ORIGINAL RESEARCH

Network Pharmacology-Based Identification of Key Pharmacological Mechanism of Shen-qi-di-huang Decoction Acting on Uremia

Xiaowen Zhang, BD; Xiao-fei Chen, MD; Wen-jia Chen, MD; Haibo Ding, MD; Bao-xia Zhang, MD

ABSTRACT

This study employs network pharmacology to uncover the pharmacological mechanisms underlying Shen-qi-di-huang decoction's efficacy in treating uremia. We identified a total of 927 differentially expressed genes (DEGs) through differential expression analysis and the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database and analysis platform, of which 607 were downregulated and 320 were upregulated. We also obtained the effective biological components and related target gene information of Chinese herbal medicines such as Renshen, Huangqi, shudihuang, Shanyao, Fuling, Mudanpi, and Shanzhuyu in Shen-qi-di-huang decoction and constructed a regulatory relationship network between molecular components and target genes in Shen-qi-di-huang decoction. We then constructed a protein-protein interaction (PPI) network of 15 targeted genes (RXRA, ND6, CYP1B1, SLPI, CDKN1A, RB1, HIF1A, MYC, HSPB1, IFNGR1, NQO1, IRF1, RASA1, PSMG1 and MAP2K4) using the STRING database and visualized the PPI network using the software Cytoscape. In addition, we revealed the key molecular functions of uremia through Gene Ontology (GO) enrichment analysis, mainly including neuron apoptotic process, cellular response to oxidative stress, regulation of neuron apoptotic process, neuron projection cytoplasm, RNA polymerase II transcription regulator complex, plasma membrane bounded cell projection cytoplasm, NADH and NADPH dehydrogenase (quinone) activity, protein kinase inhibitor and ubiquitin protein ligase binding, etc. Finally, we identified important biological pathways in uremia through Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, which mainly concentrated in Kaposi sarcoma–associated, small cell lung cancer, Gastric cancer, Hepatitis B and C, Hepatocellular carcinoma, Thyroid cancer, Bladder cancer, MAPK signaling pathway, ErbB signaling pathway, Th17 cell differentiation, HIF−1 signaling pathway, Thyroid hormone signaling pathway and Cell cycle, etc. Using integrated bioinformatical analysis, we elucidated key pharmacological mechanisms based on targeted genes, which was enable early identification of patients with uremia and would contribute to early clinical diagnosis and treatment of patients. (*Altern Ther Health Med.* 2024;30(1):44-50).

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INTRODUCTION

Uremia refers to the toxic phenomenon caused by renal failure, which prevents the excretion of body waste such as protein digestion products and urea, resulting in their retention in the body, which is the late stage of acute and chronic kidney failure and is generally known as stage 4 and stage 5 chronic kidney disease.[1] At this time, the patient's kidney cannot play a normal function, resulting in the disorder of water and electrolyte acid-base balance, renal and endocrine dysfunction; there will be metabolic end products and toxic substances in the body retention, thereby causing a series of symptoms and signs.[2] Patients with uremia need to treat the underlying disease that causes kidney damage, in addition to controlling complications and protecting residual kidney function.[3] It can cause symptoms such as fatigue, nausea, edema, and reduced urine output. If not treated promptly, it can cause neurological and psychiatric symptoms, cardiac arrest, and sudden death. Uremia caused by acute kidney disease may be cured after the cause is resolved, but chronic uremia cannot be cured currently. Usually, medications employed in the management of uremia are indicated for associated metabolic and electrolyte abnormalities. The maintenance of patients' lives mainly depends on dialysis or kidney transplantation, and the prognosis of this disease is poor. Once symptoms appear,

patients with uremia must have dialysis initiated. Hemodialysis or peritoneal dialysis is generally required, and kidney transplantation can be performed if there are conditions.[4] Therefore, it is of great significance to find the treaments and interventions for uremia to improve the patient prognosis.

Traditional Chinese medicine treatment is considered to be able to cure uremia to a certain extent. Shen-qi-di-huang decoction is a traditional Chinese medicine formula. It has the effect of supplementing Qi, nourishing Yin, nourishing the kidneys, and strengthening the spleen. Shen-qi-di-huang decoction is mainly composed of Renshen, Huangqi, Shudihuang, Shanyao, Fuling, Mudanpi, and Shanzhuyu. With its advantages of rigorous structure, exquisite compatibility and precise curative effect, it has been widely used in clinical practice, especially for kidney diseases.[5] It mainly treats the deficiency of spleen and kidney, or of Qi and Yin, whose common symptoms are dizziness, soreness and weakness of the waist and knees, burnout, fever, fatigue and night sweats, and weak pulse.[6,7] Nevertheless, the molecular and action mechanism of Shen-qi-di-huang decoction in the treatment of uremia are not fully understood, requiring further exploration.

In this study, we first selected and downloaded dataset GSE37171 and got a gene expression matrix. Then the differential expression analysis was conducted to identify uremia's differentially expressed genes (DEGs). Next, we obtained each herb's effective biological ingredients and related targets in Shen-qi-di-huang decoction, and constructed a regulation network of Shen-qi-di-huang decoction. Moreover, we used the STRING database to construct protein-protein interaction (PPI) networks of targeted genes. Finally, we analyzed the Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of targeted genes in uremia. The aim of this study was to expore the key pharmacological mechanisms of Shen- qi-di-huang decoction treating ureamia and provide theoretical support for its clinical application.

MATERIALS AND METHODS

Downloading and organizing original expression matrix

A gene expression database called Gene Expression Omnibus (GEO) was created by the National Center for Biotechnology Information (NCBI) in 2000.[8] From the GEO database, we first selected and downloaded the probe expression matrix and probe platform annotation information of dataset GSE37171 by searching the keyword "renal failure". We then used a custom Perl script to convert the probe IDs into the gene symbols, resulting in a gene expression matrix.[9]

Differentially expressed gene (DEG) analysis in uremia

In order to identify the differentially expressed genes (DEGs) between uremia and healthy control (HC) groups, differential expression analysis was conducted.[10] First, we inputed the sorted transcriptome gene expression matrix into R program (version 4.2.2, the same below) and corrected the expression value. Then, R package *limma* and function *wilcox.text* was used to compare the differences in gene expression levels between the two groups, and the DEGs expression matrix and the DEGs parameters were output.[11] Finally, we visualized DEGs using a gene heat map and a volcanic map. $P < .05$ and $|\lg FC| \geq 0.5$ were regarded as significant differences.[12,13]

Acquisition of effective ingredients and drug targets in Shen-qi-di-huang decoction

Using the Traditional Chinese Medicine Systems Pharmacology (TCMSP) Database and Analysis Platform, we obtained each herb's effective biological ingredients and related targets in Shen-qi-di-huang decoction, and sorted and matched them.[14] The filter criteria were oral bioavailability (OB) at least 30% and drug-like (DL) of at least 0.18.[15]

Construction of a regulation network of Shen-qi-di-huang decoction

The correlation matrix between Shen-qi-di-huang decoction compound ingredients and target genes and the list of DEGs were obtained from the above analysis. Then, through a customized Perl script, the genes in the two files are crossed to obtain the interaction relationship matrix and relationship node attribute list of the composite active ingredients and genes of Shen-qi-di-huang decoction. Finally, the software Cytoscape (version 3.9.1, the same below) was used to visualize the regulatory networks of Shen-qi-di-huang decoction compound components and target genes.[16,17]

Construction of protein-protein interaction (PPI) network

The protein interaction network is composed of individual proteins (genes) through interaction with each other to participate in biological signal transmission, gene expression regulation, energy and material metabolism, cell cycle regulation, and other aspects of life process.[18] We used the STRING database (http://www.string-db. org/), a protein interaction network database based on public databases and literature information that can collect multiple public databases, including UniProt, KEGG, NCBI, and Gene Ontology. We integrated these data to generate a comprehensive protein interaction network database to construct PPI networks of candidate genes. This was then visualized in Cytoscape software (http://www.cytoscape.org).[19]

Gene Ontology (GO) enrichment analysis of candidate genes in uremia

Gene Ontology (GO) is a database established by the Gene Ontology Consortium, aiming to establish a semantic vocabulary standard applicable to various species, which can define and describe the functions of genes and proteins and can be updated with continuous research.[20] First, we used R packages *clusterProfiler*, *org.Hs.eg.db*, *enrichplot* and *ggplot2* to enrich-analyze candidate genes through function enrichGO, including biological processes (BP), cellular components (CC) and molecular functions (MF).[21] The enrichment results are shown in bar charts. Adjusted $P < .05$ were considered significant.[22]

Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of candidate genes in uremia

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database is a database that systematically analyzes gene functions, genomic information and functional information, including metabolic pathways database, hierarchical classification database, gene database, genome database, etc. KEGG's pathway database is the most widely used public metabolic pathway database.[23,24] In the same way, we used R packages *clusterProfiler*, *org.Hs.eg.db*, *enrichplot* and *ggplot2* to enrich-analyse candidate genes through function enrichKEGG. The enrichment result was shown in a bubble plot.[25,26] Finally, we constructed a KEGG relationship network to visualize the link between the pathway and candidate genes.[27] Adjusted *P*-values<0.05 were considered significant.

Statistical analysis

Statistical analysis was conducted by GraphPad Prism 8. After checking data for normal distribution and variance homogeneity, continuous data were compared using multiple Student *t* tests or two-way ANOVA. All *P*-values were two-tailed, and $P < .05$ were considered significant. The data were represented as mean ± S.E.M. or as median with 10 and 90 percentiles.

RESULTS

Identification of DEGs in uremia

We collated the dataset GSE37171 (containing 75 uremia samples and 40 HC samples) and obtained a gene expression matrix containing 7319 transcripts. A total of 927 DEGs were identified by differential expression analysis, of which 607 were downregulated and 320 upregulated ($P < .05$, Figure 1a). The heat map of gene expression showed the top 20 DEGs with the most up-regulated and down-regulated expression (Figure 1b), they are respectively *FAR1*, *ZEB2*, *ACADM*, *PPIP5K2*, *YTHDF3*, *FAM3C*, *THEMIS*, *SUCLA2*, *PTPLA*, *CTD-2553C6.1*, *EPHB4*, *RPLP2*, *CCDC91*, *PRNP*, *TCERG1*, *KLF10*, *TOB1*, *RNF6*, *PTPN12*, *C16orf72*, *AKAP11*, *LYZ*, *TCEB2*, *SLC6A8*, *DPCD*, *FAH*, *TCF3*, *COX7C*, *MOSPD1*, *MYBL2*, *BPGM*, *IFI27*, *NPTN*, *SRP9*, *FECH*, *TRAK2*, *PI3*, *TSTA3*, *ND6*, and *SOD2*.

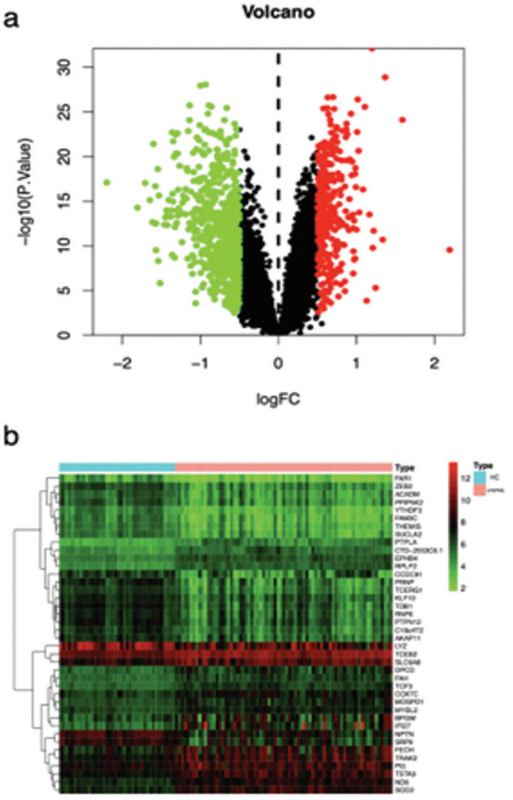
Identification of effective ingredients and drug targets in Shen-qi-di-huang decoction

Through the TCMSP database and analysis platform, we obtained the effective biological ingredients and related target gene information of mainly composed of herbs such as Renshen, Huangqi, shudihuang, Shanyao, Fuling, Mudanpi and Shanzhuyu in Shen-qi-di-huang decoction. See supplementary documents S1 and S2 for details. Then we summarized and sorted out the effective ingredients and target genes of all compound drugs in Shen-qi-di-huang decoction through Perl script, see supplementary document S3 for details.

Regulation network of Shen-qi-di-huang decoction

Through the above custom Perl script processing, we obtained the interaction matrix and relationship node attribute list between the effective molecules and target genes of each component in Shen-qi-di-huang decoction, further plot the optimal STRING PPI network using selected biological targets. All interceptive targets were imported into Cytoscape software for detecting and analyzing the parameters, as shown in the supplementary document S4. Figure 2 showed the regulatory relationship network between molecular components and targeted genes in Shen-qi-di-huang decoction.

Figure 1. (a) The volcano plot of DEGs expressed in uremia. Red dots indicated up-regulated DEGs; Green dots indicated down-regulated DEGs. FC, Fold Change. (b) The heat map of DEGs expressed in uremia. Red squares indicated up-regulated DEGs; Green squares indicated down-regulated DEGs.



PPI network of target genes in Shen-qi-di-huang decoction

We constructed a PPI network of 15 targeted genes using the STRING database (Figure 3a) and visualized the PPI network using the software Cytoscape (Figure 3b). These targeted genes were *RXRA* (Retinoid X receptor alpha), *ND6* (Mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 6), *CYP1B1* (Cytochrome P450 family 1 subfamily B member 1), *SLPI* (Secretory leukocyte peptidase inhibitor), *CDKN1A* (Cyclin dependent kinase inhibitor 1A), *RB1* (RB transcriptional corepressor 1), *HIF1A* (Hypoxia inducible factor 1 subunit alpha), *MYC* (MYC proto-oncogene, bHLH transcription factor), *HSPB1* (Heat shock protein family B (small) member 1), *IFNGR1* (Interferon gamma receptor 1), *NQO1* (NADH and NADPH quinone dehydrogenase 1), *IRF1* (Interferon regulatory factor 1), *RASA1* (RAS p21 protein activator 1), *PSMG1* (Proteasome assembly chaperone 1) and *MAP2K4* (Mitogen-activated protein kinase kinase 4).

Figure 2. Regulation network of Shen-qi-di-huang decoction. The oval nodes represented each molecular component. The different colors indicated the origin of different drugs (Green, Huangqi; Yellow, Mudanpi; Purple, Renshen; Light blue, Shanyao; Red, multi-drug). Diamond-shaped nodes represented targeted genes, and the lines between them represent regulatory relationships.

Figure 3. Protein-protein interaction (PPI) network consisting of 15 targeted genes. Nodes represented genes, and lines represented interactions between genes.

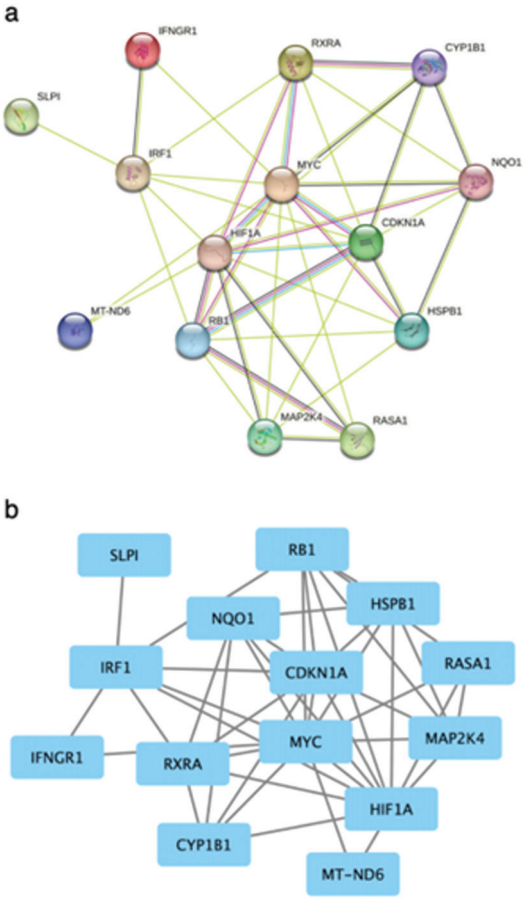
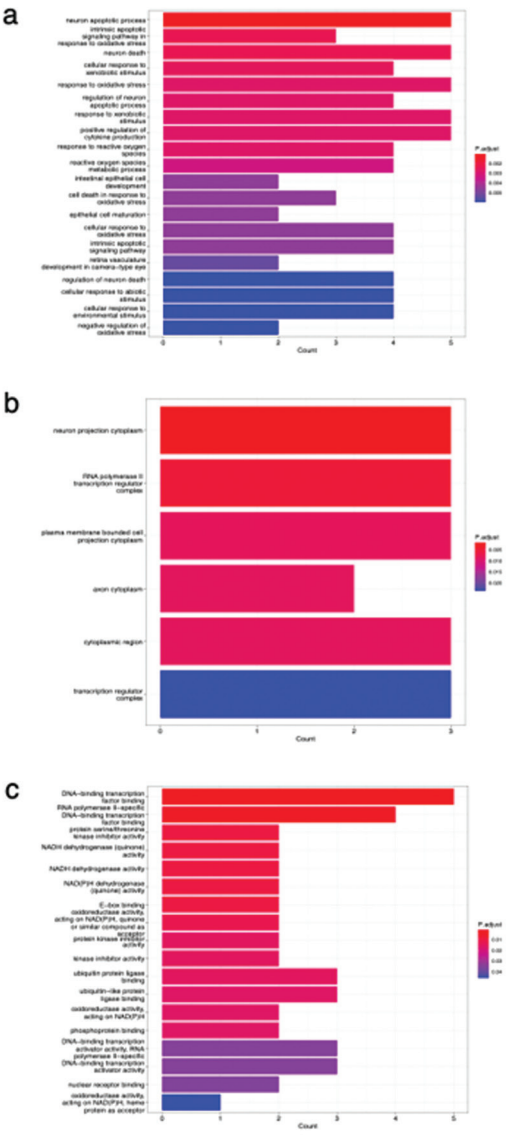


Figure 4. Bar charts for GO enrichment analysis, including BP (a), CC (b) and MF (c). The ordinate represented the description of different GO functions, and the abscissa represented the count of targeted genes enriched.



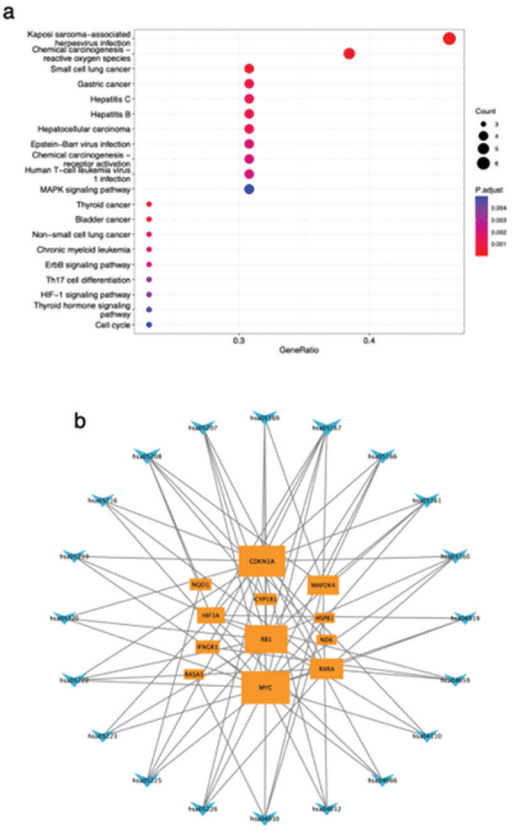
GO function enrichment of 15 targeted genes in uremia

We revealed the key molecular functions of uremia through GO enrichment analysis. As shown in Figure 4a, GO enrichment analysis showed that the functions of BP mainly included neuron apoptotic process, cellular response to oxidative stress, and regulation of neuron apoptotic process, etc ($P < .05$). As shown in Figure 4b, the function of CC was closely related to neuron projection cytoplasm, RNA polymerase II transcription regulator complex, plasma membrane bounded cell projection cytoplasm, etc ($P < .05$). As shown in Figure 4c, the function of MF was mainly concentrated in NADH and NADPH dehydrogenase (quinone) activity, protein kinase inhibitor and ubiquitin protein ligase binding, etc ($P < .05$).

KEGG pathway enrichment of 15 targeted genes in uremia

We identified important biological pathways in uremia through KEGG enrichment analysis. As shown in Figure 5a, the pathways of targeted genes were mainly concentrated in Kaposi sarcoma–associated, Small cell lung cancer, Gastric cancer, Hepatitis B and C, Hepatocellular carcinoma, Thyroid cancer, Bladder cancer, MAPK signaling pathway, ErbB signaling pathway, Th17 cell differentiation, HIF−1 signaling pathway, Thyroid hormone signaling pathway, and Cell cycle, etc ($P < .05$). Figure 5b showed the correlation between targeted genes and KEGG pathway. If more pathways were associated with a gene, the larger the volume of nodes where the gene resided. Similarly, if more genes were associated with a pathway, the larger the volume of the diamond in which the pathway resided.

Figure 5. (a) The bubble plot for KEGG enrichment analysis. The ordinate represented the description of different KEGG pathways, and the abscissa represents the ratio of genes enriched. (b) The correlation network of KEGG pathways. The orange squares represented targeted genes, and the blue diamonds represented the KEGG pathway. The more connections between them, the stronger the correlation, and the larger the volume of nodes, the more the number of related pathways or genes.



DISCUSSION

Chronic kidney disease is a public health problem faced by all countries in the world, and its incidence rate and hospitalization rate are increasing yearly, causing serious harm to patients' health. A data report shows that chronic kidney disease incidence rate is as high as 10.8%. Meanwhile, the growth rate of end-stage renal disease has accelerated in recent years, with 112 uremic patients receiving kidney replacement therapy per million people developing symptoms each year, and 442 people per million being diagnosed with uremia. Uremia refers to the inability of the human body to produce urine through the kidneys, which excretes waste products and excessive water from the body, such as glucose, protein, amino acids, sodium ions, potassium ions, sodium bicarbonate, acid-base balance disorders, etc., as well as the endocrine functions of the kidneys, such as the production of renin, erythropoietin, active vitamin D3, prostaglandins, etc, toxicity caused by metabolic disorders in kidney failure as the condition progresses. Modern medicine believes uremia is a series of complex syndromes caused by the disorder of biochemical processes within the body after the loss of kidney function. Instead of being an independent disease, it is called renal failure syndrome or kidney failure. In this study, we explored the key pharmacological mechanisms in the treatment of uremia based on network pharmacology, and found 15 key target genes such as RXRA, as well as multiple biological pathways.

Uremia is the final stage of renal failure. There is no possibility of recovery of renal function, and it cannot be completely cured and often requires dialysis or kidney transplantation to keep people alive. The main treatments for this disease include kidney transplantation, hemodialysis, and peritoneal dialysis. Due to the extremely limited renal source, most patients cannot receive kidney transplantation and can only choose dialysis treatment.[28] Even if the patient is fortunate enough to receive a kidney transplant, achieving a true cure after surgery is still difficult due to the need for long-term use of anti-rejection drugs, and a allogeneic kidney cannot guarantee the patient's normal life.[29] The cost of hemodialysis is high, and there will be low immunity, vascular calcification, bone dystrophy and other side effects.[30] Therefore, the value of TCM treatment program in the treatment of uremia is becoming increasingly prominent. However, the differences in gene expression in patients with uremia are not fully understood. Teng et al.[31] performed integrated bioinformatics analysis to identify the differentially expressed genes (DEG) and hub genes for the function and pathways in the occurrence and development of calcific aortic valve disease, which provided a new idea for the treatment of uremia.

In this study, a total of 927 DEGs were identified by differential expression analysis, of which 607 were downregulated and 320 upregulated. Next, we obtained the effective biological ingredients and related target gene information of mainly composed of herbs such as Renshen, Huangqi, Shudihuang, Shanyao, Fuling, Mudanpi and Shanzhuyu in Shen-qi-di-huang decoction through the TCMSP database and analysis platform, and constructed regulatory relationship network between molecular components and targeted genes in Shen-qi-di-huang

decoction. We then constructed a PPI network of 15 targeted genes (*RXRA*, *ND6*, *CYP1B1*, *SLPI*, *CDKN1A*, *RB1*, *HIF1A*, *MYC*, *HSPB1*, *IFNGR1*, *NQO1*, *IRF1*, *RASA1*, *PSMG1* and *MAP2K4*) using the STRING database and visualized the PPI network using the software Cytoscape. With the development and progression of kidney disease, most patients with uremia are in a microinflammatory state.[32] On the one hand, with the gradual loss of the metabolic function of the kidney, the ability of the kidney to clear inflammatory factors decreases, and the accumulation of large amounts of toxins will release inflammatory factors. In the process of chronic renal failure, the immune balance in the body is broken, therefore, different degrees of inflammation will occur in various stages of chronic renal failure. In this study, we revealed the key molecular functions of uremia through GO enrichment analysis, mainly including neuron apoptotic process, cellular response to oxidative stress, regulation of neuron apoptotic process, neuron projection cytoplasm, RNA polymerase II transcription regulator complex, plasma membrane bounded cell projection cytoplasm, NADH and NADPH dehydrogenase (quinone) activity, protein kinase inhibitor and ubiquitin protein ligase binding, etc. This indicated that Shen-qi-di-huang decoction may improve uremia by regulating the inflammatory response of the body. Finally, we identified important biological pathways in uremia through KEGG enrichment analysis, which mainly concentrated in kaposi sarcoma–associated, small cell lung cancer, gastric cancer, hepatitis B and C, hepatocellular carcinoma, thyroid cancer, bladder cancer, MAPK signaling pathway, ErbB signaling pathway, Th17 cell differentiation, HIF−1 signaling pathway, thyroid hormone signaling pathway and cell cycle, etc. This indicated that Shen-qi-di-huang decoction also has some potential role in the treatment of other diseases.

IFNGR is a type of lymphatic factor, mainly produced by activated T cells and NK cells. It is divided into ligand adsorption chain (IFNGR1) and accessory chain (IFNGR2), both of which belong to the cytokine II receptor superfamily. IFNGR complexes are widely distributed in various cells and tissues. IFNGR1 has high specificity in binding to ligands, but only when coexisting with IFNGR2 can type II interferon induce relevant signal transduction and activity. IFNGR can exist in the form of extracellular matrix connections, so cell growth can be controlled through adjacency, and it can be distributed on almost all cell surfaces except for mature red blood cells. IFNGR signals through the JAK/STAT pathway, leading to the formation of phosphorylated STAT1 homodimers, translocation of activating factors to the nucleus, and binding in the upstream promoter region of interferon-induced genes γ Activate sequence. IFNGR plays an important role in innate and adaptive immunity against viruses, certain bacteria, and protozoan infections. It is an important activator of macrophages and an inducer of MHC II expression. The abnormal expression of IFNGR is associated with many autoimmune and inflammatory diseases.[33,34] Adam M Zawada et al.[35] recruited 10 uremia patients undergoing hemodialysis and 10 healthy volunteers to perform a genome-wide analysis of DNA methylation using SuperTAG methylation-specific digital karyotype to identify genes that are differentlymethylated in chronic kidney disease (CKD). Ninety-seven candidate genes associated with immune/infectious diseases, such as IFNGR1, were found to promote uremia-induced atherosclerotic inflammatory processes. The results of this study indicated that Shen-qi-di-huang decoction mat reduce the probability of developing atherosclerotic inflammation in uremic patients by targeting the IFNGR1 gene. NADH and NADPH quinone dehydrogenase 1 (NQO1) is a member of the NADH and NADPH dehydrogenase (quinone) family and encodes a cytoplasmic 2-electron reductase.[36] Altered expression of this protein has been seen in many tumors and is also associated with Alzheimer's disease (AD).[37,38] Shen J et al.[39] quantified the expression of gene NQO1 by real-time quantitative PCR and determined the protein content by western blotting. The results showed that in advanced uremic disease, in dialysis patients (n=34), the expression of gene NQO1 was not upregulated as compared with CKD 1-5, while protein NQO1 was not upregulated. This suggested that the NRF2 pathway is activated by coexisting pathogenic mechanisms in monocytes of patients with CKD, but the effectiveness of this up-regulation is reduced in advanced uremia. Our results suggested that Shen-qi-di-huang decoction may inhibit the coexisting pathogenic mechanism by targeting NQO1 gene.

Traditional Chinese medicine is increasingly being proven to be applicable to the early or late treatment of various diseases. There are few international reports on the use of traditional Chinese medicine for the treatment of uremia, and it has been proven that uremia cannot be treated with Western medicine. However, inheriting 5000 years of traditional Chinese medicine culture may bring new hope to the suffering uremic patients. This article explores theories beyond experience through the method of network pharmacology, which indecated that Shenqi Dihuang soup might have a potential uremic therapeutic effect, but the drawback is that it did not conduct detailed experiments to further validate pathways and mechanisms. We will also further verify these key roles in designing animal experimants and cell experiments in future studies. Besides, the biological effects of other genes identified in our study and pharmacological mechanisms of Shen-qi-di-huang decoction in uremia have not been adequately reported. Therefore, further in vitro and in vivo function experiments and verification of large sample data are needed in the future.

DATA AVAILABILITY

The dataset used and/or analyzed during this study may be granted by contacting the corresponding author.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

FUNDING STATEMENT

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AUTHORS' CONTRIBUTIONS

Bao-xia zhang designed the study. Xiaowen Zhang wrote the original draft. Xiao-fei Chen collected raw data. Wen-jia Chen performed statistical and bioinformatics analyses. Haibo Ding supervised the study. Xiaowen Zhang, Bao-xia Zhang contributed equally to this work.

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