Gene expression-based analysis identified NTNG1 and HGF as biomarkers for diabetic kidney disease

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Abstract
Diabetic kidney disease (DKD) is a leading cause of end-stage renal disease. Because the molecular mechanisms of DKD are not fully understood, exploration of hub genes and the mechanisms underlying this disease are essential for elucidating the pathogenesis and progression of DKD. Accordingly, in this study, we performed an analysis of gene expression in DKD. The differentially expressed genes (DEGs) included 39 upregulated genes and 113 downregulated genes in the GSE30528 dataset and 127 upregulated genes and 18 downregulated genes in the GSE30529 dataset. Additionally, functional analyses were performed to determine the roles of DEGs using glomeruli samples from patients with DKD and healthy controls from the GSE30528 dataset and using tubule samples from patients with DKD and healthy controls from the GSE30529 dataset. These DEGs were enriched in pathways such as the Wnt signaling pathway, metabolic pathways, and the mammalian target of rapamycin signaling pathway in the GSE30528 dataset and the longevity regulating pathway and Ras signaling pathway in the GSE30529 dataset. Moreover, a protein-protein interaction network was constructed using the identified DEGs, and hub gene analysis was performed. Furthermore, correlation analyses between key genes and pathological characteristics of DKD indicated that CCR4, NTNG1, HGF, and ISL1 are related to DKD, and NTNG1 and HGF may serve as diagnostic biomarkers in DKD using the receiver–operator characteristic (ROC) curve. Collectively, our findings established 2 reliable biomarkers for DKD.

Abbreviations: DEGs = differentially expressed genes, DKD = Diabetic kidney disease, DM = diabetes mellitus, ESRD = end-stage renal disease, GEO = Gene Expression Omnibus, GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, PPI = protein-protein interaction, ROC = receiver–operator characteristic.

Keywords: bioinformatic analysis, biomarker, diabetic kidney disease, gene expression

1. Introduction
The incidence of diabetes mellitus (DM) has risen dramatically over the past 2 decades, and this disease now affects over 300 million people worldwide. Diabetic kidney disease (DKD) is a severe microvascular complication of DM, and approximately 20% to 40% of diabetic patients develop DKD, making this a leading cause of end-stage renal disease (ESRD). Individuals with ESRD secondary to DKD have an increased risk of all-cause mortality and cardiovascular disease, which causes enormous medical and socioeconomic burdens on society. DKD has 2 pathophysiological stages based on clinical manifestations and morphological abnormalities, namely, early diabetic kidney stage of glomerular hyperfiltration, microalbuminuria (30–300 mg/dl), glomerular hypertrophy and mesangial expansion, and advanced diabetic renal stage of progressive decline in glomerular filtration rate (GFR), macroalbuminuria (>300 mg/dl), glomerulosclerosis, and interstitial fibrosis.

Despite extensive studies, the prognosis of individual patients with DKD is hard to predict owing to differences in disease progression and a lack of effective prognostic parameters. In addition, current treatments, including renin-angiotensin system blockade as well as stringent glycemic, lipid, and blood pressure control, are very limited, and none of these approaches can completely prevent the progression to ESRD. Hence, it is essential to explore the molecular mechanisms of DKD and thus establish accurate diagnostic tools and treatment regimens.

Transcriptomics is a promising approach for identification of biomarkers and monitoring disease activity. Microarray technology facilitates the elucidation of mRNA profiles associated with human disease and provides a comprehensive, unbiased approach to systematically analyze disease processes. Functional genomics explores gene interactions and cellular pathways involved in disease biology that may be potential targets of newer molecular therapeutics. Ultimately, after more analysis, these biomarkers may be applied toward early diagnosis, prognosis, and prediction of therapeutic responses.
In this study, we firstly analyzed differentially expressed genes (DEGs) in glomerular and tubule tissues from patients with DKD and healthy controls using data downloaded from the Gene Expression Omnibus (GEO). Then, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to explore the molecular mechanisms of DKD. Next, protein–protein interaction (PPI) analysis was performed and hub genes were identified. Besides,
correlation analysis between hub genes and clinicopathological features in patients with DKD and in mouse models was employed, and ultimately 4 hub genes were selected for diagnostic analysis. Finally, 2 hub genes (Nertin G1, NTNG1, and Hepatocyte growth factor, HGF) were identified as key biomarkers for DKD. The NTNG1 gene is located on chromosome 1p13.3 and encodes a glycosylphosphatidylinositol protein anchored to the presynaptic membrane and HGF is known as scatter factor and tumor cytotoxic factor, is a large multidomain heterodimeric protein that belongs to the cytokine family. Our findings established 2 reliable biomarkers for DKD.

2. Materials and methods

2.1. Microarray data

Microarray data were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo).[11] The GSE30528 dataset included data on glomeruli samples from patients with DKD (n = 9) and healthy controls (n = 13) and the GSE30529 dataset included data on tubule samples from patients with DKD (n = 10) and controls (n = 12) from Homo sapiens based on the GPL571 (Affymetrix Human Genome U133A 2.0 Array) platform. The R software package was applied to process the microarray data and to normalize the unqualified files.
2.2. Identification of DEGs

DEGs were analyzed using the limma package in Bioconductor (http://www.bioconductor.org/).[12] Samples with an absolute value of log fold change greater than 1 and P value less than .05 were considered DEGs. Probe sets without corresponding gene symbols or genes with more than 1 probe set were removed or averaged, respectively. Next, identified DEGs were used for further analysis.

2.3. Functional enrichment analysis of DEGs

To investigate the biological characteristics and functional enrichment of candidate DEGs, functional enrichment analysis was performed using the DAVID (https://david.ncifcrf.gov/) online database.[13] Results with P values of less than .05 were considered significant. In addition, Circos, an information aesthetic for comparative genomics, was applied to show how genes from the input gene lists overlapped and shared GO terms.[14]

2.4. PPI network integration and module analysis

PPI networks for DEGs were investigated with the STRING database (https://string-db.org/cgi/).[15] Cytoscape (version 3.7.1) is an open source bioinformatics software platform for visualizing molecular interaction networks.[16] CytoHubba, a Cytoscape plug-in, was used to explore the hub genes in the PPI networks,[17] and the top 30 hub genes were displayed based on node degree.

2.5. Clinicopathological correlation analysis

We performed correlation analysis between hub genes and clinicopathological features in patients with DKD and in mouse models using the Nephroseq v5 database. Additionally, key hub gene expression in a DKD mouse model was analyzed using the same database.

2.6. Diagnostic analysis

We analyzed the diagnostic effectiveness of key hub genes for distinguishing patients with DKD and healthy individuals in those 2 datasets using the area under the Receiver-operator characteristic (ROC) curve.

2.7. Statistical analysis

Values were depicted as the means ± standard deviations and were considered significant when the P value < .05. Statistical analysis was performed using unpaired t test. All statistical analyses were carried out using GraphPad prism 7.0 (GraphPad Software Inc. La Jolla, CA, USA).

2.8. Ethical statement

All the data of this paper was obtained from the open-access database, we did not get these data from patients or animals directly, nor intervene these patients. So the ethical approval was not necessary.

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Figure 3. Hub gene identified from the PPI networks. (A and C) PPI network of DEGs in GSE30528 and GSE30529 data. (B and D) The top 30 hub genes of DEGs in GSE30528 and GSE30529 data. DEGs = differentially expressed genes, PPI = protein-protein interaction.
3. Results

3.1. Identification of DEGs in DKD

The microarray datasets GSE30528 and GSE30529 were standardized, and the results are shown in Figure 1A and 1B. After standardization of the microarray results, 152 DEGs were identified from the GSE30528 dataset, including 39 upregulated genes and 113 downregulated genes (Fig. 1C). Similarly, 145 DEGs were screened from the GSE30529 dataset, including 127 upregulated genes and 18 downregulated genes (Fig. 1D). The cluster heatmaps of the top 100 DEGs are shown in Figure 1E and 1F.

3.2. Functional enrichment analysis of DEGs

The overlap between ontology terms associated with DEGs in GSE30528 and GSE30529 was minimal (Fig. 2A and B); thus, it seemed logical to analyze the functional enrichment of these gene sets separately. In the GSE30528 dataset, GO analysis showed that DEGs were significantly enriched in cellular components (CCs), including proteinaceous extracellular matrix, extracellular region, phagocytic vesicle, nuclear matrix, and extracellular space. For molecular functions (MFs), DEGs were particularly enriched in GTPase activity, GTP binding, frizzled binding, chromatin binding, and quaternary ammonium group transport activity. Additionally, biological process (BP) and KEGG pathway analyses demonstrated that the DEGs were enriched in positive regulation of insulin secretion involved (BP) and KEGG pathway analyses demonstrated that the DEGs were enriched in positive regulation of insulin secretion involved. The GO analysis results showed that DEGs were significantly enriched in CCs, including cilium, nucleoplasm, nucleus, nucleolus, and intracellular membrane-bounded organelles. For MFs, DEGs were particularly enriched in RNA polymerase II core promoter proximal region sequence-specific DNA binding, growth factor activity, transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding, chromatin binding, and protein heterodimerization activity. Additionally, BP and KEGG pathway analyses demonstrated that the DEGs were enriched in positive regulation of cell growth, positive regulation of phospholipase C-activating G-protein coupled receptor signaling pathways, small GTPase-mediated signal transduction, longevity regulating pathways, metabolism of xenobiotics by cytochrome P450, the Rap1 signaling pathway, Hippo signaling pathway, and chemokine signaling pathway (Fig. 2C and D).

Similarly, functional enrichment analyses of 145 DEGs in GSE30529 were also performed using the DAVID database. GO analysis results showed that DEGs were significantly enriched in CCs, including cilium, nucleoplasm, nucleus, nucleolus, and intracellular membrane-bounded organelles. For MFs, DEGs were particularly enriched in RNA polymerase II core promoter proximal region sequence-specific DNA binding, growth factor activity, transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding, chromatin binding, and protein heterodimerization activity. Additionally, BP and KEGG pathway analyses demonstrated that the DEGs were enriched in positive regulation of cell growth, positive regulation of phospholipase C-activating G-protein coupled receptor signaling pathways, small GTPase-mediated signal transduction, longevity regulating pathways, metabolism of xenobiotics by cytochrome P450, the Rap1 signaling pathway, Hippo signaling pathway, and chemokine signaling pathway (Fig. 2C and D).

3.3. Identification of hub genes from the PPI networks

Here, PPI networks were generated with STRING database (Fig. 3A and C), and hub genes were identified using CytoHubba from the PPI network. The top 30 hub genes in 2 datasets were constructed using Cytoscape (Fig. 3B and D), and their corresponding node degrees were listed in Table 1. Finally, the top 20 hub genes in 2 datasets, including HIST1H2BN, BUB1, CENPF, KIF2A, HIST1H4D, HIST1H4J, SMARCA5, THY1, CENPQ, WNT11, POLR2J, CD52, MRT04, KIFC1, KDM4A, NTNG1, GPLD1, EMG1, BMS1, CCR4, MPHOSPH10, BWSL, BOP1, DMT1, NOP58, D1E5F, TEX10, INS, CDC42, H2AFX, HAND2, HGF, CSF2, HIST3H3, ISL1, EED, HIST1H4I, HBEGF, POLR2J, and COP8 were selected for following analysis.

3.4. Clinicopathological correlation analysis of hub genes

The aforementioned hub genes associated with DKD were further analyzed with Nephroseq v5 database. We found that the mRNA levels of C-C motif chemokine receptor 4 (CCR4) and NTNG1 were positively related to GFR in patients with DKD, whereas the mRNA levels of HGF and Insulin gene enhancer binding protein-1 (ISL1) were negatively related to GFR in DKD patients (Fig. 4). CCR4 is the receptor 2 two CC chemokine ligands (CCLs)-CCL17 (also called thymus- and activation-regulated chemokine) and CCL22 (macrophage-derived chemokine). ISL1 is a subtype of the LIM homologous domain transcription factors. The rest of hub genes mRNA levels were not significantly correlated to GFR (data not shown). Additionally, we verified the transcriptional levels of CCR4, NTNG1, HGF, and ISL1 in DKD mice model using the same database. Compared with those in non-DKD mice, expression of NTNG1 and CCR4 were down-regulated in eNOS-deficient C57BLKS db/db mice (Fig. 5A and B). Furthermore, we analyzed the correlations between NTNG1 and CCR4 mRNA levels and other clinical parameters, such as weight, fasting blood glucose (FBG), and urinary albumin to creatinine.
ratio (UACR). Correlation results showed that NTNG1 mRNA levels was positively related to FBG and negatively related to weight (Fig. 5C and D), while CCR4 mRNA levels was negatively related to FBG and positively related to weight (Fig. 5E and F). NTNG1 and CCR4 mRNA levels were not significantly correlated to UACR (data not shown). The data of HGF and ISL1 in mice models were not available.

3.5. Diagnostic analysis

We finally tested whether these 4 hub genes associated with GFR can serve as diagnostic biomarkers using ROC curves. ROC curve analysis revealed that the AUC was 0.9658 (95% CI, 0.8995–1.032) for NTNG1, 0.5897 (95% CI, 0.315–0.8645) for CCR4, 0.65 (95% CI, 0.4166–0.8834) for ISL1 (Fig. 6), suggesting NTNG1 and HGF can serve as diagnostic biomarkers for distinguishing patients with DKD from healthy controls with the AUC exceeded 0.80.

4. Discussion

DKD is a heterogeneous disease with various clinicopathological stages and therapeutic responses. Approximately half of patients with type 2 diabetes and one-third of patients with type 1 diabetes will develop DKD.[18,19] Moreover, it is difficult to precisely identify DKD in epidemiology or clinical practice, particularly in patients with type 2 diabetes. Indeed, high mortality rates associated with type 1 and type 2 diabetes are largely confined to those with DKD.[20–22] Consequently, there is a need for innovative treatment strategies for preventing, treating, and even reversing DKD. Current evidence has revealed that genetic factors may explain why some individuals develop and some do not.[23] In addition, transcriptional and bioinformatics analyses have expanded our understanding of the molecular mechanisms of disease pathogenesis and progression, which is essential for identifying genetic alternations and establishing potential diagnostic biomarkers. However, the exact molecular mechanisms of DKD have not been fully elucidated. Accordingly, in the
current study, 2 mRNA microarray datasets (GSE30528 and GSE30529) were analyzed to obtain DEGs from glomerular and tubule tissues of patients with DKD and healthy controls. We identified many nonoverlapping DEGs in the 2 datasets and showed that DEGs in GSE30528 were significantly enriched in extracellular space, organic cation transport, Wnt signaling pathway, metabolic pathways, mTOR signaling pathway, Hippo signaling pathway, and chemokine signaling pathway.

Some evidences have demonstrated that Wnt/β-catenin signaling plays an essential role in the development of DKD,
including induction of podocyte dysfunction. Activation of Wnt signaling in podocytes contributes to glomerular basement membrane abnormalities, decreases nephrin expression, induces detachment of podocytes, and disrupts the epithelial-mesenchymal transition, ultimately causing albuminuria and glomerulosclerosis. The mTOR signaling cascade has effects on cellular growth, survival, and metabolism. Podocyte-specific loss of mTOR causes proteinuria and progressive glomerulosclerosis, and podocyte-specific genetic inhibition of mTOR activation prevents progressive glomerular diseases. The Hippo pathway regulates cell proliferation, apoptosis, and stemness in response to a wide range of extracellular and intracellular signals, including cell-cell contact, mechanical cues, ligands of G-protein-coupled receptors, and cellular energy status. A previous study indicated that crosstalk between the epidermal growth factor (EGF) receptor signaling pathway and the Hippo pathway may be an important underlying mechanism mediating the development and progression of DKD.

Although most studies have focused on glomerular damage, tubules are also known play a pivotal role in the pathogenesis of DKD. Moreover, there is a good correlation between tubulo-interstitial changes and renal function. Functional analysis using the GSE30529 database demonstrated that DEGs were mainly enriched in growth factor activity, the longevity regulating pathway, metabolism of xenobiots by cytochrome P450, the Rap1 signaling pathway, the RAS signaling pathway, and mineral absorption. Rap1 is a small GTPase that regulates cell adhesion, migration, proliferation, and cell survival. The expression and activity of Rap1 were decreased in patients with DKD and in animal models, and activation of Rap1 ameliorates renal tubular injury by regulation of mitochondrial dysfunction. Interestingly, hyperactivity of intrarenal RAS has been strongly implicated in both the onset and progression of DKD, and pharmacological RAS inhibition has been reported to prevent microtubular changes.

Next, the top 30 hub genes were identified from PPI networks, and correlation analysis was performed between transcriptional levels of hub genes and clinicopathological parameters. Among these genes, only NTNG1, CCR4, HGF, and ISL1 mRNA levels were significantly correlated to clinical parameters. NTNG1, an axon guidance molecule, contains laminin-type EGF-like domains and is bound to the plasma membrane through a GPI anchor. Chemo-kines and their receptors play a crucial role in the immune homeostasis and inflammatory response of renal diseases. CCR4, highly expressed T-helper type 2 (Th2) cells, and regulatory T cells, involves in Th1/Th2 regulation. A recent study suggests that CCR4 and its ligand CCL22 axis facilitated Th22 cells recruited into mesangial cells and tubular epithelial cells, and thus contributes to proteinuria in IgA nephropathy. The functional role of CCR4 in DKD still await to be elucidated. In the present study, NTNG1 and CCR4 mRNA level was decreased in DKD patients and mouse models, and positively related to GFR in DKD patients. In DKD mice model, NTNG1 mRNA level was positively related to FBG and negatively related to weight; CCR4 mRNA level was negatively related to FBG and positively related to weight. These results suggest that NTNG1 and CCR4 may serve as a predictive biomarker in glomerular injury.

HGF, a protein that binds to the hepatocyte growth factor receptor to regulate cell growth, cell motility and tissue regeneration, is extensively investigated in diabetes and diabetic
complications, such as DKD. Evidences revealed that exogenous HGF can attenuate proteinuria and tubulointerstitial fibrogenesis via inhibiting TGF-β expression and accelerating kidney repair in murine models.[47–49] In concordance with previous studies, our results shown that HGF mRNA level was downregulated in patients with DKD, seemingly suggesting HGF plays a renoprotective role in DKD. ISL-1, a member of the LIM/homeodomain family of transcription factors, regulates insulin gene expression.[100] Our results shown that ISL-1 mRNA level was overexpression and negatively correlated to GFR in DKD patients compared to healthy samples, and further researches are needed to unravel its mole mechanisms in DKD. Furthermore, we examined whether NTNG1, CCR4, HGF, and ISL-1 may server as reliable biomarkers using ROC curve. Finally, NTNG1 and HGF were defined as diagnostic biomarkers with high sensitivity and specificity. Significant progress has been made to broaden our understanding about the mechanisms of DKD based on transcriptomic analysis.

Several limitations should also be considered in interpreting study results. First, transcriptional alterations and downstream changes in protein function do not always correspond, and thus predicted hub genes at protein level should be detected using western blot or immunohistochemistry. Second, hub genes and pathways were identified based on small sample sizes by bioinformatic analysis. Therefore, further research with large sample sizes is needed to verify the molecular mechanisms of the identified genes in DKD using loss-of-function and gain-of-function study.

5. Conclusion

Taken together, our findings provided an integrative analysis of candidate genes and pathways in human DKD and identified 2 hub genes (NTNG1 and HGF) may server as reliable biomarkers in DKD.

Author contributions

Data curation: Yun-Liang Tang.
Methodology: Xiao-Yang Dong.
Supervision: Yun-Liang Tang.
Writing – original draft: Zhen-Guo Zeng.
Writing – review and editing: Zhen Feng.

Correction

The affiliation for Drs. Xiao-Yang Dong and Zhen Feng is now appearing as affiliation a.

References

[1] Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus–present and future perspectives. Nat Rev Endocrinol 2011;8:228–36.
[2] Wild S, Roglic G, Green A, et al. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes care 2004;27:1047–53.
[3] Lind M, Wedel H, Rosengren A. Excess mortality among persons with type 2 diabetes. N Engl J Med 2016;374:788–9.
[4] Zhang L, Wang F, Wang L, et al. Prevalence of chronic kidney disease in China: a cross-sectional survey. Lancet (London, England) 2012;379: 815–22.
[5] Matsushita K, van der Velde M, Astor BC, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet (London, England) 2010;375:2073–81.
[6] Wen CP, Cheng YL, Tsai MK, et al. Cause mortality attributable to chronic kidney disease: a prospective cohort study based on 462 293 adults in Taiwan. Lancet (London, England) 2008;371:2173–82.
[7] Marshall SM. Recent advances in diabetic nephropathy. Postgrad Med J 2004;80:624–33.
[8] Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 2009;10:57–63.
[9] Hoheisel JD. Microarray technology: beyond transcript profiling and genotype analysis. Nat Rev Genet 2006;7:200–10.
[10] Luca F, Kupfer SS, Knights D, et al. Functional genomics of host-microbiome interactions in humans. Trends Genet 2018;34:30–40.
[11] Barrett T, Wilhite SE, Dodou D, et al. NCBI GEO: archive for functional genomics data sets–update. Nucleic Acids Res 2013;41:D991–995.
[12] Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43:e47.
[13] Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37:1–3.
[14] Yu Y, Ouyang Y, Yao W. shinyCircos: an RShiny application for interactive creation of Circos plot. Bioinformatics (Oxford, England) 2018;34:1229–31.
[15] Sklarczak D, Gable AL, Lyon D, et al. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019;47:D607–d613.
[16] Smoot ME, Ono K, Ruscheinski J, et al. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics (Oxford, England) 2011;27:431–2.
[17] Chiu CH, Chen SH, Wu HH, et al. cyt3dHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 2014;8 (Suppl 4):S11.
[18] Thomas MC, Weekes AJ, Broadley OJ, et al. The burden of chronic kidney disease in Australian patients with type 2 diabetes (the NEFRON study). Med J Aust 2006;185:140–4.
[19] Dwyer JP, Parving HH, Hunsicker LG, et al. Renal dysfunction in the presence of normoalbuminuria in type 2 diabetes: results from the demand study. Cardiorenal Med 2012;2:1–0.
[20] Bruno G, Merletti F, Bargero G, et al. Estimated glomerular filtration rate, albuminuria and mortality in type 2 diabetes: the Casale Monferrato study. Diabetologia 2005;48:1947–50.
[21] Groop PH, Thomas MC, Moran JL, et al. The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. Diabetes 2009;58:1651–8.
[22] Afkarian M, Sachs MC, Kestenbaum B, et al. Kidney disease and increased mortality risk in type 2 diabetes. J Am Soc Nephrol 2013;24:302–8.
[23] Thomas MC, Groop PH, Tryggvason K. Towards understanding the inherited susceptibility for nephropathy in diabetes. Curr Opin Nephrol Hypert 2012;21:193–202.
[24] Li SY, Huang PH, Tarng DC, et al. Four-and-a-Half LIM domains protein 2 is a coactivator of wnt signaling in diabetic kidney disease. J Am Soc Nephrol 2015;26:3072–84.
[25] Bose M, Almas S, Prabhakar S. Wnt signaling and podocyte dysfunction in diabetic nephropathy. J Invest Med 65:1093–101.
[26] Dai C, Stodz DB, Kiss LP, et al. Wnt/beta-catenin signaling promotes podocyte dysfunction and albuminuria. J Am Soc Nephrol 2009;20: 1997–2008.
[27] Kato H, Gruenwald A, Suh JH, et al. Wnt/beta-catenin pathway in podocytes integrates cell adhesion, differentiation, and survival. J Biol Chem 2011;286:26003–15.
[28] Kato H, Susztak K. Repair problems in podocytes: Wnt, Notch, and glomerulosclerosis. Semin Nephrol 2012;32:350–6.
[29] Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. Cell 2017;168:960–76.
[30] Inoki K, Mori H, Wang J, et al. mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. J Clin Invest 2011;121:2181–96.
[31] Yu FX, Zhao B, Guan KL. Hippo pathway in organ size control, tissue homeostasis, and cancer. Cell 2015;163:811–28.
[32] Chen J, Harris RC. Interaction of the EGF receptor and the hippo pathway in the diabetic kidney. J Am Soc Nephrol 2016;27:1689–700.
[33] Magri CJ, Fava S. The role of tubular injury in diabetic nephropathy. Eur J Intern Med 2009;20:551–5.
[34] Tang SC, Yiu WH, Lin M, et al. Diabetic nephropathy and proximal tubular damage. J Renal NutrV 25 2015;230–3.

[35] Tramonti G, Kanwar YS. Review and discussion of tubular biomarkers in the diagnosis and management of diabetic nephropathy. Endocrine 2013;43:494–503.

[36] Satrapo B. Tubulointerstitial biomarkers for diabetic nephropathy. J Diabetes Res 2018;2852398.

[37] Bos JL, de Rooij J, Reedquist KA. Rap1 signalling: adhering to new models. Nat Rev Mol Cell Biol 2001;2:569–77.

[38] Xiao L, Zhu X, Yang S, et al. Rap1 ameliorates renal tubular injury in diabetic nephropathy. Diabetes 2014;63:1366–80.

[39] Fujisawa T, Iekami H, Kawaguchi Y, et al. Meta-analysis of association of insertion/deletion polymorphism of angiotensin I-converting enzyme gene with diabetic nephropathy and retinopathy. Diabetologia 1998;41:47–53.

[40] Ng DP, Tai BC, Koh D, et al. Angiotensin-I converting enzyme insertion/deletion polymorphism and its association with diabetic nephropathy: a meta-analysis of studies reported between 1994 and 2004 and comprising 14,727 subjects. Diabetologia 2005;48:1008–16.

[41] Nakashita T, Ikeda T, Nishimura S, et al. Netrin-G1: a novel glycosyl phosphatidylinositol-linked mammalian netrin that is functionally divergent from classical netrins. J Neurosci 2000;20:6540–50.

[42] Zhu Y, Yang H, Bi Y, et al. Positive association between NTNG1 and schizophrenia in Chinese Han population. J Genet 2011;90:499–502.

[43] Lin L, Lesnick TG, Maraganeo DM, et al. Axon guidance and synaptic maintenance: preclinical markers for neurodegenerative disease and therapeutics. Trends Neurosci 2009;32:142–9.

[44] Segerer S, Nelson PJ. Chemokines in renal diseases. Sci World J 2005;5:835–44.

[45] Berlato C, Khan MN, Schioppa T, et al. A CCR4 antagonist reverses the tumor-promoting microenvironment of renal cancer. J Clin Invest 2017;127:801–13.

[46] Gan L, Zhou Q, Li X, et al. Intrinsic renal cells induce lymphocytosis of Th22 cells from IgA nephropathy patients through B7-CTLA-4 and CCL-CCR pathways. Mol Cell Bio Chem 2018;441:191–9.

[47] Dai C, Yang J, Bastacky S, et al. Intravenous administration of hepatocyte growth factor gene ameliorates diabetic nephropathy in mice. J Am Soc Nephrol V 15 2004;2637–47.

[48] Kagawa T, Takemura G, Kosai K, et al. Hepatocyte growth factor gene therapy slows down the progression of diabetic nephropathy in db/db mice. Nephron Physiol 2006;102:92–102.

[49] Flaquer M, Franquesa M, Vidal A, et al. Hepatocyte growth factor gene therapy enhances infiltration of macrophages and may induce kidney repair in db/db mice as a model of diabetes. Diabetologia 2012;55:2059–68.

[50] Karlsson O, Thor S, Norberg T, et al. Insulin gene enhancer binding protein Isl-1 is a member of a novel class of proteins containing both a homeo- and a Cys-His domain. Nature 1990;344:879–82.