IJKD KIDNEY DISEASES

Identification of PHACTR4 as A New Biomarker for Diabetic Nephropathy and Its Correlation with Glomerular Endothelial Dysfunction and Immune Infiltration

Baixue Yu,[1,2] Mei Meng,[1,2] Tingting Li,[1,2] Yi Shi[1,2]

[1]Shanghai Key Laboratory of Organ Transplantation, Fudan University, Shanghai, China
[2]Institute of Clinical Science, Zhongshan Hospital, Fudan University, Shanghai, China

Keywords. diabetic nephropathy, weighted gene co-expression network analysis, PHACTR4, biomarkers

Original Paper

Introduction. The pathophysiology of diabetic nephropathy (DN) is fundamentally dependent on glomerular endothelial cells (GECs), which are a crucial portion of the glomerular filtration barrier. This study aimed to identify biomarker candidates associated with GECs dysfunction in DN by combining microarray and single-cell sequencing dataset analysis.

Methods. Microarray dataset GSE30528 was downloaded from the Gene expression omnibus (GEO) database. Key gene sets for diabetic kidney disease (DKD) were selected by using weighted gene co-expression network analysis (WGCNA). Biomarker candidates were then identified using least absolute shrinkage and selection operator (LASSO) logistic regression. The single-cell sequencing data (GSE131882) was used to explore the biological functional differences in glomerular endothelium between the control and DKD groups. The diagnostic efficiency of the selected biomarker was tested in the Receiver operating characteristic (ROC) curve. Moreover, we used the single-sample gene set enrichment analysis (ssGSEA) to compare immune cell infiltration between DKD and control groups. RT-PCR was used to validate the selected gene expression in cultured glomerular endothelial cells under high glucose stimulation.

Results. Phosphatase and actin regulator 4 (PHACTR4) was ultimately selected as the key GEC-related biomarker in DKD. Significantly downregulated PHACTR4 mRNA expression was further validated in human glomerular endothelial cells (HGECs) under high glucose stimulation by using RT-PCR. The decreased PHACTR4 was found to be associated with abnormal endothelial proliferation and neo-angiogenesis. Additionally, immune infiltration analysis revealed that PHACTR4 was negatively associated with inflammatory infiltration, especially pro-inflammatory cells including activated CD4 and CD8 T cells, B cells, and Mast cells, indicating PHACTR4 downregulation may exacerbate inflammatory reaction.

Conclusion. PHACTR4 is a potential diagnostic marker for DKD and plays an essential role in aberrant glomerular endothelial proliferation and inflammation in DKD.

IJKD 2023;17:314-26
www.ijkd.org
DOI: 10.52547/ijkd.7858

INTRODUCTION

Diabetic nephropathy (DN) or diabetic kidney disease (DKD) is known as one of the most serious diabetic microvascular complications, leading to approximately 50% of all end-stage kidney diseases (ESKD) worldwide.[1] Globally, it is estimated that

nearly 463 million individuals are suffering from diabetes, among which two-fifths of them are likely to develop DN.[2] Clinically, DN is highly associated with progressive kidney impairment characterized by increased albumin permeability, declined glomerular filtration rate (GFR), hypertension, infections, cardiovascular, disease (CVD), and ESKD, imposing a heavy burden on individuals and the health care system.[3]

Glomerular endothelial cells (GECs) are key components of the glomerular filtration barrier (GFB). Unlike endothelial cells from conductive large vessels, GECs possess transmembrane pores of 50 to 80 nm and are covered with a thick layer of the glycocalyx which helps to maintain the negative charge of the barrier membrane.[4] Glomerular endothelial dysfunction has been increasingly identified as an early and detrimental hallmark of DN, leading to GFB damage and elevated albuminuria secretion.[5,6] Reduced thickness of glycocalyx and mean percentage of fenestration in GECs have been observed in DN, which was correlated with impaired glomerular filtration rate and consequent albuminuria.[7,8] However, the primary drivers and underlying mechanisms of GECs dysfunction in DN are still uncertain.

Weighted gene co-expression network analysis (WGCNA) is a powerful tool for exploring essential pathological mechanisms and identifying diagnostic and therapeutic biomarkers.[9] The study was performed to gain a deeper insight into the underlying mechanism of DN by bioinformatic analyses and explore potential diagnostic markers. In the study, we first analyzed gene expressions between DKD and control individuals and then explored key genes of DKD by WGCNA combined with the machine learning algorithm. In addition, the differently expressed genes (DEGs) in glomerular endothelial cells between the control and DKD group were identified using single-cell RNA analysis. The phosphatase and actin regulator 4 (PHACTR4) was identified as a critical diagnostic biomarker and its downregulation was further validated in human GECs exposed to high glucose stimulation. Inflammatory infiltration levels were assessed using the single sample gene set enrichment analysis (ssGSEA) method. Overall, we have unveiled a potential GEC-related biomarker for the progression of DKD, which may contribute to better prevention and treatment of DKD.

MATERIALS AND METHODS

Data Collection and Preprocessing

The clinical traits and gene expression profiling of microarray data GSE30528 were downloaded from Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), which contains glomerular tissues from 13 non-DKD individuals and 9 patients with Human Diabetic Kidney Disease. All data were processed using the R project (version 4.0.2) and normalized with the "limma" package (version).[10] Differently expressed genes (DEGs) were selected based on the threshold of | log2FoldChange | greater than 1 and adjusted *P* value less than .05.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

GO function annotation and KEGG pathway enrichment analysis were performed using the "enrichGO" and "enrichKEGG" functions in the "clusterProfiler" package (version "3.10.1")[11] to better understand the underlying molecular mechanism of diabetic nephrology.

Weighted Gene Co-expression Network Analysis (WGCNA)

Correlation networks are increasingly being used to analyze large, high-dimensional data sets. In the gene expression profiling of GSE30528, a total of 12,644 genes were identified from control and DKD glomeruli samples. And the top 5,000 genes with the greatest standard deviation were selected for subsequent weighted correlation network analysis using the "WGCNA" R package.[9] Gene co-expression similarity between genes i and j (Sij) was defined by Pearson's correlation coefficient cor (i, j). A further power function was used to correlate the adjacency of genes: Amn = power (Smn, β). The optimal soft threshold β was screened out using a gradient method (β ranging from 1 to 20) to acquire the scale-free network with a degree of independence greater than 0.85. Additionally, the adjacency matrix was transformed into a topological overlap matrix, modules detection was conducted by hierarchical average linkage clustering analysis as previously described. Finally, modules with a correlation of above 0.75 were merged.

Selection of Key Modules Corresponding to Clinical Traits

Module eigengene (ME) is considered a representative of the gene expression profiles in each module. After the construction of gene modules, we summarized and used them for the component analysis of the corresponding module. Module-trait relationships were assessed by the association between MEs and clinical traits. Coexpression modules with the highest correlation coefficient among all modules indicate the important clinical significance and are defined as the key module for further analysis.

Key Genes Identified by Machine Learning Algorithm

The machine learning algorithm least absolute shrinkage and selection operator (LASSO) logistic regression was used to construct the DKD genetic classification models and identify the optimal feature genes. LASSO is a regression analysis method that implements both variable selection and regularization to enhance the prediction accuracy and interpretability of the resulting statistical model. In this study, LASSO classification models were constructed using the "glmnet" package. The efficacy of the biomarker was further evaluated by the receiver operating characteristic (ROC) curve.

Single-cell Sequencing Data

The online single-cell sequencing data (GSE131882) were processed as previously described.[12] Since our study mainly focused on diabetic glomerulopathy, only glomerular endothelial cells, podocytes, inflammatory cells, and mesangial cells were selected for further analysis. DEGs in GECs between the control and DKD groups were identified by a P value < .01.

Immune Infiltration Analysis

The immune infiltration landscape of microarray data (GSE30528) was analyzed by the single-sample gene set enrichment analysis (ssGSEA) score using the Gene Set Variation Analysis (GSVA) R package. Marker genes for immune cell types were obtained from Bindea's study.[13] Further two-dimensional principal component analysis (PCA) was performed to investigate the differences in the immune filtration landscape between the control and DKD group using "FactoMineR" and "factoextra" packages. "corrplot" R package was used to assess the association of key genes with immune cell infiltration and other genes involved in the pathogenesis of DKD.

Cell Culture

Human Glomerular Endothelial Cells (HGECs) were purchased from ScienCell, America. Cells were grown in humidified air (5% CO_2 at 37 °C). High glucose (44.4 mM) and isotonic control mannitol (44.4 mM) were administrated for 3 days and cells were harvested for further mRNA detection.

Real-time Quantitative Polymerase Chain Reaction (RT-PCR)

PHACTR4 mRNA expression in control and high-glucose–treated HGECs was evaluated by RT-PCR. Cellular RNA was obtained by Trizol regents (Takara Bio Inc, Shiga, Japan), followed by reverse transcribed to cDNA using PrimeScript RT Master Mix (Takara Bio Inc, Shiga, Japan). cDNA aliquots were amplified with primers specific for PHACTR4 and housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in a QuantStudio 5 Dx Real-Time PCR System (Applied Biosystems Life Technologies, Foster City, Calif).

Statistical Analysis

Data from the experimental part were compared with the one-way ANOVA method, followed by Tukey post hoc testing for pairwise comparison. $P < .05$ was taken to indicate a statistically significant difference.

Soft Threshold

Weighted correlation network analysis, also known as weighted gene co-expression network analysis (WGCNA), is a widely used data mining method especially for studying biological networks based on pairwise correlations between variables. While it can be applied to most high-dimensional data sets, it has been most widely used in genomic applications. It allows one to define modules (clusters), intramodular hubs, and network nodes with regard to module membership, to study the relationships between co-expression modules, and to compare the network topology of different networks (differential network analysis). The network construction gives highly robust results with respect to different choices of the soft threshold.

In our study, the optimal soft threshold is 10.

RESULTS

Different Expression Genes (DEGs) in DKD

We initially explored the molecular pathogenesis of DKD, 12644 genes from the GSE30528 dataset were analyzed in DKD and control glomerular samples. A total of 461 DEGs were observed between the control and DKD groups, with 119 upregulated genes and 342 downregulated genes (Figure 1A and B). Furthermore, the potential function of these screened DEGs was identified by utilizing GO and KEGG enrichment analysis. GO analysis revealed that these DEGs are mainly enriched in the biological process including urogenital system development, kidney development, renal system development, cell-substrate adhesion, and nephron development (Figure 1C), and KEGG analysis showed that these DEGs were highly relevant to pathways including PI3K-AKT, cell adhesion, complement, and coagulation cascades were significantly enriched (Figure 1D).

DKD-related Modules Are Identified by WGCNA

WGCNA was performed to elucidate critical gene clusters associated with DKD. In the present study, 10 were selected as the optimal soft threshold by the "pickSoft Threshold" function (Figure 2A, B).

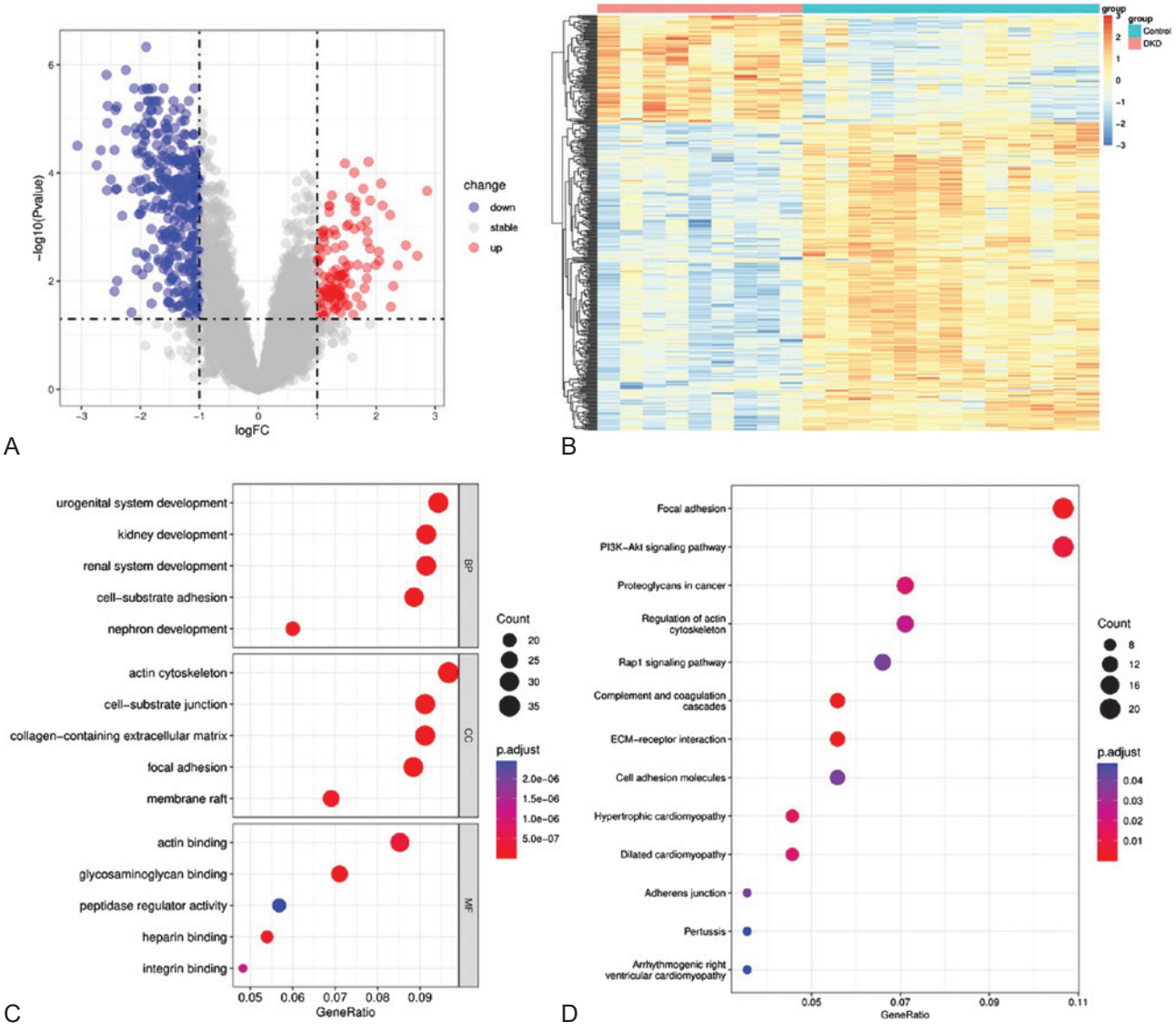


Figure 1. Different Expression Genes (DEGs) Analysis Between DKD and Control [(A) Volcano plots and (B) Heatmap of DEGs in DKD compared to control; (C) GO biological process (BP), cellular component (CC), and molecular function (MF) enrichment analysis. (D) KEGG pathway analysis results]

Subsequently, the co-expression modules were plotted in the clustering dendrograms and 18 modules were identified (Figure 2C, D). As shown in Figure 3 A and B, the MEblack module was the most relevant module related to DKD traits, which was selected as the pivotal module for further analysis. A close correlation was also observed between genes in the MEblack module and gene significance for DKD (Figure 3C). 279 overlapped genes were obtained from the MEblack module and DEGs (Figure 3D).

We further performed GO and KEGG enrichment analyses to better understand the biological significance of overlapped genes. The bubble plot shows the functional features of the MEblack module with an adjusted *P* value < .05. As shown in the GO enrichment (Supplementary Figure 1A), those genes were mainly enriched in terms like cell–substrate adhesion, urogenital system development, kidney development, renal system development, and nephron development. Furthermore, KEGG analysis showed overlapped genes were highly related to pathways including PI3K-AKT signaling, focal adhesion, complement and coagulation cascades, and cell adhesion molecules (Supplementary Figure 1B). Twenty genes

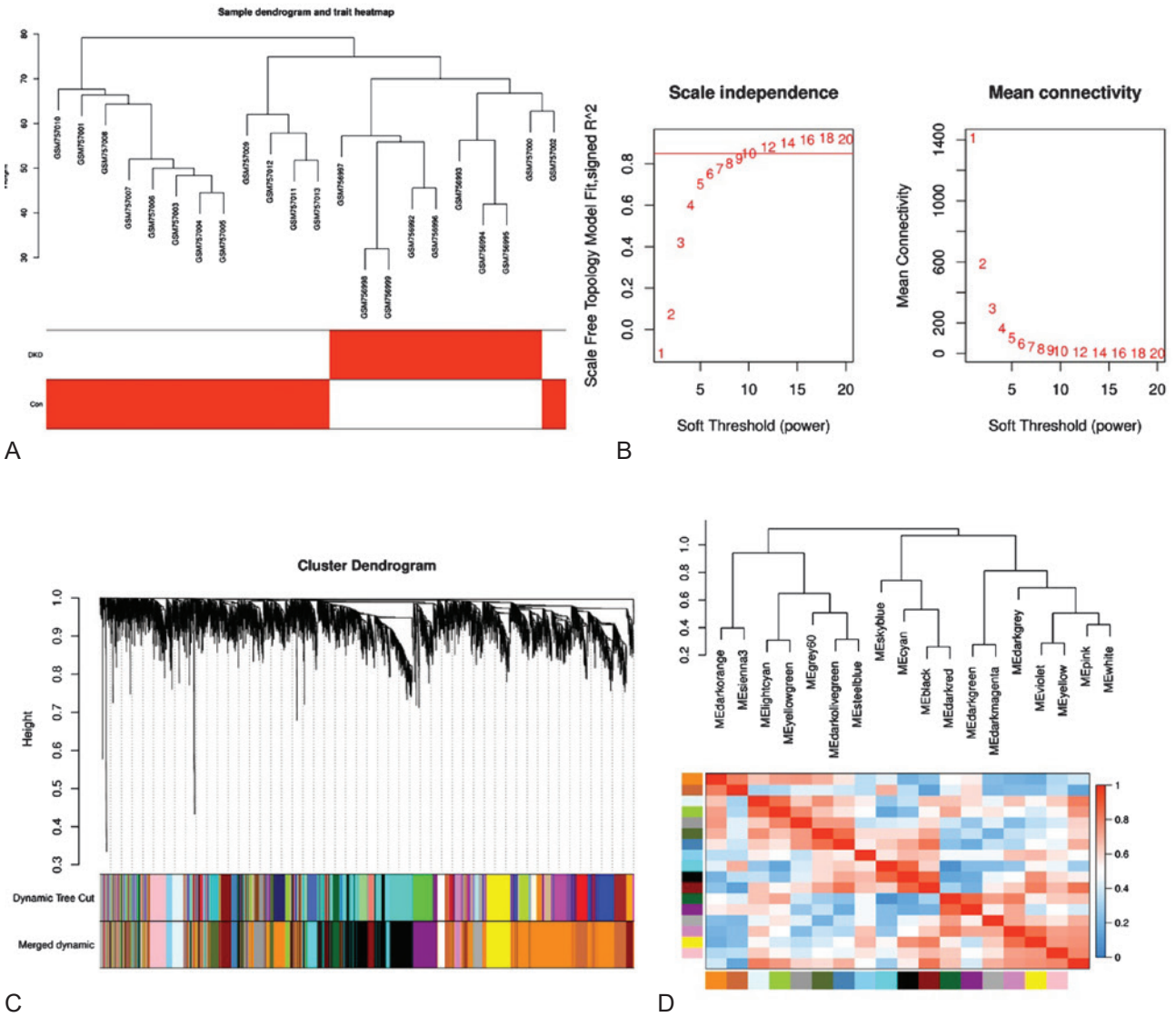


Figure 2. Sale-free Networks Construction and Genes Clustering by WGCNA [(A) Sample dendrogram and trait heatmap used for detecting outliers; (B) Scale independence and mean connectivity for each power value between 1 to 20; (C) Dendrogram of clustered genes based on selected soft threshold power; and (D) Hierarchical module clustering and the eigengene adjacencies heat map plot]

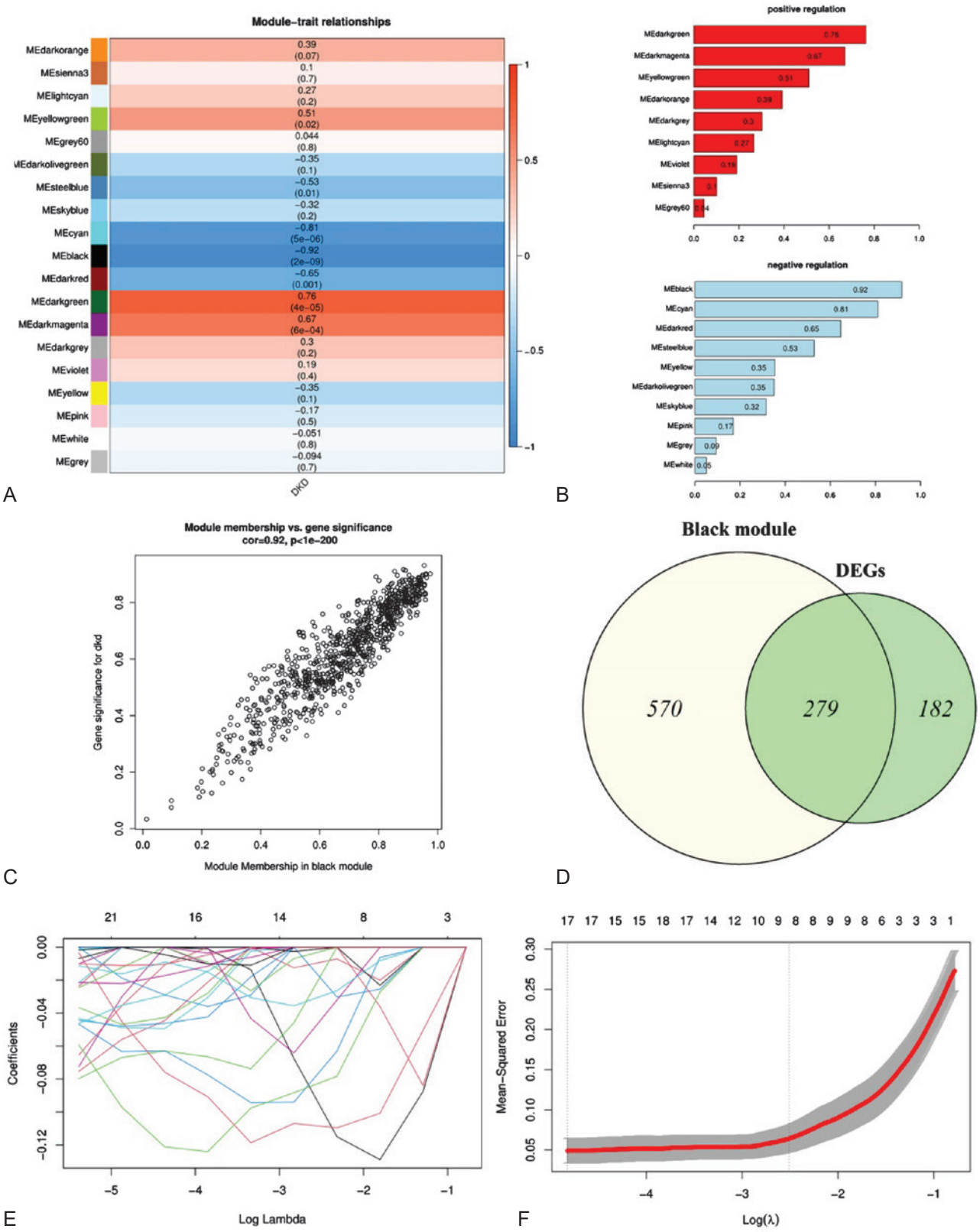
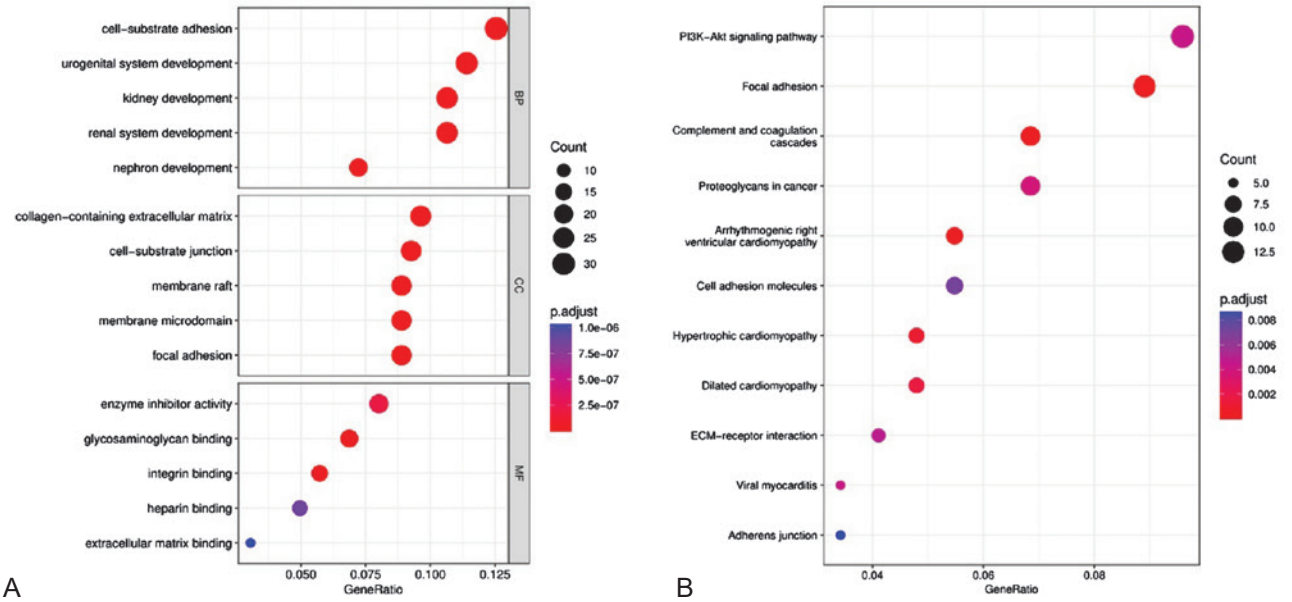


Figure 3. Gene Modules Mining Based on WGCNA [(A) Correlation and *P* values between different modules and DKD; (B) The positive and negative correlation of each module with DKD; (C) Scatter plot of the relationship between eigengene in black module and clinical traits of DKD; (D) Overlapped genes between MEblack module and DEGs; and (E, F) LASSO penalized model was used to screen potential DKD Biomarkers]



Supplementary Figure 1
GO Enrichment (A) and KEGG Analysis (B) of Genes in the MEblack Module

of potential diagnostic markers were obtained by using the LASSO regression algorithm (Figure 3E, F), namely ANXA2, NME7, MED4, SOBP, NFE2L3, PHACTR4, CEL, CD200, CMAHP, SERP1NE2, RETREG1, WT1-AS, DPYSL3, HRG, NPHS1, GOL1M4, HS3ST3A1, DACH1, LRRC2 and IGF1.

Functional Differences of GECs in the DKD Group

We previously identified 15 types of cells in the single-cell sequencing dataset (GSE131882).[12] Glomerular endothelial cells, podocytes, inflammatory cells, and mesangial cells were selected to explore the crosstalk inside glomeruli. The t-SNE plot of glomerular cells was shown in Figure 4A. In addition, there is no significant difference in the cell percentages of podocytes, GECs, macrophage 0, and macrophage 1 between the control and DKD groups. Of note, Macrophage 0 and 1 are the clusters we annotated in this scRNA analysis. However, the DKD group exhibited a significant increase in cell percentages of T cells, monocyte, B cells, and plasma cells. Since GECs play a crucial role in the pathogenesis of diabetic nephrology, further analysis was performed to explore the biological function differences of GECs between groups. DEGs in GECs between the control and DKD group were identified by *P* value < .01. GO term analysis showed the DEGs of GECs between groups were mainly enriched in cell migration, proliferation, and adhesion (Figure 4C). And KEGG analysis revealed these DEGs were highly related to pathways including MAPK signaling pathway, focal adhesion, and Rap1 signaling pathway.

PHACTR4 Is Associated with GECs Dysfunction and Increased Immune Infiltration in DKD

PHACTR4 was ultimately identified based on the results from the LASSO regression algorithm and DEGs of GECs between groups. The ROC curve analysis further indicated the high diagnostic efficacy of PHACTR4 with an AUC of 0.974 (95% CI: 0.924 to 1.000, Figure 5A). Since accumulating evidence highlighted the involvement of inflammation in the development of DKD, we further explored the correlation between PHACTR4 and inflammation infiltration in DKD. The immune infiltration landscape in the control and DKD groups was analyzed by ssGESA (Figure 5B). And a great abundance of immune infiltration was observed in the DKD group, especially activated B cells, activated CD4 T cells, activated CD8 T cells, central memory CD8 T cell, T follicular helper cell, and mast cell (Figure 5C), indicating the activation of inflammation in DKD process. PCA analysis indicated a significant difference in immune infiltration between groups (Figure 5D).

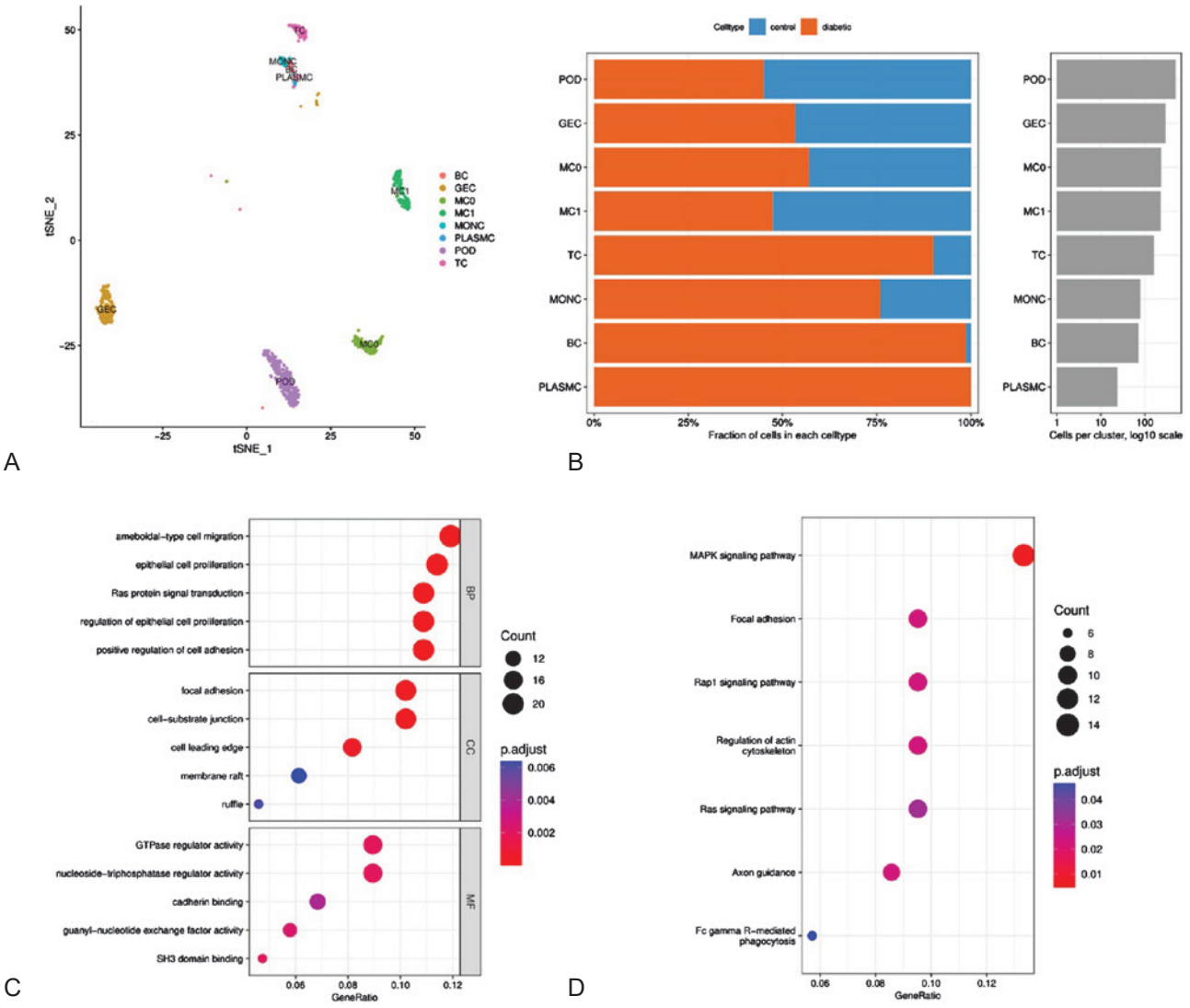


Figure 4. Functional Differences of GECs in the DKD Group [(A) t-SNE diagram of major glomerular cell clusters which was annotated using marker expression. GEC, glomerular endothelial cells; MC0 and MC1, two subgroups of macrophages; MONC, monocytes; PLASMC, plasma cell; POD, podocytes; TC, T cells; BC, B cells; (B) Cell fraction of each cell type in healthy and DKD patients (left), and cell numbers of each cell type in total samples (right); (C) GO; and (D) KEGG analysis results of different expressed genes in GECs between the control and DKD groups]

In addition, the expression of PHACTR4 was significantly downregulated in the DKD group (Figure 5E). Subsequently, we investigated the association between PHACTR4 and the infiltration levels of pro-inflammatory cells. As shown in Figure 6E, PHACTR4 showed a negative association with most inflammatory cells (Figure 5F). And the significant downregulation of PHACTR4 was further verified in high glucose-administrated HGECs (Figure 6).

DISCUSSION

In the present study, gene expression profiles from both control and DKD samples (GSE30528) were analyzed by using WGCNA methods to identify critical biomarkers for diagnosis and treatment of DKD. MEblack modules were found to be highly correlated with DKD and overlapped genes from MEblack modules and DEGs were selected for further analysis. Afterward, the LASSO regression algorithm was used to identify the characteristic genes for DKD. DEGs in GECs between groups were analyzed using single-cell sequencing data (GSE131882). PHACTR4 was ultimately identified as the key biomarker after overlapping the LASSO results and endothelial

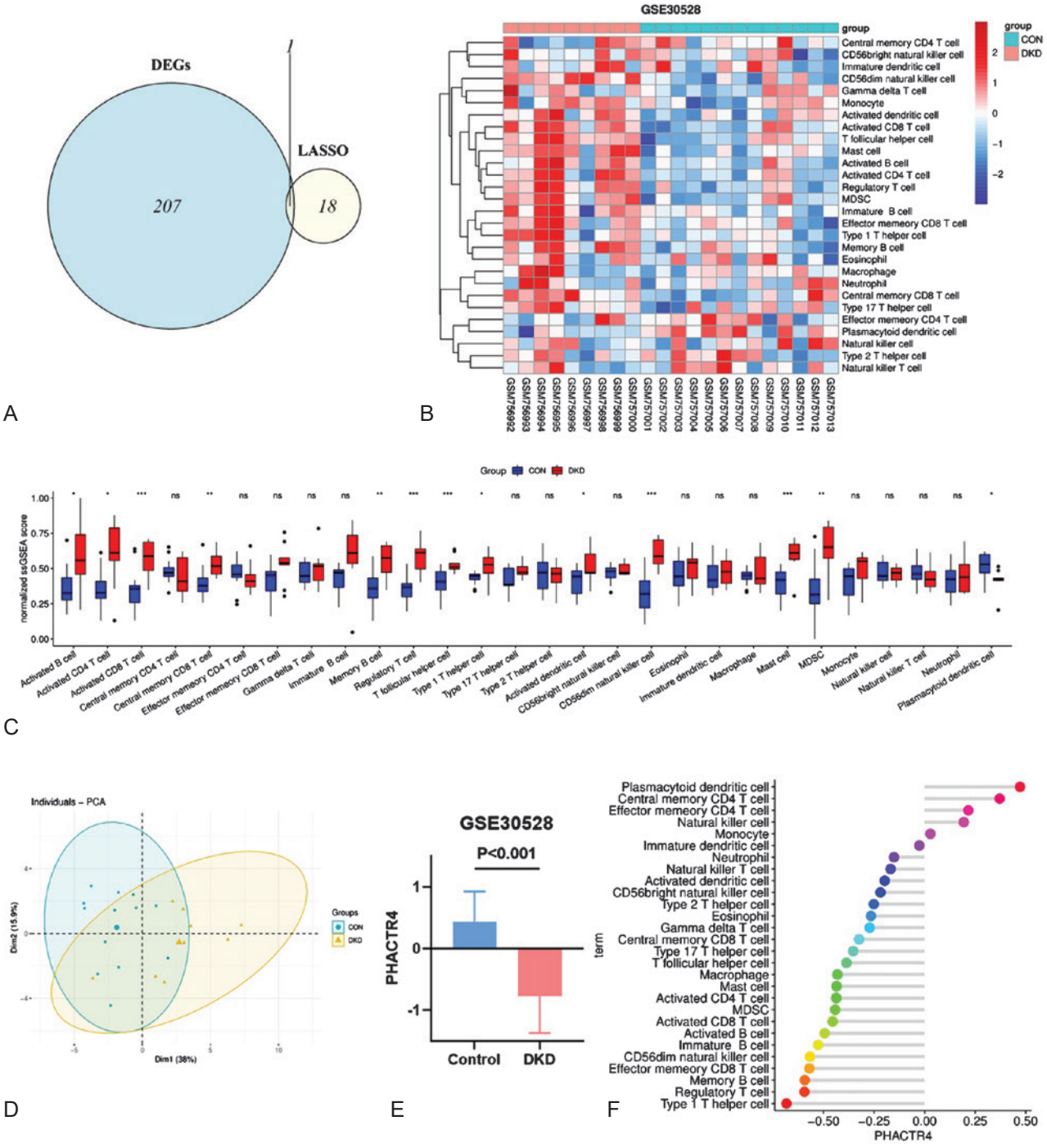


Figure 5. PHACTR4 is associated with inflammation [(A) Venn diagram of the intersecting genes from the Lasso algorithm model and DEGs of GECs between groups; (B) The landscape of twenty-eight immune cells infiltration between healthy and DKD samples; (C) Different proportions of immune cells between DKD and control; (D) PCA analysis of immune cell infiltration of DKD and control samples; (E) Different expression of PHACTR4 in DKD compared with control; and (F) Correlation between PHACTR4 and immune cells}.
Abbreviations: ns, not significant ($P > .05$; *$P < .05$; **$P < .01$)

DEGs, and RT-PCR analysis found PHACTR4 mRNA expression was significantly suppressed in high glucose-stimulated HGECs. In addition, PHACTR4 was also highly related to immune cell infiltration in DKD, which indicated the clinical significance of PHACTR4 in the diagnosis and

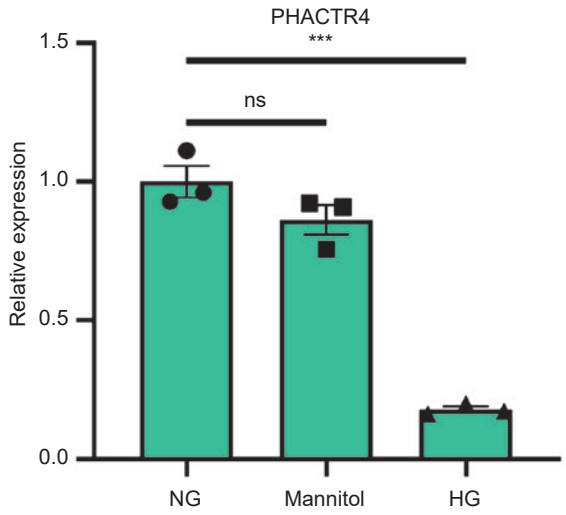


Figure 6. PHACTR4 mRNA expression is highly suppressed in HGECs with high glucose stimulation. Expression levels of PHACTR4 mRNA in high glucose (44.4mM) and osmotic control mannitol (44.4 mM) and control (5.5 mM).
Abbreviations: ns, not significant ($P > .05$; ***$P < .0001$)

treatment of DN.

While metabolism and hemodynamics have been considered the primary factors contributing to DKD, a growing body of research has revealed that DKD is marked by chronic inflammation infiltration, which further worsens renal damage and proteinuria.[14] Our results showed that 461 DEGs between the control and DKD groups were highly related to pathways including PI3K-AKT, cell adhesion, complement, and coagulation cascades (Figure 1D). Weighted gene co-expression network analysis (WGCNA) is a powerful tool for exploring essential pathological mechanisms and identifying diagnostic and therapeutic biomarkers. Consistent with the above results, KEGG analysis displayed that overlapped genes in the MEblack module and DEGs were also mainly enriched in pathways including PI3K-AKT signaling, focal adhesion, complement and coagulation cascades and cell adhesion molecules, which highlights the role of chronic inflammation in the pathogenesis of diabetic nephropathy.

Immune cells and inflammatory regulatory factors play key roles in the development and progression of DKD. Recent evidence has revealed that pro-inflammatory cytokines were significantly increased in blood samples from DKD patients compared with healthy control,[15] and immune cells including macrophages, T cells, B cells, dendritic cells, and mast cells were abundantly infiltrated in renal biopsy of DKD.[16-18] Likewise, our results from the single-cell sequencing data also showed a significant increase in inflammatory cells in the DKD group (Figure 4B). In addition, the single-sample gene set enrichment analysis (ssGSEA) was used for characterizing and quantifying immune cell infiltration in DKD. Compared with the healthy control group, DKD glomeruli were more abundant in various immune cells including T cells (activated CD4+ T cell, activated CD8+ T cells, central memory CD8 T cell, T follicular helper cell, regulatory T cell, and Type1 T helper cell), B cells (Activated B cells, memory B cell), activated dendritic cell, mast cell and myeloid-derived suppressor cells (MDSC). These results were supported by prior studies that activated CD4+ and CD8+ T cells were infiltrated in the kidney of both diabetic patients and DKD mice,[19,20] while suppressor of T cells activation significantly reduced albuminuria levels.[21] A marked increase in B cells and mast cells was found in the interstitium of DKD patients, and the greater abundance of B cells and mast cells infiltrating the interstitium was significantly associated with increased albuminuria.[20,22,23]

The highly fenestrated characteristics combined with covered glycocalyx endow the GECs sieving property, which could function as the glomerular filtration barrier and support renal vasculature.[24,25] In addition, the endothelial protective mechanism (especially angiogenesis) could be initiated when exposed to different stimuli (including inflammatory cells or pro-inflammatory cytokines), followed by endothelial phenotypic switching and renal dysfunction.[26] Accumulating evidence has revealed aberrant glomerular angiogenesis and impaired endothelial fenestration in both human and mouse diabetic nephropathy.[27-29] Lai *et al.* reported that TGF-β could cause diabetic glomerulopathy by activating endothelial pro-inflammatory and proliferative signaling pathways.[27] However, the underlying mechanism of endothelial cell proliferation and neo-angiogenesis remains a moot point. In our study, we analyzed the online single-cell sequencing data to identify DEGs in glomerular endothelium between the control and DKD groups. GO term analysis showed endothelial

DEGs were mainly enriched in cell proliferation and adhesion (Figure 4C). In addition, KEGG analysis revealed that these DEGs were highly related to pathways including MAPK signaling and focal adhesion, which also emphasizes the pro-inflammatory and pro-proliferative signaling pathways in glomerular endothelium.

We further screened the key gene for diabetic nephropathy and endothelial dysfunction, and the overlapped gene PHACTR4 was identified in endothelial DEGs and LASSO results. PHACTR4, a member of the PHACTR family, is known as one of the suppressors of tumorigenesis and/or proliferation genes. with functions in protein phosphatase 1 (PP1) localization and Rb dephosphorylation,[30] PHACTR4 plays a role in PP1 localization and Rb dephosphorylation, inhibiting cellular proliferation and transformation. Studies reported that a defect in the PHACTR4 gene disrupts the interaction between PHACTR4 with PP1, resulting in the inhibitory phosphorylation of PP1 and enhanced cellular proliferation.[31] The defect and mutation in PHACTR4 have been described in multiple tumor subtypes.[31-34] Moreover, tumor cell lines with PHACTR4 defect show suppressor hypersensitivity with PHACTR4 supplement.[30] Whether PHACTR4 is involved in diabetic nephrology, however, remains unknown. A prior study discovered PHACTR4 served as a novel circulating immune biomarker in lupus nephritis using immunoproteomics.[35] In our study, the considerable downregulation of PHACTR4 was observed in glomerular tissue in diabetic nephrology. The ROC curve analysis further indicated the high diagnostic efficacy of PHACTR4 with an AUC of 0.974 (95% CI: 0.924 to 1.000, Figure 5A). Consistently, we found a markedly decreased expression of PHACTR4 in glomerular endothelium in the DKD group compared with the control group. These results indicated the PHACTR4 defect in glomerular endothelium may induce abnormal endothelial proliferation and promote neo-angiogenesis, indicating PHACTR4 may be a potential biomarker for the diagnosis and treatment of DKD.

Further analysis revealed that the downregulation of PHACTR4 transcript was highly related to the abundance of infiltrated immune cells including activated CD4 and CD8 T cells, activated B cells, and Mast cells. These findings indicate that PHACTR4 may promote the initiation and development of DKD by regulating the inflammatory reaction, and thus targeting PHACTR4 may be a novel therapeutic approach to attenuate the progression of DKD.

LIMITATIONS

The main limitations of this study were as follows: (1) our research mainly relied on bioinformatical mining which lacks clinical validation. (2) We focused on exploring genes on mRNA levels without conducting the non-coding RNA. Therefore, large clinical cohorts are needed to validate our results in the future.

CONCLUSION

In conclusion, we identified that PHACTR4 is a potential diagnostic marker for diagnosis of DKD by using WGCNA, machine learning algorithm, and single-cell sequencing analysis. In addition, PHACTR4 is shown to correlate with both abnormal endothelial proliferation and immune cell infiltration. Our findings provide a potential candidate for diagnosing DKD, inspiring researchers for clinical validation, and developing new biomarkers in DKD.

AUTHORS' CONTRIBUTION

Baixue Yu and Yi Shi designed the experiments.

Baixue Yu, Mei Meng and Tingting Li performed the experiments.

Baixue Yu, Mei Meng, Tingting Li and Yi Shi analyzed the data and drafted the manuscript.

ETICAL CONSIDERATION

Gene Expression Omnibus (GEO) is an international public repository that archives and freely distributes microarray, next-generation sequencing, and other forms of high-throughput functional genomics data submitted by the research community. The GEO database is designed to provide and encourage access within the scientific community to the most up to date and comprehensive gene expression and hybridization array data. Therefore, NCBI places no restrictions on the use or distribution of the GEO data.

ACKNOWLEDGEMENT

We would like to thank Dr. Jin Han for his kind support in bioinformatics analysis.

REFERENCES

1. GROSS J L, DE AZEVEDO M J, SILVEIRO S P, et al. Diabetic nephropathy: diagnosis, prevention, and treatment [J]. Diabetes care, 2005, 28(1): 164-76.
2. JAGER K J, KOVESDY C, LANGHAM R, et al. A single number for advocacy and communication-worldwide more than 850 million individuals have kidney diseases [J]. Kidney Int, 2019, 96(5): 1048-50.
3. ALICIC R Z, ROONEY M T, TUTTLE K R. Diabetic Kidney Disease: Challenges, Progress, and Possibilities [J]. Clin J Am Soc Nephrol, 2017, 12(12): 2032-45.
4. MAEZAWA Y, TAKEMOTO M, YOKOTE K. Cell biology of diabetic nephropathy: Roles of endothelial cells, tubulointerstitial cells and podocytes [J]. J Diabetes Investig, 2015, 6(1): 3-15.
5. FU J, LEE K, CHUANG P Y, et al. Glomerular endothelial cell injury and cross talk in diabetic kidney disease [J]. Am J Physiol Renal Physiol, 2015, 308(4): F287-97.
6. LASSéN E, DAEHN I S. Molecular Mechanisms in Early Diabetic Kidney Disease: Glomerular Endothelial Cell Dysfunction [J]. Int J Mol Sci, 2020, 21(24).
7. SATCHELL S C. The glomerular endothelium emerges as a key player in diabetic nephropathy [J]. Kidney Int, 2012, 82(9): 949-51.
8. RAMNATH R D, BUTLER M J, NEWMAN G, et al. Blocking matrix metalloproteinase-mediated syndecan-4 shedding restores the endothelial glycocalyx and glomerular filtration barrier function in early diabetic kidney disease [J]. Kidney Int, 2020, 97(5): 951-65.
9. LANGFELDER P, HORVATH S. WGCNA: an R package for weighted correlation network analysis [J]. BMC Bioinformatics, 2008, 9: 559.
10. RITCHIE M E, PHIPSON B, WU D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies [J]. Nucleic Acids Res, 2015, 43(7): e47.
11. YU G, WANG L G, HAN Y, et al. clusterProfiler: an R package for comparing biological themes among gene clusters [J]. Omics, 2012, 16(5): 284-7.
12. LI T, SHEN K, LI J, et al. Glomerular Endothelial Cells Are the Coordinator in the Development of Diabetic Nephropathy [J]. Front Med (Lausanne), 2021, 8: 655639.
13. BINDEA G, MLECNIK B, TOSOLINI M, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer [J]. Immunity, 2013, 39(4): 782-95.
14. RAYEGO-MATEOS S, RODRIGUES-DIEZ R R, FERNANDEZ-FERNANDEZ B, et al. Targeting inflammation to treat diabetic kidney disease: the road to 2030 [J]. Kidney Int, 2023, 103(2): 282-96.
15. PERLMAN A S, CHEVALIER J M, WILKINSON P, et al. Serum Inflammatory and Immune Mediators Are Elevated in Early Stage Diabetic Nephropathy [J]. Ann Clin Lab Sci, 2015, 45(3): 256-63.
16. NGUYEN D, PING F, MU W, et al. Macrophage accumulation in human progressive diabetic nephropathy [J]. Nephrology (Carlton), 2006, 11(3): 226-31.
17. KLESSENS C Q F, ZANDBERGEN M, WOLTERBEEK R, et al. Macrophages in diabetic nephropathy in patients with type 2 diabetes [J]. Nephrol Dial Transplant, 2017, 32(8): 1322-9.
18. LIU J L, ZHANG L, HUANG Y, et al. Epsin1-mediated exosomal sorting of Dll4 modulates the tubular-macrophage crosstalk in diabetic nephropathy [J]. Mol Ther, 2023, 31(5): 1451-67.
19. LIM A K, MA F Y, NIKOLIC-PATERSON D J, et al. Lymphocytes promote albuminuria, but not renal dysfunction or histological damage in a mouse model of diabetic renal injury [J]. Diabetologia, 2010, 53(8): 1772-82.
20. MOON J Y, JEONG K H, LEE T W, et al. Aberrant recruitment and activation of T cells in diabetic nephropathy [J]. Am J Nephrol, 2012, 35(2): 164-74.
21. HERRERA M, SöDERBERG M, SABIRSH A, et al. Inhibition of T-cell activation by the CTLA4-Fc Abatacept is sufficient to ameliorate proteinuric kidney disease [J]. Am J Physiol Renal Physiol, 2017, 312(4): F748-f59.
22. OKOŃ K, STACHURA J. Increased mast cell density in renal interstitium is correlated with relative interstitial volume, serum creatinine and urea especially in diabetic nephropathy but also in primary glomerulonephritis [J]. Pol J Pathol, 2007, 58(3): 193-7.
23. SUR S, NGUYEN M, BOADA P, et al. FcER1: A Novel Molecule Implicated in the Progression of Human Diabetic Kidney Disease [J]. Front Immunol, 2021, 12: 769972.
24. LOCATELLI M, ZOJA C, CONTI S, et al. Empagliflozin protects glomerular endothelial cell architecture in experimental diabetes through the VEGF-A/caveolin-1/PV-1 signaling pathway [J]. J Pathol, 2022, 256(4): 468-79.
25. MOHANDES S, DOKE T, HU H, et al. Molecular pathways that drive diabetic kidney disease [J]. J Clin Invest, 2023, 133(4).
26. JOURDE-CHICHE N, FAKHOURI F, DOU L, et al. Endothelium structure and function in kidney health and disease [J]. Nat Rev Nephrol, 2019, 15(2): 87-108.
27. LAI H, CHEN A, CAI H, et al. Podocyte and endothelial-specific elimination of BAMBI identifies differential transforming growth factor-β pathways contributing to diabetic glomerulopathy [J]. Kidney Int, 2020, 98(3): 601-14.
28. HONG Q, ZHANG L, FU J, et al. LRG1 Promotes Diabetic Kidney Disease Progression by Enhancing TGF-β-Induced Angiogenesis [J]. J Am Soc Nephrol, 2019, 30(4): 546-62.
29. NAKAGAWA T, KOSUGI T, HANEDA M, et al. Abnormal angiogenesis in diabetic nephropathy [J]. Diabetes, 2009, 58(7): 1471-8.
30. SOLIMINI N L, LIANG A C, XU C, et al. STOP gene Phactr4 is a tumor suppressor [J]. Proc Natl Acad Sci U S A, 2013, 110(5): E407-14.
31. CAO F, LIU M, ZHANG Q Z, et al. PHACTR4 regulates proliferation, migration and invasion of human hepatocellular carcinoma by inhibiting IL-6/Stat3 pathway [J]. Eur Rev Med Pharmacol Sci, 2016, 20(16): 3392-9.
32. LI VOLTI G, ZANGHì A, ZANGHì G. Editorial - PHACTR4/STAT3 axis as a possible target therapy for hepatocellular carcinoma treatment [J]. Eur Rev Med Pharmacol Sci, 2016, 20(19): 3953-4.

33. ZHANG Y, KIM T H, NISWANDER L. Phactr4 regulates directional migration of enteric neural crest through PP1, integrin signaling, and cofilin activity [J]. Genes Dev, 2012, 26(1): 69-81.
34. KIM T H, GOODMAN J, ANDERSON K V, et al. Phactr4 regulates neural tube and optic fissure closure by controlling PP1-, Rb-, and E2F1-regulated cell-cycle progression [J]. Dev Cell, 2007, 13(1): 87-102.
35. TANG C, FANG M, TAN G, et al. Discovery of Novel Circulating Immune Complexes in Lupus Nephritis Using Immunoproteomics [J]. Front Immunol, 2022, 13: 850015.

Correspondence to:
Yi Shi
Zhongshan Hospital, Fudan University, 180 Fenglin Road, Xuhui, Shanghai 200032, P.R. China
E-mail: Shiyi_8@126.com, 18211210067@fudan.edu.cn

Received July 2023
Revised August 2023
Accepted October 2023