CFAP45 is a Potential Predictor of Mitochondrial Dysfunction in Diabetic Kidney Disease

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Research

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Abstract

Background: Cilia and Flagella Associated Protein 45 (CFAP45) is known to be involved in the regulation of ciliary motility. However, its potential role in diabetic kidney disease (DKD) remains unknown. This study demonstrated the role of CFAP45 in the development of DKD based on the Gene Expression Omnibus database.

Methods: First, we investigated the expression of CFAP45 in a whole-genome expression microarray (GSE30122). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, as well as Gene Set Enrichment Analysis (GSEA), were then conducted to identify the key signaling pathways associated with CFAP45. The networks between transcript factors and hub genes were then constructed by Cytoscape software.

Findings: The expression levels of CFAP45 mRNA were reduced in DKD samples as compared to normal samples. Receiver operating characteristic (ROC) curve analysis suggested the significant discriminatory power (area under curve [AUC] = 0.811) of CFAP45 between DKD and normal tissues. Low expression levels of CFAP45 were correlated with apoptosis, senescence and the induction of mast cell infiltration. Function enrichment analysis indicated that CFAP45 is involved in the most significant hallmarks of mitochondrial metabolism dysfunction, including glycolysis and gluconeogenesis, fatty acid metabolism and degradation, ubiquitin-dependent protein catabolic processes, the tricarboxylic acid cycle (TCA) cycle, the action of oxidoreductase on Nicotinamide adenine dinucleotide (NADH), and the peroxisome proliferator-activated receptor (PPAR) signaling pathways located in the mitochondrial matrix and the lysosomal membrane. The transcription factor SMAD4 was found to negatively regulate the PPAR γ coactivator 1 α (PGC1 α).

Interpretation: CFAP45 may regulate mitochondrial metabolic activity by affecting the function of the primary cilium on the kidney epithelial cells. CFAP45 may serve as a potential biomarker for the diagnosis and treatment of DKD.

Introduction

Diabetic kidney disease (DKD) is one of the most common chronic microvascular complications and has a prevalence of 30–40% in patients with diabetes. Ultimately, DKD will lead to chronic renal failure and end-stage kidney disease (ESRD), thus seriously shortening life expectancy and reducing life quality.^{1,2} Although aggressive early interventions can improve prognosis, only a limited number of therapies are currently available. Moreover, the efficacy of these therapies in reversing the progression of disease remains limited. Given the high levels of morbidity and mortality associated with chronic kidney disease, it is imperative that we explore new therapeutic and prognostic targets for patients with DKD.

The pathogenesis of DKD is complex and multi-factorial, involves hemodynamic changes, metabolic disorders, oxidative stress, inflammation, and fibrosis.³ As kidneys are mitochondrial-rich and highly metabolic organs, they require a large amount of ATP to maintain normal function. However, diabetes is associated with abnormalities of fatty acids and oxygen delivery that lead to changes in metabolic fuel sources. These changes then influence the production of ATP and contribute to renal hypoxia.⁴ Genes that exert impact on mitochondrial dysfunction and energy metabolism may also be an important risk factor for DKD. Therefore, it may be possible to identify new targets by screening gene networks for changes related to DKD.

CFAP45 (also known as NESG1, CCDC19) was first reported in 1999⁵ and encodes the cilia and flagella associated protein 45.⁶ Previous studies indicate that CFAP45 is a potential tumor suppressor in nasopharyngeal carcinoma (NPC) and non-small cell lung cancer (NSCLC).⁶ Lower expression levels of CFAP45 have been reported in NPC and NSCLC tissues compared to normal tissues, and the induced overexpression of CFAP45 was shown to significantly suppress cell proliferation and cell cycle transition from G1 to S.^{7,8} CFAP45 has recently been reported to be associated with motile ciliopathy; researchers found that CFAP45 mediates adenine nucleotide homeostasis *via* AMP binding.⁹ This finding suggests CFAP45 plays an important role in ciliary motility and energy metabolism processes. However, the underlying mechanisms of CFAP45 in the progression of DKD remain unclear.

In the present study, we aimed to explore the expression differences and potential mechanism of CFAP45 in patients with DKD. To do this, we analyzed a Gene Expression Omnibus (GEO) dataset and investigated how CFAP45 interacts with other genes by performing Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, and Gene Set Enrichment Analysis (GSEA); we also performed Protein-protein interaction (PPI) network analysis. We hypothesized that CFAP45 might influence the development and progression of DKD. The findings of our study should enhance our understanding of the molecular mechanisms of DKD and may help to identify new therapeutic targets.

Methods

1.1 Data Processing

A total of 69 gene expression profiles from DKD patients (n = 19), and healthy controls (n = 50) were downloaded from the GEO dataset (GSE30122^{10,11} files, <https://www.ncbi.nlm.nih.gov/geo/>). The Affymetrix Human Genome U133A 2.0 Array data were normalized using the robust multi-array average (RMA) method as implemented in the affy package¹². CFAP45 gene expression data was obtained from Cancer Cell Line Encyclopedia (CCLE) (<https://portals.broadinstitute.org/ccle/about>). There was no need for ethics approval or informed consent, as all data used in this study were obtained from GEO and CCLE datasets.

1.2 Biological pathway analysis by ssGSEA

The Hallmark gene sets of biological pathways were downloaded from the GeneCards database (<http://www.genecards.org>). Pathway analysis was performed using a single sample Gene Set Enrichment Analysis (ssGSEA)¹³, an extension of GSEA, which calculates separate enrichment scores of every gene set for each sample. Correlation between the expression level of CFAP45 and the activity of biological pathways was examined.

1.3 The correlation between CFAP45 expression and immune infiltration

To estimate the proportion of immune cells in DKD samples, CIBERSORT¹⁴ was used, with the LM22 set representing 22 immune cell subtypes (including B cells, T cells, natural killer cells, macrophages, etc.). CIBERSORT is a gene expression-based deconvolution algorithm, it uses a set of barcode gene expression values (a “signature matrix” of 547 genes) for characterizing immune cell composition.¹⁴ Association between CFAP45 and immune cell infiltration was assessed.

1.4 Differentially Expressed Gene (DEG) Analysis

Gene expression data were then divided into high- and low- groups according to the median CFAP45 expression level. PCA was carried out using the R package FactoMineR. Then we used the Bioconductor limma package to identify DEGs between sample groups.¹⁵ The criteria of adjusted *P*-value < 0.05 and |logFC| > 1.0 were applied to screen the DEGs. Heatmaps and volcano plots were used to visualize gene expression patterns.

1.5 Functional Enrichment Analysis and Gene Set Enrichment Analysis

GO and KEGG pathway analysis were performed on the DEGs between high- and low- CFAP45 mRNA expression groups with the clusterProfiler R package (version 3.14.3)¹⁶. False discovery rate (FDR) cutoff of 0.05 was used for statistical significance.

To elucidate the enrichment outcome between high- and low- CFAP45 groups, GSEA was further performed using the molecular signature database (MSigDB) collections: c2.cp.kegg.v6.2.symbols by R package clusterProfiler.^{16,17} The expression level of CFAP45 was used as a phenotype label. Normalized enrichment scores (|NES| > 1), adjusted *P*-values < 0.1 and FDR *q* value < 0.25 were considered statistically significant.

1.6 Protein-protein interaction analysis and Transcriptional Regulatory Network (TRN) construction

The protein-protein interaction network of candidate genes was constructed in the STRING (<http://string-db.org>) database. A medium confidence score of 0.4 was used as minimum required interaction score and the resulting network was analyzed with Cytoscape software (version 3.72)¹⁸. The Cytohubba plug-in¹⁹ was used to select and identify the top 10 hub genes according to the Maximal Clique Centrality (MCC) method. Subsequently, transcription factors were selected according to their expression correlation with DEGs. Pearson correlation coefficient was calculated, and 0.8 was defined as the threshold to construct the network. Finally, transcriptional regulatory networks were visualized using Cytoscape.

1.7 Statistical Analysis

We used the Mann-Whitney U test or Student t test for continuous variables depending on the normality of distribution. Pearson χ^2 or Fisher exact test was used to analyze the association between CFAP45 expression and categorical variables. Correlation between genes were analyzed using the Pearson correlation test. AUC was computed using the R package “pROC”²⁰. All statistical analysis was conducted with R (version 4.0.2) and *P* values < 0.05 were considered statistically significant.

Results

2.1 Expression pattern of CFAP45 mRNA in DKD

The characteristics of DKD patients in GSE30122 have been reported previously.¹⁰ As shown in Fig. 1A, we compared the expression levels of CFAP45 in 19 DKD tissues and 50 normal tissues using the Wilcoxon's rank sum test. The expression levels of CFAP45 mRNA in DKD tissues were significantly lower than those in normal tissues ($p = 7.59 \times 10^{-5}$) (Fig. 1A). Next, we performed receiver operating characteristic (ROC) analysis to measure the discriminatory ability of CFAP45. The area under curve (AUC) of the ROC curve was 0.811, thus representing a moderate ability to discriminate cases of DKD from a normal kidney (Fig. 1B). In addition, we used the CCLE database to analyze the expression levels of CFAP45 mRNA in different cell lines from various tissues (Fig. 1C). We observed high expression levels of CFAP45 in cell lines associated with meningioma, breast, and the urinary tract. Low levels of CFAP45 expression were identified in soft tissue and cell lines associated with B-cell_ALL and neuroblastoma.

2.2 The correlation between CFAP45 expression and different biological processes

Analysis of the GeneCards database identified 178 apoptosis-related genes, 152 autophagy-related genes, and 117 senescence-related genes. Pathway enrichment analysis was then performed with ssGSEA. High expression levels of CFAP45 were significantly associated with higher levels of apoptosis ($P < 0.01$, Fig. 2A) and higher senescence scores ($P < 0.05$, Fig. 2C). The expression levels of CFAP45 were not significantly correlated with the extent of autophagy (Fig. 2B).

2.3 The correlation between CFAP45 expression and immune infiltration

Pearson correlation was used to analyze the correlation between CFAP45 expression levels and the relative abundance of 22 immune cell subtypes (quantified by CIBERSORT) in DKD samples (Fig. 3A). Analysis showed that CFAP45 expression was positively correlated with activated mast cells (Pearson's correlation = 0.855, $P < 0.001$, Fig. 3B) and resting dendritic cells (Pearson's correlation = 0.491, $P = 0.033$, Fig. 3C), and negatively correlated with resting mast cells (Pearson's correlation = -0.514, $P < 0.017$, Fig. 3D).

2.4 Identification of DEGs in between high- and low- CFAP45 groups

PCA analysis (Fig. 4A) identified a large difference between high- and low- CFAP45 groups. Next, R package limma was performed to compare the gene expression profiles between the two groups. A total of 349 DEGs, including 59 up-regulated genes and 290 down-regulated genes, were detected (adjusted P -value < 0.05 and $|\log FC| > 1.0$). The relative expression levels of DEGs were illustrated by Volcano plot (Fig. 4B) and Heat map (Fig. 4C).

2.5 Functional enrichment of CFAP45 related DEGs in DKD patients

To predict the functional enrichment information of CFAP45-associated DEGs in DKD, we performed GO enrichment analysis using the ClusterProfiler R package. GO results showed that CFAP45-associated DEGs were mainly located in the mitochondrial matrix and the lysosomal membrane (Fig. 5A and Table 1). The biological processes (BPs) and molecular functions (MFs) of CFAP45-associated genes included the regulation of protein modification by small protein conjugation or removal, the tricarboxylic acid cycle, proteasome-mediated ubiquitin-dependent protein catabolic processes, oxidoreductase activity acting on NAD(P)H, phospholipid transporter activity, intramembrane lipid transporter activity, and I-SMAD binding (Fig. 5B-C and Table 1). Next, we performed KEGG pathway enrichment analysis and identified several pathways that were influenced by CFAP45, including the citrate cycle (TCA cycle), carbon metabolism, valine, leucine and isoleucine degradation, fatty acid metabolism, endocytosis, glycolysis and gluconeogenesis, amino sugar and nucleotide sugar metabolism, the PPAR signaling pathway, and peroxisomes (Fig. 5D and Table 2).

Table 1
Gene Ontology (GO) analysis of differentially expressed genes (DEGs)

ONTOLOGY	ID	Description	Count	P value	Gene
BP	GO:1903320	regulation of protein modification by small protein conjugation or removal	15	8.46E-06	UFL1/HMG20A/BAG5/RWDD3/HSP90AB1/TRIP12/CTNNB1/BAG2/ISG15/TOPORS/HSPA5/DCUN1D1/RAB1A/NDFIP1/HERPUD1
BP	GO:0050792	regulation of viral process	14	1.13E-05	LGALS1/IFI27/STAU1/IGF2R/ISG15/IFITM2/NELFCD/IFITM3/SNF8/MPHOSPH8/POLR2J/CCL4/IFITM1/FCN3
BP	GO:0006099	tricarboxylic acid cycle	6	1.88E-05	DLAT/NNT/DLD/SUCLA2/SDHA/SUCLG2
CC	GO:0005759	mitochondrial matrix	21	3.25E-05	NME4/CBR4/CLPX/DLAT/IARS2/OXCT1/DLD/HSPA9/VDAC1/SUCLA2/NARS2/HSPD1/ACAD8/ETFA/TFB2M/SUCLG2/ACSM3/MRPL19/GLUD1/ALDH7A1/MIPEP
CC	GO:0005925	focal adhesion	19	4.14E-05	LIMA1/KLF11/HNRNPK/PPP1CB/TRIOBP/RRAS/CTNNB1/TGFB1I1/HSPA9/RAB21/IGF2R/CPNE3/CYBA/ZNF185/FLRT3/HSPA5/EHD3/DPP4/CNN3
CC	GO:0030055	cell-substrate junction	19	5.21E-05	LIMA1/KLF11/HNRNPK/PPP1CB/TRIOBP/RRAS/CTNNB1/TGFB1I1/HSPA9/RAB21/IGF2R/CPNE3/CYBA/ZNF185/FLRT3/HSPA5/EHD3/DPP4/CNN3
MF	GO:0043903	regulation of interspecies interactions between organisms	14	2.34E-05	LGALS1/IFI27/STAU1/IGF2R/ISG15/IFITM2/NELFCD/IFITM3/SNF8/MPHOSPH8/POLR2J/CCL4/IFITM1/FCN3
MF	GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process	19	8.73E-05	UFL1/TBL1XR1/BAG5/HSP90AB1/CD2AP/IFI27/WWP1/CTNNB1/DNAJB14/RNF14/MTM1/TOPORS/UBE2W/PTTG1/HSPA5/NEDD4L/HERPUD1/PSMB10/PSMA4
MF	GO:0046596	regulation of viral entry into host cell	5	0.000128	LGALS1/IFITM2/IFITM3/IFITM1/FCN3

Table 2
Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of differentially expressed genes (DEGs)

ID	Description	Count	P value	Gene
hsa00020	Citrate cycle (TCA cycle)	5	0.00019	DLAT/DLD/SUCLA2/SDHA/SUCLG2
hsa01200	Carbon metabolism	8	0.001409	DLAT/DLD/SUCLA2/PGK1/SDHA/SUCLG2/EHHADH/GLUD1
hsa00650	Butanoate metabolism	4	0.001575	OXCT1/L2HGDH/ACSM3/EHHADH
hsa00280	Valine, leucine and isoleucine degradation	5	0.001755	OXCT1/DLD/ACAD8/EHHADH/ALDH7A1
hsa00640	Propanoate metabolism	4	0.003277	DLD/SUCLA2/SUCLG2/EHHADH
hsa01212	Fatty acid metabolism	5	0.003762	CBR4/SCP2/ACSL1/CPT2/EHHADH
hsa04144	Endocytosis	11	0.005765	ARFGAP3/WWP1/SNX2/AGAP1/IGF2R/ RAB11FIP1/EHD3/SNF8/NEDD4L/ARPC1A/SNX4
hsa00010	Glycolysis / Gluconeogenesis	5	0.007501	MINPP1/DLAT/DLD/PGK1/ALDH7A1

Gene set enrichment analysis was also used to identify signaling pathways that were involved in DKD by comparing between data sets with high and low levels of CFAP45 expression. GSEA revealed significant differences ($|\text{NES}| > 1$) in the enrichment of MSigDB collections (c2.cp.kegg.v6.2.symbols). Four pathways showed significant differential enrichment in the low- CFAP45 expressed phenotype, including nicotinate and nicotinamide metabolism, tryptophan metabolism, selenoamino acid metabolism, and the adipocytokine signaling pathway (Fig. 6A, 6B and Table 3), thus indicating the potential role of CFAP45 in the metabolic disorder associated with DKD.

Table 3
Gene set enrichment analysis (GSEA) analysis parameters.

Name	Size	Enrichment Score	NES	P value	Leading edge
KEGG_NICOTINATE_AND_NICOTINAMIDE_METABOLISM	16	-0.6427	-1.48595	0.017682	tags = 50%, list = 15%, signal = 59%
KEGG_TRYPTOPHAN_METABOLISM	33	-0.6979	-1.39764	0.087891	tags = 67%, list = 16%, signal = 79%
KEGG_SELENOAMINO_ACID_METABOLISM	19	-0.5416	-1.36996	0.095703	tags = 63%, list = 24%, signal = 83%
KEGG_BETA_ALANINE_METABOLISM	21	-0.73829	-1.36531	0.116142	tags = 71%, list = 16%, signal = 85%
KEGG_ADIPOCYTOKINE_SIGNALING_PATHWAY	62	-0.38756	-1.36028	0.057769	tags = 23%, list = 11%, signal = 25%

2.6 PPI Network analysis and Transcriptional Regulatory Network (TRN) construction

All CFAP45-associated DEGs were then imported into the STRING database for protein-protein interaction network analysis; the confidence score was set to 0.4 (Fig. 7A). Next, the top ten hub genes were ranked using the MCC method, including *WWP1*, *FBXW2*, *NEDD4L*, *HERC6*, *RNF14*, *TRIP12*, *PJA2*, *UBE2W*, *RNF138*, and *UFL1* (Fig. 7B).

Changes in the patterns of gene expression are known to be affected by regulatory events at the transcriptional and post-transcriptional levels. Therefore, we selected potential transcription factor (TF) targets to further explore the mechanisms that might underlie the progression of DKD. As shown in Fig. 7C and Table S1, there was a strong correlation between TFs and DEGs (Pearson's correlation > 0.8 , $P < 0.0001$). We introduced this data into Cytoscape and created a transcriptional regulatory network (TRN) featuring key transcription factors, including CTNNB1, KLF11, RCOR1, SMAD4, SNAPC2, and TBL1XR1.

Discussion

CFAP45 encodes the Cilia and Flagella Associated Protein 45 and is located in chromosomal region 1q23.2. Previous studies have established the importance of CFAP45 as a tumor suppressor in nasopharyngeal carcinoma (NPC) and non-small cell lung cancer (NSCLC). CFAP45 acts in

these scenarios by blocking cell growth *via* the PI3K/AKT/C-Jun pathway.^{6–8} However, recent study has shown that axonemal dynein ATPases are able to direct ciliary and flagellar beating by linking to CFAP45 in order to mediate axonemal adenine nucleotide homeostasis. Therefore, CFAP45 deficiency contributes to a motile ciliopathy that presents as left-right asymmetry abnormalities, dyskinetic sperm flagella, and chronic upper and lower respiratory disease in human.⁹ Although the motile function of primary cilia in such cases are absent, the sensory and signal transduction features are retained.²¹ It is well known that primary cilium (PC) are found on kidney epithelial cells (KECs); however, little is known about the expression of CFAP45 and its potential role in kidney disease. We conducted the present study to investigate how CFAP45 might be involved in DKD and to identify the mechanisms involved.

In this study, we acquired expression profile data related to diabetic kidney disease from the GEO database and demonstrated that CFAP45 was significantly downregulated in DKD tissues compared with normal tissues. Furthermore, CFAP45 had significant potential to discriminate between DKD and normal tissues (AUC from ROC analysis: 0.811). In addition, we found that low levels of CFAP45 expression were correlated with apoptosis and senescence and may, therefore, promote the progression of disease. It is also worth noting that CFAP45 induced an immune response resulting in the infiltration of mast cells. The enzyme chymase, present in mast cells, has been reported to be a potent anti-inflammatory factor in renal injury by limiting neutrophil hyperactivation, recruitment, and associated damage.²²

To further investigate the function of CFAP45 in DKD, 349 CFAP45-associated DEGs were subjected to GO and KEGG pathway enrichment analysis. Analysis indicated that mitochondrial metabolic dysfunction may play an important role in the development of DKD because glucose (glycolysis/gluconeogenesis, amino sugar, and nucleotide sugar metabolism), fatty acids (phospholipid transporter activity, intramembrane lipid transporter activity, and fatty acid metabolism), amino acids (ubiquitin-dependent protein catabolic processes, valine, leucine, and isoleucine degradation), and other metabolic substrates (e.g., the TCA cycle) all represent chemical fuels that are used by the mitochondria to make ATP (oxidoreductase activity acting on NADH).⁴ We also used GEO data for GSEA analysis and found that nicotinate and nicotinamide metabolism, amino acid metabolism (tryptophan, selenoamino), and the adipocytokine signaling pathway, in DKD tissues were differentially enriched in the phenotype that was associated with low expression levels of CFAP45. This suggests that CFAP45 serves as a potential biomarker for predicting mitochondrial dysfunction in DKD patients. Since CFAP45 affects mitochondrial metabolic function in DKD, it is also possible that CFAP45 may also represent a potential therapeutic target.

As a highly metabolic organ, the kidney has a dense population of mitochondria and is very reliant on the production of mitochondrial energy.²³ A recent study showed that shear stress originating from urinary flow is sensed by the primary cilia of proximal tubular KECs and stimulates mitochondrial oxidative metabolism *via* the nutrient-sensing kinase AMPK-PGC1 α signaling cascade.^{24,25} In the present study, GO and KEGG pathway analysis demonstrated that CFAP45 may play an important role in mitochondrial metabolism *via* the peroxisome proliferator-activated receptor (PPAR) signaling pathway. In addition, we found that the SMAD4 transcription factor negatively regulates PPAR γ coactivator 1 α (PGC1 α) *via* the construction of transcriptional regulation network (Table S1). According to previous studies, hyperglycemia induces Smad4 localization to mitochondria can induce diabetic nephropathy by reducing glycolysis and oxidative phosphorylation (OXPHOS).²⁶ Furthermore, stimulation of AMP-activated protein kinase (AMPK) has been shown to possess a powerful ability to inhibit the nuclear translocation of Smad4 in diabetic kidneys.²⁷ In addition, drugs that could re-establish the homeostasis of mitochondrial energy are promising drugs for the restoration of DKD, including orally active synthetic adiponectin receptor agonist²⁸, berberine²⁹ and mitochondria-targeted antioxidant MitoQ³⁰. These findings suggest that CFAP45 may exert significant influence on the progression of DKD. Based on pathway analysis, SMAD4 participates in the regulation of mitochondrial energy metabolism by negatively regulating CFAP45-related DEGs through the PPAR-PGC1 pathway. Additional experiments are now needed to demonstrate the biological impact of CFAP45 in DKD and to further clarify the underlying regulatory mechanism.

Although this study enhanced our understanding of the relationship between CFAP45 and DKD, there are still some limitations that need to be considered. Firstly, it is important to incorporate a range of other parameters in our analysis, including gender, age, eGFR, and proteinuria. However, these parameters were not available in the public database we used for our analysis. Secondly, our sample size was insufficient; additional studies are needed to validate our findings. Thirdly, this was a retrospective study and therefore lacked some clinical data. Future studies should follow a prospective design to avoid bias. Finally, the regulatory network between CFAP45-related DEGs and transcriptional factors was predicted using online databases and therefore requires further validation.

In summary, we found that the expression levels of CFAP45 were significantly downregulated in DKD tissues. Our analysis suggests that CFAP45 may play an important role in the regulation of mitochondrial metabolic activity *via* its function on the primary cilium of kidney epithelial cells. Our analysis also showed that one of the mechanisms underlying these effects may involve the downregulation of PGC1 α by the SMAD4 transcription factor. Our study enhances our understanding of how CFAP45 is involved in the progression of DKD and provides a potential biomarker for the diagnosis and treatment of DKD.

Declarations

Ethics approval and consent to participate

There was no need for ethics approval or informed consent, as all data used in this study were obtained from GEO and CCLE datasets.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available in the GEO dataset. GSE30122 files, <https://www.ncbi.nlm.nih.gov/geo/>

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YC performed data analysis and wrote the manuscript. MS validated the results and helped with the data visualization. YS and LJ helped to prepare for data collection and the literature search. YG, QF helped with the manuscript review and editing. XYX, KFX worked on the methodology and supervision. TY designed the study and is the guarantor of this work as such TY has full access to all the study data and takes responsibility for the integrity of the data and the accuracy of the data analysis. All the authors critically revised and approved the final manuscript.

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Figures

Fig. 1

Figure 1

Expression pattern of CFAP45 mRNA in Diabetic kidney disease (DKD). (A) The expression levels of CFAP45 mRNA were significantly lower in DKD tissues than in normal tissues ($p = 7.59 \times 10^{-5}$). (B) Receiver operating characteristic (ROC) analysis of CFAP45 expression levels showed a promising discriminatory power between DKD and normal tissues. The horizontal axis represents the false positive rate while the vertical axis represents the true positive rate. (C) CFAP45 expression levels across different cell lines from Cancer Cell Line Encyclopedia (CCLE) data sets.

Fig. 2

Figure 2

The association between CFAP45 expression levels and different biological processes. High expression levels of CFAP45 were significantly associated with apoptosis (A) and senescence (C) pathways, but not autophagy (B).

Fig. 3

Figure 3

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The association between CFAP45 expression and immune cell infiltration in DKD. (A) Forest plot showing the correlation between CFAP45 and immune cell subsets. The size of the dots represents the absolute value of Pearson's correlation coefficient. The correlation between CFAP45 expression and the relative enrichment score of activated mast cells (B), resting dendritic cells (C), and resting mast cells (D).

Fig. 4

Figure 4

The identification of differentially expressed genes (DEGs) based on CFAP45 mRNA expression. Principle component analysis (A), Volcano plot (B) and Heat map (C) of the differential gene profiles between groups exhibiting high and low levels of CFAP45 expression.

Fig. 5

Figure 5

Significantly enriched Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway terms of CFAP45-associated genes in DKD. Interactive networks, including cellular component (A), biological process (B), and molecular function (C), of the top five enrichment terms colored by P-values. (D) Heat map of the KEGG pathways enriched by CFAP45-associated DEGs.

Fig. 6

Figure 6

Gene set enrichment analysis (GSEA) based on CFAP45 expression. (A) Several pathways and biological processes were differently enriched in CFAP45-associated genes in DKD. GSEA indicated that low expression levels of CFAP45 were correlated with nicotinate and nicotinamide metabolism, tryptophan metabolism, selenoamino acid metabolism, and beta alanine metabolism (B).

Fig. 7

Figure 7

Protein-protein interaction (PPI) network analysis and transcriptional regulatory network (TRN) construction. (A) A PPI network was constructed using the STRING database. The nodes represent genes and the edges represent PPI. (B) A total of ten hub genes were identified via MCC algorithm analysis. Red and yellow nodes indicate hub genes. (C) Transcriptional regulation network of the differentially expressed genes (DEGs) and transcription factors (TFs). The triangular nodes represent TFs while the oval nodes represent DEGs. Red edges indicate positive regulation, while green edges indicate downregulation. For detailed results, see Supplementary Table 1.

Supplementary Files

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- [SupplementalTable1.xlsx](#)