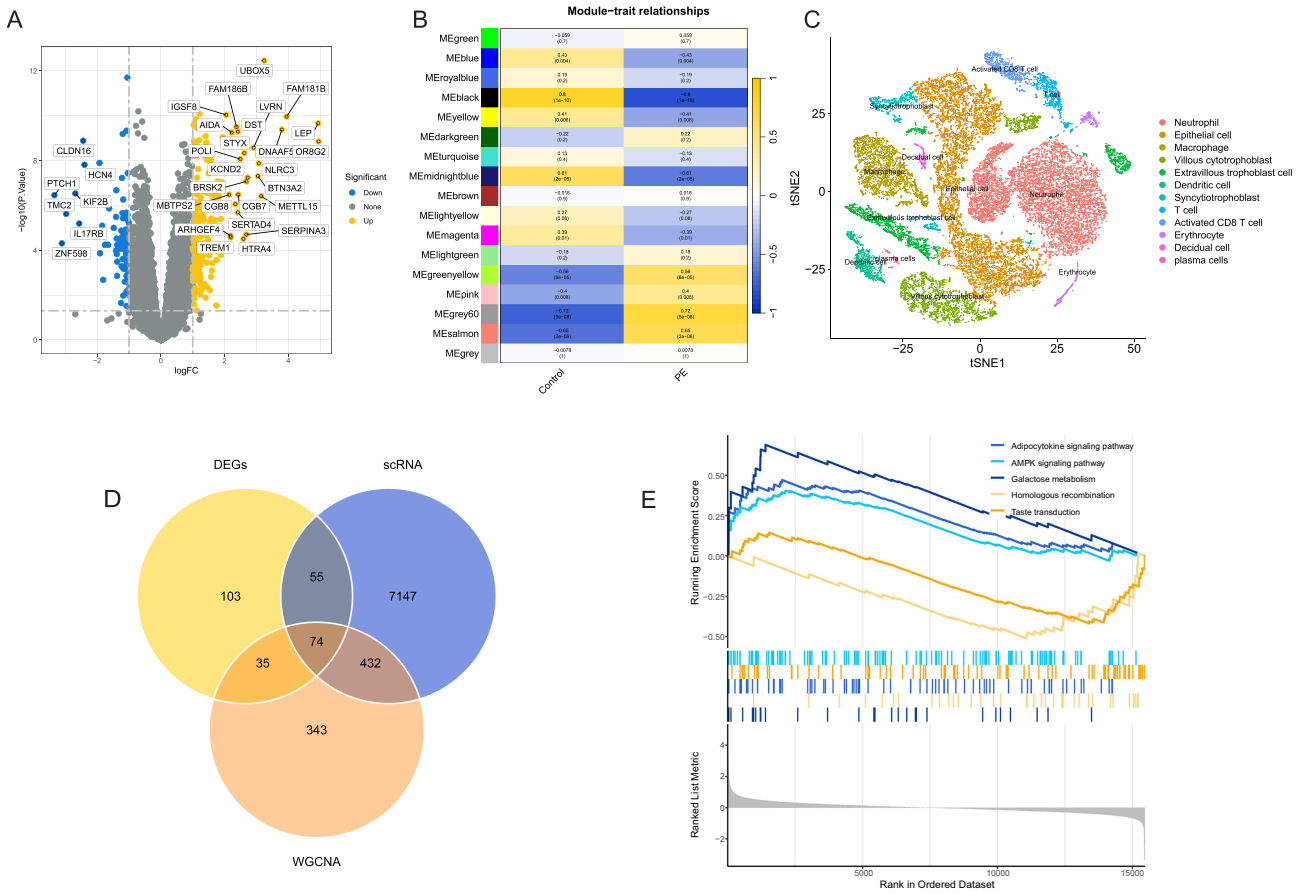

Figure 2 Gene screening and gene set enrichment analysis. (**A**) Volcano plot: differentially expressed genes were labeled with yellow (up-regulated in PE) and blue (down-regulated in PE). (**B**) Weighted gene co-Expression network analysis: Correlation between gene modules and groups, the darker the color, the stronger the correlation. (**C**) Identifying cell types and differential expression markers in GSE173193. (**D**) Venn diagram to obtain intersection genes. (**E**) Gene set enrichment analysis.

Immune Infiltration and Pathway Enrichment

To understand the relationships between immunity and PE and between immunity and the hub genes, we performed immune infiltration analysis. The results revealed two major categories of immune cells, regulatory T cells ($p<0.05$) and activated NK cells ($p<0.01$), which were significantly different between the control group and the PE group in GSE10588 (Figure 4A and B). And the fraction of activated NK cells decreased, and the number of regulatory T cells increased in PE group (Figure 4B). Activated NK cells, regulatory T cells, gamma delta T cells, resting dendritic cells, and CD8+ T cells were significantly correlated with the hub genes (Figure 4D). Furthermore, CITED2 was positively correlated with regulatory and CD8 T cells and negatively correlated with resting dendritic cells, activated NK cells and gamma-delta T cells (Figure 4D). There was an almost positive correlation between the expression of the hub genes (Figure 4C). The enrichment results suggested that many pathways were enriched among the groups, while the glycolysis, hypoxia, and NOTCH signaling pathways were most significantly different between the groups (Figure 4E). The hub genes were significantly related to several pathways, including hypoxia, glycolysis, NOTCH signaling, and the apical surface (Figure 4F).

Gene Expression and Validation

The relation between PE and NOTCH3 and SASH1 has been disclose by many studies, while there are no or few relevant reports on the BANF1, CITED2, PLEKHG2 and PLEC genes. Consequently, their mRNA expression and validation were measured using GSE10588 dataset and clinical samples. BANF1, CITED2, PLEKHG2 and PLEC had significantly higher expression levels in the PE group in the GSE10588 cohort (Figure 5A). RT-PCR revealed that the

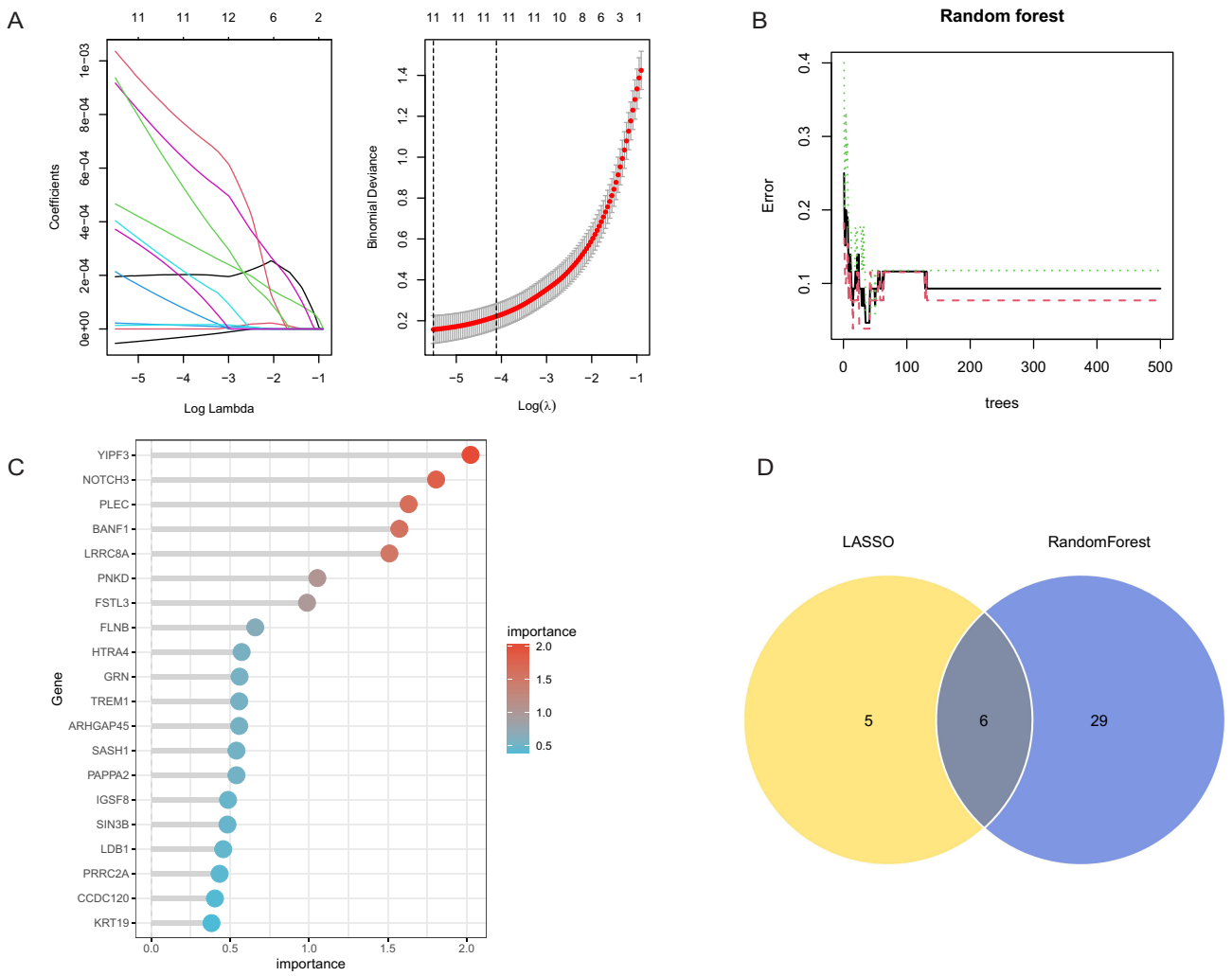

Figure 3 Machine learning algorithm to screen hub genes. (**A**) Intersection genes were further screened by LASSO regression. (**B** and **C**) Randomforest to screen genes. (**D**) Six hub genes (BANF1, CITED2, PLEKHG2, PLEC, SASH1, NOTCH3) were obtained by the intersection of venn diagram.

mRNA expression levels of BANF1, CITED2 and PLEKHG2 were different between the PE (n=14) and control (n=10) groups, and the expression was upregulated in the PE group, whereas there was no statistical difference on PLEC expression (Figure 5B). Combined with the relevant literature, we further studied the relationship between CITED2 and PE.

CITED2 Analysis and Protein Expression

Single-gene GSEA was carried out to investigate the primary signaling pathways associated with CITED2. The results suggested that the gene were related mainly to DNA replication, cell cycle, oocyte meiosis, spinocerebellar ataxia, proteasome, carbon metabolism, and Parkinson's disease, etc (Figure 6A). And Parkinson's disease, proteasome and spinocerebellar ataxia were the top 3 enriched pathways (Figure 6A). Moreover, according to the ROC analysis, CITED2 demonstrated good performance (AUC>0.85) in the GSE10588 cohort, indicating that it may be strongly correlated with PE (Figure 6B). In addition, the average CITED2 protein expression level was different between the control (n=10) and PE (n=14) groups, the PE group was higher than the control group (Figure 6C and D). According to the clinical specimens in our study, compared with those in the control group, both the protein and mRNA expression levels of CITED2 in the PE placenta tissues were upregulated.

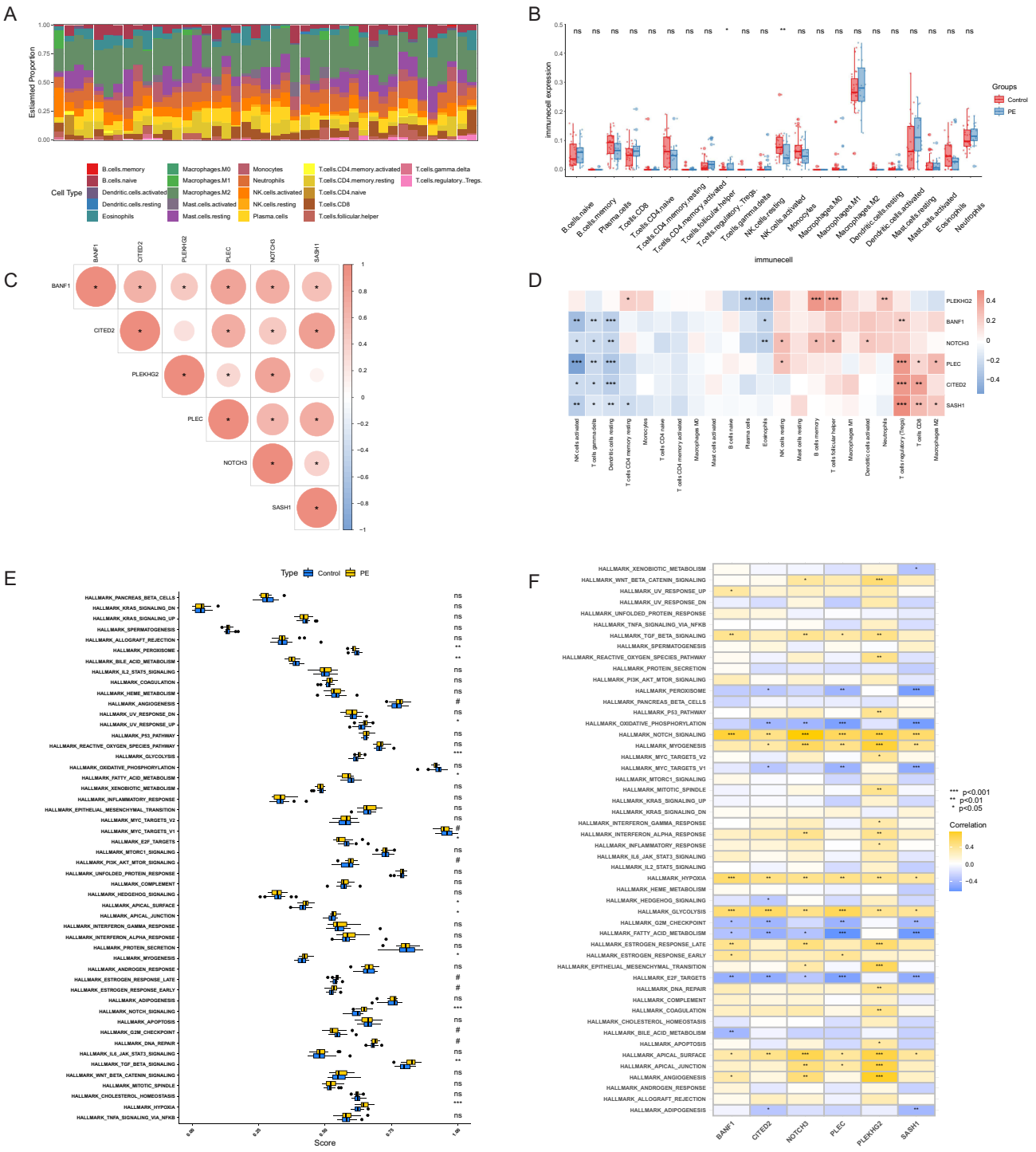

Figure 4 Immune infiltration and pathway enrichment analysis. (**A**) The proportion of 22 types of immune cells in different samples of GSE10588. (**B**) Correlation between 22 types immune cells and groups. (**C**) The correlation between hub genes. (**D**) The correlation between immune cells and hub genes. (**E** and **F**) Single-sample gene set enrichment analysis to explore the correlation between enrichment pathways and groups and the enrichment pathways and hub genes. *p<0.05, **p<0.01, ***p<0.001, # 0.05≤p<0.2, ns, p≥0.2.

CITED2 Silencing

RT–PCR and WB were used to verify the silencing effect of the three designed siRNAs on CITED2. The expression levels of CITED2 mRNA in the three siRNA-silenced cell lines were significantly different from those in the control group (Figure 6E). In terms of protein expression levels, the si-1 and si-2 groups exhibited significant differences

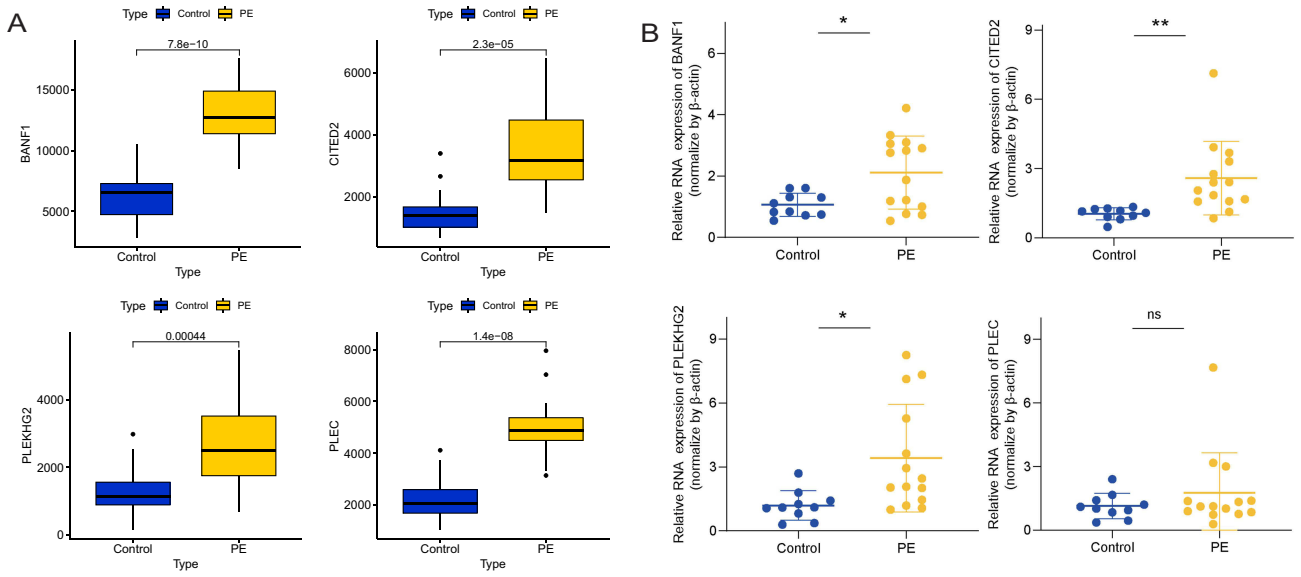

Figure 5 Gene's mRNA expression and clinical sample validation. (**A**) The mRNA expressions of BANF1, CITED2, PLEKHG2, PLEC in GSE10588 samples. (**B**) The mRNA expressions of BANF1, CITED2, PLEKHG2, PLEC in 24 clinical samples, including control group (n=10) and PE group (n=14). The data shown as mean ± SD and *p < 0.05; **p < 0.01; ns, p>0.05.

compared with those of the si-NC group, but there was no significant difference between the protein expression levels of the si-3 group and those of the si-NC group (Figure 6F and G). The expression of both the mRNA and protein was mostly reduced in the si-2 group.

CITED2 Silencing Inhibited the Proliferation of HTR8-SVneo Cells

According to the results of the cell proliferation assay, the OD values of cells in the siRNA groups were significantly lower than those in the si-NC and control groups (Figure 7A). The OD values of the si-2, si-1, si-3, si-NC and control groups increased successively, which indicated that their proliferation levels increased successively. This order corresponded to the corresponding mRNA and protein expression levels in the CITED2-silenced cells. The results suggested that silencing CITED2 expression may inhibit the proliferation of HTR8-SVneo cells. In addition, CITED2 was most significantly silenced by si-2, which could be used in subsequent wound healing assays, transwell invasion assays and detection of the cell apoptosis rate.

CITED2 Silencing Suppressed the Migration and Invasion Ability of HTR8-SVneo Cells

The healing area of the si-CITED2 group was smaller than that of the si-NC and control groups (P<0.001), and there was no significant difference between the si-NC group and the control group (P>0.05) (Figure 7B and C). These findings indicate that silencing CITED2 in HTR8-SVneo cells may inhibit their migration ability. In addition, there were significantly fewer transmembrane cells in the si-CITED2 group than in the si-NC and control groups (P<0.01), but the number of transmembrane cells was not significantly different between the si-NC group and the control group (P>0.05) (Figure 7D and E). It suggested that silencing CITED2 expression in HTR8-SVneo cells may inhibit their invasion ability.

CITED2 Silencing Promotes HTR8-SVneo Cell Apoptosis

At 24 hours after transfection, the proportion of apoptotic cells in the si-CITED2 group was significantly higher than that in the si-NC and control groups (P<0.001), and there was no significant difference between the si-NC and control groups (P>0.05) (Figure 7F and G). These results suggested that inhibiting CITED2 expression in HTR8-SVneo cells may promote apoptosis.

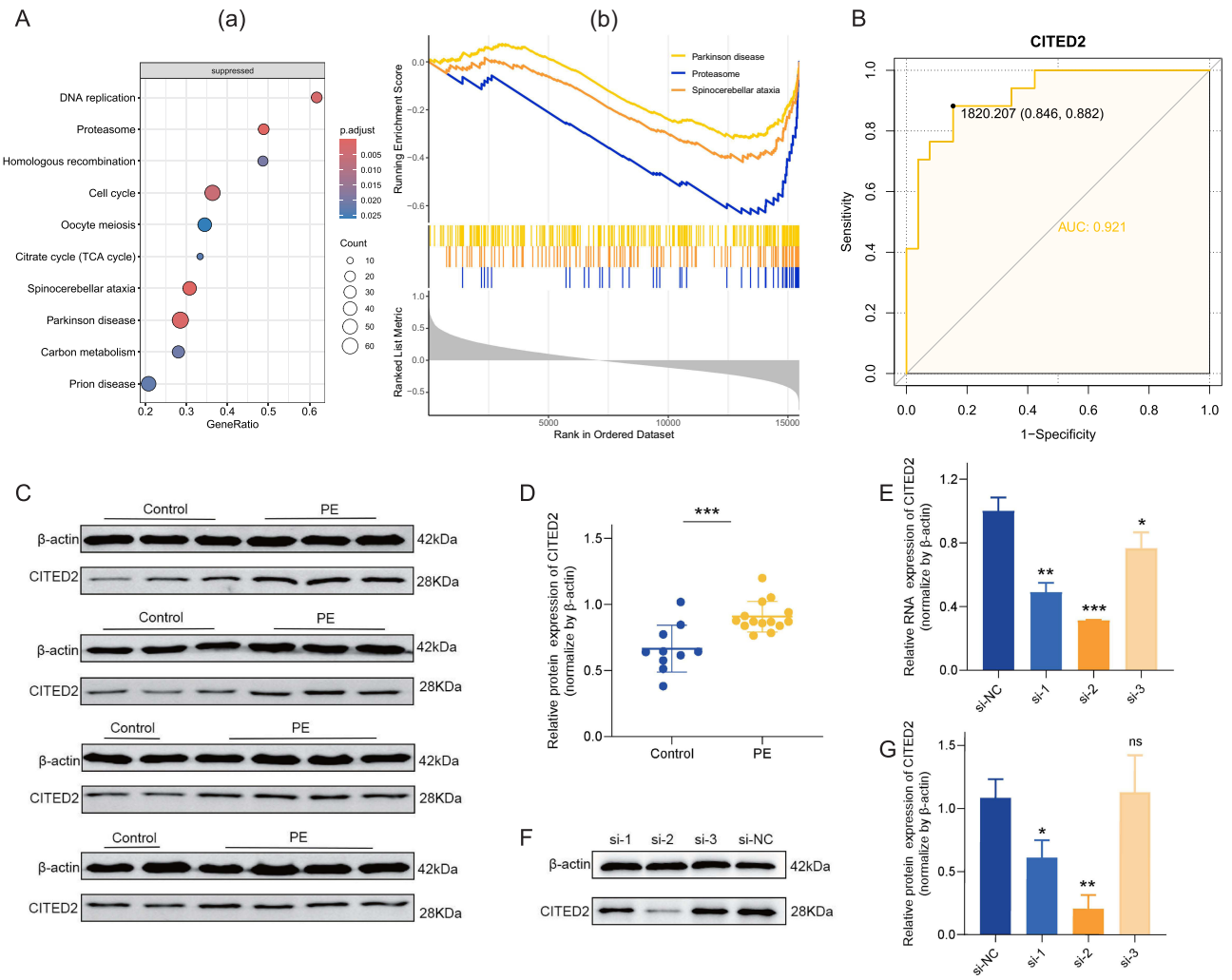

Figure 6 Analysis and siRNA silencing efficiency validation of CITED2. (**A**) Single gene GSEA enrichment analysis of CITED2, (a) dot plot shows the top 10 enrichment pathways, (b) line plot shows the top 3 enrichment pathways. (**B**) Receiver operating characteristic curve of CITED2. (**C** and **D**) The expression level of CITED2 protein in 24 clinical samples, including control group (n=10) and PE group (n=14) samples. (**E**–**G**) The silencing efficiency of CITED2 was verified by mRNA and protein expression. The data shown as mean ± SD, and *p < 0.05; **p < 0.01; ***p < 0.001; ns, p>0.05.

Medicines Targeting CITED2

The Enrichr website was used to find drugs that target CITED2. We found a total of 26 kinds of drugs that met our criteria. Several of these drugs, such as pioglitazone and troglitazone, belong to the class of litazone. These drugs may be involved in the treatment of PE by targeting CITED2. The information for the drugs was shown in Table 5.

Discussion

In recent years, an increasing number of studies have been conducted to explore biomarkers of PE and search for therapeutic targets via bioinformatics. However, the pathogenesis of PE has not yet been clarified, and no effective treatment has been found. In this report, we identify six genes (BANF1, CITED2, PLEKHG2, PLEC, NOTCH3, and SASH1) associated with PE through bioinformatics. Multiple signaling pathways and immune cells are related with these genes. Previous studies have shown that NOTCH3 and SASH1 affected the proliferation, migration, and invasion of placental trophoblast cells. In vitro, the overexpression of SASH1 can inhibit trophoblast cell growth, migration, and invasion and cause death.[20] Placental tissue from PE patients has increased NOTCH3, and miR-16 influences the development of PE by controlling the biological activity of trophoblast cells via the Notch family.[21] However, the association of the other four genes with PE has not been elucidated by reports. In this study, we provide data supporting

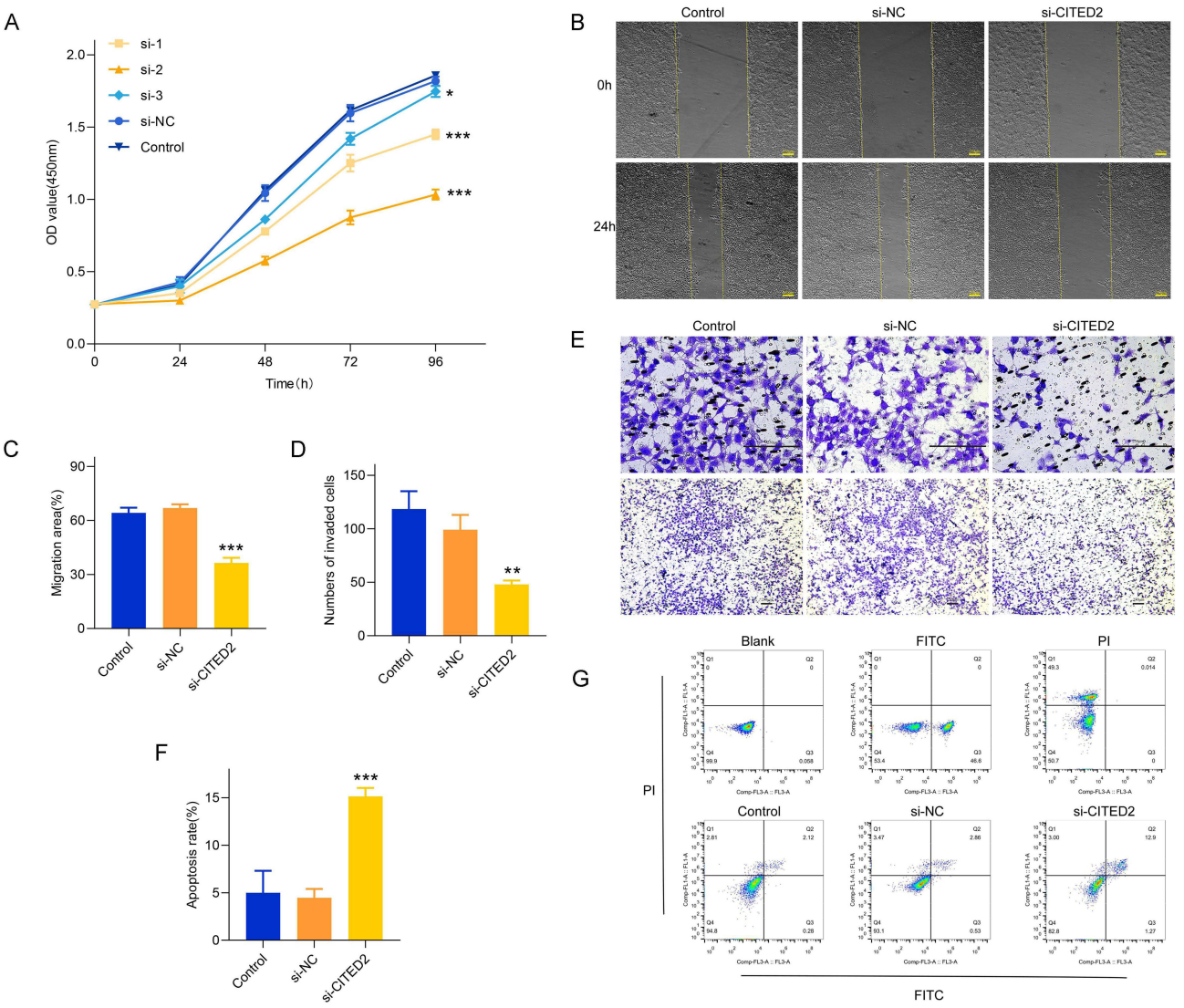

Figure 7 The effects of CITED2 on trophoblastic function. (**A**) Cell proliferation assays, CITED2 silencing resulted in decreased proliferation of HTR8 cell line. (**B** and **C**) Wound healing assays, images taken with microscope magnification of 40x, the migration area of cells was observed after 24 hours of culture and CITED2-silenced HTR8 cell line has worse healing ability. (**D** and **E**) Cell invasion assay, images taken with microscope magnification of 40x (down) and 200x (up), the silencing group had fewer transmembrane cells after 36 hours of culture. (**F** and **G**) The apoptosis rate was detected by flow cytometry, it was higher in the CITED2 silencing group. The data shown as mean ± SD and represent 3 independent experiments, and *p < 0.05; **p < 0.01; ***p < 0.001.

BANF1, CITED2 and PLEKHG2 may be associated with PE, and the relationship between CITED2 and PE is further investigated.

The proportions of activated NK cells and regulatory T cells are significant difference between the control and PE groups (Figure 4B). Six hub genes are associated with activated NK cells, regulatory T cells, gamma delta T cells, resting dendritic cells and CD8+ T cells (Figure 4D). Many studies have shown that immune cell dysfunctions, such as regulatory T cells, macrophages, NK cells, and neutrophils, are important in the pathogenesis of PE.[22,23] The number of NK cells is lower in PE patients than in healthy individuals,[24,25] which is consistent with our findings. Furthermore, the glycolysis, NOTCH signaling, and hypoxia pathways are significantly enriched (Figure 4F). One known characteristic of PE and a significant cause of its development is hypoxia in placental tissue.[26] Hunkapiller et al also demonstrate that the NOTCH signaling pathway affects vascular infiltration in trophoblasts, contributing to PE.[27] And aberrant glycolysis influences trophoblast invasion contributes to the development of PE.[28] Changes in PE placental tolerance and immunity are induced by the NOTCH/NOTCH ligand.[29] In addition, the transforming growth factor-β (TGF-β) signaling pathway and the peroxisome and bile acid metabolism signaling pathways are also differentially enriched between the PE and

Table 5 Drugs Prediction of CITED2

Drugs	P-value	Adjusted P-value	Odds Ratio	Combined Score
ZERANOL CTD 00007009	0.001699967	0.034932912	19,966	127,326.1054
indometacin PC3 UP	0.002349959	0.034932912	19,953	120,782.6407
pioglitazone PC3 UP	0.002449958	0.034932912	19,951	119,939.1172
troglitazone PC3 UP	0.003099951	0.034932912	19,938	115,169.2467
tretinoin HL60 DOWN	0.003499947	0.034932912	19,930	112,704.3007
rosiglitazone PC3 UP	0.003649945	0.034932912	19,927	111,851.1105
vanoxerine PC3 UP	0.003849943	0.034932912	19,923	110,765.8407
hyoscyamine HL60 DOWN	0.004049941	0.034932912	19,919	109,734.8241
ellipticine PC3 UP	0.004049941	0.034932912	19,919	109,734.8241
gedunin CTD 00003449	0.004449938	0.034932912	19,911	107,815.3803
azacyclonol PC3 UP	0.004499937	0.034932912	19,910	107,587.5039
ciclopirox PC3 UP	0.004899934	0.034932912	19,902	105,849.455
celastrol CTD 00002186	0.005099932	0.034932912	19,898	105,032.1506
Decabromodiphenyl oxide CTD 00000574	0.005199931	0.034932912	19,896	104,635.2494
colforsin MCF7 UP	0.005299931	0.034932912	19,894	104,245.7845
4-PHENYLBUTYRIC ACID CTD 00002847	0.00539993	0.034932912	19,892	103,863.4786
menadione PC3 UP	0.005649928	0.034932912	19,887	102,937.3495
azacitidine PC3 UP	0.005999925	0.034932912	19,880	101,706.2448
perhexiline MCF7 UP	0.006149924	0.034932912	19,877	101,200.0793
colchicine HL60 DOWN	0.006499921	0.034932912	19,870	100,064.6284
thimerosal CTD 00006868	0.006549921	0.034932912	19,869	99,907.33811
phenoxybenzamine PC3 UP	0.007699913	0.039199557	19,846	96,581.47674
parthenolide MCF7 DOWN	0.00809991	0.039443042	19,838	95,537.86936
parthenolide HL60 DOWN	0.009049904	0.041399588	19,819	93,248.41635
ciglitazone CTD 00001835	0.009499902	0.041399588	19,810	92,244.74636
thioridazine PC3 UP	0.0097999	0.041399588	19,804	91,601.08681

control groups (Figure 4E). The association of these signaling pathways with PE also has been demonstrated in previous studies. TGF-β signaling plays a key role in regulating extravillous trophoblasts[30] and invasion[31] during human placental development. Peroxisome activity is reduced in the placenta of PE animals.[32] And intrahepatic cholestasis of pregnancy increases the risk of PE in pregnant women.[33] In brief, these results further raise the possibility that the hub genes are associated with PE.

CITED2, a transcription factor widely expressed in the body, plays an important role in maintaining the normal biological functions of tissues and cells. Dysregulation of CITED2 expression is associated with many diseases, including tumor and nontumor diseases. A number of studies have shown that this gene is related to the occurrence and development of a variety of tumors and affects the biological function of tumor cells. In different tumor types, the expression trend of CITED2 and its effect on tumor cells are also different. The immunoreactivity of CITED2 is significantly increased in breast cancer tissues, and increasing its expression can significantly improve the proliferative activity and migratory performance of breast cancer cells.[34] However, after knockdown of the gene, the migration and invasion ability of cells are significantly reduced, and the establishment of metastasis is also significantly reduced.[35] In recent years, studies have shown that CITED2 expression is increased in breast and prostate cancer tissues, and its expression level is negatively correlated with the survival rate of patients and has a role in promoting prostate cancer metastasis.[36,37] Our study revealed that the migration and invasion ability of HTR8-SVneo cells decrease significantly after CITED2 expression is knocked down. The expression of CITED2 promotes tumor growth in nude mice, while CITED2 knockdown results in tumor shrinkage and improves overall survival in mice, and CITED2 can promote the proliferation of lung cancer tumor cells and that the survival rate of nude mice increases after CITED2 knockdown.[38] Our data revealed that the proliferation ability of HTR8-SVneo cells after CITED2 knockdown is also lower than that of the control and normal cells. Recent studies have shown that the expression of CITED2 is increased in leukemia patients and

that knocking down CITED2 can promote the apoptosis of leukemia cells.[39] We found that knocking down CITED2 expression may increase the apoptosis rate of HTR8-SVneo cells. CITED2 has certain effects on the function of trophoblast cells. These effects are in the same direction as the effects of CITED2 on some tumor cells. In nontumor diseases, CITED2 inhibits the hypoxia response and reduces renal clearance by negatively regulating HIF target genes, leading to renal and cardiovascular dysfunction.[40] In animal models of type 2 diabetes, upregulation of CITED2 expression leads to angiogenesis disorders.[11] The overexpression of CITED2 separates P300/CBP from the promoters of angiogenic genes, thus leading to angiogenic defects and affecting normal development.[41] This protein is significantly increased after ischemic injury and promoted neuronal death under ischemic stress.[42] It has been further shown that CITED2 deficiency affects the vascularization of the placenta in mice.[16] Dysangiogenesis is a cause of PE, but whether CITED2 is involved in the development of it by affecting vascular endothelial cells needs further study in the future.

Previous studies have reported that CITED2 plays an important role in cell differentiation and is involved in various signal transduction pathways, hypoxia regulation and coagulation processes, and the regulation of trophoblast differentiation.[14,15] CITED2 is important for the proliferation, survival and self-renewal of mouse embryonic stem cells and is required for the reprogramming of mouse embryonic fibroblasts into induced pluripotent stem cells.[43] Fernandes et al also reported that CITED2 has a high affinity for the CH1 domain of the transcription coactivator CBP/p300, and after binding to this domain, CITED2 can act as a positive or negative regulator of transcription.[9] CBP and P300 have histone 3 lysine 27 (H3K27) acetyltransferase activity and are associated with trophoblastic differentiation and disorders associated with placental diseases, including PE and intrauterine growth restriction.[44,45] Although many previous studies have indicated the importance of CITED2 for placental and trophoblast cells development, its correlation with PE is unclear.

CITED2 is widely expressed in developing embryos and placentas,[16] as a transcriptional coactivator necessary for their normal development, and is differentially expressed in pathological placentas.[19] Downregulation or deletion of CITED2 can lead to abnormal mouse embryonic development.[46] Activation of endoplasmic reticulum stress downregulates CITED2 expression in trophoblastic cells.[19] In addition, study has shown that although the translation of most mRNA is inhibited under hypoxia conditions, the translation of CITED2 mRNA is still effective.[47] However, few previous studies have clarified the expression of CITED2 in human PE placental tissue. In this study, our results showed that the expression levels of CITED2 mRNA and protein in placental tissues from the PE group are higher than those in placental tissues from the control group. Hypoxia is a feature of the PE placenta, and it has been shown that CITED2 expression in trophoblast cells under hypoxic conditions undergoes elevation, which limits the recruitment of p300/CBP to the complex.[18] Collett et al found that the CITED2 protein is suppressed in the endoplasmic reticulum stress model of BeWo cells,[19] in contrast to our finding of elevated CITED2 expression in human PE placenta. In this instance, we think it could be because of different kinds of experimental materials—placental tissue and cell specimens. In addition, while endoplasmic reticulum stress is present in PE placenta, varying stress levels can also affect the production of the CITED2 protein. Kuna et al demonstrated the significance of CITED2 for the proper development of the placenta through animal experiments, and CITED2 knockdown would prevent trophoblast cells from differentiation and invasion ability.[14,16] Our study also investigated that CITED2 suppression can lower trophoblast cells' invasion capacity at the cellular level. Furthermore, we also explore the effects of CITED2 on the proliferation, migration and apoptosis functions of trophoblast cells. We found this has not yet been seen to be reported.

When CITED2 is silenced, the function of HTR8 cells exhibit different changes compared with that of si-NC and control cells. Compared to those of si-NC and normal control cells, the proliferation, migration and invasion ability of si-CITED2 cells are lower, but the degree of apoptosis is higher. These findings suggested that CITED2 may promote the proliferation, migration and invasion ability of trophoblast cells and inhibit their apoptosis during the development of placental tissue to maintain the normal development of placental tissue. Reports have shown that CITED2 can bind to the HIF-1-TAz1 complex as a molecular switch for fine-tuning the hypoxia response, resulting in HIF-1 dissociation and termination of the hypoxia reaction to prevent irreversible damage to tissue due to uncontrolled oxidative stress response.[48] Overall, these findings, combined with those from previous studies, indicate that the onset of PE is related to insufficient invasion of the placenta and damage caused by maternal and fetal interface oxidative stress, and the overexpression of CITED2 can eliminate the stress of endoplasmic reticulum and apoptosis induced by high glucose;[49] thus, CITED2 may play a role in resisting the occurrence of

PE. This finding also suggested that CITED2 may be further explored as a therapeutic target for PE in the future. Therefore, we use online websites to predict the drugs targeted by CITED2 and there are a variety of drugs that can act on CITED2, among which the most common drug is the drug in the class of litazone (Table 5). In the future, if additional evidence is able to prove the correlation between CITED2 and PE and the underlying mechanism, these drugs may be used to target CITED2 in the treatment of PE. Although our study reveals the effects of CITED2 on the proliferation, migration, invasion and apoptosis of trophoblast cells, its specific mechanism of action still needs to be further studied.

The present study still had some limitations. Due to the different platforms for single-cell RNA sequencing and bulk RNA sequencing, differences in primers and sample preparation may occur for different platforms. Hence, we analyzed them individually to get the differential genes and then intersected them. To address the batch effect inherent to single-cell sequencing data, Seurat v.5.1.0 was employed in this study to reduce the dimensionality of the original data set and to identify the data features of interest. Consequently, data alignment was realized and the impact of batch effect was reduced. Despite our best efforts to harmonize the two datasets through rigorous data preprocessing, normalization, and batch correction techniques, the combination of single-cell RNA sequencing data and bulk RNA sequencing data from different datasets may have introduced confounding factors that could potentially confound our results. However, we concentrated on the most robust and consistent findings across both datasets and corroborated our results with experimental data. Moreover, additional molecular studies are required to elucidate the potential mechanism of CITED2 on PE.

Conclusion

In summary, our study screened six PE-related genes that are enriched mainly in glycolysis, hypoxia, and NOTCH signaling pathways. And activated NK cells and regulatory T cells may play important roles in this disease. CITED2 is differentially expressed in placental tissues from PE patients. Moreover, CITED2 is found to affect the proliferation, migration, invasion and apoptosis of trophoblast cells and may be a therapeutic target for PE in the future.

Abbreviations

PE, preeclampsia; CITED2, CBP/p300-interacting transactivator with glutamic acid/aspartic acid-rich carboxyl-terminal domain 2; NK cells, natural killer cells; BANF1, BAF nuclear assembly factor 1; PLEC, plectin; PLEKHG2, pleckstrin homology and RhoGEF domain containing G2; NOTCH3, notch receptor 3; SASH1, SAM and SH3 domain containing 1; GEO, Gene Expression Omnibus; scRNA-seq, single-cell RNA sequencing; UMI, unique molecular identifiers; PCs, principal components; DEGs, differentially expressed genes; WGCNA, weighted gene co-expression network analysis; GSEA, gene set enrichment analysis; ssGSEA, single-sample gene set enrichment analysis; ROC, receiver operating characteristic; CIs, confidence intervals; RT-PCR, real-time polymerase chain reaction; WB, Western blotting; SD, standard deviation.

Data Sharing Statement

Two datasets analysed during the current study are available in the GEO database (https://www.ncbi.nlm.nih.gov/geo/). And the data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

The study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, Guangxi, China (2024-E184-01). This study was performed in accordance with the ethical standards as laid down in the Declaration of Helsinki and its later amendments or comparable ethical standards.

Funding

The research was supported by Joint Project on Regional High-Incidence Diseases Research of Guangxi Natural Science Foundation [Grant number 2024GXNSFAA010368]; National Natural Science Foundation of China [grant number 82160295]; Guangxi medical and health appropriate technology development and application project [Grant number S2022080]; the Natural Science Foundation of Guangxi [grant number 2021GXNSFAA196018]; "Medical Excellence Award" Funded by the Creative Research Development Grant from the First Affiliated Hospital of Guangxi Medical University; Youth Science Foundation of Guangxi Medical University [Grant number GXMUYSF202311]; Self-funded

Research Project of Health Commission of Guangxi Zhuang Autonomous Region [Grant number Z-A20230572]; and Project for Enhancing Middle-aged and Young Teacher's Research Basis Ability in Colleges of Guangxi [Grant number 2024KY0110]. The funding body did not participate in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Disclosure

The authors have no relevant financial or non-financial interests to disclose for this work.

References

1. Dimitriadis E, Rolnik DL, Zhou W, et al. Pre-eclampsia. *Nat Rev Dis Primers*. 2023;9(1):8. doi:10.1038/s41572-023-00417-6
2. Phipps EA, Thadhani R, Benzing T, Karumanchi SA. Pre-eclampsia: pathogenesis, novel diagnostics and therapies. *Nat Rev Nephrol*. 2019;15(5):275–289. doi:10.1038/s41581-019-0119-6
3. Poon LC, Shennan A, Hyett JA, et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: a pragmatic guide for first-trimester screening and prevention. *Int J Gynaecol Obstet*. 2019;145(Suppl 1):1–33. doi:10.1002/ijgo.12802
4. Chappell LC, Cluver CA, Kingdom J, Tong S. Pre-eclampsia. *Lancet*. 2021;398(10297):341–354. doi:10.1016/s0140-6736(20)32335-7
5. Rana S, Lemoine E, Karumanchi SA, Karumanchi SA, GrangerJ P. Preeclampsia: pathophysiology, challenges, and perspectives. *Circ Res*. 2019;124(7):1094–1112. doi:10.1161/circresaha.118.313276
6. Roberts JM, Rich-Edwards JW, McElrath TF, Garmire L, Myatt L. Subtypes of preeclampsia: recognition and determining clinical usefulness. *Hypertension*. 2021;77(5):1430–1441. doi:10.1161/hypertensionaha.120.14781
7. Myatt L. The prediction of preeclampsia: the way forward. *Am J Obstet Gynecol*. 2022;226(2s):S1102–S1107.e8. doi:10.1016/j.ajog.2020.10.047
8. MacDonald TM, Walker SP, Hannan NJ, Tong S, Kaitu'u-Lino TJ. Clinical tools and biomarkers to predict preeclampsia. *EBioMedicine*. 2022;75:103780. doi:10.1016/j.ebiom.2021.103780
9. Fernandes MT, Calado SM, Mendes-Silva L, Bragança J. CITED2 and the modulation of the hypoxic response in cancer. *World J Clin Oncol*. 2020;11(5):260–274. doi:10.5306/wjco.v11.i5.260
10. Kunkemoeller B, Chen K, Lockhart SM, Wang X, Rask-Madsen C. The transcriptional coregulator CITED2 suppresses expression of IRS-2 and impairs insulin signaling in endothelial cells. *Am J Physiol Endocrinol Metab*. 2021;321(2):E252–e259. doi:10.1152/ajpendo.00435.2020
11. Wang X, Lockhart SM, Rathjen T, et al. Insulin downregulates the transcriptional coregulator CITED2, an inhibitor of proangiogenic function in endothelial cells. *Diabetes*. 2016;65(12):3680–3690. doi:10.2337/db16-0001
12. Lou X, Sun S, Chen W, et al. Negative feedback regulation of NF-κB action by CITED2 in the nucleus. *J Immunol*. 2011;186(1):539–548. doi:10.4049/jimmunol.1001650
13. Gonzalez YR, Zhang Y, Behzadpoor D, et al. CITED2 signals through peroxisome proliferator-activated receptor-gamma to regulate death of cortical neurons after DNA damage. *J Neurosci*. 2008;28(21):5559–5569. doi:10.1523/jneurosci.1014-08.2008
14. Kuna M, Dhakal P, Iqbal K, et al. CITED2 is a conserved regulator of the uterine-placental interface. *Proc Natl Acad Sci U S A*. 2023;120(3):e2213622120. doi:10.1073/pnas.2213622120
15. Imakawa K, Dhakal P, Kubota K, et al. CITED2 modulation of trophoblast cell differentiation: insights from global transcriptome analysis. Article. *Reproduction*. 2016;151(5):509–516. doi:10.1530/rep-15-0555
16. Withington SL, Scott AN, Saunders DN, et al. Loss of Cited2 affects trophoblast formation and vascularization of the mouse placenta. *Dev Biol*. 2006;294(1):67–82. doi:10.1016/j.ydbio.2006.02.025
17. Moreau JL, Artap ST, Shi H, et al. Cited2 is required in trophoblasts for correct placental capillary patterning. *Dev Biol*. 2014;392(1):62–79. doi:10.1016/j.ydbio.2014.04.023
18. Gobble RM, Groesch KA, Chang M, Torry RJ, Torry DS. Differential regulation of human PlGF gene expression in trophoblast and nontrophoblast cells by oxygen tension. *Placenta*. 2009;30(10):869–875. doi:10.1016/j.placenta.2009.08.003
19. Collett GP, Redman CW, Sargent IL, Vatish M. Endoplasmic reticulum stress stimulates the release of extracellular vesicles carrying danger-associated molecular pattern (DAMP) molecules. *Oncotarget*. 2018;9(6):6707–6717. doi:10.18632/oncotarget.24158
20. Liu S, Jiang S, Huang L, Yu Y. Expression of SASH1 in Preeclampsia and Its Effects on Human Trophoblast. *Biomed Res Int*. 2020;2020:5058260. doi:10.1155/2020/5058260
21. Yuan Y, Wang X, Sun Q, Dai X, Cai Y. MicroRNA-16 is involved in the pathogenesis of pre-eclampsia via regulation of Notch2. *J Cell Physiol*. 2020;235(5):4530–4544. doi:10.1002/jcp.29330
22. Miller D, Motomura K, Galaz J, et al. Cellular immune responses in the pathophysiology of preeclampsia. *J Leukoc Biol*. 2022;111(1):237–260. doi:10.1002/jlb.5ru1120-787rr
23. Deer E, Herrock O, Campbell N, et al. The role of immune cells and mediators in preeclampsia. *Nat Rev Nephrol*. 2023;19(4):257–270. doi:10.1038/s41581-022-00670-0
24. Milosevic-Stevanovic J, Krstic M, Radovic-Janosevic D, Popovic J, Tasic M, Stojnev S. Number of decidual natural killer cells & macrophages in pre-eclampsia. *Indian J Med Res*. 2016;144(6):823–830. doi:10.4103/ijmr.IJMR_776_15
25. Lockwood CJ, Huang SJ, Chen CP, et al. Decidual cell regulation of natural killer cell-recruiting chemokines: implications for the pathogenesis and prediction of preeclampsia. *Am J Pathol*. 2013;183(3):841–856. doi:10.1016/j.ajpath.2013.05.029
26. Tong W, Allison BJ, Brain KL, et al. Chronic hypoxia in ovine pregnancy recapitulates physiological and molecular markers of preeclampsia in the mother, placenta, and offspring. *Hypertension*. 2022;79(7):1525–1535. doi:10.1161/hypertensionaha.122.19175
27. Hunkapiller NM, Gasperowicz M, Kapidzic M, et al. A role for Notch signaling in trophoblast endovascular invasion and in the pathogenesis of pre-eclampsia. *Development*. 2011;138(14):2987–2998. doi:10.1242/dev.066589
28. Yang M, Li H, Rong M, Zhang H, Hou L, Zhang C. Dysregulated GLUT1 may be involved in the pathogenesis of preeclampsia by impairing decidualization. *Mol Cell Endocrinol*. 2022;540:111509. doi:10.1016/j.mce.2021.111509

29. Cao W, Wang X, Chen T, et al. The expression of Notch/Notch Ligand, IL-35, IL-17, and Th17/Treg in preeclampsia. *Dis Markers*. 2015;2015:316182. doi:10.1155/2015/316182
30. Haider S, Lackner AI, Dietrich B, et al. Transforming growth factor-β signaling governs the differentiation program of extravillous trophoblasts in the developing human placenta. *Proc Natl Acad Sci U S A*. 2022;119(28):e2120667119. doi:10.1073/pnas.2120667119
31. Li Y, Yan J, Chang HM, Chen ZJ, Leung PCK. Roles of TGF-β superfamily proteins in extravillous trophoblast invasion. *Trends Endocrinol Metab*. 2021;32(3):170–189. doi:10.1016/j.tem.2020.12.005
32. Santana-Garrido A, Reyes-Goya C, Espinosa-Martín P, et al. Oxidative and inflammatory imbalance in placenta and kidney of sFlt1-induced early-onset preeclampsia rat model. *Antioxidants*. 2022;11(8):1608. doi:10.3390/antiox11081608
33. Zhang L, Tang C, Ye C, Huang L, Wu Y. Intrahepatic cholestasis of pregnancy can increase the risk of metabolic disorders: a meta-analysis. *J Med Biochem*. 2022;41(4):549–558. doi:10.5937/jomb0-33222
34. Minemura H, Takagi K, Sato A, et al. CITED2 in breast carcinoma as a potent prognostic predictor associated with proliferation, migration and chemoresistance. *Cancer Sci*. 2016;107(12):1898–1908. doi:10.1111/cas.13081
35. Jayaraman S, Doucet M, Lau WM, Kominsky SL. CITED2 modulates breast cancer metastatic ability through effects on IKKα. *Mol Cancer Res*. 2016;14(8):730–739. doi:10.1158/1541-7786.Mcr-16-0081
36. Shin SH, Lee GY, Lee M, et al. Aberrant expression of CITED2 promotes prostate cancer metastasis by activating the nucleolin-AKT pathway. *Nat Commun*. 2018;9(1):4113. doi:10.1038/s41467-018-06606-2
37. Lau WM, Doucet M, Huang D, Weber KL, Kominsky SL. CITED2 modulates estrogen receptor transcriptional activity in breast cancer cells. *Biochem Biophys Res Commun*. 2013;437(2):261–266. doi:10.1016/j.bbrc.2013.06.063
38. Chou YT, Hsieh CH, Chiou SH, et al. CITED2 functions as a molecular switch of cytokine-induced proliferation and quiescence. *Cell Death Differ*. 2012;19(12):2015–2028. doi:10.1038/cdd.2012.91
39. Mattes K, Berger G, Geugien M, Martens JHA, Vellenga E, Schepers H. CITED2 affects leukemic cell survival by interfering with p53 activation. meeting abstract. *Blood*. 2016;128(22):1542. doi:10.1182/blood.V128.22.1542.1542
40. Tanaka T, Yamaguchi J, Higashijima Y, Nangaku M. Indoxyl sulfate signals for rapid mRNA stabilization of Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 2 (CITED2) and suppresses the expression of hypoxia-inducible genes in experimental CKD and uremia. *FASEB j*. 2013;27(10):4059–4075. doi:10.1096/fj.13-231837
41. AlAbdi L, He M, Yang Q, Norvil AB, Gowher H. The transcription factor Vezf1 represses the expression of the antiangiogenic factor Cited2 in endothelial cells. *J Biol Chem*. 2018;293(28):11109–11118. doi:10.1074/jbc.RA118.002911
42. Huang T, González YR, Qu D, et al. The pro-death role of Cited2 in stroke is regulated by E2F1/4 transcription factors. *J Biol Chem*. 2019;294(21):8617–8629. doi:10.1074/jbc.RA119.007941
43. Kranc KR, Oliveira DV, Armesilla-Diaz A, et al. Acute loss of cited2 impairs nanog expression and decreases self-renewal of mouse embryonic stem cells. Article. *Stem Cells*. 2015;33(3):699–712. doi:10.1002/stem.1889
44. Paauw ND, Lely AT, Joles JA, et al. H3K27 acetylation and gene expression analysis reveals differences in placental chromatin activity in fetal growth restriction. *Clin Epigenet*. 2018;10(1):85. doi:10.1186/s13148-018-0508-x
45. van Uitert M, Moerland PD, Enquobahrie DA, et al. Meta-analysis of placental transcriptome data identifies a novel molecular pathway related to preeclampsia. *PLoS One*. 2015;10(7):e0132468. doi:10.1371/journal.pone.0132468
46. Lopes Floro K, Artap ST, Preis JI, et al. Loss of Cited2 causes congenital heart disease by perturbing left-right patterning of the body axis. *Hum Mol Genet*. 2011;20(6):1097–1110. doi:10.1093/hmg/ddq554
47. van den Beucken T, Magagnin MG, Savelkouls K, Lambin P, Koritzinsky M, Wouters BG. Regulation of Cited2 expression provides a functional link between translational and transcriptional responses during hypoxia. *Radiother Oncol*. 2007;83(3):346–352. doi:10.1016/j.radonc.2007.04.026
48. Berlow RB, Dyson HJ, Wright PE. Hypersensitive termination of the hypoxic response by a disordered protein switch. *Nature*. 2017;543(7645):447–451. doi:10.1038/nature21705
49. Gu H, Yu J, Dong D, et al. High glucose-repressed CITED2 expression through mir-200b triggers the unfolded protein response and endoplasmic reticulum stress. Article. *Diabetes*. 2016;65(1):149–163. doi:10.2337/db15-0108