

Decoding the Pharmacological Secrets of Shenqi Dizhi membrane kidney formula attenuates membranous nephropathy: A network pharmacology and transcriptomics approach

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Abstract

Introduction: Idiopathic membranous nephropathy (IMN) is a challenging autoimmune kidney disease. Based on the traditional Chinese medicine (TCM) theory of "dual deficiency of qi and yin" and "blood stasis", our group developed Shenqi Dizhi Compound Formula (SQDZ). This study aimed to systematically investigate the pharmacological effects and underlying mechanisms of SQDZ in treating IMN.

Methods: We integrated a passive Heymann nephritis (PHN) rat model with UPLC-MS/MS-based chemical profiling, network pharmacology, and renal transcriptomic analysis to identify the active components, potential targets, and mechanisms of SQDZ.

Results: UPLC-MS/MS analysis identified 588 compounds in SQDZ, including active ingredients like baicalin and astragaloside II. Network pharmacology mapped these to 261 potential therapeutic targets. In PHN rats, SQDZ significantly reduced urinary protein, improved lipid metabolism, and alleviated renal injury. Mechanistically, it modulated immune-inflammatory pathways (e.g., IL-6/STAT3) and regulated complement and coagulation cascades (involving *Serpine1*, *F10*, *Itgal*). It also restored podocyte function and reduced inflammation via downregulation of *Ccr2* and *Cd38*. Transcriptomics consolidated six core genes: *Cd38*, *F10*, *Slc5a2*, *Itgal*, *Ccr2*, and *Serpine1*.

Discussion: The findings indicate that SQDZ acts through synergistic, multi-targeted modulation of both immune-inflammatory and coagulation pathways, rather than a single target. This mechanism aligns well with the TCM principles of replenishing "qi and yin" and resolving "blood stasis," providing a systems-level explanation for its efficacy.

Conclusion: This study demonstrates that SQDZ exerts multi-target therapeutic effects on IMN, providing a robust pharmacological basis for its clinical application.

1. INTRODUCTION

Idiopathic membranous nephropathy(IMN)is classified as an autoimmune-mediated podocytopathy of unknown etiology.In this disease,circulating autoantibodies are produced due to various endogenous or exogenous triggers,or external antigens may become planted on the surface of podocytes.These antigens form complexes with circulating antibodies,resulting in subepithelial deposition of immune complexes along the glomerular basement membrane(GBM),which subsequently activates the complement cascade.This process subsequently induces the assembly of membrane attack complexes,culminating in podocyte damage,as characterized by structural disruption and functional compromise.Recent epidemiological observations have demonstrated a marked escalation in IMN incidence rates.Research in mainland China revealed that the proportion of IMN among all glomerular diseases has increased from 12.2%to 24.9%¹.Notably,multinational epidemiological investigations have documented a progressive increase in IMN incidence over the past decade².IMN exhibits a prolonged clinical course with heterogeneous outcomes,where spontaneous complete remission occurs in 30–

33% of all cases, and partial remission is attained by an additional 30% of the patients. However, 30–40% of the patients experience progressive renal function decline, which eventually develops into an end-stage renal disease³. Therefore, strategies to slow down the progression of IMN remain a major clinical concern.

As per the Kidney Disease: Improving Global Outcomes (KDIGO) clinical recommendations, immunosuppressive regimens constitute the cornerstone therapeutic strategy for disease management. These guidelines recommend the use of rituximab, tacrolimus, glucocorticoids, and cyclophosphamide for patients with membranous nephropathy who exhibit severe comorbidities, declining renal function, or persistent proteinuria. While immunosuppressive agents demonstrate clinical efficacy, their administration entails clinically significant adverse events and elevated relapse rates, which remain major obstacles in the therapeutic management of IMN^{4,5}. Traditional Chinese medicine (TCM), which has accumulated centuries worth of empirical knowledge and holistic regulatory mechanisms, demonstrates unique therapeutic potential through its multicomponent synergism and multitarget modulation, thereby expanding the therapeutic repertoire for comprehensive management of IMN^{6,7}.

Shen-Qi-Di-Huang Decoction (SQDHD) is one of the classic prescriptions in TCM, originally recorded in *Za Bing Yuan Liu Xi Zhu* during the Qing Dynasty. It is primarily used for replenishing qi and nourishing yin, tonifying the kidneys, and strengthening the spleen. Recent studies have demonstrated its therapeutic potential in managing various renal diseases, including lupus nephritis, diabetic nephropathy, and uremia^{8–11}. Clinical research indicates that SQDHD protects the GBM, alleviates oxidative stress damage in the kidneys, and reduces renal fibrosis⁸. In addition, SQDHD inhibits USP14 to induce podocyte autophagy, thereby increasing K63-linked ubiquitination of Beclin1 and improving membranous nephropathy⁹. Based on evidence-based medicine and expert consensus, the research team identified qi-yin deficiency syndrome as the core pattern of IMN and selected Shenqi Dihuang Decoction (Level III evidence) as the foundational formula^{12,13}. By incorporating the pathological characteristics of "renal collateral stasis" in IMN and the blood-activating effects of leech, the optimized "Shenqi Dizhi Moshen Formula" was developed. Preliminary clinical studies confirmed that this compound formula combined with Western medicine demonstrates significantly superior efficacy compared to Western medicine alone in treating IMN patients with qi-yin deficiency syndrome¹⁴.

In recent years, network pharmacology and transcriptomics have emerged as powerful tools for elucidating the complex pharmacodynamics of multicomponent therapies such as TCM formulations¹⁵. In this study, a passive Heymann nephritis (PHN) rat model was established to simulate IMN. First, the phenotypic effects of SQDZ on IMN were evaluated through laboratory, histopathological, and molecular analyses. Next, a combination of transcriptomic and network pharmacology approaches was used to identify the key molecular targets and signaling pathways involved in the therapeutic actions of SQDZ. Finally, the predicted targets and pathways were validated using reverse transcription polymerase chain reaction (RT-

PCR),immunofluorescence(IF)immunohistochemistry(IHC),and western blotting(WB).This study aimed to provide a theoretical and scientific foundation for advancing the application of TCM in the treatment of IMN.

2. MATERIALS AND METHODS

2.1. Animals and ethics statement

Male Sprague–Dawley rats($n = 60$;weight:180–220g)and male New Zealand white rabbits were procured from Hangzhou Medical College,Zhejiang Province,China.The rats were maintained under specific pathogen-free(SPF)conditions,and the rabbits were housed in a clean-grade animal laboratory.

2.2. Preparation of SQDZ

SQDZ formula was composed of Tai Zi Shen(*Pseudostellaria heterophylla*(Miq.)Pax),Huang Qi(*Astragalus membranaceus* Bunge),Di Huang(*Rehmannia glutinosa*(Gaertn.)DC.),Shan Zhu Yu(*Cornus officinalis* Siebold&Zucc),Shan Yao(*Dioscorea oppositifolia* L.),Bai Zhu(*Atractylodes Macrocephala* Koidz.),Fu Ling(*Poria cocos*(Schw.)Wolf),Shui Zhi(*Whitmania pigra* Whitman),Jing Ying Zi(*Rosa laevigata* Michx),and Qian Shi(*Euryale ferox* Salisb).Ten herbs were purchased from the Zhejiang Academy of TCM.The abovementioned herbs were immersed in distilled water(10times the herbal weight)for 1h,heated,and decocted for 30min.When the temperature reached 100°Cand the pressure reached 0.1MPa,the mixture was continued to heat and cooked for 1h and the liquid was poured out.After filtration,the remaining herbs were boiled in distilled water with 8 times the weight of the herbs.The two liquid extracts were then combined and concentrated to 4.48 g/mL concentration by micro-fire and stored at 4°C.

2.3. HPLC-MS analysis of SQDZ

The components in SQDZ were detected by UPLC-MS.In shortly,800μL of methanol was added into 200μL of SQDZ solution,then vortexed and mixed for 10 minutes,centrifuge at 13000 rpm for 10 minutes.The supernatant was taken and analyzed by UPLC-MS.

HPLC-MS conditions were as following:Welch AQ-C18(2.1×150 mm,1.8μm)liquid chromatographic column.Mobile phase A was composed of 0.1%formic acid aqueous solution,the mobile phase B was composed of a methanol solution,with gradient elution(0–1.0min,98%A;1–5.0min,98–80%A;5–10 min,80–50%A;10–15min,50–20%A;15–20min,20–5%A;20–27min,5–5%A;27–28min,5–98%A;28–30 min,98–98%A.The flow rate was set to 0.3 mL/min,and the column temperature was 35°C.The injection volume was 5μL.

Mass spectrometry conditions:the ionization mode was ESI,the scan range was m/z 100.0–1500.0,the spray voltage was 3.2kV,the ion source temperature was 300°C,collision gas was high-purity argon(purity $\geq 99.999\%$),collision energy(n)CE was 30,40,60.The sheath gas was nitrogen(purity $\geq$

99.999%)with 40Arb.The auxiliary gas was nitrogen(purity $\geq 99.999\%$)with 15 Arb,350°C.Data acquisition time:30.0 min.The details were as described by Lei et al¹⁶.

2.4. IMN model renal and groups

The PHN model was employed to simulate IMN through experimental preparations conducted in strict accordance with previously established protocols¹⁷.The successful induction of the IMN model was confirmed when the 24-h urinary albumin excretion reached ≥ 40 mg/24 h,whereafter the validated model rats were randomly allocated to the following five experimental groups:Model(MG),SQDZ-low(SQDZ-L,11.2g·kg⁻¹·d⁻¹),SQDZ-middle(SQDZ-M,22.4g·kg⁻¹·d⁻¹),SQDZ-high(SQDZ-H,44.8g·kg⁻¹·d⁻¹),Benazepril(BNPL,10mg·kg⁻¹·d⁻¹),n = 10 in each group.In addition,10 of the healthy Sprague–Dawley rats were selected as normal controls(NG).The normal and model groups received an equivalent volume of distilled water.SQDZ and BNPL were continuously administrated for 4 weeks.

After these 4 weeks,the 24-h urine was collected from the metabolic cages.Following sodium pentobarbital anesthesia,abdominal aortic blood sampling was performed,with subsequent serum isolation performed through centrifugation under standardized conditions(3500 rpm,4°C,10 min).Following tissue harvesting,the renal cortical tissues were subjected to dual-processing:specimens designated for histopathological and IHC examination were immersion-fixed in 4%paraformaldehyde,while the residual tissues were cryopreserved in liquid nitrogen for subsequent storage at-80°C in ultra-low temperature freezers.

2.5. Biochemical analysis

Following a 4-week therapeutic intervention,standardized biological sampling was conducted,which included systematic collection of both serum specimens and 24-h urinary outputs for subsequent biomarker analyses.The quantification of 24-h urinary protein excretion and serum biomarkers was performed using an AU5800 automated biochemistry system(Beckmann,East China,China),with comprehensive analysis covering:(1)Lipid metabolism parameters-total cholesterol(TC),triglycerides(TG),low-density lipoprotein(LDL);(2)Hepatic-renal function markers-albumin(ALB),creatinine(Cr),blood urea nitrogen(BUN),and alanine aminotransferase(ALT).

2.6. Renal histopathological assessment

Renal specimens underwent standardized histological processing comprising immersion-fixation in 4%paraformaldehyde(for 24h),paraffin-embedding,and microtome-sectioning at 4-μm thickness for microscopic evaluation.The sections were then stained with hematoxylin and eosin(H&E)to evaluate glomerular injury.Histopathological evaluation was performed of H&E-stained sections and graded as per the semi-quantitative scoring of tubulointerstitial damage severity using the following scale:intact architecture=Grade 0,<25%involvement=Grade 1,25–50%lesion area(Grade 2),51–75%pathology=Grade 3,and > 75%structural compromise=Grade 4).A higher score indicated more severe tubular injury.In addition,Image J software was used for semi-quantitative analysis of H&E-stained sections by

measuring the glomerular diameters, with the average width and height calculated for individual glomeruli.

Masson staining was performed to evaluate renal fibrosis, with collagen deposition quantified as the percentage of stained area per field of view. The periodic acid-Schiff (PAS) staining technique was employed to evaluate the severity of tubulointerstitial injury.

2.7. Immunofluorescent examination of IgG

Paraffin-embedded rat renal sections were baked, dewaxed, and hydrated, and subjected to antigen repair using citrate buffer (or pepsin). The sections were permeabilized with 0.5% Triton-X-100 for 10 min at room temperature and blocked by bovine serum ALB (Solarbio) for 30 min at 37°C, followed by incubating overnight with FITC goat anti-IgG antibody (F6258-2ML, 1/100; Sigma-Aldrich) overnight at 4°C. Following washing procedures, FITC-conjugated secondary antibody (1:100) was applied dropwise and incubated at 37°C for 45 min. After DAPI counterstaining, the sections were mounted and examined by fluorescence microscopy (CKX53, Olympus, Tokyo).

2.8. Network pharmacology analysis

Chemical constituents and their corresponding targets for SQDZ were sourced from the TCMSP database and PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) (Kim et al., 2019; Stelzer et al., 2016; Yue et al., 2017). The inclusion criteria were established as follows: compounds must exhibit an oral bioavailability (OB) of > 30% and demonstrate a drug-likeness (DL) index of > 0.18. Given the absence of hirudin records in the TCMSP database, its three-dimensional structural data in the SDF format were acquired through systematic retrieval using "Hirudin" as the primary search identifier. The SDF structures of the screened substances and hirudins were uploaded to the Swiss Target Prediction database, specifying "Homo sapiens" as the species and setting the probability threshold to > 0.1 to identify the potential compound targets.

2.9. RNA extraction and transcriptome analysis

Total RNA isolation from renal tissues was performed using Trizol reagent (ThermoFisher, 15596018). RNA concentrations and purities were assessed through Bioanalyzer 2100 and RNA6000 Nano LabChip Kit (5067 – 1511, Agilent, CA, USA). Only samples with a RIN (RNA integrity number) > 7.0 were selected for subsequent library preparation. cDNA libraries were generated through standardized protocols using the pooled RNA from <sample description> samples of <research species> followed by sequencing on the Illumina Novaseq™ 6000 platform. Principal component analysis (PCA) was conducted utilizing the princomp function of R software (<http://www.r-project.org/>) in this experience. Transcriptomic distinctions among Control, DN, and DN pGLP samples were assessed by PCA. Differentially expressed genes (DEGs) were identified with the "DESeq2" R package, based on the false discovery rate (FDR) of < 0.05 and an absolute fold change of ≥ 2 .

2.10. Building protein–protein interaction (PPI) networks and key targets enrichment analysis

A cohort of 242 differentially expressed target genes underwent systematic PPI analysis via the STRING database(<https://cn.string-db.org/>), followed by topological network construction and gene regulatory mapping through Cytoscape software(version 3.10).The core regulatory nodes within the gene interaction network were subjected to topological analysis employing the CytoMCED computational module.

Functional enrichment analysis encompassing Gene Ontology(GO)terms and Kyoto Encyclopedia of Genes and Genomes(KEGG)pathways was systematically conducted on a cohort of 242 target DEGs through the DAVID bioinformatics platform(<https://david.ncifcrf.gov>).Functionally significant annotations(FDR-corrected $p < 0.05$)were screened for subsequent pathway analysis,with enriched GO terms and KEGG pathways undergoing systematic visualization through the Chiplot bioinformatics platform(<https://www.chiplot.online/>).

2.11. Docking of molecules

The PubChem database was used to download the abovementioned key component structure files(SDF format)as ligands.Using the PDB database(<https://www.rcsb.org/>),six core target protein 3D structures were downloaded(in PDB format):namely,SERPINE1(3LW2),F10(4ETY),ITGAL(7KC3),CCR2(2BDN),CD38(4CMH),and SLC5A2(8HEZ)as receptors.The binding energy was calculated by molecular docking via the cb-dock2 online website¹⁸.

2.12. WB assay

Frozen renal tissue samples were subjected to total protein extraction using RIPA buffer,and the protein concentration was determined with the BCA Protein Assay Kit.The denatured protein was subjected to 10%SDS-PAGE.Proteins were transferred onto PVDF membranes(IPVH00010,Millipore)following SDS-PAGE and blocked with 5%nonfat milk.The primary antibody(podocin,synaptopodin,CD2-associated protein[CD2AP],IL-6,STAT3,p-STAT3,and C5b-9)underwent overnight incubation at 4°C, followed by a 2-h room temperature incubation of the secondary antibody on the PVDF membrane.The PVDF membrane was equilibrated with a chemiluminescent substrate and processed through an ultra-high sensitivity chemiluminescent imaging system for signal acquisition(anon-5200,Shanghai Tianneng Technology Co.).Finally,the protein bands were quantified using the Image J software.

2.13. Real-time PCR

Total RNA was extracted by using the RNA rapid extraction kit(SparkJade,Shandong,China)in accordance with the manufacturer's instructions.Complementary DNA synthesis was performed by using an RT kit(SparkJade),followed by quantitative PCR amplification with SYBR Green chemistry(SparkJade)to quantify the target gene expressions. β -actin mRNA was used as the control. All primer sequences utilized in this study are detailed in Table 1.The relative gene expression changes were calculated by using the $2^{-\Delta\Delta Ct}$ method.

Table 1
Primer sequences used in the study

Gene name	Forward	Reverse
F10	CATCCTCACTGCCGCCCATTTG	GCCGTCTTCCTGTTCCGTGTTC
CD38	TCCTGCTCCTGGTCGCCTTG	GGCAGCGTCCCAAGATGATGTC
ITGAL	TGACCCAGGCTACCCGCTTG	GAACCCACCAGGCTTCCCATTG
SERPINE1	GCGTCTTCCTCCACAGCCATTC	TCTCTGTTGGATTGTGCCGAACC
SLC5A2	AACAGCAGCAGCACACTCTTCAC	AACACCACCCAAAGCCTTCCAAC
CCR2	TCCTGCCCCTACTTGTCATGGTC	TGAGCCTCACAGCCCTATGCC

2.14. IHC staining and IF

The changes in the expressions of F10,ITGAL1,FABP3,and FABP4 proteins in the renal tissues of the rats after exposure to SQDZ were determined by IHC staining,as described previously(Lei et al.,2024).The tissue sections were visualized under a microscope.The regions that were stained yellow indicated the positive expression of the proteins.Image J software was utilized to analyze semi-quantitatively.

2.15. Statistical analysis

Data analysis and visualization were performed using SPSS 25.0 and GraphPad Prism 8.0 software.Continuous variables are expressed as the mean±standard deviation(SD).For comparisons among multiple groups,a one-way analysis of variance was applied for data with normal distribution and homogeneous variance,while the Mann–Whitney U-test was applied for non-normally distributed data or unequal variance.For comparisons between two groups,an independent samples t-test was used for normally distributed data with equal variance,whereas the Mann–Whitney U-test was used for non-normal distributions or unequal variance. $p < 0.05$ was considered to indicate statistical significance.

3. RESULTS

3.1. Therapeutic effects of SQDZ on biochemical parameters in PHN rats

At weeks 2 and 4,the 24-hour urinary total protein(24-hUTP)levels in the MG group were significantly higher than those in the NG group($p < 0.05$).The benazepril(BNPL),medium-dose SQDZ(SQDZ-M),and high-dose SQDZ(SQDZ-H)treatment groups showed significantly reduced 24-hour urinary protein levels compared to the MG group($p < 0.05$,Fig. 1A).There were no significant differences in serum BUN,ALB,or Cr levels among the groups,suggesting preserved renal function(Figs. 1C–E).The MG group exhibited significantly elevated ALT,TC,TG,and LDL-c levels compared to the NG group,while SQDZ-M and SQDZ-H treatment dose-dependently reversed these increases($p < 0.05$,Figs. 1F–I).BNPL had no such effects.

3.2. SQDZ improved renal pathological manifestations in PHN rats

H&E staining revealed normal renal structure in the NG group, while the MG group exhibited significant pathological alterations including inflammatory cell infiltration, tubular epithelial cell detachment, and protein cast formation, along with significantly increased glomerular diameter compared to NG. Treatment with SQDZ-M, SQDZ-H, and BNPL markedly ameliorated these pathological changes and reduced glomerular diameter, with SQDZ-L showing moderate improvement (Figs. 2A and E).

PAS staining demonstrated distinct glycogen accumulation patterns. No glycogen deposition was observed in NG renal tissues, whereas MG exhibited substantial glycogen accumulation. Treatment with SQDZ-M, SQDZ-H, and BNPL significantly reduced both glycogen deposition and tubular injury (Figs. 2B and F).

Masson's trichrome staining revealed renal fibrotic alterations (Figs. 2C and G). The NG group showed no collagen fiber proliferation, while the MG group exhibited extensive collagen deposition, indicating severe renal fibrosis. Both SQDZ-M and SQDZ-H groups demonstrated significantly reduced collagen fiber content compared to the MG and BNPL groups.

Immunofluorescence (IgG) staining further validated the therapeutic efficacy of SQDZ (Figs. 2D and H). While no IgG deposition was detected in the NG group, substantial IgG accumulation was observed in the MG group. Treatment with SQDZ-M, SQDZ-H, and BNPL significantly attenuated IgG deposition. **Fig. 2.** SQDZ alleviates renal pathological injury in PHN rats. (A) H&E staining by confocal microscopy ($\times 400$, scale bar = $50\mu\text{m}$) of rats in each group. (B) PAS staining (scale bar = $50\mu\text{m}$). (C) Masson staining (scale bar = $50\mu\text{m}$). (D) IgG expression in nephridial tissues (scale bar = $50\mu\text{m}$). (E) Statistical results of glomerulus diameter. (F) Tubular injury scores in different groups. (G) Statistical results of the renal fibrosis area. (H) The mean fluorescence intensity of IgG fluorescence (green) in each group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ when compared to the normal group (NG). $^*p < 0.05$ and $^{**}p < 0.01$ when compared to the model group (MG).

3.3. The components and related targets analysis in SQDZ by database

By integrating data from the DisGeNET, GeneCards, and OMIM databases, a total of 1,939 IMN-related genes were identified. Simultaneously, 677 potential targets of SQDZ were predicted using the SwissTargetPrediction database (Fig. 3A&C). From these, 242 overlapping genes were identified as potential therapeutic targets of SQDZ in the treatment of IMN (Fig. 3F). Subsequently, a compound–target (C–T) interaction network was constructed based on the compounds associated with these 242 genes. As shown in Fig. 3F, the C–T network comprised 360 nodes (i.e., 118 candidate compounds and 242 targets) and 1,869 edges.

3.4. The components and related targets analysis in SQDZ by HPLC-MS

Through UPLC-MS, a total of 588 compounds were identified in SQDZ under both positive- and negative-ion modes (Fig. 3B). Among them, Baicalin, Astragaloside II, and Verbascoside, which are known bioactive components of *Astragalus membranaceus* Bunge, were detected. The markers of *Cornus officinalis* Siebold & Zucc including Loganin, Morroniside were also detected. Furthermore, atractylodin which have been reported to be major ingredients found in *Atractylodes Macrocephala* Koidz. The top 20 most abundant compounds are listed in Table 2.

Subsequently, we input the 588 chemical components detected by HPLC-MS into the TCMSP database, setting OB greater than 30% and DL greater than or equal to 0.18 as screening criteria, and found 36 components. We then further searched the TCMSP database to identify the targets corresponding to the active components. We obtained the intersection genes between IMN-related genes and SQDZ-HPLC/MS-related genes (Figure 3E), the C-T network shown in Fig. 3G. In the C-T network consisted of 79 nodes (including 25 candidate compounds and 54 target proteins) and 215 interaction edges.

Finally, there were 261 interaction targets which SQDZ were related to IMN (Fig. 3H). These results highlighted multi-target and systemic therapeutic potential of SQDZ in managing IMN.

Table 2
Characterization of the top 20 active compounds in SQDZ detected by UPLC-MS.

Name	Formula	m/z	RT [min]	Ration (%)
Baicalin	C ₂₁ H ₁₈ O ₁₁	447.0914	14.684	4.77
(1R,2R,5S,8R,10R,14R)-20-hydroxy-1,2,14,18,18-pentamethyl-17-oxo-8-(prop-1-en-2-yl)pentacyclo-henicosane-5-carboxylic acid	C ₃₀ H ₄₆ O ₄	453.33536	18.47	4.66
Glycyrrhizic Acid, Ammonium Salt	C ₄₂ H ₆₂ O ₁₆	821.39587	18.483	3.54
C18(Plasm)-18:1 PC	C ₄₄ H ₈₆ NO ₇ P	284.29428	22.651	2.74
(S)-MALATE	C ₄ H ₆ O ₅	133.01286	1.702	2.72
Glutamic acid	C ₅ H ₉ NO ₄	148.06015	1.271	2.61
L-(+)-Arginine	C ₆ H ₁₄ N ₄ O ₂	175.11873	1.064	2.27
(2S,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[(5-hydroxy-8-methoxy-4-oxo-2-phenyl-4H-chromen-7-yl)oxy]oxane-2-carboxylic acid	C ₂₂ H ₂₀ O ₁₁	461.10696	15.338	2.25
Nuciferine	C ₁₉ H ₂₁ NO ₂	296.16403	11.054	2.12
5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	127.03909	5.553	2.05
L-PROLINE	C ₅ H ₉ NO ₂	116.07079	1.38	1.82
L-Glutamic acid	C ₅ H ₉ NO ₄	146.04449	1.281	1.67
5-Hydroxy-1-tetralone	C ₁₀ H ₁₀ O ₂	163.07506	18.19	1.47
L-ASPARAGINE	C ₄ H ₈ N ₂ O ₃	133.06065	1.216	1.34
Phytosphingosine	C ₁₈ H ₃₉ NO ₃	318.29959	14.902	1.31
D-(-)-quinic acid	C ₇ H ₁₂ O ₆	191.0551	1.508	1.30
Phellodendrine chloride	C ₂₀ H ₂₄ ClNO ₄	342.16946	8.759	1.29
CITRATE	C ₆ H ₈ O ₇	191.01886	2.49	1.20
Ligustilide	C ₁₂ H ₁₄ O ₂	191.10651	16.733	1.19
Hesperetin-7-O-rutinoside	C ₂₈ H ₃₄ O ₁₅	387.11392	1.459	1.02

3.5. Pathway enrichment analysis and PPI analysis

A PPI network of the 261 identified targets was constructed using the STRING database by integrating the SQDZ-associated targets with the IMN-related genes. The network was visualized using Cytoscape software, revealing a PPI network consisting of 226 nodes and 1,756 edges (Fig. 4A). To identify the key targets, topological parameters were calculated using Cytoscape, while hub genes were screened and analyzed with the CytoMCED plugin. As shown in Fig. 5C, nodes with a degree centrality ≥ 30.00 were considered the hub targets. Among them, IL-6 and STAT3 were identified as the core hub genes.

KEGG pathway enrichment analysis suggested that SQDZ alleviates kidney injury in IMN by modulating multiple signaling pathways, including the PI3K-Akt-signaling pathway, NF- κ B-signaling pathway, and necroptosis-related pathway (Fig. 4B). GO enrichment analysis demonstrated that putative target-associated biological processes were predominantly involved in cellular migration activation, RNA polymerase II-driven transcriptional upregulation, and inflammatory signaling pathways. Cellular component enrichment was predominantly associated with the plasma membrane, while molecular function enrichment was primarily related to protein binding (Fig. 4C). These findings demonstrated that SQDZ exerts its therapeutic action in IMN through the modulation of multiple signaling pathways.

3.6. Effects of SQDZ on the transcriptome of rat nephridial tissues

To further investigate the mechanism through which SQDZ alleviates renal pathological injury in PHN rats, RNA-sequencing (RNA-seq) was performed to analyze the gene expression in renal tissues from the MG and SQDZ-M groups. When compared with MG, the SQDZ-M group exhibited 274 DEGs ($p < 0.05$, $|\log_2 \text{fold change}| > 1$), with 94 upregulated and 180 downregulated genes (Fig. 5A). GO enrichment analysis of these DEGs is shown in Fig. 6B. The significantly enriched terms were primarily involved in immune responses, leukocyte-dependent defense mechanisms, and the modulation of inflammatory signaling cascades. The enriched molecular functions involved immune activity and cytokine receptor activity, while the enriched cellular components were mainly associated with the external side of the plasma membrane. The KEGG pathway analysis (Fig. 5C) demonstrated that the DEGs showed significant enrichment in the biological pathways such as cytokine–cytokine receptor interaction, cell-adhesion molecules, hematopoietic cell lineage, and complement and coagulation cascades. These findings suggest that SQDZ alleviates renal injury in PHN rats primarily by modulating immune and inflammatory responses as well as the complement and coagulation cascades.

To further identify the core hub genes targeted by SQDZ in PHN rats, the intersection between the DEGs and SQDZ-related targets was analyzed. This finding revealed nine common genes, including *Cd38*, *F10*, *Slc5a2*, *Itgal*, *Ccr2*, and *Serpine1* (Fig. 5D). A heatmap of six significantly downregulated genes—*Cd38*, *F10*, *Slc5a2*, *Itgal*, *Ccr2*, and *Serpine1*—demonstrated marked downregulation following SQDZ treatment (Fig. 5E).

3.7. Molecular docking analysis of SQDZ to the intersection genes

Molecular docking validation was conducted between the active compounds in SQDZ and the six identified core protein targets. The analysis revealed that 44 compound components were capable of docking with these targets (Fig. 6). The binding energies between the active compounds and the protein targets ranged from -9.6 to -6.5 kcal/mol, with the majority of interactions exhibiting binding energies of ≤ -7.0 kcal/mol, indicating strong binding affinities between the core constituents of SQDZ and the key target proteins. Among these, the compounds demonstrating the highest binding affinities to the six targets included telocinobufagin, cerevisterol, gemin D, gallic acid-3-O-(6'-O-galloyl)-glucoside, 5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside, and kadsurenone.

3.8 Effect of SQDZ on immune and inflammatory pathway via regulation of the IL-6/STAT3 signaling axis in the PHN model

Podocyte injury is a key pathological feature of IMN, with inflammatory pathway activation playing a central role in its pathogenesis. KEGG pathway enrichment analysis indicated that SQDZ primarily modulates immune and inflammatory responses to ameliorate IMN. Both network pharmacology and RNA-seq analyses identified *IL-6*, *STAT3*, *CCR2*, and *CD38* as the core target genes through which SQDZ exerts its therapeutic effects. In this study, we further investigated podocyte injury markers and the IL-6/STAT3-related inflammatory pathway using WB and RT-PCR, the results were shown in Fig. 7.

WB results revealed that, relative to NG, the expressions of C5b-9, podocin, synaptopodin, and CD2AP in the renal tissues of MG rats were markedly reduced. Treatment with SQDZ significantly upregulated the protein levels of C5b-9, podocin, synaptopodin, and CD2AP when compared with MG. Additionally, the protein levels of IL-6 and the p-STAT3/STAT3 ratio were markedly increased in MG compared with NG, while these were significantly reduced following SQDZ-M treatment. RT-PCR analysis revealed that *ccr2*, *cd38*, and *tnf* mRNA expressions were significantly upregulated in the renal tissues of MG rats when compared with that in the NG rats. SQDZ-M treatment effectively downregulated the expression of *ccr2* and *cd38*.

The experimental evidence demonstrated that SQDZ protects podocytes and alleviates IMN by suppressing IL-6/STAT3-mediated immune and inflammatory activation.

3.9 Effect of SQDZ on complement and coagulation cascades pathway via regulation of F10/Serpine1/Itgal in the PHN model

IHC and IF staining were utilized to interrogate SQDZ-mediated modulation of complement and coagulation cascades in PHN rat models. As shown in Fig. 8, IMN mice exhibited significantly increased renal expressions of F10, PAI1, and Itgal in contrast with that in the normal control mice. However, the administration of SQDZ markedly reduced the expression of F10, Serpine1, and Itgal in the renal tissues when compared with that in the model group. Furthermore, RT-PCR analyses showed concordance with IHC and IF data, suggesting that SQDZ administration significantly suppressed F10, Serpine1, and Itgal mRNA levels.

3.10. Effect of SQDZ on SLC5A2 in the PHN model

IHC and RT-PCR analyses were conducted to assess SLC5A2 expression. As shown in Fig. 9, when compared with NG, both mRNA and protein expressions of SLC5A2 in the renal tissues of MG rats demonstrated a marked elevation. However, treatment with SQDZ significantly reduced the SLC5A2 expression in contrast with that by MG.

4. DISCUSSION

We used a PHN rat model to evaluate how SQDZ treats IMN. These findings further confirmed that SQDZ not only improved renal dysfunction (as evidenced by decreased 24-hUTP, ameliorated dyslipidemia, and regulation of lipid imbalances such as high cholesterol) but also alleviated renal tissue lesions (including reduced GBM thickening, IgG deposition, and tubulointerstitial fibrosis) in IMN model rats. Pathway enrichment analysis revealed that the therapeutic effects of SQDZ in IMN may be mediated through the regulation of immune and inflammatory responses, as well as the complement and coagulation cascade pathways.

Podocyte injury is a central pathological feature in the development of membranous nephropathy and is considered a primary target in IMN. Podocytes are terminally differentiated cells located in the glomerular septum, where they regulate glomerular filtration via the slit diaphragm. Key structural proteins of the slit diaphragm—including podocin, synaptopodin, C5b-9, and CD2AP—are essential for maintaining the integrity of the glomerular filtration barrier. Persistent podocyte injury can lead to podocyte detachment and death, ultimately resulting in progressive renal damage and renal failure. In this study, SQDZ significantly reduced the expression of podocyte injury markers, including C5b-9, podocin, synaptopodin, and CD2AP.

Aberrant immune and inflammatory responses are known to exacerbate podocyte injury. Previous network pharmacology analysis identified IL-6 and STAT3 as the key therapeutic targets of SQDZ in IMN. Past research has flagged IL-6 and STAT3 as SQDZ's main targets^{19,20}. IL-6, a pleiotropic cytokine abundantly expressed in both immune cells and resident renal cells, demonstrated a significant upregulation across diverse nephrotic syndromes. This cytokine initiates downstream JAK2/STAT3 signaling through dual activation mechanisms—classical membrane-bound receptor signaling and soluble receptor-mediated trans-signaling—thereby exerting pivotal regulatory control over cellular proliferation, apoptotic processes, immune homeostasis, and inflammatory responses²¹. In trans-signaling, IL-6 binds to its soluble receptor to form an IL-6/soluble interleukin-6 receptor complex that activates STAT3 phosphorylation, triggering intracellular signaling²². This event leads to the upregulation of downstream effector molecules such as CCR2²³, which amplifies inflammatory signaling and promotes the release of pro-inflammatory cytokines, including TNF- α ²⁴. CD38, a multifunctional transmembrane glycoprotein, is predominantly expressed in natural killer cells, dendritic cells, and B cells. It is also recognized as a principal source of autoantibodies against PLA2R and other nephritogenic autoantibodies²⁵. The activation of the JAK-STAT1 pathway has been shown to upregulate the CD38 expression, while increased enzymatic activity of CD38 can further activate the NF- κ B pathway. This

cascade promotes IL-6 expression,enhances B-cell differentiation,and stimulates the production of autoantibodies²⁶,thereby intensifying the autoimmune response.Transcriptome and WB analyses confirmed significantly elevated expression of IL-6,p-Stat3/Stat3,Ccr2,Cd38,and TNF in the renal tissues of PHN rats.Notably,SQDZ treatment markedly normalized the expressions of these critical genes and proteins.These findings strongly indicate that SQDZ exerts its nephroprotective effects by mitigating podocyte injury caused by immune complexes and inflammatory mediators through the inhibition of the IL-6/STAT3 pathway,thereby delaying IMN progression.

The risk of a hypercoagulable state and thrombosis is common in IMN and is associated with massive proteinuria,hypoalbuminemia,endothelial injury,and platelet activation²⁷.Hypoalbuminemia stimulates compensatory hepatic synthesis of high-molecular-weight procoagulant proteins.Moreover,the compromise of the glomerular filtration barrier induces urinary excretion of low-molecular-weight anticoagulant proteins,including antithrombin III and plasminogen,which consequently diminishes endogenous anticoagulant activity.*Serpine1* encodes plasminogen activator inhibitor-1(PAI-1),primarily produced by endothelial cells.PAI-1 is a known risk factor for thrombosis²⁸;it exerts regulatory control over the fibrinolytic system through dual inhibition of both tissue-type plasminogen activator(tPA)and urokinase-type plasminogen activator(uPA),thereby suppressing plasminogen activation and fibrin degradation,ultimately promoting thrombosis²⁹.F10 plays a central role in the coagulation cascade.Its active form,coagulation factor Xa,not only directly induces hypercoagulability by activating platelets via thrombin but also indirectly stimulates protease-activated receptors on glomerular endothelial cells.This event leads to increased PAI-1 secretion,reduced fibrinolytic activity,and a heightened risk of thrombosis and renal injury³⁰.ITGAL binds to its primary ligand,intercellular adhesion molecule-1,facilitating leukocyte adhesion,and migration across the vascular endothelium.This interaction further upregulates PAI-1 expression and suppresses fibrinolysis³¹.In our study,the KEGG pathway enrichment analysis indicated that SQDZ may exert regulatory effects on the complement and coagulation cascades.Further verification experiments revealed that SQDZ downregulated the gene and protein expression of *Serpine1*,F10,and *Itgal* in the renal tissues of PHN rats.These findings suggested that SQDZ alleviates the hypercoagulable state in IMN by inhibiting the *Serpine1*/F10/*Itgal* pathway,thereby enhancing fibrin degradation.

SLC5A2,located on human chromosome 16p11.2,encodes the sodium-glucose cotransporter 2(SGLT2),which is predominantly expressed in the S1 and S2 segments of the renal proximal tubule.SGLT2 facilitates the reabsorption of glucose and sodium ions,limiting their urinary excretion,which contributes to glomerular hyperperfusion and hyperfiltration,thereby exacerbating proteinuria and renal impairment^{32–34}.In this study,RNA-seq and IHC results demonstrated that SQDZ significantly reversed the elevated expression of *SLC5A2* in the renal tissues of PHN model rats,suggesting a renal protective effect akin to that of SGLT2 inhibitors.Furthermore,molecular docking analysis revealed that several active compounds in SQDZ—including 5'-hydroxyiso-muronulato1-2',5'-di-O-glucoside,9,10-dimethoxypterocarpan-3-O-β-D-glucoside,isomnucronulato1-7,iridoids,2'-di-O-glucosioleand,cornin,and loganin—exhibited a strong binding affinity to SGLT2.These constituents may

represent the key active ingredients in SQDZ responsible for its beneficial effects on the hyperosmotic state observed in IMN.

5. Conclusions

This study, integrating RNA-seq, network pharmacology, molecular biology, and animal experiments, provides compelling evidence that SQDZ mitigates IMN by targeting multiple mechanisms. These mechanisms include modulation of immune and inflammatory pathways, regulation of complement and coagulation cascades, and the inhibition of renal reabsorption processes. SQDZ primarily acts on the expression of CD38, F10, SLC5A2, Itgal, CCR2, and Serpine1, highlighting its multifaceted therapeutic potential in the treatment of IMN.

Declarations

Ethics statement

The animal study was approved by Animal experiments were conducted in compliance with the regulations of the Experimental Animal Ethics Committee of the Zhejiang Academy of Traditional Chinese Medicine (approved No.: 2025023). And were performed under the national standards of Institutional Animal Care and Use Committee. The study was conducted in accordance with the local legislation and institutional requirements.

CRediT authorship contribution statement

LZY: Conceptualization, Methodology, Formal analysis, Visualization, Writing-original draft, Writing-review and editing. **SL**: Conceptualization, Methodology, Formal analysis, Visualization, Writing-original draft, Writing-review and editing. **SSL**: Conceptualization, Methodology, Formal analysis, Visualization, Writing-original draft, Writing-review and editing. **MC**: Conceptualization, Methodology, Formal analysis, Visualization, Writing-original draft, Writing-review and editing. **YJZ**: Funding acquisition, Supervision, Visualization, Writing-original draft, Writing-review and editing. **QC**: Funding acquisition, Project administration, Writing-original draft, Writing-review and editing.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

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Figures

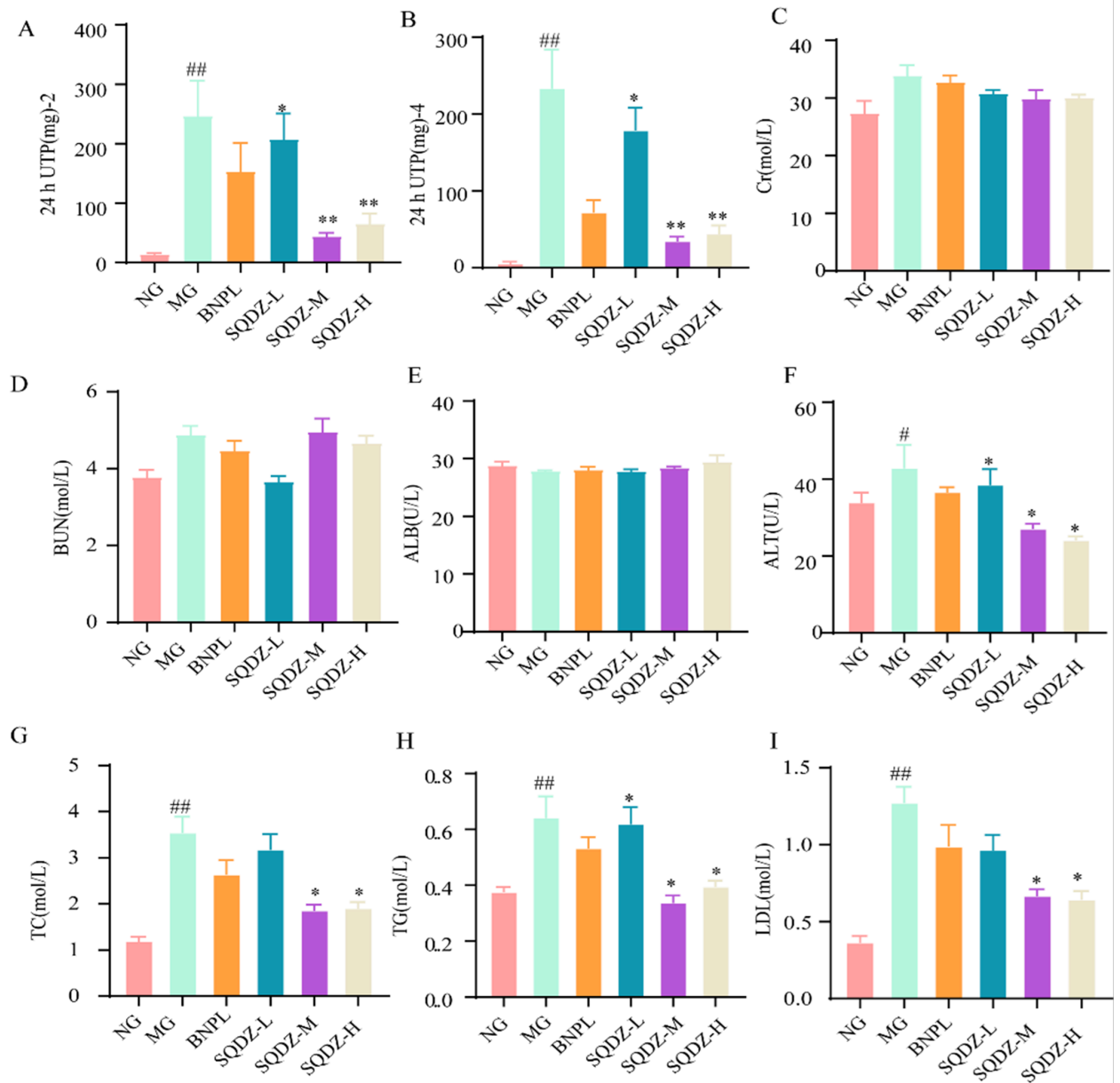


Figure 1

Therapeutic effects of SQDZ on biochemical parameters in IMN rats. **(A)** 24-h urinary protein-2 (24-h UTP) for 2nd week. **(B)** 24-h urinary protein (24-h UTP) for 4th week. **(C)** Serum creatinine (Cr). **(D)** Serum Urea Nitrogen (BUN). **(E)** Serum albumin (ALB). **(F)** Serum alanine aminotransferase (ALT). **(G)** Serum total cholesterol (TC). **(H)** Serum triglycerides (TG). **(I)** Serum low-density lipoprotein (LDL). # $p < 0.05$ and ## $p < 0.01$ when compared to the normal group (NG). * $p < 0.05$ and ** $p < 0.01$ when compared to the model group (MG), $n = 10$.

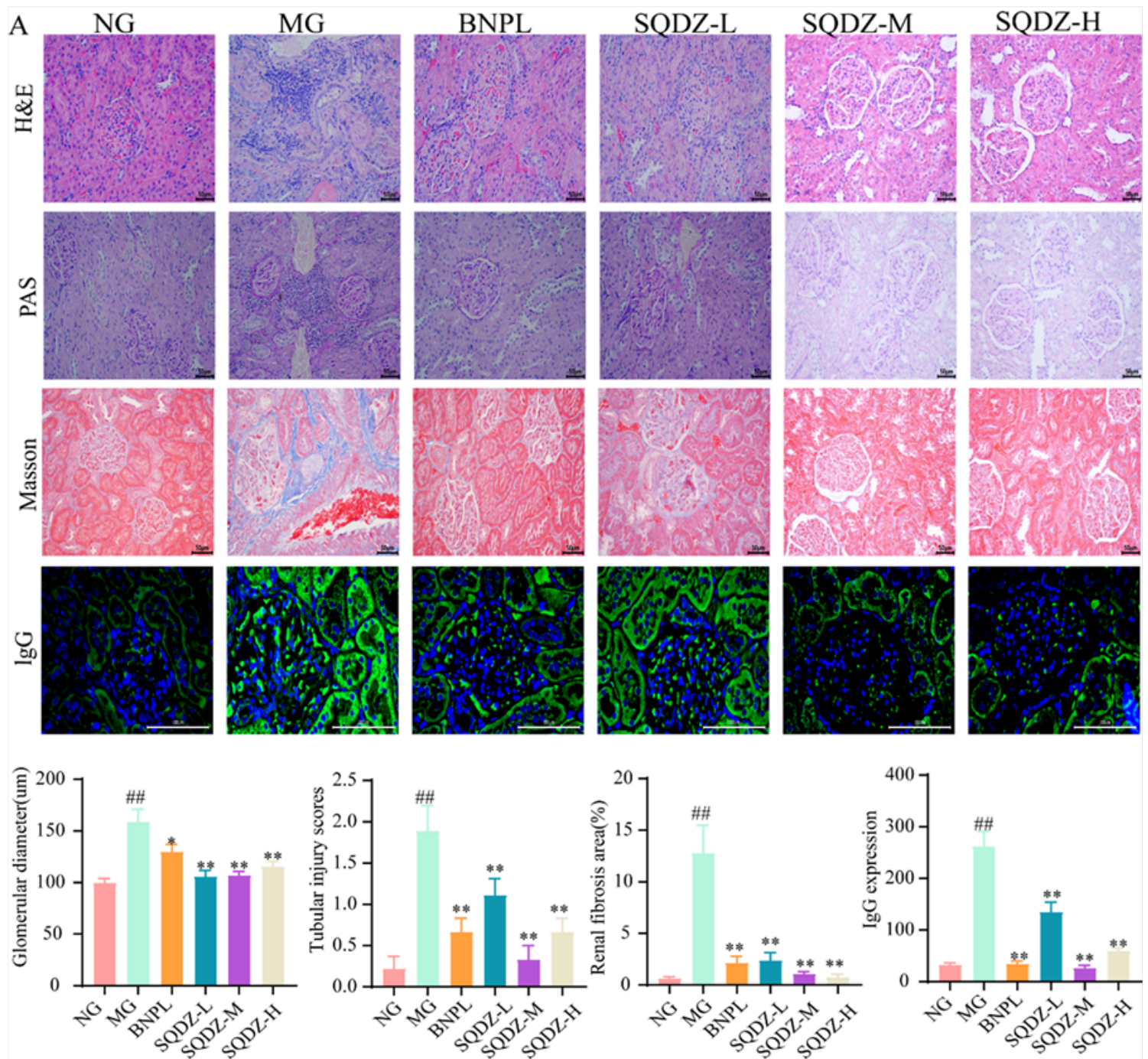


Figure 2

SQDZ alleviates renal pathological injury in PHN rats. **(A)** H&E staining by confocal microscopy ($\times 400$, scale bar = $50\mu\text{m}$) of rats in each group. **(B)** PAS staining (scale bar = $50\mu\text{m}$). **(C)** Masson staining (scale bar = $50\mu\text{m}$). **(D)** IgG expression in nephridial tissues (scale bar = $50\mu\text{m}$). **(E)** Statistical results of glomerulus diameter. **(F)** Tubular injury scores in different groups. **(G)** Statistical results of the renal fibrosis area. **(H)** The mean fluorescence intensity of IgG fluorescence (green) in each group. [#] $p < 0.05$ and ^{##} $p < 0.01$ when compared to the normal group (NG). ^{*} $p < 0.05$ and ^{**} $p < 0.01$ when compared to the model group (MG).

database in SQDZ to IMN-related target by database.(E)C-T network diagram about compounds detected by HPLC-MS in SQDZ to IMN-related target.(H)Venn diagram about merged IMN-related target to SQDZ.

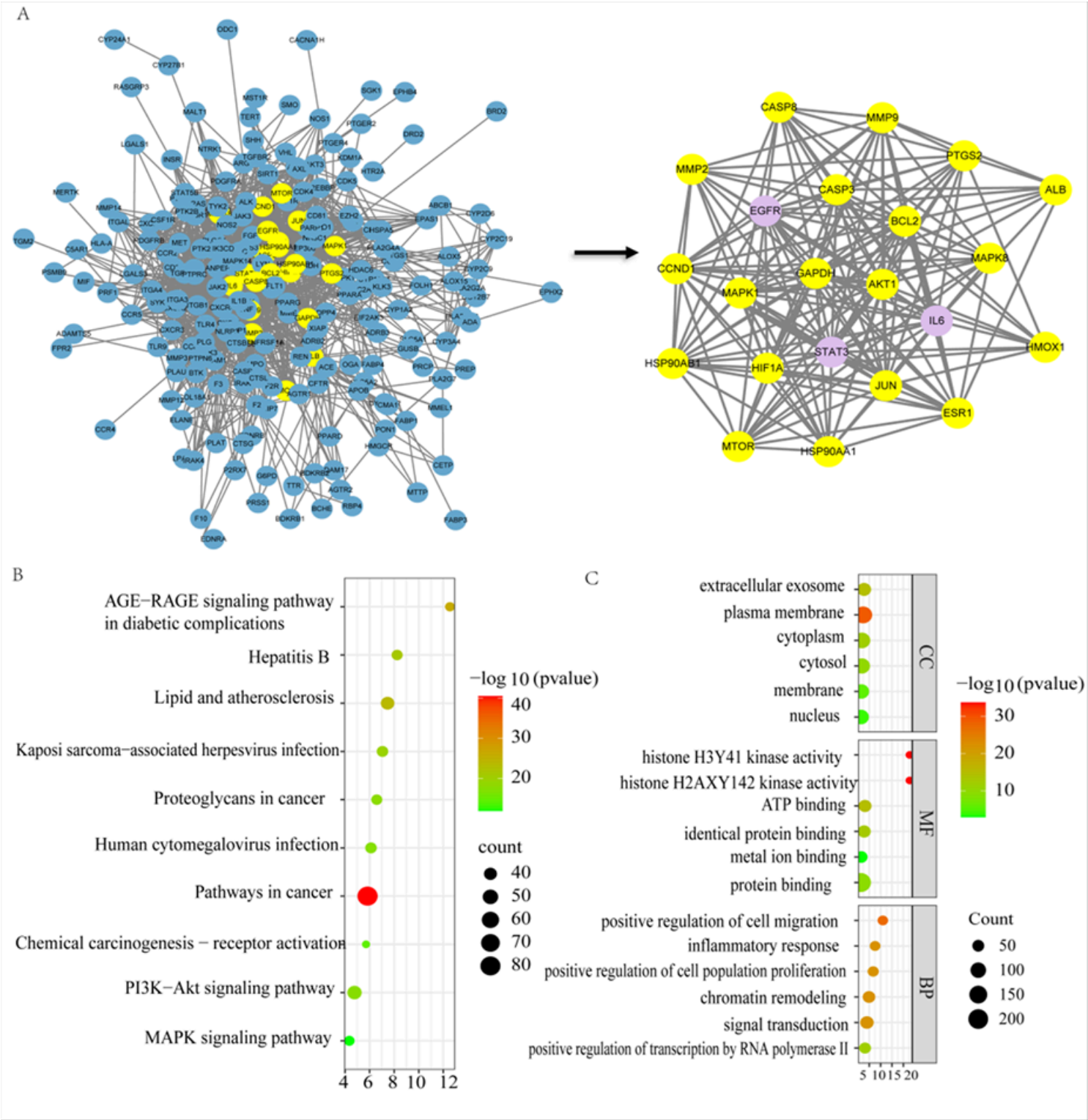


Figure 4

PPI analysis and pathway enrichment analysis. **(A)**PPI network and hub genes about the SQDZ-associated targets with the IMN-related targets. **(B)**KEGG enrichment analysis bubble chart for anti-IMN targets of QSDZ. **(C)**GO enrichment analysis of the anti-IMN targets of SQDZ.

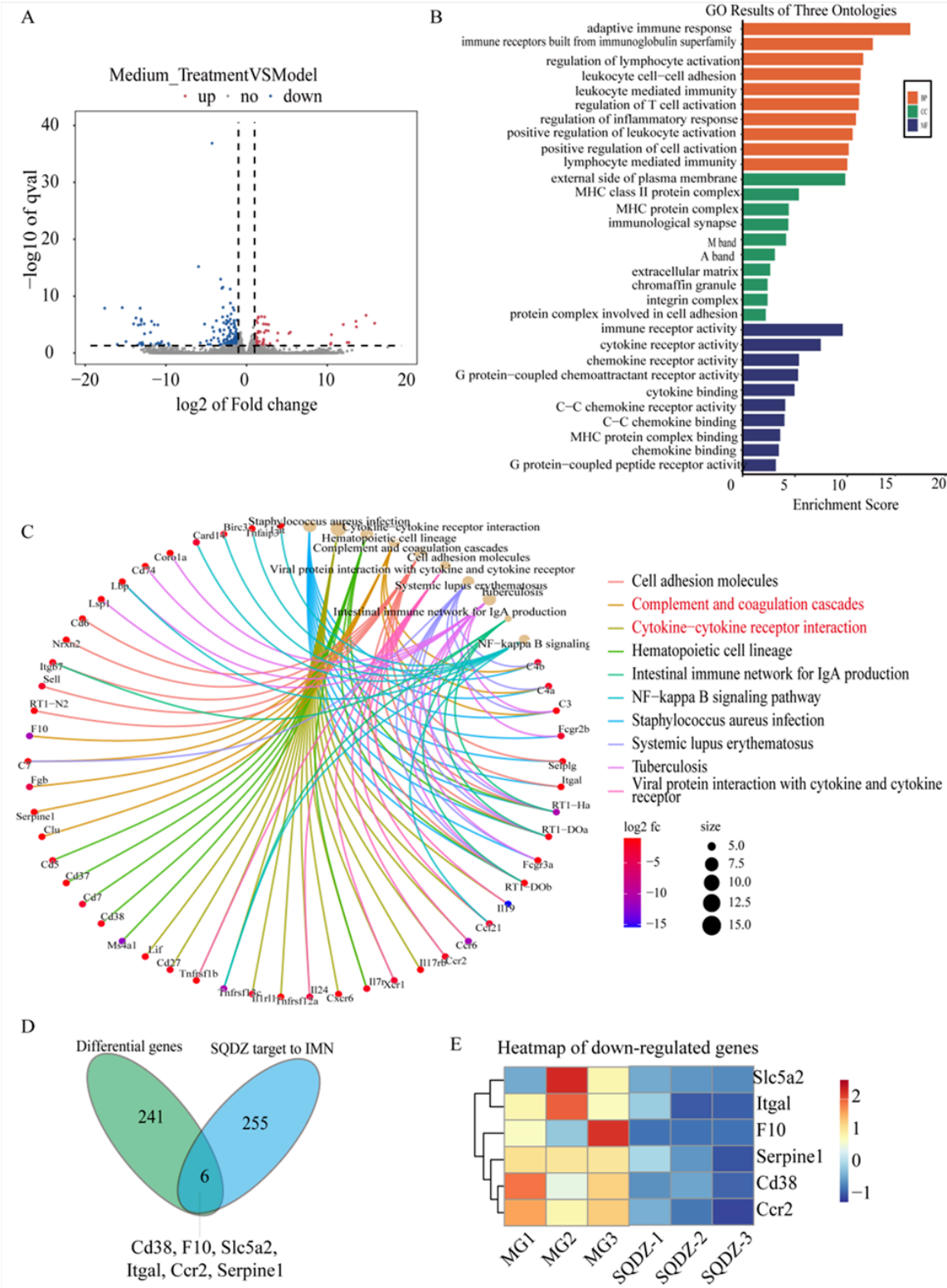


Figure 5

Effect of SQDZ on the transcriptome of rat renal tissues. **(A)**Enrichment analysis of differentially expressed genes between the MG and SQDZ-M groups. **(B)**Results of GO enrichment analysis(first 10 pathways). **(C)**Results of KEGG enrichment analysis(first 10 pathways). **(D)**Intersection of different genes by RNA-seq and SQDZ-related genes by network pharmacology in Wynn diagram. **(E)**The heatmap about the six genes between the MG and SQDZ-M groups

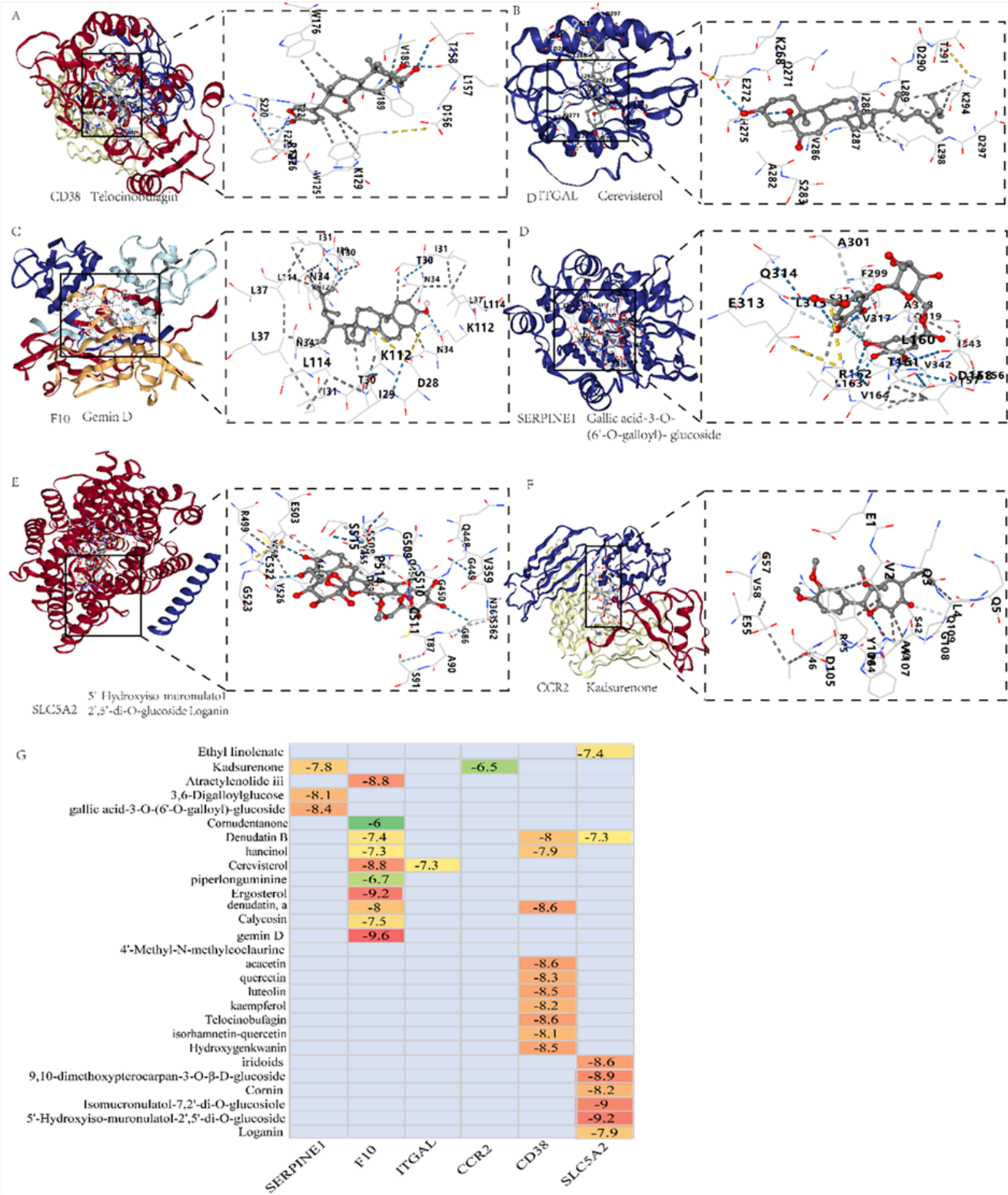


Figure 6

Molecular docking analysis of SQDZ to the intersection genes. **(A–F)** The highest docking scores were between active compounds and six targets. **(G)** The heat map presents molecular docking results between the hub targets and active compounds.

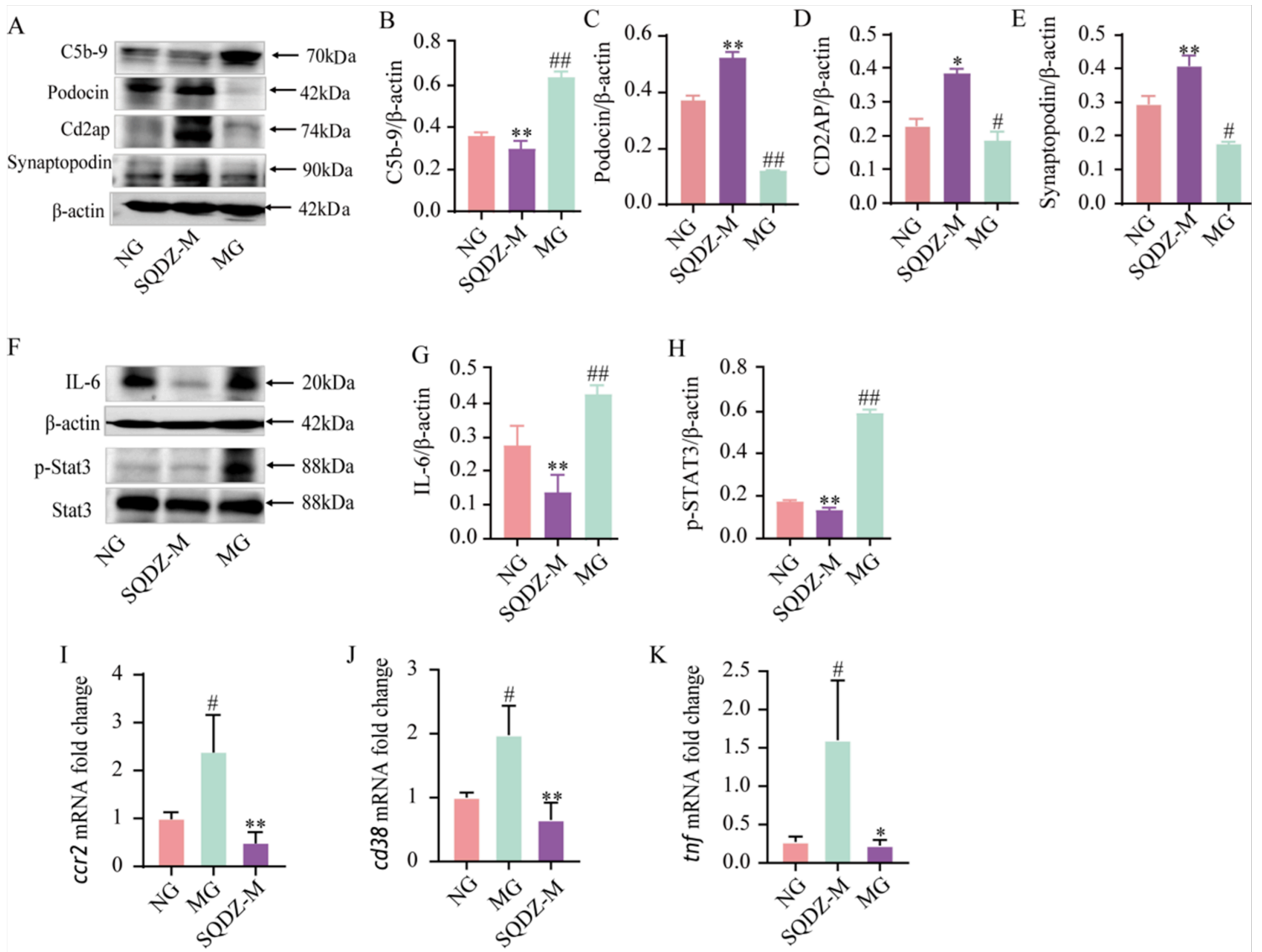


Figure 7

The effect of SQDZ on renal podocyte markers in PHN rats. **(A)** Protein bands of C5b-9, podocin, synaptopodin, and CD2AP in the renal tissues. **(B–E)** Semi-quantitative analysis of podocyte markers expression. **(F)** A protein band of IL-6, p-STAT3/STAT3 in the renal tissues. **(G–H)** Semi-quantitative analysis of IL-6, p-STAT3/STAT3 expression. **(J–K)** *ccr2* and *cd38* mRNA expression in renal tissues. [#] $p < 0.05$ and ^{##} $p < 0.01$ when compared to the normal group (NG). ^{*} $p < 0.05$ and ^{**} $p < 0.01$ when compared to the model group (MG).

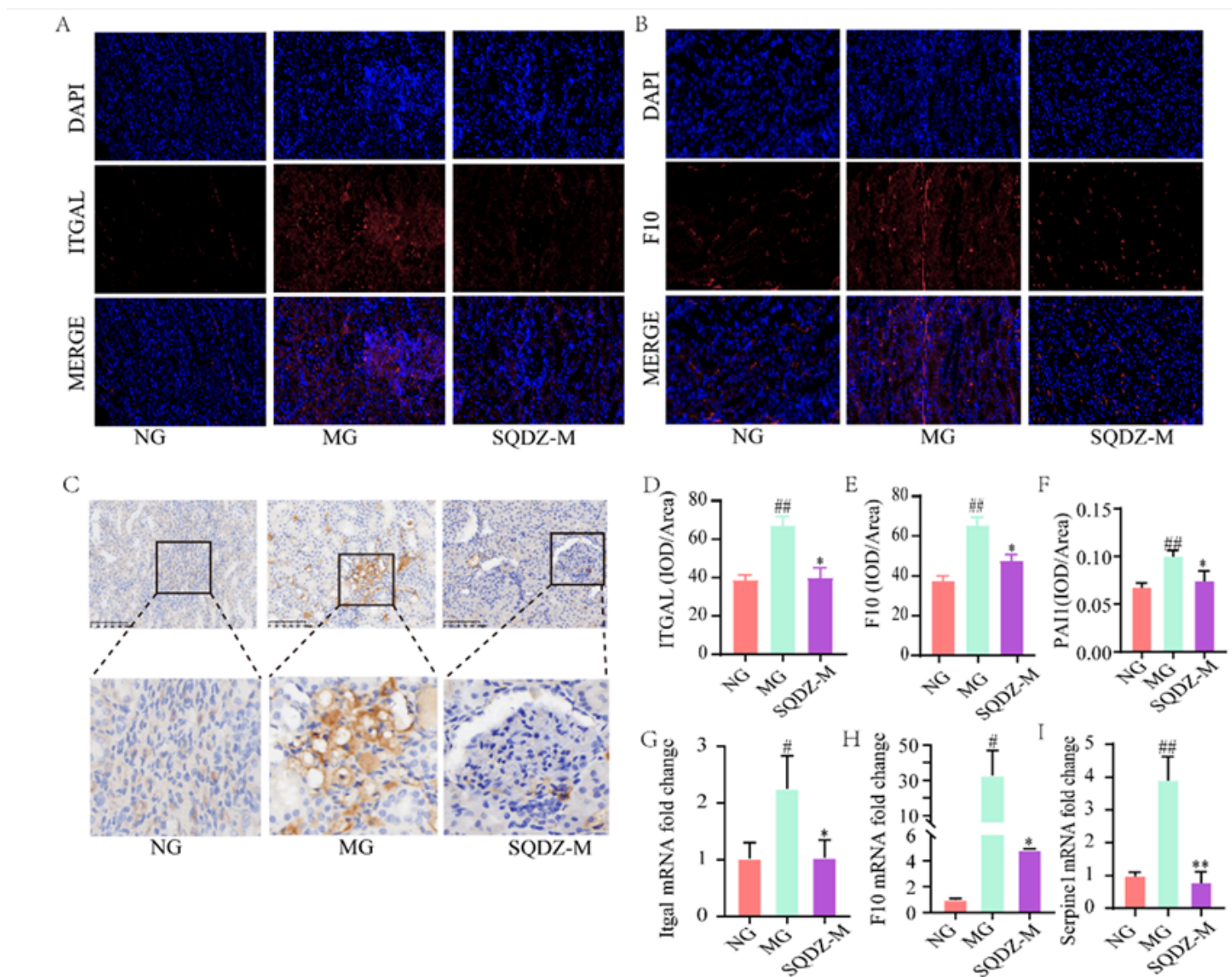


Figure 8

The effect of SQDZ on regulating F10/Serpine1/Itgal in the PHN model. **(A)** The ITGAL protein expression was performed by immunofluorescence (200×). **(B)** The F10 protein expression was performed by immunofluorescence (200×). The red and blue fluorescence represents the expression of F10/ITGAL, and DAPI, respectively. **(C)** The PAI1 protein expression was performed by immunohistochemistry (200×). **(D–F)** Semi-quantitative analysis of Itgal, F10, and Serpine1 by IF/IHC in renal tissues. **(G–H)** The *Itgal*, *F10*, and *Serpine1* mRNA expression by RT-PCR in renal tissues. [#] $p < 0.05$ and ^{##} $p < 0.01$ when compared to the normal group (NG). ^{*} $p < 0.05$ and ^{**} $p < 0.01$ when compared to the model group (MG).

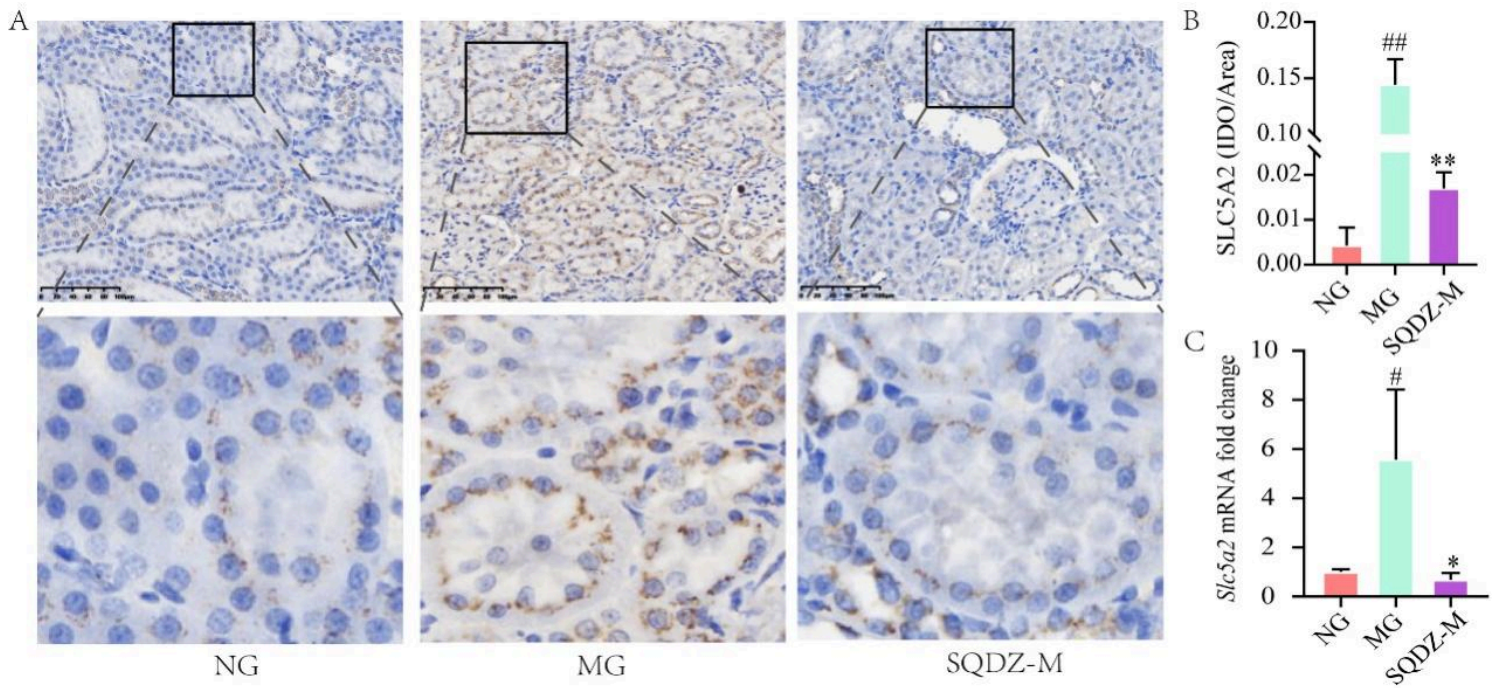


Figure 9

The effect of SQDZ on SLC5A2 expression in the PHN model. **(A)** SLC5A2 expression by IHC staining ($\times 200$, scale bar = $100\mu\text{m}$) of rats in each group. **(B)** Semi-quantitative analysis of SLC5A2. **(C)** *Slc5a2* mRNA expression in renal tissue. # $p < 0.05$ and ## $p < 0.01$ when compared to the normal group (NG). * $p < 0.05$ and ** $p < 0.01$ when compared to the model group (MG).

Supplementary Files

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