Based on Network Pharmacology and Molecular Docking Technology to Explore the Mechanism of Coptis in the Treatment of Diabetic Nephropathy

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Keywords: Huanglian, Diabetic Nephropathy, Network Pharmacology, Molecular Docking.

Abstract: Objective Based on network pharmacology and molecular docking to explore the mechanism of Coptis in the treatment of diabetic nephropathy. Methods Search and screen the main active ingredients in Coptis chinensis through the TCM System Pharmacology Database and Platform (TCMSP) and obtain the corresponding targets. Through the four databases of GeneCards, OMIM, PharmGkb, and TTD, the genes related to diabetic nephropathy are searched and merged, and the target genes of the effective component target and the disease-related gene intersection are obtained through the R language. Cytoscape 3.8.0 software was used to construct a drug component-target-disease regulation network, and a protein interaction network was constructed through the STRING online website. Using R language, GO enrichment analysis and KEGG enrichment analysis were performed on the potential targets of Huanglian in the treatment of diabetic nephropathy. In AutoDockTools-1.5.6, the molecular docking of key target proteins and main active ingredients is realized. As a result, 10 active ingredients of Coptidis for treating diabetic nephropathy were obtained, including: berberine, quercetin, etc.; corresponding to 104 target genes, including: PTSG2, CCL2, MAPK1, etc. Among them, PTSG2 is the core of the PPI network Protein; KEGG pathway enriched to obtain 166 pathways, including: IL-17 signaling pathway, TNF signaling pathway, NF-kappa B signaling pathway, VEGF signaling pathway, etc. The results of molecular docking showed that berberine (berberine) has binding properties to PTSG2. Conclusion Through network pharmacology, the target and mechanism of Coptidis in the treatment of diabetic nephropathy are predicted.1 Introduction.

1 INTRODUCTION

1.1 Diabetes Pathogenesis and Treatment Research

Diabetes (diabetes mellitus, DM) is a metabolic disease caused by insufficient insulin secretion or the inability of insulin to act. Continued maintenance of high blood sugar and long-term metabolic disorders may cause damage to the whole body tissues and organs, especially the kidneys, and their dysfunction and failure.

Diabetic kidney disease (DKD) is the most common microvascular complication of diabetes. It is a kidney disease caused by DM. (Neal, 2017); (Xing, 2021) It has become the leading cause of end-stage kidney disease globally (Perkovic, 2019); (Verma, 2018); (Xing, 2021), and its prevalence is increasing year by year (Liu, 2013). Clinical manifestations are generally proteinuria, hypertension, edema, etc. (Ritz, 2010); (Xing, 2021), and in severe cases, it can even cause renal failure and life-threatening. The pathogenesis of DKD is complicated. Modern research believes that the occurrence of DKD may be related to oxidative stress, inflammation, metabolic status, activation of NF-κB, and activation of the renin-angiotensin-aldosterone system. (Haraguchi, 2020); (Xing, 2021) Among them, the inflammatory

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response plays an important role in the pathogenesis of DKD. (Friedman, 2004); (Li, 2008) Inflammatory factors include IL, NF-κB, TNF-α, TGF-β1, etc. (Lu, 2012); inflammatory factors not only cause kidney Damage can also activate some signal channels to aggravate the inflammatory response (Lu, 2010), which further stimulates the development of DKD. At present, Western medicine lacks effective treatment for DKD, and studies have shown that Chinese and Western medicine treatment will be the development trend of the treatment of DKD.

1.2 Research Purpose and Introduction

From ancient times to the present, there have been many discussions on Xiaoke in the literature and classics, and the earliest relevant discussion appears in the "Huangdi Neijing" (Wang, 2011); (Zhang, 2017). Diabetic nephropathy belongs to the category of "diabetes" or "nephropathy" in traditional Chinese medicine, and Huanglian is used to treat diabetic a lot." Huanglian has a bitter taste and a cold nature. It has the effects of clearing heat and dampness, purging fire and detoxification. It is used for damp heat, fullness of damp heat, vomiting and sourness, red eyes, diminishing thirst, etc. Materia Medica Justice" says: "Coptis rhizome has great bitterness and severe cold, bitterness and dampness, cold overcomes heat, and can vent all excess damp and fire, and the residual heat of heart, spleen, liver, and kidney; the fire of gallbladder, stomach, large intestine, and small intestine, nothing will be incurable." (Peng, 2018)

Modern pharmacological studies (Fu, 2021); (WANG, 2019) have shown that Coptidis has anti-inflammatory, hypoglycemic, antitoxin, and anti-tumor effects. Berberine, the main component of Coptis, can effectively treat diabetic nephropathy. (Li, 2016); (Yang, 2019)

Therefore, this article intends to use network pharmacology methods to collect and analyze relevant data from major databases. Through methods such as drawing, tabulation, screening, and docking, we will explore and verify the target of Coptidis on DKD at the molecular level, and predict its mechanism of action.

2 MATERIAL AND METHODS

2.1 Database and Software Preparation

Commonly used software: PERL (strawberry-perl-5.32.1.1-64bit), R language (R-4.0.4-win), Cytoscape (Cytoscape_v3.8.0), ChemOffice (Chem3D.exe), PyMOL, AutoDockTools-1.5.6, vina and other Databases: TCMSP, GeneCard, OMIM, PharmGKB, TTD

2.2 Acquirement of the Effective Components of Coptis and Its Target

The TCSMP database was used to search for Coptidis Rhizoma Coptidis, and the effective components of Coptidis Rhizoma Coptidis and its corresponding target data were screened based on the criteria of oral bioavailability (OB) ≥ 30% and druglikeness (DL) ≥ 0.18 in the TCMSP database. Obtain the target name of the corresponding person in the UniProt database.

2.3 Acquisition and Screening of Diabetic Nephropathy Related Genes

Search with "Diabetic Kidney Disease" and "diabetic nephropathy" in GeneCards, OMIM, TTD, and PharmGKB databases to obtain DKD-related gene sets and make their intersection Venn diagrams.

2.4 Obtain the Intersection of the Target of the Effective Component of Coptis and DKD-Related Genes

Use R language and corresponding R language scripts to perform online analysis on the target of Coptidis active ingredient and DKD disease-related genes, draw the Venn diagram of the intersection of drug ingredient targets and disease-related genes, and get the intersection genes. This intersection gene is the target of Coptidis for DKD.

2.5 Constructing the Mechanism Network of Coptis Chinensis in Regulating DKD

Upload the relevant files to the graphical display and analysis software Cytoscape_v3.8.0, use Cytoscape to construct a drug-component-target network relationship diagram, and analyze the corresponding relationship between the effective components and

the target, and the results are usually displayed by Degree. The higher the degree value of the target gene, the greater the number of connected nodes, which means that this node is more important in the network relationship graph.

2.6 Construct (PPI) Protein Interaction Network and Screen Core Genes

Upload the intersection gene file of the effective component target of Coptis chinensis and DKD disease-related genes to the STRING online website, select the species as "Human", enter the website to select all genes, set the minimum required interaction score to lowest confidence (0.4000), and then analyze, Get the PPI protein interaction network and related documents. Finally, the file was imported into Cytoscape to obtain the final core gene through three screenings.

2.7 Enrichment Analysis of GO and KEGG Pathways

Using R and its scripts, set qvalueFilter=0.05 (P value≤0.05) and working directory of related files. Then perform GO (gene ontology) enrichment analysis and KEGG (Kyoto encyclopedia of genes and genomes) pathway enrichment analysis in R-4.0.4-win respectively, Obtain available enrichment bubble chart and pathway enrichment histogram.

2.8 Molecular Docking Verifies the Binding Relationship Between the Active Ingredient and the Target

Download the 2D structure of the small molecule ligand from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/), import it into ChemOffice to convert the 2D structure of the small molecule ligand into a 3D design, and optimize it to the minor free energy structure, Get the 3D structure of small molecule ligand. Download the protein receptor structure of the selected active ingredient from the PDB database (http://www.rcsb.org), import the PyMOL software to remove water molecules and small molecule ligands to obtain the protein receptor file. Use AutoDockTools-1.5.6 software to hydrogenate the protein receptor obtained in the previous step and then convert the small molecule ligand and protein receptor file format to determine the functional pockets on the protein receptor roughly. Use vina to perform molecular docking between the target and the target protein to obtain the score of the molecular docking result. The lower the free energy, the better the binding.

3 RESULTS

3.1 Screening and Intersection of Active Ingredient Targets and Disease-Related Genes

Through the TCM System Pharmacology Database and Platform (TCMSP), we searched and screened 14 main active ingredients in Coptis and 148 corresponding targets. In the GeneCards, OMIM, TTD, and PharmGKB databases, 3506 DKD (DN) disease-related genes (after deduplication) were explored. The Venn diagram is drawn by the intersection of the active ingredient targets and disease-related genes (see Figure 1), and ten active ingredients of Coptidis for treating diabetic nephropathy include: berberine, etc., and 104 corresponding target genes include: PTSG2, MMP3, MAPK1, etc

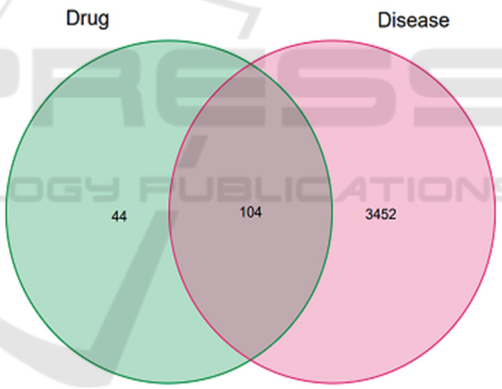


Figure 1: Venn diagram of the intersection of the corresponding target of the practical components of Coptidis and DKD-related genes.

3.2 The Mechanism Network of Coptidis Regulating DKD

The analysis software Cytoscape_v3.8.0 was used to construct and visualize the drug component-target network of Coptidis regulated DKD (see Figures 2). There are 277 nodes, including ten active ingredients, 104 target genes, and 163 edges. Among them, PTGS2, ESR1, AR, and PTGS1 have slightly more connections, and the Degree value is higher, which may play an essential role in the treatment.

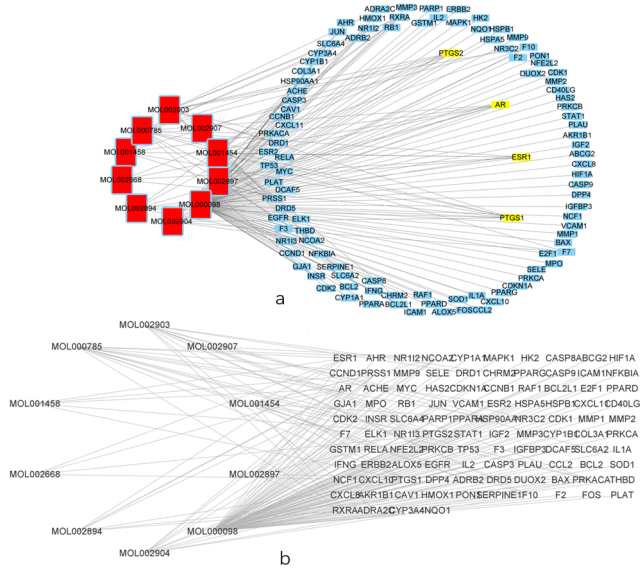
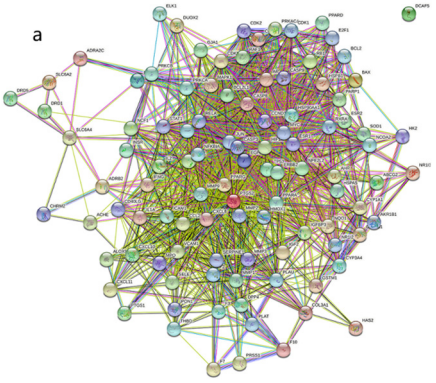


Figure 2: a: Rhizoma Coptidis regulates the DKD network, in which genes PTGS1, PTGS2, ESR1, AR, etc. are more connected b: Coptis Rhizoma controls the DKD network, in which the active ingredients MOL000098, MOL000758, MOL001454, etc. are more connected.

3.3 PPI Protein Interaction Network

The lowest confidence (0.4000) was screened through the STRING online website to construct and visualize the protein interaction network of the target genes for the treatment of diabetic nephropathy (DKD) in Coptis Chinensis (see Figure 3 a). There are 103 nodes and 1466 edges in the PPI network. The result of importing the network into Cytoscape is visualized as network 1. Select DC (Betweenness), CC (Closeness), DC (Degree), EC (Eigenvector), and LAC which are all greater than the median value. Thirty-eight nodes generate network 2, and 17 nodes generate network three by repeating the screening criteria (see Figure 3 bcd). Network 3 is the core network gene. Among them, the target genes may be related to the treatment of diabetic nephropathy (DKD) by Huanglian, from which genes can be selected for molecular docking.



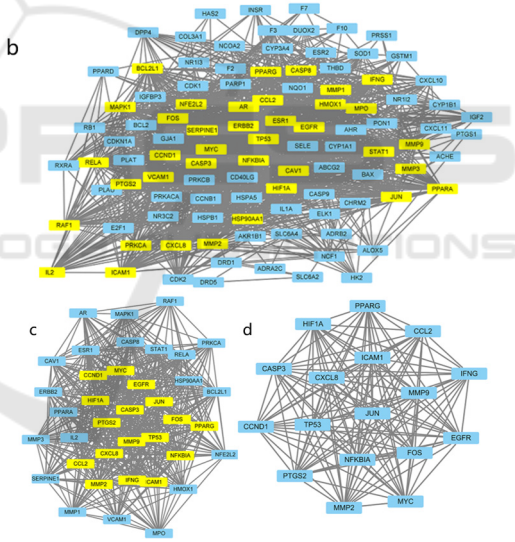


Figure 3: a: The PPI network of Coptis treatment of DKD targets, where each sphere represents a protein or gene, and the number of lines with different colors represents the relationship b: the PPI network of a picture is imported into the visualized network diagram of Cytoscape c: the gene network is shown in b Diagram of the first-level core network screened by specific criteria d: Diagram b: Diagram of the core gene network filtered by specific criteria

3.4 GO and KEGG Enrichment Analysis

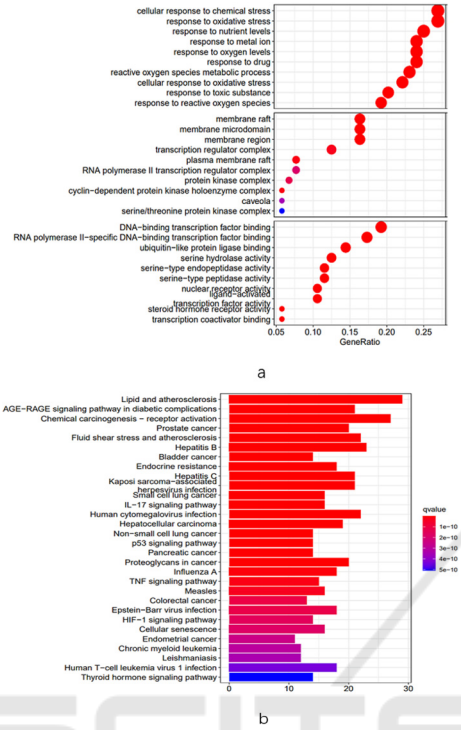


Figure 4: a: GO enrichment analysis bubble chart, the larger the value of generation, the more significant the enrichment, the redder the bubble color, the more relevant b: the histogram of KEGG pathway enrichment analysis, the longer the column indicates that the gene is in this pathway, The more significant the enrichment, the redder the color suggests, the more relevant

The results of GO enrichment analysis (see Figure 4a) show that BP mainly includes cellular Response to chemical stress, Response to oxidative stress, Response to nutrient levels, Response to the drug, etc.

KEGG pathway enrichment analysis has 165 pathways, and the top 30 pathways are plotted as a histogram (see Figure4b). The results showed that Lipids and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, Prostate cancer, IL-17 signaling pathway, TNF signaling pathway, NF-kappa B signaling pathway, and other metabolic pathways have significant gene enrichment. Screen the IL-17 signaling pathway, TNF signaling pathway, NF-kappa B signaling pathway, and VEGF signaling based on the selected PTGS2 and refer to relevant literature VEGF signaling pathway and draw a table (see Table 1)

Table 1: According to the specific data of the four signal pathways determined by PTGS2, including name, number of genes, specific gene names, etc.

Serial number	*Pathway*	*Number of genes*	*Gene*
hsa04657	*IL-17 signaling pathway*	*16*	PTGS2/HSP90AA1/MMP3/RELA/FOS/MMP9/MAPK1/JUN/CASP3/NFKBIA/CASP8/MMP1/CCL2/CXCL8/IFNG/ *CXCL10*
hsa04668	*TNF signaling pathway*	*15*	PTGS2/MMP3/RELA/FOS/MMP9/MAPK1/JUN/CASP3/NFKBIA/CASP8/ICAM1/CCL2/ *SELE/VCAM1/CXCL10*
hsa04064	*NF-kappa B signaling pathway*	12	PTGS2/RELA/BCL2/BCL2L1/PLAU/NFKBIA/ICAM1/VCAM1/CXCL8/PRKCB/PARP1/ *CD40LG*
hsa04370	*VEGF signaling pathway*	7	PTGS2/CASP9/MAPK1/RAF1/PRKCA/PRKCB /*HSPB1*

3.5 Molecular Docking Results

Table 2: PTGS2 molecular docking result scoring table (select the first five docking positions with the lowest free energy)

mode	affinity(kcal/mol)	dist from best mode (rmsd l.b.)	dist from best mode (rmsd u.b.)
1	-9.2	0.000	0.000
2	-9.0	37.418	38.338
3	-8.9	47.593	48.959
4	-8.8	2.188	3.850
5	-8.7	1.948	7.680

The molecular docking technology was used to verify the binding degree of the docking between the corresponding receptor protein corresponding to the receptor protein berberine and the related small-molecule ligand of PTGS2, and the minimum free energy was selected and visualized with PyMOL (see Figure 5). The docking results show that the free

energy of the receptor protein and the corresponding small molecule ligand is low, indicating that the target protein has a better binding ability than the small molecule (see Table 2)

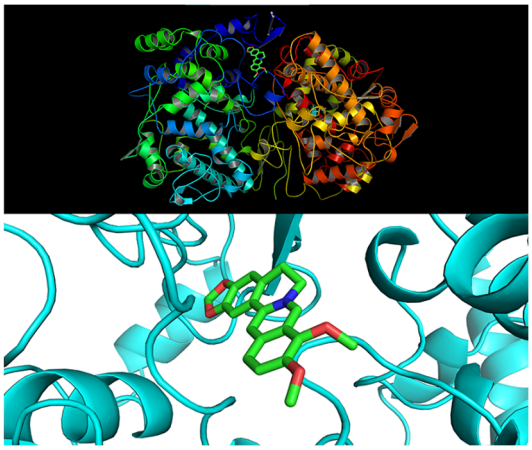


Figure 5: Select the minimum free energy docking form, and use PyMOL to visualize the panorama and details.

3.6 PTSG2, CCL2 Gene Mutation Analysis

It is predicted that the genes related to DKD may be PTGS2 and CCL2. Studies have shown that increased expression of renal tubules MCP-1/CCL2 will promote kidney damage, and the level of urine MCP-1/CCL2 will gradually increase with the progress of DKD, and the severity of it will deepen. (Morii, 2003); (Zhu, 2013). It shows that CCL2 and its pathway are closely related to DKD. They are analyzed through the online website (http://www.cbioportal.org/). The analysis results are shown in Figure 6: a: PTSG2 has Missense Mutation, Truncating Mutation unknown gene mutation, and CCLI2 has a strange gene mutation in Missense Mutation. b: PTGS2 may have genetic mutations in Kidney Renal Clear Cell Carcinoma, Renal Clear Cell Carcinoma, Kidney Renal Papillary Cell Carcinoma, and Kidney Renal Papillary Cell Carcinoma. Among them, Kidney Renal Clear Cell Carcinoma and Renal Clear Cell Carcinoma are more likely to occur. Nearly 40% of the genetic mutations in Renal Clear Cell Carcinoma will be amplified. CCL2 is less likely to be mutated in Kidney Renal Clear Cell Carcinoma, but nearly 50% of the possible genetic mutations will be strengthened after the genetic mutation.

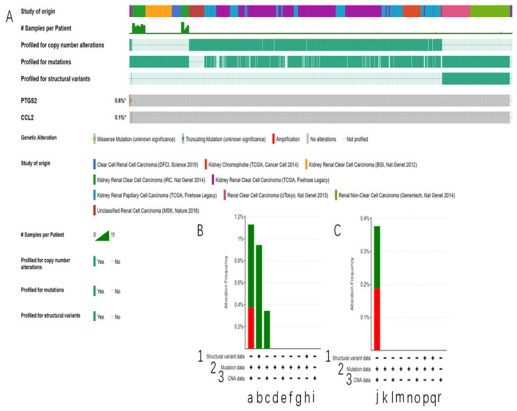


Figure 6: A: Comparative analysis of gene mutations of PTGS2 and CCL2 in kidney-related cancers. B: Analysis of PTGS2 mutations [1:Structural variant data 2:Mutation data 3:CAN data a:Kidney Renal Clear Cell Carcinoma (TCGA, Firehose Legacy) b:Renal Clear Cell Carcinama (UTOkyo, Nat Genet 2013) c:Kidney Renal Papillary Cell Carcinama (TCGA, Firehose Legacy) d:Clear Cell Renal Cell Caricinoma (DFCI, Science 2019) e:Kidney Chromophobe(TCGA, Cancer Cell 2014) f:Kidney Renal Clear Cell Carcinoma(BGI, Nat Genet 2012) g: Kidney Renal Clear Cell Carcinoma (IRC, Nat Genet 2014) h:Renal Non-Clear Cell Carcinoma (Genentech, Nat Genet 2014) i:Unclassificied Renal Cell Cacinoma (MSK, Nature 2016) C: Analysis of CCL2 mutations. [1:Structural variant data 2:Mutation data 3:CAN data j: Kidney Renal Clear Cell Carcinoma(TCGA, Firehose Legacy) k: Clear Cell Renal Cell Caricinoma(DFCI, Science 2019) l: Kidney Chromophobe(TCGA, Cancer Cell 2014) m: Kidney Renal Clear Cell Carcinoma(BGI, Nat Genet 2012) n: Kidney Renal Clear Cell Carcinoma(IRC, Nat Genet 2014) o: Kidney Renal Papillary Cell Carcinama(TCGA, Firehose Legacy) p: Renal Clear Cell Carcinama(UTOkyo, Nat Genet 2013) q: Renal Non-Clear Cell Carcinoma(Genentech, Nat Genet 2014) r: Unclassificied Renal Cell Cacinoma(MSK, Nature 2016)] (In B and C, the green part represents Mutation, and the red part represents Amplification)

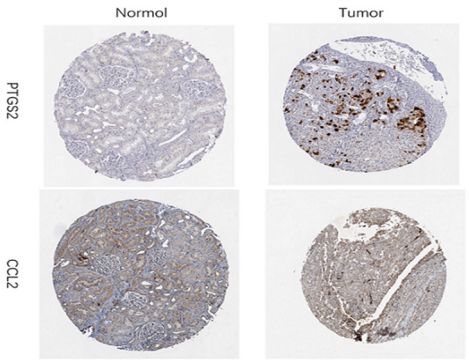


Figure 7: Comparison of the expression map of the two genes in normal kidney tissue and tumor tissue. Image is taken from Human Protein Atlas (http://www.proteinatlas.org) online database

4 DISCUSSIONS

Through network pharmacology data collection, screening, and analysis, some key genes (Figure 3d) and signal pathways (Figure 4b) were obtained. In the process of searching the literature, we learned that berberine can treat DKD renal insufficiency (Niksic L, 2005) and protect the kidneys (Lan, 2010). After screening the core gene and the corresponding target of berberine to take the intersection, it was finally determined to select the only intersection gene PTGS2. Four related signal pathways were chosen from the first 30 pathways with significant KEGG enrichment (Table 1). Finally, PTGS2 was molecularly docked (Figure 5). The results showed that the lowest score was -9.2 (kcal/mol) (Table 2), indicating that PTGS2 and its related pathways may be essential genes and pathways regulating berberine treatment of DKD.

Studies have shown that the occurrence of DKD may be related to inflammation, metabolic status, activation of NF-κB, etc. Inflammation plays a vital role in the pathogenesis of diabetic nephropathy, causing kidney damage and activating some signal channels to exacerbate inflammation reactions. Inflammatory factors include interleukin (IL), nuclear factor-κB (NF-κB), tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1), etc. Among them, IL may be related to IL- 17 signaling pathway, TNF-α is related to TNF signaling pathway, and NF-κB is associated with NF -kappa B signaling pathway. Berberine is likely to regulate these inflammatory factors to regulate the DKD metabolic pathway to protect the kidney and treat DKD.

Studies have shown that berberine has a relatively apparent anti-inflammatory effect, mainly by inhibiting the production and activity of inflammatory factors. It can reduce the activity of neutrophil phospholipase A2 and reduce the production of prostaglandin E2 in inflammatory tissues (Hu, 2014). Prostaglandin-endoperoxide synthase (PTGS), also known as cyclooxygenase, is a key enzyme in prostaglandin biosynthesis, closely related to the synthesis of prostaglandin E2, and PTGS2 is one of the two types of PTGS Inducible may be involved in the synthesis of prostaglandin E2. Based on this speculation, berberine may regulate PTGS2 to regulate related metabolic pathways to achieve anti-inflammatory effects and reduce kidney damage.

In addition, the gene mutation analysis of PTGS2 and CCL2 in section 2.6 (Figure 6) also shows that PTGS2 is more likely and more prone to gene mutations in related kidney diseases than CCL2, which also illustrate the relationship of PTGS2 and pathogenesis of DKD is closer.

5 CONCLUSIONS

In summary, based on network pharmacology, predictive analysis verified that PTGS2 and its related pathways may be important genes and pathways regulating berberine treatment of DKD. Berberine is likely to further inhibit the synthesis and release of some inflammatory factors (such as IL, NF-κB, TNF-α, TGF-β1, etc.) by inhibiting PTGS2, thereby achieving regulation of DKD metabolic pathways (TNF signaling pathway, IL-17 signaling pathway, NF-kappa B signaling pathway, VEGF signaling pathway) to achieve anti-inflammatory effects, and ultimately reduce kidney damage, protect the kidneys, and achieve the effect of treating DKD.

In this study, a series of methods were used to find the relationship between berberine and the DKD disease gene PTGS2, the effective component of Coptidis Rhizome, and to verify the feasibility of Coptis Rhizoma (berberine) for regulating PTGS2 in the treatment of DKD. It provides a new idea for the research on the target and mechanism of Coptidis in the treatment of diabetic nephropathy in the future.

It is hoped that this study can provide reference for other researchers who are starting to develop the mechanism of action of Coptis in the treatment of DKD in the future.

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