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Research Article

Research on Potential Pathogenesis of Advanced Diabetic Nephropathy based on Bioinformatic Analysis

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Abstract: ***Objective:*** Through the bioinformatic analysis of gene chips related to advanced diabetic nephropathy in the GEO database, the key genes of advanced diabetic nephropathy are screened, whose biological functions and signal pathways are predicted as well. ***Methods:*** The gene chips related to advanced diabetic nephropathy from the GEO expression profile database was downloaded, and the differentially expressed genes in patients with advanced diabetic nephropathy and normal people were analyzed through R. For the screened differentially expressed genes, the biological function of GO and the enrichment analysis of KEGG signal pathway were used to predict their biological functions and related signal pathways. In addition, a protein-protein interaction network was constructed, so as to screen core pathogenic genes utilizing STRING database and Cytoscape. ***Results:*** By analyzing the chip GSE142025, 301 differential genes were obtained, including 197 up-regulated genes and 104 down-regulated genes. Both GO annotation and enrichment analysis suggested that differential genes were mainly involved in immune-inflammatory response and cytokine action. Furthermore, KEGG pathway analysis suggested that the most important pathway related to advanced diabetic nephropathy was MAPK signaling pathway. Through protein-protein interaction network and module analysis, C3, CCR2, CCL19, and SAA1 were selected as the core sites of the interaction. ***Conclusions:*** Differential genes participate in the pathogenesis of advanced diabetic nephropathy through the KEGG pathway, the immune inflammatory response and cytokine action, which provides new ways for the diagnosis and treatment of advanced diabetic nephropathy.

Key words: Diabetic nephropathy; Bioinformatics; C3; CCR2; CCL19; SAA1

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Diabetic nephropathy (DN) is a crucial global health concern, whose prevalence gradually increases with the occurrence of diabetes[1]. DN is one of the most common complications of diabetes as well as the main cause of end-stage renal disease, accounting for about 50% of its cases in developed countries[2-3]. In China, patients usually progress to advanced stage when diagnosed, with main renal changes including glomerulosclerosis and tubular interstitial fibrosis, whose clinical features are renal failure, massive proteinuria, etc. [4-5]. Therefore, it is of great importance to predict its development into the end-stage, while the pathogenesis of DN is not yet clear. In recent years, with the rapid development of bioinformatics and gene chip technology, as well as the establishment and improvement of many disease databases, a certain theoretical basis for revealing the pathogenesis of human diseases and new

therapeutic targets has been provided. The study uses bioinformatic technology, downloads GSE142025 chip data from the National Center for Biotechnology Information (NCBI) and Gene Expression Omnibus (GEO), and screens the differentially expressed genes in patients with advanced DN (DEGs), so as to explore the possible mechanisms of DEGs' biological functions and signaling pathways in the pathogenesis of advanced DN.

1 Materials and methods

1.1 Data collection

The advanced DN-related gene expression profile data used in this study is the GEO database from NCBI (https://www.ncbi.nlm.nih.gov/geo/). In the GEO database, "advanced diabetic nephropathy" is used as the keyword to obtain the original human kidney tissue gene chip GSE142025[6]. The mRNA data set is based on the gene expression data of the Illumina HiSeq 4000 Human Platform GPL20301. The chip contains 9 normal kidney tissue data (GSM4217808~GSM4217816) and 21 advanced DN kidney tissue data (GSM4217781~GSM4217801).

1.2 Data processing and differential expression analysis

The data set downloaded from the GEO database is read and normalized by R. The standardized chip expression profile was analyzed by limma package, and the Bayesian method was used for multiple test correction. DEGs were screened with | log2FC |> 2 and P<0.05 as the standard. Then, cluster analysis was used on DEGs and heat maps were drawn using gplots package.

1.3 GO enrichment analysis of differential genes and KEGG pathway analysis

The DAVID database (https://david.ncifcrf.gov/) [7] was used to analyze the screened DEGs, and gene annotation enrichment analysis was performed based on gene ontology (GO). Then, molecular function (MF), cellular components (CC) and biological process (BP) were used for data analysis. Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to annotate DEGs, mainly including gene function, biological pathways, cell location, signal pathways, etc. In the study, we analyzed the KEGG signaling pathway through the KOBAS database[8], whose screening condition was P<0.05.

1.4 Analysis of protein-protein interaction network on differential genes

The protein-protein interaction(PPI) network and modular analysis was conducted through STRING database (http://string-db.org/)[9] and Cytoscape software[10]. DEGs were imported into the STRING database to analyze the PPI for differential genes, and then module analysis was performed using the degree plug-in of Cytoscape. The most closely connected modules in the PPI was determined, the interaction among the DEGs encoded proteins was predicted, and the most critical gene was screened out accordingly.

2 Results

2.1 Screening of differentially expressed genes

A total of 301 differential genes were obtained from the data set GSE142025 using | log2FC |> 2 and P<0.05 as the standard, including 197 up-regulated genes and 104 down-regulated genes, as shown in Figure 1. The 10 genes with the largest differences are: C3, PNOC, TIMP1, SAA1, ALB, CCR2, CCR7, CCL21, CCL19, and GPR55, respectively, of which 9 are up-regulated genes and 1 is down-regulated gene, as shown in Table 1. The cluster analysis was conducted using gplots package to visualize the differential genes, as shown in Figure 2.

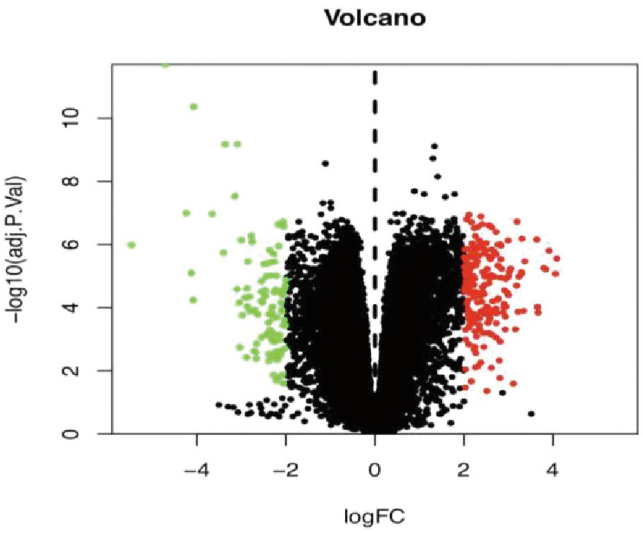

Figure 1. The volcano map of DEGs (Green represents for down-regulated genes, red represents for up-regulated genes, black represents for indifferent genes)

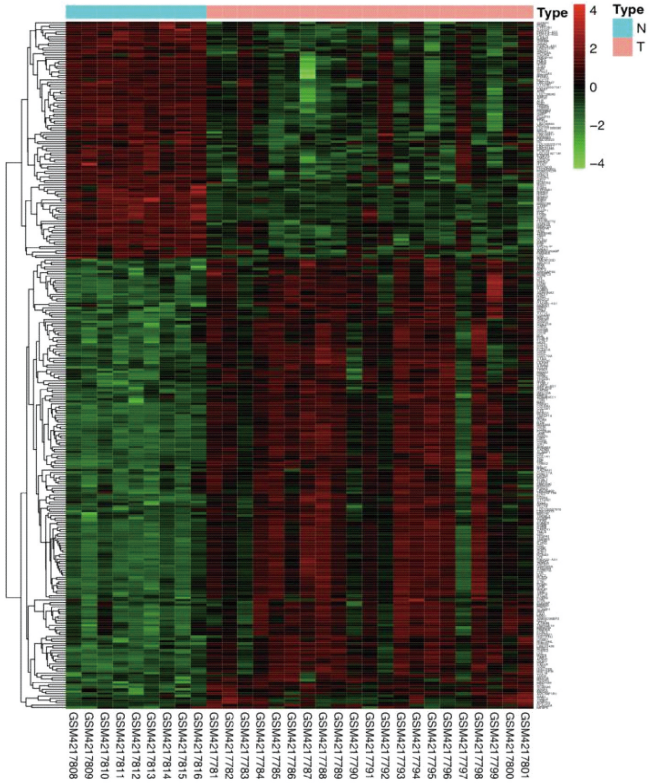

Figure 2. The heat map of DEGs (Green represents for down-regulated genes, red represents for up-regulated genes)

2.3 GO enrichment analysis and KEGG pathway analysis of differential genes

The DAVID database was used to perform GO biological function enrichment analysis on 301 significant differential genes. In terms of molecular function, the differential genes were mainly enriched in cytokine activity, extracellular matrix structural components, CCR chemokine receptor binding, and chemokine receptor binding. As for cellular components, differential genes were mainly attached to the extracellular matrix containing collagen, blood particles, platelet alpha particles, etc. In biological processes, differential genes were mainly involved in the regulation of both T cell activation and lymph cell activation, humoral immune response as well as other processes, as shown in Figure 3. The enrichment analysis of the KEGG signaling pathway indicated that the significant differential genes were mainly enriched in the MAPK signaling pathway, as shown in Figure 4. The results of GO functional biological function enrichment analysis and KEGG signal pathway enrichment analysis showed that the biological functions related to advanced DN were immune inflammatory response and cytokine effects.

Table 1. DEGs between the normal group and the advanced DN group

Gene	logFC	AveExpr	*t*	P.Value	adj.P.Val	B
C3	2.51815	12.67013	6.63329	2.38E-07	6.00E-06	6.97045
PNOC	2.21043	5.17251	6.35275	5.17E-07	9.99E-06	6.21429
TIMP1	2.21960	11.80685	7.69056	1.38E-08	1.10E-06	9.74725
SAA1	2.79222	5.99085	3.45250	1.67E-03	4.78E-03	-1.60462
ALB	-2.71351	11.44703	-3.76738	-3.76738	2.36E-03	-0.80282
CCR2	2.72019	8.78864	8.41395	2.13E-09	4.02E-07	11.5674
CCR7	2.08602	7.65469	5.45996	6.31E-06	5.83E-05	3.77362
CCL21	3.30081	10.20805	8.03036	5.69E-09	6.60E-07	10.6109
CCL19	4.05322	9.12909	6.43895	4.07E-07	8.52E-06	6.44736
GPR55	2.27502	5.05444	4.89509	3.12E-05	1.93E-04	2.22082

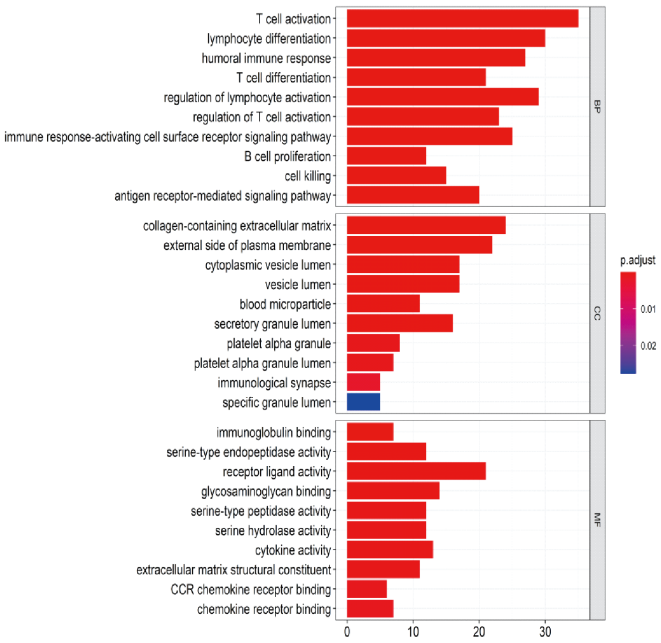

Figure 3. The GO biological function enrichment analysis of DEGs

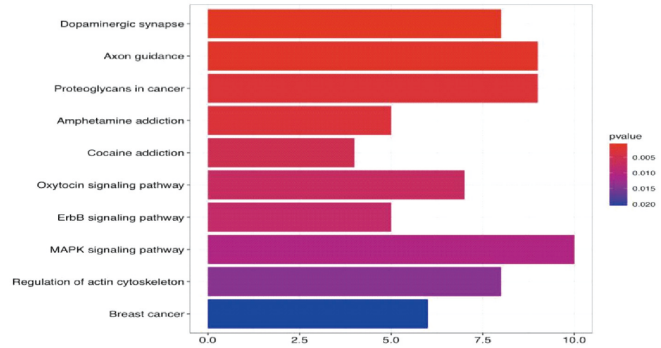

Figure 4. The KEGG signaling pathway analysis of DEGs

2.4 Screening of key genes in the protein-protein interaction network

In order to screen out the differential genes highly related to advanced DN, we used STRING database and Cytoscape software to construct the protein-protein interaction network of 301 differential genes, as shown in Figure 5. Among which, the top 10 core genes were further screened, as shown in Figure 6, which were C3, PNOC, TIMP1, SAA1, ALB, CCR2, CCR7, CCL21, CCL19, and GPR55, respectively. The results indicated that C3, CCR2, CCL19, and SAA1 proteins were closely related to other proteins.

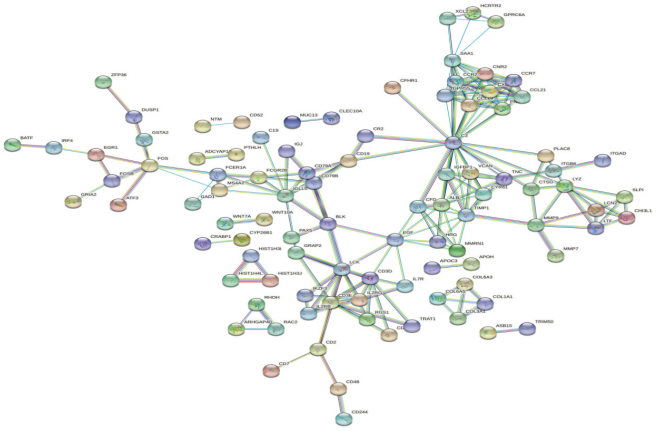

Figure 5. The protein-protein interaction network of KEGs

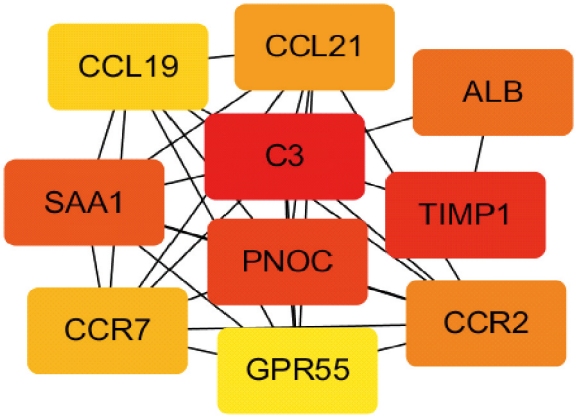

Figure 6. Core genes in the protein-protein interaction network of KEGs (The darker the color, the higher the ranking)

3 Discussion

As one of the most common chronic metabolic diseases, diabetes has affected the health of about 500 million people worldwide. With the continuous development of social economy and quality of life, the incidence of diabetes is increasing year by year[11]. According to statistics from the International Diabetes Association, it is estimated that the number of patients with diabetes will increase to 629 million in 2045 worldwide [12]. DN is a common complication of type 1 and type 2 diabetes, which is a syndrome characterized by urinary albumin excretion, diabetic glomerulopathy and decreased glomerular filtration rate in diabetic patients. In most countries, the prevalence of diabetes has made DN the most

common cause of end-stage renal disease [13]. The occurrence and development of DN is a long-term, multi-factor, multi-step complex pathological process, whose specific molecular mechanism has not yet been elucidated, and there is still a lack of effective treatment methods. Therefore, finding specific target biomarkers to guide clinical diagnosis is the focus and challenge of studying DN nowadays.

With the rapid development of high-throughput sequencing technology and gene chip technology, the use of bioinformatics to conduct in-depth discovery of sequencing data or gene chips has made the analysis of the expression profile of whole genome mRNA more extensive [14]. It can further explore the related target genes and interactions that affect the occurrence and development of advanced DN, provide theoretical basis and new ideas for the potential molecular mechanism, treatment and prognosis of DN. In this study, the mRNA data set GSE142025 was downloaded from the GEO database, 9 cases of normal kidney tissue and 21 cases of advanced DN kidney tissues of the chips were obtained and analyzed. Through the database, 301 DEGs were screened in the kidney tissues of patients with advanced DN and that of the control group, followed by GO biological function enrichment, KEGG signal pathway enrichment and protein-protein interaction network analysis. A total of 10 DEGs were screened to be the most significant, which were C3, PNOC, TIMP1, SAA1, ALB, CCR2, CCR7, CCL21, CCL19, and GPR55, respectively. Except for the down-regulation of ALB, the other 9 genes were up-regulated in the advanced DN group. These DEGs protein products were mainly located in the extracellular matrix, the outer side of the plasma membrane, immune synapses and other cellular components. The main molecular functions included cytokine activity, chemokine receptor binding, and structural components of the extracellular matrix, which were involved in the regulation of T cell activation, regulation of lymphocyte activation, humoral immune response and other processes. The protein-protein interaction network analysis revealed that C3, CCR2, CCL19, SAA1 gene-coded proteins were closely related to other proteins, thus playing an important role in the pathogenesis of advanced DN.

Inflammation is considered as a new mechanism related to the development of DN[15]. During the development of DN, glomerular sclerosis and renal fibrosis are caused by the deposition of extracellular matrix, which are mainly because of the infiltration of immune cells, inflammatory cells, chemokines and cytokines in the kidney[15]. The main systems that can cause inflammation are cytokines and chemokines. In our study, target genes related to glomerular and tubular interstitial inflammation were up-regulated, including cytokine SAA1, chemokine CCL19, chemokine receptor CCR2, and complement C3. As the most sensitive inflammatory cytokine, SAA1 can disrupt the body's coagulation system and cause vascular endothelial dysfunction, which in turn promotes thrombosis and endothelial cell damage. Meanwhile, it can activate the MAPK signaling pathway, resulting in glomerular sclerosis and tubular fibrosis [16]. Central chemokine CCR2 induces lymphocyte recruitment and vascular damage[17], while CCL19 induces T cell recruitment [18-19], which is consistent with related pathology researches that T cells and lymphocytes are the main inflammatory infiltration of DN [20]. It has been reported that new treatments targeting central chemokine CCR2 inhibitors can successfully reduce proteinuria in DN [21]. In addition, in our study, C3 is listed as the most important key gene and highly interacts with pro-inflammatory factors in PPI. In addition to complement activation, it can also aggravate DN inflammation through other pathways, which is consistent with previous studies that increased C3 transcriptome and protein levels in diabetic patients with impaired renal function [22]. The positivity of C3 in renal histopathology is related to severe damage, thus blocking it during signal transduction can improve the prognosis of the kidney [22-23]. The above studies are consistent with our GO enrichment results, cytokine activity, CCR chemokine receptor binding, and chemokine receptor binding. The results showed that the synthesis of cytokines, chemokines, and C3 may play a key role in advanced DN.

GO analysis is used to analyze the biological processes and cellular components that edcoded proteins of DEGs may involve. In our study, the GO annotation of the up-regulation of glomerular and tubular interstitial DEGs indicated that the structural components of the extracellular matrix and the extracellular matrix containing collagen were enriched, revealing the abnormally active fibrosis process in advanced DN. The main pathological feature of DN is the accumulation of diffuse

extracellular matrix. In advanced DN, the glomerular mesangial matrix increases and enlarges, which leads to nodular sclerosis [24], and tubulointerstitial fibrosis. In addition, the extracellular matrix containing collagen also increases the interstitial volume [25]. It has been reported that the non-dissociative fibrosis process of DN is the pathogenic mechanism leading to advanced DN [26]. In addition, GO analysis showed that both glomerular and tubular interstitial DEGs were enriched in genes located in blood particles and platelet alpha particles. Previous studies showed that the level of kidney-derived blood particles in diabetic patients was elevated, which had a pro-fibrotic effect on mesangial and renal tubular cells [27-28]. Platelet particles in patients with DN may cause endothelial damage through external transduction [29]. The results in our study showed that the structural components of extracellular matrix, extracellular matrix containing collagen, blood particles, platelet alpha particles, T cell activation, and increased DEGs in lymphocyte differentiation, mediated the process of inflammation and fibrosis in advanced DN.

A large number of in vivo and in vitro experiments have shown that multiple signaling pathways play a key role in the pathogenesis of DN. In this study, the enrichment analysis of KEGG signaling pathway indicated that DEGs were mainly enriched in MAPK signaling pathway. Mitogen activated protein kinase (MAPK) is a group of serine and threonine proteases that can be activated by different extracellular signals and transduce intracellularly, and then mediate different biological effects [30]. MAPK exerts a variety of cell pathophysiological functions such as coordinated regulation of cell growth and development, inflammation, etc. It is mainly divided into four sub-families, namely ERK1/2, P38MAPK, JNK, and ERK5/BMK1, of which ERK1/2, P38MAPK, and JNK are directly involved in the development of DN [31-32], which mainly causes the activation of macrophages and recruits as well as activates other immune cells, producing a variety of immune inflammatory factors [33], and ultimately leading to nodular sclerosis of the kidney tissue.

In conclusion, conventional treatments that strictly control blood pressure, blood sugar, and protein cannot prevent renal failure in patients with advanced DN. However, our study reveals the pathogenic genes and ways to accelerate the process of DN from the perspective of bioinformatics, and emphasizes the importance of preventing the development of kidney inflammation and fibrosis. The four core proteins screened out in our study are mainly based on the MAPK signaling pathway, which participate in the occurrence and development of advanced DN by regulating the release of cytokines and chemokines. The biological functions show that the deposition of extracellular matrix. and the elevated blood particles are the main pathogenesis of advanced DN. In summary, these genes and pathways may be potential targets for the treatment of DN.

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