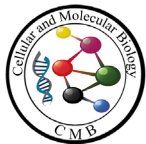

Cellular and Molecular Biology

E-ISSN : 1165-158X / P-ISSN : 0145-5680

www.cellmolbiol.org

C M B Association Publisher

Bioinformatic analysis of CHEK1 as a marker of glomerular epithelial cell injury in diabetic nephropathy

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ARTICLE INFO

Original paper

Article history:
Received: April 10, 2023
Accepted: August 12, 2023
Published: August 31, 2023

Keywords:

CHEK1, diabetic nephropathy, bioinformatics analysis

ABSTRACT

Diabetic nephropathy (DN) is considered to be a kidney disease caused by diabetes. In recent years, the incidence of DN has been on the rise, which is also a major challenge in the treatment and prognosis of the disease. Therefore, the search for new biomarkers of DN is urgent and has important clinical significance for reducing the morbidity and mortality of DN. In this study, two datasets GSE1009 and GSE142153 were selected to extract expression profile-based data from DN glomerular samples, and 238 differentially expressed genes (DEGs) were screened. Then, through enrichment analysis, the biological function of DEGs involved in DN disease was preliminarily explored. Subsequently, the STRING website was used to construct a protein-protein interaction map (PPI) to find 10 key genes (CHEK1, ITGB3, COL4A2, COL4A5, COL4A3, COL4A4, CCNB2, CCNB1, TPX2, KIF11), Which play an important role in the progression of DN disease and are closely related to other genes. CHEK1 was the focus of this study, and the expression level of CHEK1 in glomerular epithelial cell models was verified by qRT-PCR. Our results suggest that CHEK1 is a potential biomarker of the degree of damage to DN glomerular epithelial cells.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.8.32 Copyright: © 2023 by the C.M.B. Association. All rights reserved.

Introduction

It is widely believed that the kidney damage caused by diabetic nephropathy is closely related to diabetes. Although the main lesion site of DN is confined to the glomerular tissue, its consequences are very serious. Clinically, hypertensive nephropathy caused by DN is common and even progresses to acute renal failure, which brings a great burden to DN patients and their families. In addition, studies suggest that end-stage renal disease caused by DN accounts for 30%-50% of the total number of cases (1). In fact, the basic units of the kidney include the glomerulus, glomerular capsule, and urinary tubules. Among them, the glomerulus plays the main role of filtration, and the glomerular epithelial cells are the important defense line of the filtration barrier. At present, more and more scholars have found that glomerulus also plays a key role in the progression of DN (2,3). Therefore, relevant studies on the role of glomerular epithelial cells in the progression of DN need to be improved.

With the deepening of research, more and more biomarkers were found, including vascular endothelial growth factor (VEGF), growth differentiation factor-15 (GF-15), and bilirubin, which were mainly related to structural kidney lesions in urine, serum, or plasma (4-8). However, for clinical scientific research, a single biomarker is not enough to provide adequate diagnostic conditions. Therefore, in order to improve the diagnostic accuracy of DN disease, more biomarkers need to be continuously mined and more biomarkers should be combined as far as possible to make joint diagnosis. The search for and validation of new biomarkers can help in the treatment and prevention of DN, as well as play an important role in exploring the occurrence of the disease.

In this study, Two microarray data of GSE1009 and GSE142153 from the GED database were used. The key genes were screened by bioinformatics analysis. Kidney tissue-related data and control data were selected to screen out differentially expressed genes (DGEs). GO, KEGG pathway enrichment analyses were conducted to explore the biological effects of DEGs, which participate in the course of DN. PPI was then constructed using the STRING online website and Cytoscape software to search for important genes. The clinical characteristics of these genes were reexamined to screen the most important key genes in the course and development of DN. In addition, a glomerular epithelial cell model was constructed to extract RNA and verify the expression level of the key gene CHEK1 by qRT-PCR, to confirm the reliability of CHEK1 again. Finally, we conclude that CHEK1 has the potential to be a new biomarker for evaluating the degree of glomerular epithelial cell injury during DN.

Materials and Methods

Data sources

Microarray data were downloaded from GEO (http://www.ncbi.nlm.nih.gov/geo). Therefore, this study does not require medical ethical review. We obtained gene chip expression profiles of kidney tissue and peripheral samples of DN mice from two datasets, GSE1009 and GSE142153. And then R software (version 4.0.3) was used for probe ID

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conversion and data standardization.

Analysis of differential expression

The "limma" packets were used for differential expression analysis of all gene data. Screening DEGs condition is: $|\ Log_2FC| > 1$ and p values < 0.05. The differential genes of diabetic nephropathy mice and the control group were obtained.

GO, KEGG pathway enrichment analyses

In order to preliminarily explore the main biological effects of DEGs and the biological processes involved in the course of diabetic nephropathy, the "clusterProfiler" package of R software was used for gene ontology (GO) enrichment analysis, and then for Genome Encyclopedia (KEGG) enrichment analysis through DAVID online database. Finally, two R software packages, "ggplot2" and "enrichplot", were used to enrich the bubbles to visualize the data.

PPI network analyses and hub gene identification

All DEGs involved in the process of diabetic nephropathy were placed on the STRING online website, to construct the protein-protein interaction (PPI) diagram, the minimum interaction score of 0.7 was set. PPI Related data were uploaded to Cytoscape software (version 3.7.2), then data were visualized and saved. The MCODE plug-in Cytoscape software was used to perform basic module analysis on the data, under the following conditions: MCODE score ≥ 6, node score cutoff $= 0.2$, degree cutoff $= 2$, k-score $= 2$ and max depth $= 100$.

In vitro glomerular epithelial cell culture and high glucose stimulation

Immortalized mouse glomerular epithelial cells were purchased from Wuhan Pronosai Life Science and Technology Co. (Wuhan, China) and cultured according to a previous study (9).

Two groups were designed for the experiment, the high-glycemic group (HG) and the normal-glycemic group (NG). Among them, the glomerular epithelial cells in the HG group were cultured with high glucose DMEM (glucose concentration was 30.0 mmol/L), while the glomerular epithelial cells in the normal glucose group were cultured with DMEM (glucose concentration was 5.3 mmol/L). After 72 hours, the glomerular epithelial cells in the HG and NG groups were collected, and mRNA was extracted respectively for subsequent qRT-PCR experiments.

Validation of qRT-PCR on treated glomerular epithelial cells

In order to reverse transcribe mRNA into cDNA, we applied Primer Script Real-Time Kit (TaKaRa Biotechnology, Dalian, China). According to the kit instructions, qPCR experiments were conducted using Power SYBR Green PCR Master Mix (TaKaRa Biotechnology, Dalian, China). Specific primers of each index were added into the system, to detect the relative expression levels of mRNA of different indexes, $2^{-\Delta\Delta Ct}$ was used for analysis. β-actin forward 5'-TGGCACCCAGCACAATGAA-3', reverse 5'-CTAAGTCATAGTCCGCCTAGAAGCA-3'; CHEK1 forward 5'-CCGTTATTAGCGTAGATG-3', reverse 5'-TATGTGATGTGGCTTAGAAT-3'.

Statistical analysis

Student's t-test was used to compare the relative expression level of CHEK1 between the high glucose group and the normal glucose group.

Results

Identification of DEGs in the GSE1009 and GSE1421153 dataset

Through bioinformatics analysis, 238 differential genes were discovered to be involved in the development of diabetic nephropathy (Figures 1 and 2). It is worth noting that 153 genes showed an up-regulated trend and 85 genes showed a down-regulated trend.

Enrichment analysis result of DEGs in DN

The results of the KEGG enrichment analysis suggested that the interaction between cytokines and their receptors was more significant (Figure 3a). it is suggested that DEGs mainly participate in many signaling pathways, such as

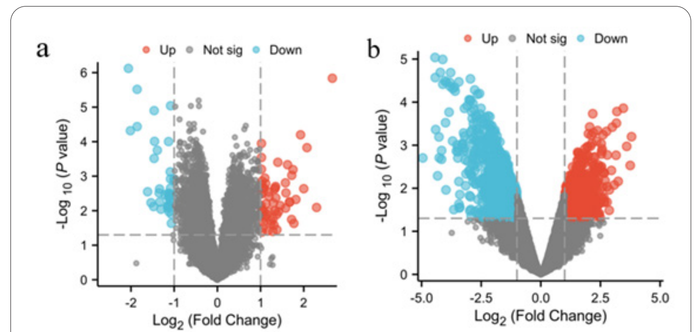

Figure 1. Transcriptome volcano map analysis of normal subjects and diabetic nephropathy patients. (a) Comparison of peripheral blood specimens. (b) Tissue from a portion of the kidney.

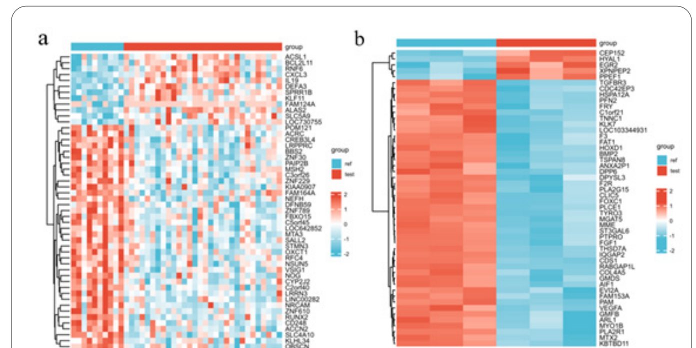

Figure 2. Cluster analysis of normal group and diabetic nephropathy patients. (a) Comparison of peripheral blood specimens. (b) Tissue from a portion of the kidney.

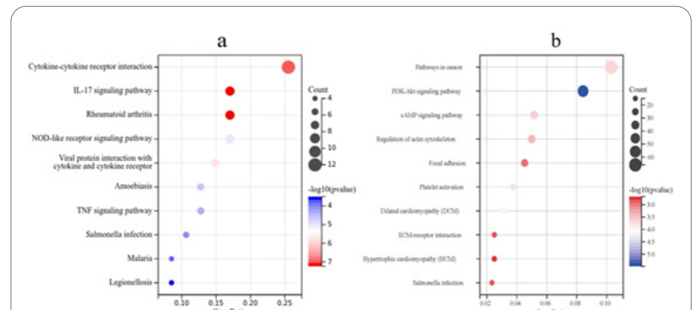

Figure 3. KEGG analysis of DEGs between normal group and diabetic nephropathy patients. (a) DEGs for peripheral blood specimens. (b) DEGs for tissue from a portion of the kidney.

IL-17, PI3K-Akt and TNF (Figure 3b). In terms of cellular components, GO enrichment analysis results showed that cellular response to a chemical stimulus is the most important function (Figure 4a). DEGs is also involved in system development, cellular response to an organic substance, and other biological functions (Figure 4b).

PPI network construction and hub genes selection

With the standardized processing and statistical analysis of R software, important DEGs involved in DN disease were obtained, and we constructed a PPI network using STRING. The confidence level of the interactions in this was 0.7.

A module (MCODE score = 7.0) was obtained through the MCODE package and further analysed for its biological function. The module consisted of 10 genes that interacted strongly with each other (Figure 5).

In order to further discover the most important target genes in the process of diabetic nephropathy, the CytoHubba plug-in was utilized to conduct network analysis on the important nodes in the PPI map, and the most important target gene CHEK1 was identified through the construction of cross-analysis model (Figure 5a). Sequencing all the genes according to the algorithm score, and then assigned a score of 1-30 based on the ranking. Relying on the scores, analysis of transcriptomic data from kidney tissue, the first 10 genes (CHEK1, ITGB3, COL4A2, COL4A5, COL4A3, COL4A4, CCNB2, CCNB1, TPX2, KIF11) were identified. CHEK1 and ITGB3 were located in the important module and scored high (Figure 5a). Relying on the scores, Analysis of transcriptome data from peripheral blood, the first 10 genes (IL10, IL1β, CXCL8, CXCL1, CXCL2, CXCL3, CXCL20, CCL7, CCL2, MMP) were identified (Figure 5b). According to the present results, inflammatory factors and chemokine disorders in peripheral blood may be exacerbated by exacerbating the inflammatory response in diseased tissues. In summary, these genes are central to the network and are thought to play a potential role in DN.

Validation of CHEK1 as a biomarker of DN glomerular epithelial cell injury

Based on the results of bioinformatics analysis, we selected CHEK1 as a candidate biomarker. To validate the role of CHEK1 in DN, treated glomerular epithelial cells were tested by qRT-PCR. CHEK1 mRNA expression was increased in high glucose-stimulated cells (Figure 6). These results suggest that CHEK1 can be considered as a biomarker for the early detection of DN.

Discussion

Diabetic nephropathy is considered to be one of the important factors leading to end-stage renal disease. In particular, the incidence of diabetic nephropathy shows an increasing trend year by year, which will increase the potential risk of end-stage renal disease (10). Recently, for the development of DN, significant progress has been made in the study of metabolic pathways, and the emerging therapies are exciting. However, the pathogenesis of diabetic nephropathy needs to be supplemented. The specific molecular mechanism of glomerular cell injury and its role in the process of DN, even the pathogenic mechanism involved in renal function injury, and induction of

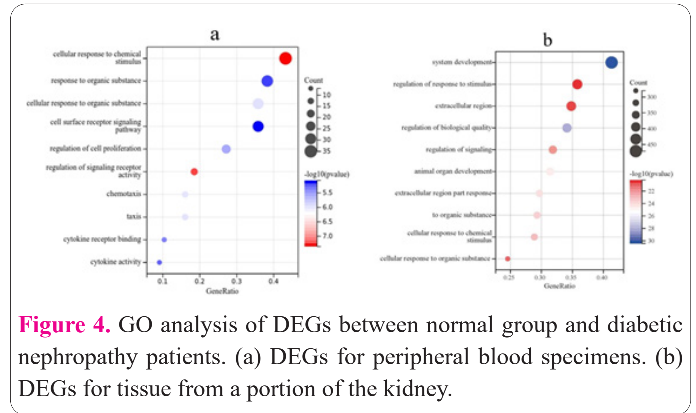

Figure 4. GO analysis of DEGs between normal group and diabetic nephropathy patients. (a) DEGs for peripheral blood specimens. (b) DEGs for tissue from a portion of the kidney.

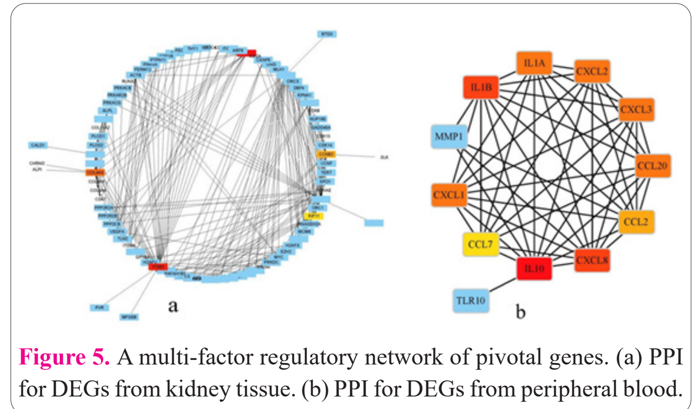

Figure 5. A multi-factor regulatory network of pivotal genes. (a) PPI for DEGs from kidney tissue. (b) PPI for DEGs from peripheral blood.

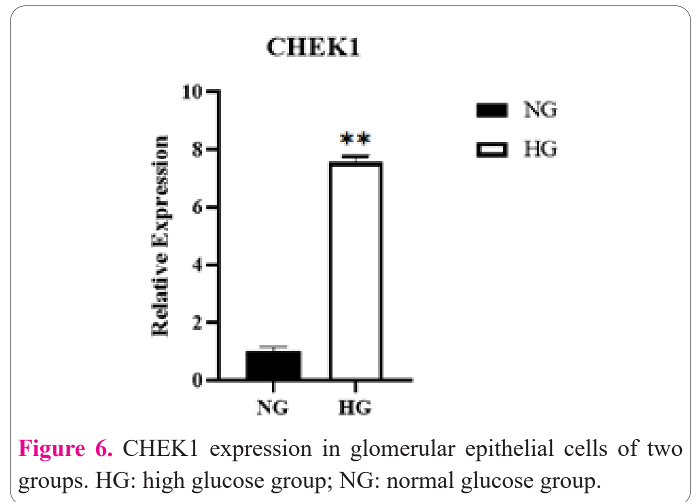

Figure 6. CHEK1 expression in glomerular epithelial cells of two groups. HG: high glucose group; NG: normal glucose group.

renal failure, are still unknown. As a result, there are still uncertain risk factors in the clinical treatment of DN (11). Therefore, further exploration of the pathogenesis of DN, and looking for novel, effective, and specific biomarkers for diagnosis and treatment of DN disease have important clinical significance.

As public database analysis technologies have been put into the medical scientific research system continuously, such as high-throughput sequencing and bioinformatics, new understandings have been gained on the occurrence and development of DN. More and more biomarkers have become research hotspots. These studies constantly reveal the biological pathways and biological effects of various biomarkers in the course of DN. There is great significance for us to re-understand diabetic nephropathy. Therefore, this study also attempts to screen DEGs and identify important key genes through biological information methods.

We conducted an enrichment analysis to preliminarily explore the main biological effects of DEGs: GO enrichment analysis and KEGG enrichment analysis. Our ana-

lysis results are consistent with many scholars, which provides a reliable basis for further analysis and research. Firstly, many studies have proposed that inflammation is an important process involved in the development of DN, and our DEGs are also significantly enriched in inflammation. A growing number of studies and pathological examinations suggest that inflammation is the main pathogenesis of DN, involving a complex network of molecular regulation (12). Furthermore, there is a recurring theme regarding chemokines, such as cytokine-mediated signalling pathways and chemokine activity, suggesting that chemokines play an important role in DN. role in DN. Clinical studies and some histopathological studies have shown that chemokines are associated with the development of DN (13,14), there is reason to think that chemokines in DEGs should be paid more attention. Some terms, such as atherosclerosis and ischaemia, have also emerged suggesting that these diseases are in some ways similar to DN and may be a complication of diabetes mellitus (15,16).

Based on the MCODE algorithm, we got a high-score module, in which CHEK1 (17), as the core gene, caught our attention. Reviewing the enrichment analysis results indicated that the module was highly correlated with cellular chemotaxis and cellular responses to chemokines, and other proteins in the module had strong interactions with CHEK1. indicating that CHEK1 are pivotal genes. Some genes associated with chemosynthetic genes, such as COL4A2, COL4A5, COL4A3, and COL4A4, as well as other factors, e.g., CCNB1 and CCNB2. Through topological algorithms in bioinformatics analysis, they are all contained in top-level hub genes. The results of this part of our research are consistent with the results of our enrichment analysis, it is also consistent with the understanding of many scholars on the DN pathogenesis. This also provides some basic theories for subsequent studies on the pathogenesis and molecular mechanism of DN. In particular, the research on the 10 central genes is worth further discussion (CHEK1, ITGB3, COL4A2, COL4A5, COL4A3, COL4A4, CCNB2, CCNB1, TPX2, KIF11), and will become our focus in our subsequent research

Based on previous research results, we reasonably believe that CHEK1 is a key gene involved in the DN, and it is important for biological processes, such as cell chemotaxis, and has the potential to develop into a biomarker of the disease. Therefore, in order to further verify the expression level of CHEK1 in glomerular cells, we constructed a cell model, constructed mouse glomerular epithelial cells in vitro, and stimulated them with high-concentration glucose to simulate the biological environment of diabetic nephropathy. The results suggested that CHEK1 was more expressed in cells with high glucose concentration, and the difference in CHEK1 expression level was statistically significant. At present, Kevin Schulte et al. proposed that glomerular epithelial cells can migrate to the capsule of the glomerulus, under inflammatory or pathological conditions (18), and Let Bariety et al. labeled glomerular epithelial cells specifically, and the positive rate reached 76.6% in pathological sections of the Bowman's capsule (19). These results suggest that glomerular epithelial cells have chemotaxis, and CHEK1 may be involved in the way.

At present, some researchers have proposed that CHEK1 expression significantly increased can effectively activate the mTORC1 signaling pathway, which will lead to dysfunction of glomerular endothelial cells, and further participate in the pathogenic process (20). CHEK1 is secreted primarily by macrophages and has been considered an important activator of neutrophils. Many researchers have found CHEK1 expression in a variety of cell species (21). The literature on CHEK1 expression in renal tubular epithelial cells is insufficient, and our study is to provide more evidence to support this conclusion. In addition, Bastl et al. proposed that the increased expression of CHEK1 is due to reduced catabolism in chronic renal failure (CRF) (22). However, the increased expression level of CHEK1 will lead to the corresponding VEGF increase, which is of great significance for glomerular hypertrophy (21,23). CHEK1 deficiency is mainly manifested as reduced vascular permeability, which will cause neutrophils to participate in the inflammatory response and angiogenesis (24,25). In fact, CHEK1 is closely related to membranous nephropathy, IgA nephropathy and hypertensive nephropathy (26-32), all of which suggest that CHEK1 is highly likely to be involved in the pathogenesis of diabetic nephropathy.

In conclusion, this study extracted gene expression profile data related to diabetic nephropathy from the GEO database, and used bioinformatics analysis to explore key genes involved in the development of DN, and revealed the biological processes and effect pathways of these genes. Subsequently, a renal epithelial cell model was constructed in vitro, to simulate the biological microenvironment of diabetic nephropathy. qRT-PCR was used to verify the relative expression level of CHEK1 in the cells. Based on the results of bioinformatics analysis and experiments, we believe that CHEK1 has the potential to be a new biomarker for diabetic nephropathy.

Conflict of Interests

The authors declared no conflict of interest.

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