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ORIGINAL RESEARCH

Sanqi Qushi Granule Alleviates Proteinuria and Podocyte Damage in NS Rat: A Network Pharmacology Study and in vivo Experimental Validation

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Background: Nephrotic syndrome (NS) and its numerous complications remain the leading causes of morbidity and mortality globally. Sanqi Qushi granule (SQG) is clinically effective in NS. However, its potential mechanisms have yet to be elucidated.

Methods: A network pharmacology approach was employed in this study. Based on oral bioavailability and drug-likeness, potential active ingredients were picked out. After acquiring overlapping targets for drug genes and disease-related genes, a component-target-disease network and protein–protein interaction analysis (PPI) were constructed using Cytoscape, followed by GO and KEGG enrichment analyses. Adriamycin was injected into adult male Sprague-Dawley (SD) rats via the tail vein to establish NS model. Kidney histology, 24-hr urinary protein level, creatinine (Cr), blood urea nitrogen (BUN), triglyceride (TG), total cholesterol (TC), and low-density lipoprotein (LDL-C) level were assessed. Western blotting, immunohistochemistry, and TUNEL staining were applied.

Results: In total, 144 latent targets in SQG acting on NS were screened by a network pharmacology study, containing AKT, Bax, and Bcl-2. KEGG enrichment analysis suggested that PI3K/AKT pathway was enriched primarily. In vivo validation results revealed that SQG intervention ameliorated urine protein level and podocyte lesions in the NS model. Moreover, SQG therapy significantly inhibited renal cells apoptosis and decreased the ratio of Bax/Bcl-2 protein expression. Moreover, we found that Caspase-3 regulated the PI3K/AKT pathway in NS rats, which mediated the anti-apoptosis effect.

Conclusion: By combining network pharmacology with experimental verification in vivo, this work confirmed the treatment efficacy of SQG for NS. SQG protected podocyte from injury and inhibited kidney apoptosis in NS rats via the PI3K/AKT pathway at least partially.

Keywords: Sanqi Qushi granule, nephrotic syndrome, podocyte injury, kidney apoptosis, network pharmacology, PI3K/AKT pathway

Introduction

Nephrotic syndrome (NS) manifests a range of clinical conditions of massive proteinuria, edema, hypoproteinemia, and hyperlipidemia.[1] Patients with NS are generally more susceptible to hypertension, thrombotic events, and serious infections.[2] Prednisone is often the first-line therapy for NS.[3] Management of NS is very challenging because 10%–20%of patients develop steroid-resistant nephrotic syndrome (SRNS).[3] Moreover, an overwhelming majority of individuals with SRNS progress to Chronic Kidney Disease (CKD) and End-Stage Renal Disease (ESRD) within a few

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years.[2,4] Currently, there is no specific treatment to curb disease progression into ESRD.[2] Hence, it is urgent to develop effective drugs for NS.

Sanqi Qushi Granule (SQG) is derived from Sanqi oral solution, which is a hospital preparation for clinical use in Guangdong Provincial Hospital of Chinese Medicine.[5] Our group confirmed that Sanqi oral solution could exert protective effects in podocyte and mitigate proteinuria.[6,7] Further, the Sanqi Qushi Granule was tolerable, and clinically effective in lowering proteinuria for individuals with NS. However, the underlying mechanisms of SQG on NS are yet to be elucidated.

Network pharmacology is a novel approach for complicated mechanistic studies integrating systems biology, bioinformatics, cheminformatics, and other associated domains. It can disclose the central action targets and herb compounds with construction of drug-gene-disease network.[8,9] In this study, we aim to validate the efficacy of SQG on NS rats and investigate the potential mechanisms with network pharmacology and experimental verification.

Methods and Reagents

Acquisition of Active Ingredients in SQG

The active ingredients of SQG were obtained from the Traditional Chinese Medicine Systems Pharmacology database (TCMSP, http://tcmspw.com) and existing literature. TCMSP has access to Chinese herbal medicines system information, encompassing drugs, targets, and their relations. Oral bioavailability (OB) and drug-likeness (DL) are two of main pharmacokinetic parameters for drugs properties. According to the selection criteria (an OB value of ≥30% and a DL value of ≥0.18), the screened compounds were allowed for further analysis.

Identification of NS-Related Genes

The targets associated with NS in humans were collected from Online Mendelian Inheritance in Man database (OMIM, http://omim.org/, updated in 2022), DisGeNET Database (https://www.disgenet.org/) and Gene Cards database (https://www.genecards.org/). "Nephrotic syndrome" was indicated to search as the keyword in this study. After taking the intersection of the predicted genes from three databases, target genes can be obtained.

PPI Network Analysis

The PPI network analysis was performed using the STRING database (http://string-db.org) to explore a functional interaction between proteins. Afterward, these interactions were visualized by importing all data into Cytoscape 3.9. 1 software. Within this network, nodes were indicated as targets and edges represented interaction relationships.

GO and KEGG Pathway Enrichment Analysis

Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were carried out using Metascape (http://www.metascape.org/) database. The results of both two enrichment analyses focus on elucidating the core signaling pathway of SQG acting on NS.

Animals and Experiments

A group of adult male Sprague-Dawley (SD) rats weighting 180–220g were provided by Animal Research Laboratory of Guangdong Province (Guangzhou, China). The ethical approval was given by Research Institute of the Animal Protection and Use Committee of Guangdong Provincial Hospital of Chinese Medicine [No.2018069]. Adaptive feeding for 7 days, six randomly selected rats were assigned to the blank control group (Control) and were given 0.9% saline through the tail vein. Adriamycin (#HY-15142/CS-1239, MCE, United States) was injected into the tail vein of the remaining 12 rats only once to construct a NS model. After 3 weeks, the 12 rats were randomly assigned to the Model group (Model) and the SQG intervention group (SQG). Subsequent experimental interventions of a 4-week period are presented below. In case of model group and control group, the rats were only administered with saline. The daily dose of SQG decoction (11.02 g/Kg) was equal to the clinical dosage. This optimal dosage was screened based on a previous in vivo efficacy

experiment, in which the results showed the efficacy of low-dose group was better and data are not yet published as shown in Figure S1.

SQG Aqueous Extract Preparation

All the raw herb of SQG were purchased from pharmacy in Guang Dong Provincial Hospital of Chinese Medicine, conforming with the criteria of 2020 edition Chinese Pharmacopoeia. The dosage proportions of *Hedysarum Multijugum Maxim*. (No.200800061, Gansu, China; 30 g), *Panax Notoginseng (Burk.) F. H. Chen Ex C. Chow* (No.2101011631, Yunnan, China; 5 g), *Isaria cicadae Miquel* (No.2208011101, Jiangsu, China; 10 g), *Atractylodes Macrocephala Koidz*. (2208011101, Jiangsu, China; 10 g), *Radix Paeoniae Rubra* (No. 2011004, Inner Mongolia, China; 10 g), and *Smilacis Glabrae Rhixoma* (2210001, Guangdong, China; 30 g), *Curcumae Rhizoma* (200602321, Guangxi, China; 10 g). The seven herbs were mixed and decocted twice, adding 8 times the amount of distilled water, each time for 2 h, followed by filtration. Subsequently, the aqueous extract of SQG was concentrated through an evaporator (IKA, Germany).

Biochemistry of Blood and Urine

Urine samples were collected employing metabolic cages for 24 hr. Rats were fasted with free access to water at time of urine collection. The volumes of the urine were calculated and recorded. After 15-min centrifugation at room temperature (3000 rpm), the supernatant was kept at −80°C for subsequent test. The blood was obtained via an abdominal aorta and left standing for 1 hr. After 5-min centrifugation (12,000 rpm, 4°C), the serum was isolated and preserved at −80°C to perform biochemical assays. All blood and urine samples were assayed in the Department of Clinical Laboratory in Guangdong Provincial Hospital of Chinese Medicine by an automatic biochemical analyzer (Roche cobas C702).

Histology

Kidney tissue of rats was fixed using 10% paraformaldehyde for 24 hr, followed by embedding with paraffin. Then, they were cutting into 3μm thick sections. Next, renal slices were deparaffinized and rehydrated in xylene and alcohol series, respectively. Finally, paraffin sections were stained with hematoxylin and eosin.

Immunohistochemistry

The slices of kidney tissue were dewaxed and rehydrated, followed by antigen retrieval with Tris-EDTA (PH9.0) at a high pressure. Hydrogen peroxide (3%) was used to block the endogenous peroxidase for 15 min. After 30-min blocking with 10% goat serum at 37°C, the renal slices were incubated using following primary antibodies nephrin (1:250, ab216341, Abcam, UK) overnight at 4°C. Next, 1-hr secondary antibody incubation was performed. Then, the renal tissue sections were detected using Max Vision^{TM} HRP kit (MXB Biotechnologies, Fujian, China) and stained with hematoxylin (Leagene, Beijing, China). The stainings were visualized by microscope (Bio-Rad, Laboratories, Hercules, CA, United States), and quantitation was conducted by Image J.

Western Blot

The total protein was extracted from kidney tissues by adding a RIPA lysis buffer mixed with PMSF and phosphatase inhibitor cocktail. The Pierce™ BCA protein assay kit (23227, Thermo Fisher Scientific, Rockford, IL, United States) was used to detect the protein concentration of samples by following kit instruction manual. In equal amounts, the total protein was separated on the 10% SDS-PAGE gel and transferred onto the 0.45μM polyvinylidene fluoride membrane (Millipore, Burlington, United States). After blocking with 5% non-fat skimmed milk at room temperature, the membranes undergent overnight incubation with the corresponding primary antibody at 4°C (1:1000, p-PI3K, ab191606, Abcam; 1:1000, PI3K, 4257, CST; 1:1000, p-AKT, 4060, CST; 1:1000, AKT, 4685, CST; 1:1000, Bax, 2772, CST; 1:500, Bcl-2, sc-7382, Santa Cruz Biotechnology; 1:1000, Podocin, sc-518088, Santa Cruz Biotechnology; 1:250, Nephrin, ab216341, Abcam, UK; 1:5000, β-actin, AC038, ABclonal, China). The next day, the membranes were washed with TBST buffer three times, followed by 1-hr incubation at room temperature with a related secondary antibody. Subsequently, ECL reagent was used to visualize the protein bands (Millipore, Burlington, United States).

TUNEL Staining

In nucleus, genomic DNA fragmentation is a sign of apoptosis. TUNEL relies on the binding of the exposed 3'-OH ends of DNA to fluorescent probe Cy3 labeled dUTP, in the catalytic effect of Terminal Deoxynucleotidyl Transferase (TdT). Apoptosis DNA fragments in renal tissue were assessed by the TUNEL (Beyotime, Shanghai, China) assay kit, in accordance with the kit's instructions. In brief, the slices (kidney, 3μm) were dewaxed, rehydrated, following by a 30-min incubation using protease K (no DNase 20μg/mL, Roche, Germany) at 37°C. After washing with PBS 3 times, the renal slices were incubated in a TUNEL reaction solution at 37°C avoiding light for 60 min. Subsequently, the staining was viewed under a microscope (Olympus, Japan).

Statistical Analysis

Results were graphed with GraphPad Prism 8.4.3. All data in this study were indicated as mean ± s.d. One-way ANOVA analysis was applied for data analysis. A p value < 0.05 was considered statistically significant.

Results

Bioactive Compounds in SQG

Among seven drugs of Huangqi, Sanqi, Baizhu, Chanhua, Chishao, Ezhu, Tufuling, 16, 7, 4, 22, 13, 1 and 15 compounds, respectively, were obtained by ADME screening (Supplementary Table S1). After removing duplicates, 69 active ingredients in SQG remained.

Active Ingredient-NS Target Network

Targets of the main ingredients in SQG were predicted in SwissTarget Prediction database and TCMSP database. After removing duplicates, the predicted targets contained 466 genes in total (Supplementary Table S2). Meanwhile, we also searched three different databases and identified therapeutic targets for NS: 2051 from GeneCard, 181 from OMIM, and 384 from Disgenet. Subsequently, 2262 unduplicated disease targets for NS were acquired in total (Supplementary Table S3). Ultimately, we obtained 144 overlapping targets, by taking the intersection of genes for NS and SQG (Figure 1A and Supplementary Table S4). We constructed "Active ingredient-NS Target Network" to clarify the potential mechanism of SQG against NS using Cytoscape software (Figure 1B). In this network, quercetin (MOL000098) is most closely related to the genes of NS, and the remaining compounds followed in turn: beta-sitosterol (MOL000358), stigmasterol (MOL000449), baicalein (MOL002714), naringenin (MOL004328), which are ordered by their degrees.

PPI Analysis

We imported 144 overlapping targets into the STRING database to build the PPI network and visualized it using Cytoscape (Figure 2). The PPI network displayed 534 proteins nodes and 2365 edges. The higher the protein degree is, the more critical role the protein plays in this process. The proteins ranked in the top 10 with high degree value were identified as hub genes, containing TP53, AKT1, MAPK1, HSP90AA1, SRC, MAPK14, RELA, TNF, ESR1, MAPK8.

GO and Pathway Enrichment Analysis

GO and KEGG pathway enrichment analyses were conducted by importing 144 overlapping genes into Metascape Database. The GO enrichment analysis results were presented, covering 1633 biological processes (BPs), 146 molecular functions (MFs), and 92 cellular components (CCs) term. The top 10 most enriched BP, CC, and MF terms are plotted in Figure 3A. BP enrichment analysis contains responses to oxidative stress, cellular responses to chemical stress, responses to peptides, responses to lipopolysaccharide, and so on. The CC terms mainly enriched membrane raft, membrane microdomain, vesicle lumen, and so on. The terms of MF contain steroid binding, DN−binding transcription factor binding, heme binding, and so on. The KEGG analysis yielded 165 pathways, of which the top 30 are displayed in Figure 3B. Further, the results indicated PI3K-Akt signaling pathway and apoptosis may be the underlying mechanism of SQG against NS.

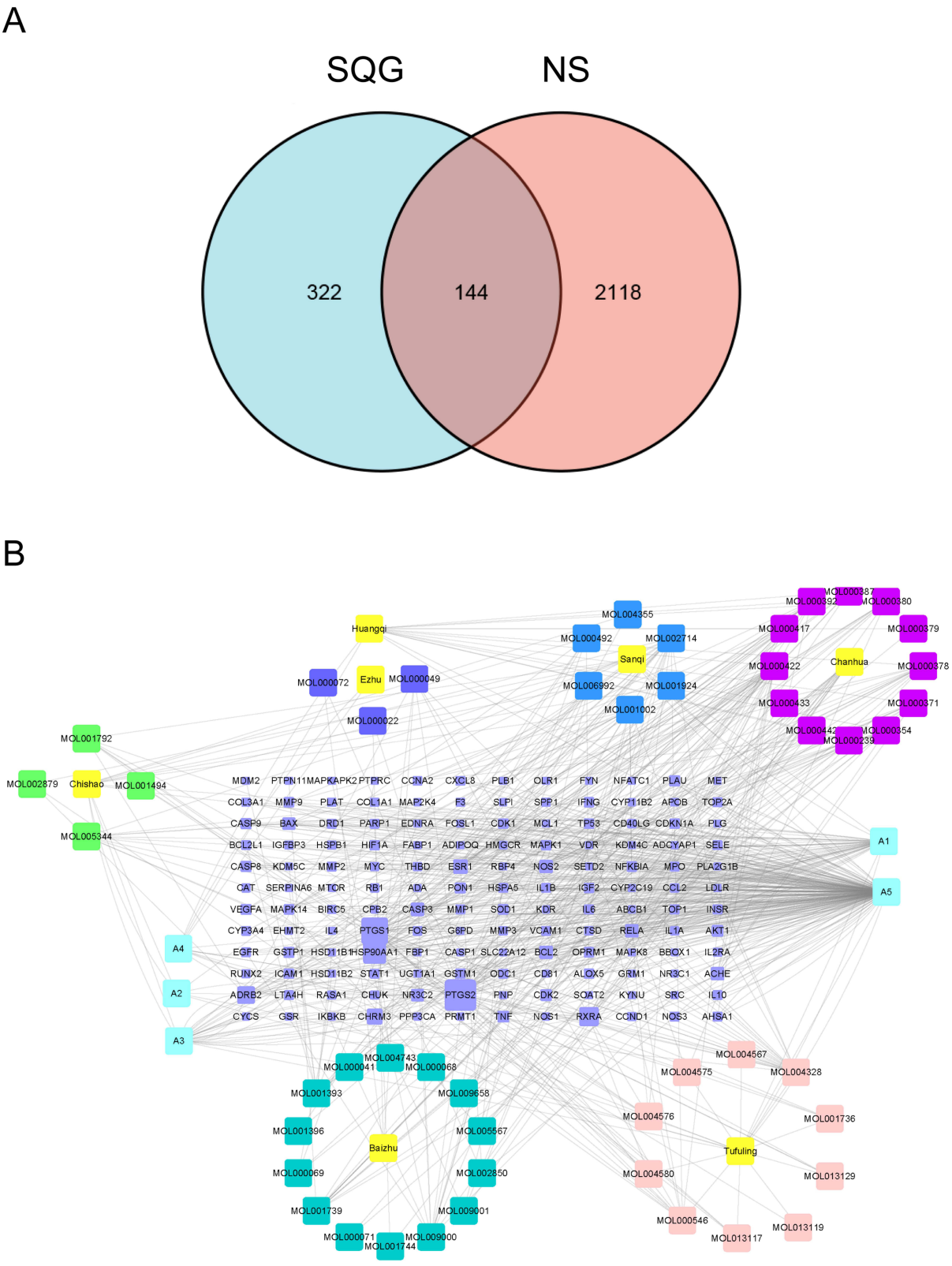

Figure 1 Active ingredient-NS Target Network (**A**) Intersection SQG-related targets with NS targets; (**B**) The drug-bioactive ingredient gene-disease network of SQG for NS. Herbs share overlapped ingredients, labeled as A1- A5. A1 is for beta-sitosterol; A2 is for sitosterol; A3 is for Stigmasterol; A4 is for hederagenin; A5 is for quercetin.

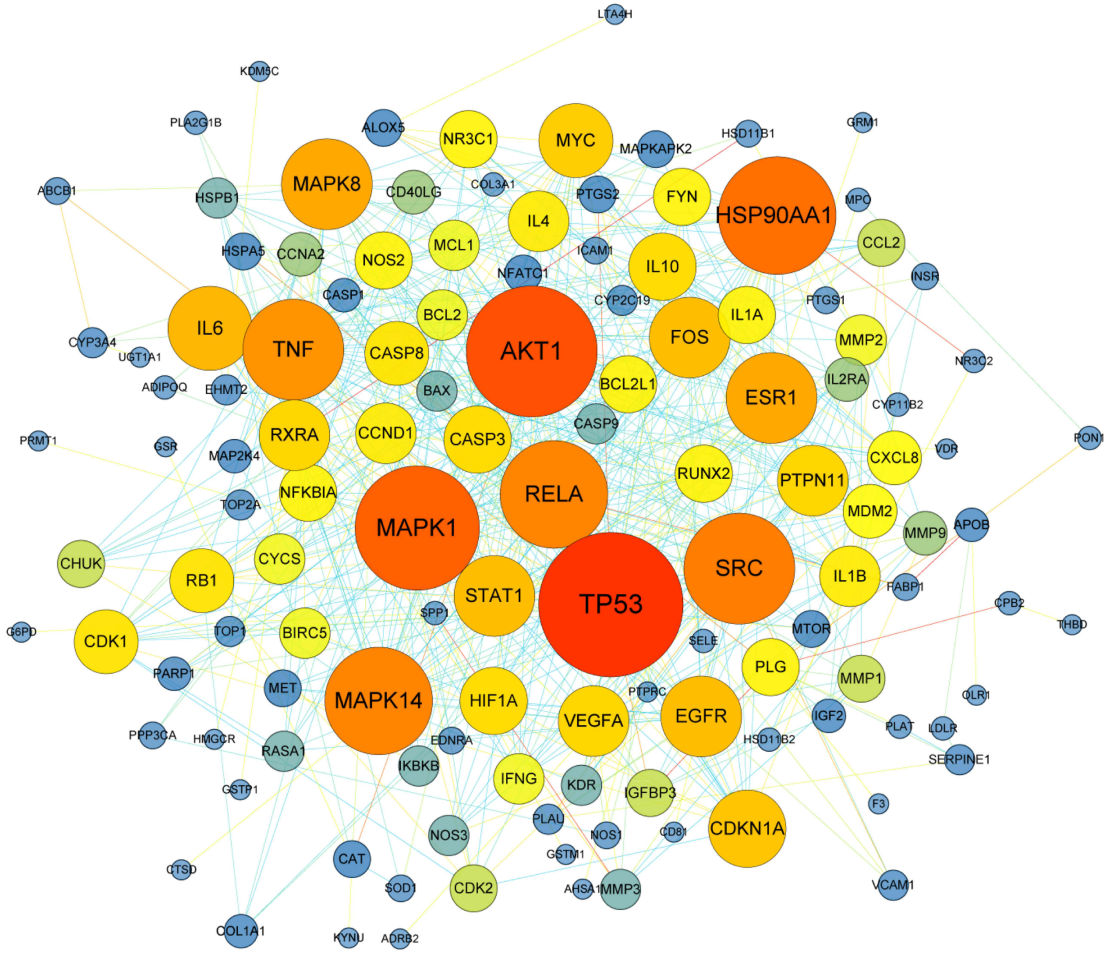

Figure 2 The PPI network based on SQG-NS common targets. Overlapping proteins are marked as nodes in this network and the interactions between the proteins are labeled as lines connecting nodes. The bigger nodes represent higher degree with darker color.

Urine Protein Quantitation and Serum Biochemical Indexes

Proteinuria is a key feature of nephrotic syndrome. As illustrated in Figure 4A, 24-h urinary protein levels with the exception of control group were increased significantly and ADR-treated rats developed heavy proteinuria. The SQG invention reduced proteinuria markedly ($p < 0.01$). After SQG treatment, Cr, BUN, TG, TC, and LDL-C levels distinctly decreased, compared with the control group in Figure 4B–F. The above results revealed that SQG had a therapeutic effect on NS rats similarly.

Histopathological Analysis

The pathological changes in the kidney were visualized by light microscopy. As shown in Figures 4G, there was no visible kidney injury in control group. Whereas, inflammatory infiltrates, tubular dilatation, and edema were significantly observed in rats with NS. While with SQG intervention, less inflammatory cell infiltrates were found, compared with NS rats without SQG treatment. The SQG group showed tubular dilatation and edema distinctly diminished, too. It can be seen that SQG could attenuate renal damages in NS rats induced by ADR.

SQG Attenuated Glomerular Podocyte Lesions in NS Rats

Podocytes, namely the visceral glomerular epithelial cell, play a role in maintaining glomerular filtration function. Once podocyte damage has occurred, proteinuria developed directly. Nephrin and podocin were expressed in a podocyte slit-pore diaphragm, which contributes to maintaining podocyte morphology. Decreased levels of nephrin were observed in

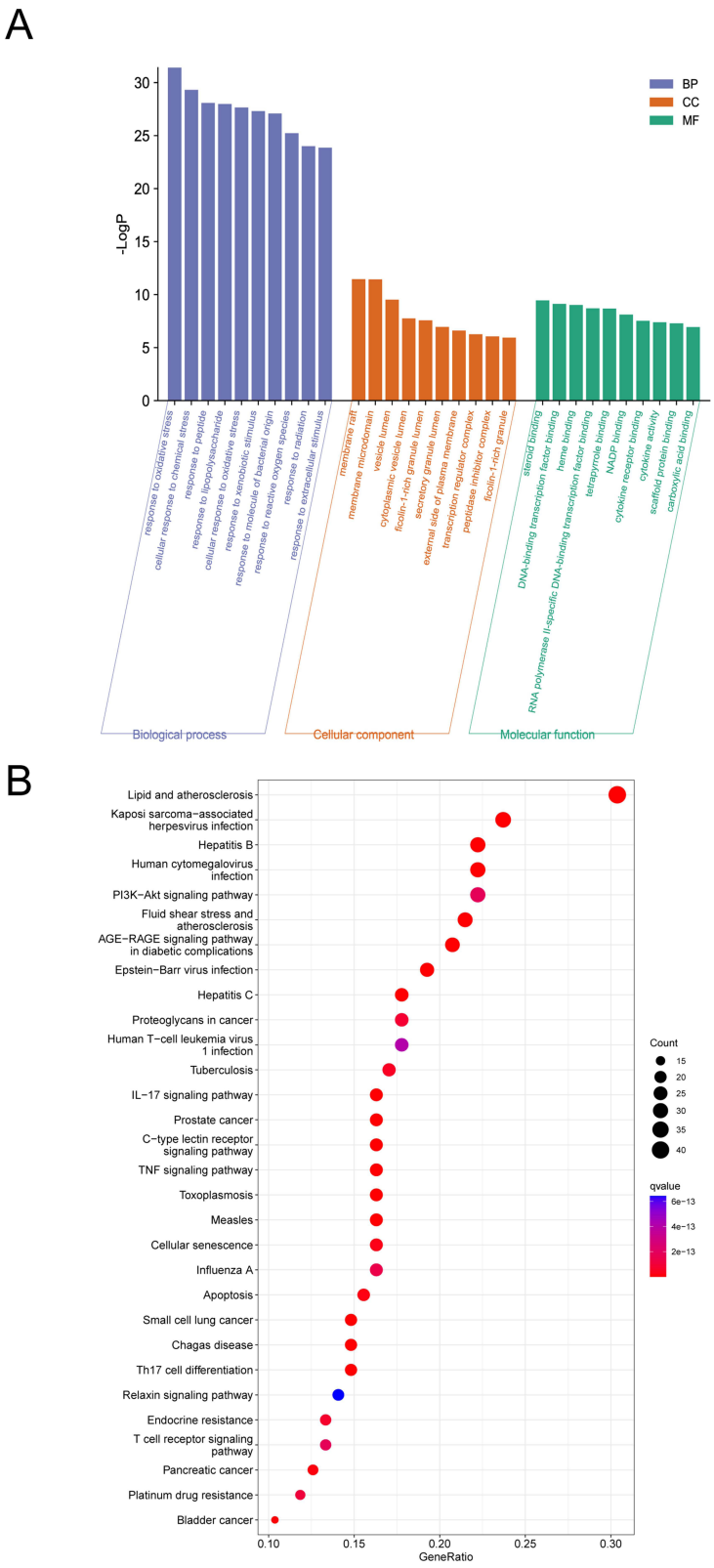

Figure 3 GO and pathway enrichment analysis of SQG active components in the treatment of common targets of NS. (**A**) The GO enrichment analysis. (**B**) KEGG pathway enrichment analysis.

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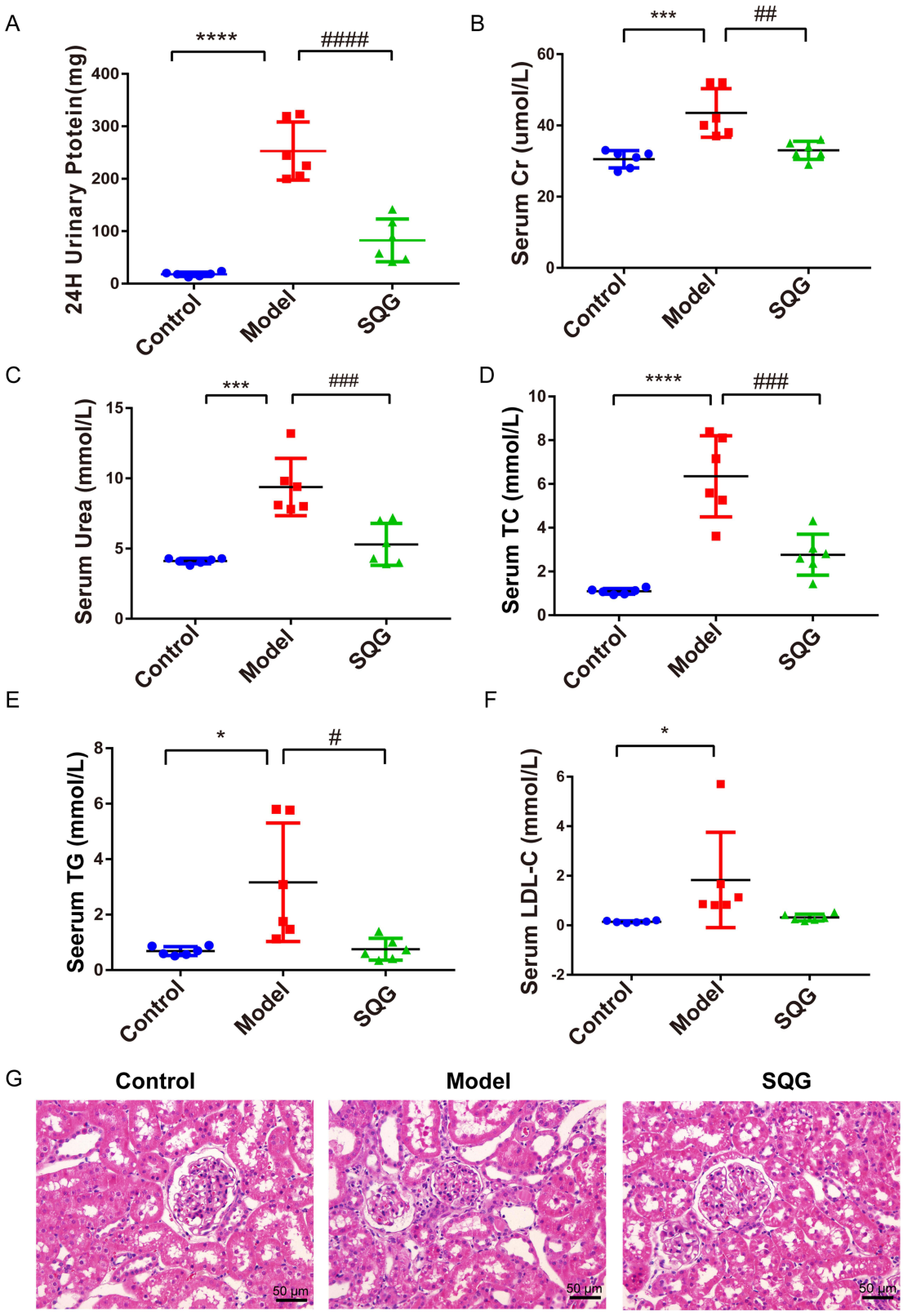

Figure 4 SQG ameliorated indicators of kidney function and pathological lesions in NS rat. (**A**–**E**) The biochemical indicators of urine and blood after 4 weeks of SQG treatment. (**A**) 24 hours urine protein quantitation (**B**) Serum creatinine concentration (**C**) Serum urea nitrogen concentrations (**D**) Serum total cholesterol concentrations (**E**) Serum total triglyceride concentrations (**F**) Serum low-density lipoprotein cholesterol concentrations (**G**) Representative images of H&E-stained sections of the kidney (scale bar: 50 μm). Data in (**A**–**E**) are presented as the mean ± s.d. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$, vs Control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$, vs Model group. n = 6 samples per group.

NS rats with immunochemical staining, as shown in Figure 5A and B. However, after SQG administration, the expression of nephrin was significantly restored. The Western blot analysis of nephrin and podocin protein expression demonstrated similar trends in Figure 5C–E. Collectively, SQG treatment protected podocyte from damage in rats with NS.

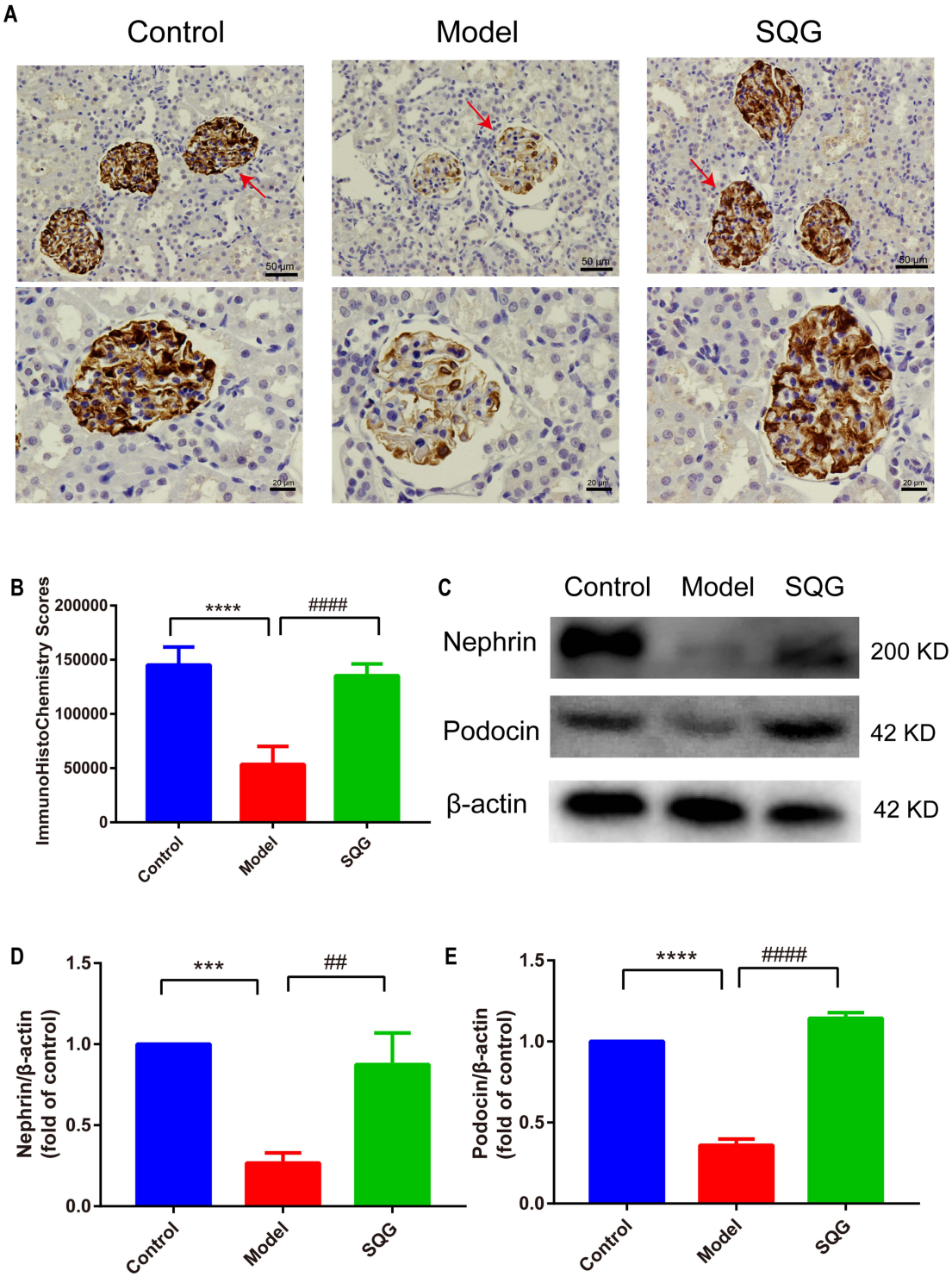

Figure 5 SQG ameliorates glomerular podocyte damage in NS rats, nephrin and podocin expression as examples. (**A**) Nephrin immunohistochemical staining images (scale bar: 50μm and 20μm). (**B**) Immunohistochemistry analysis of Nephrin. (**C–E**) Representative Western blot analysis of nephrin and podocin levels in kidney tissue. Data are presented as the mean ± s.d. ***$p < 0.001$, ****$p < 0.0001$, vs Control group. ##$p < 0.01$, ####$p < 0.0001$, vs Model group. n = 3 samples per group.

Effects of SQG on Kidney Podocyte Apoptosis

Kidney tissues were subjected to TUNEL staining in this study (Figure 6A and B). The results exhibited considerable glomerular podocyte apoptosis in all NS rats. Compared with the model group, podocyte apoptosis in glomerulus was pronouncedly inhibited

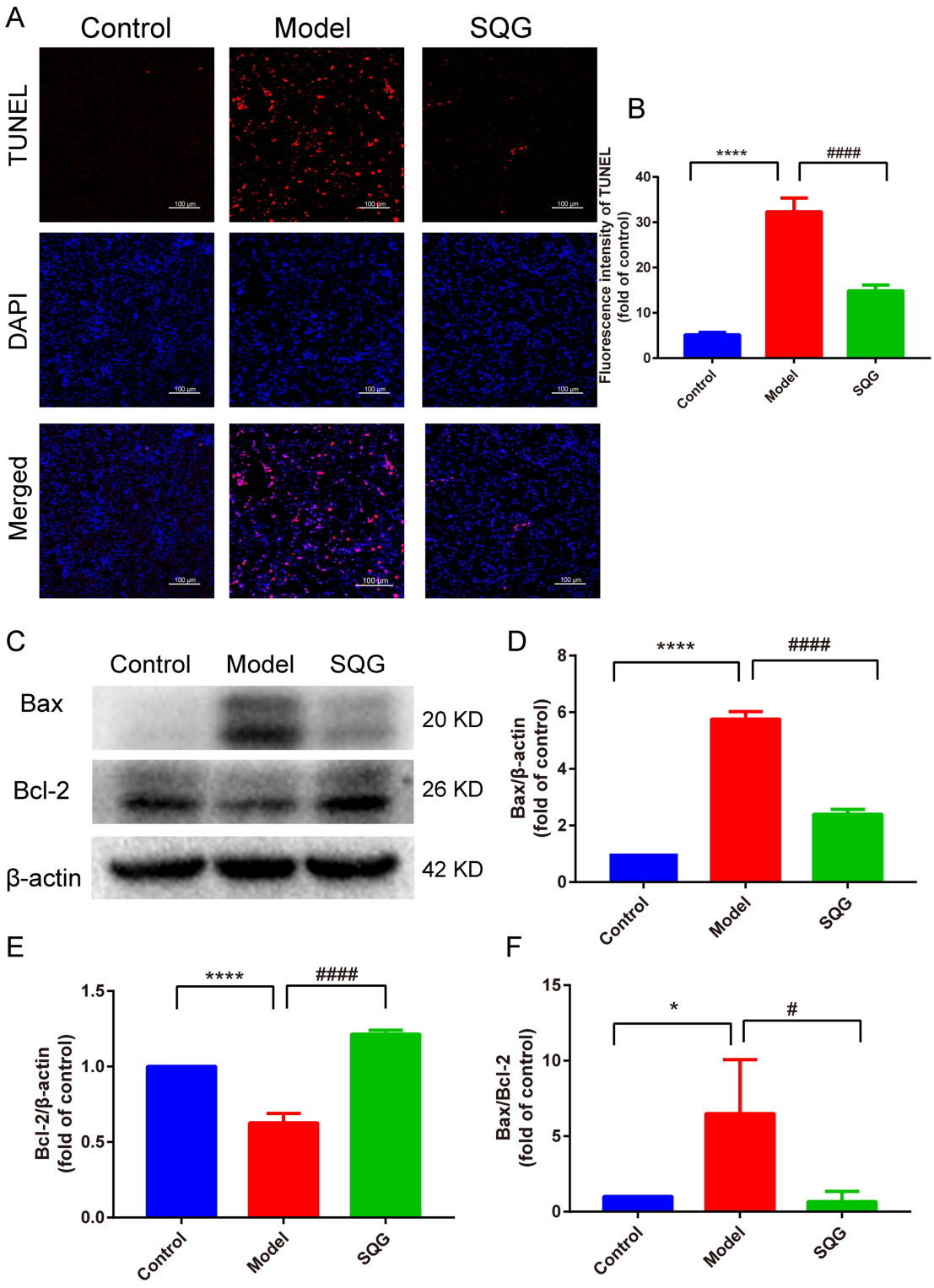

Figure 6 SQG blunted the activation of the apoptosis pathway and inhibited kidney cells apoptosis in NS rats. (**A**). Kidney slices were stained by TUNEL assay (scale bar: 100μm). (**B**) Fluorescent intensity analysis of the TUNEL staining. (**C**–**E**) Representative Western blot analysis of Bax, Bcl-2 levels in kidney tissue. Data are represented as mean ± s.d. from independent groups. *$p < 0.05$, ****$p < 0.0001$, vs control group. $^{\#}p < 0.01$, $^{\#\#\#\#}p < 0.0001$, vs Model group. n = 3 samples per group.

with SQG treatment. We further performed Western blot analysis for apoptosis-associated protein expressions (Bax, Bcl-2) in kidney tissues from three groups. As shown in Figures 6C–F, rats in model group had higher protein expression of Bax, together with reduced protein expression of Bcl-2, than rats in control group. This predicted apoptotic signaling pathway was activated, triggering cell death. In contrast, SQG administration downregulated protein expression of Bax and upregulated protein expression of Bcl-2, leading to inhibition of apoptosis.

Activated PI3K/AKT Pathway by SQG

To explore the anti-apoptotic effects of SQG on ADR-induced rats of NS, we detected whether the PI3K/AKT signaling pathway is activated by WB. As indicated in Figure 7, the protein of p-PI3K and p-AKT were down-regulated in model group, and these were restored after SQG treatment.

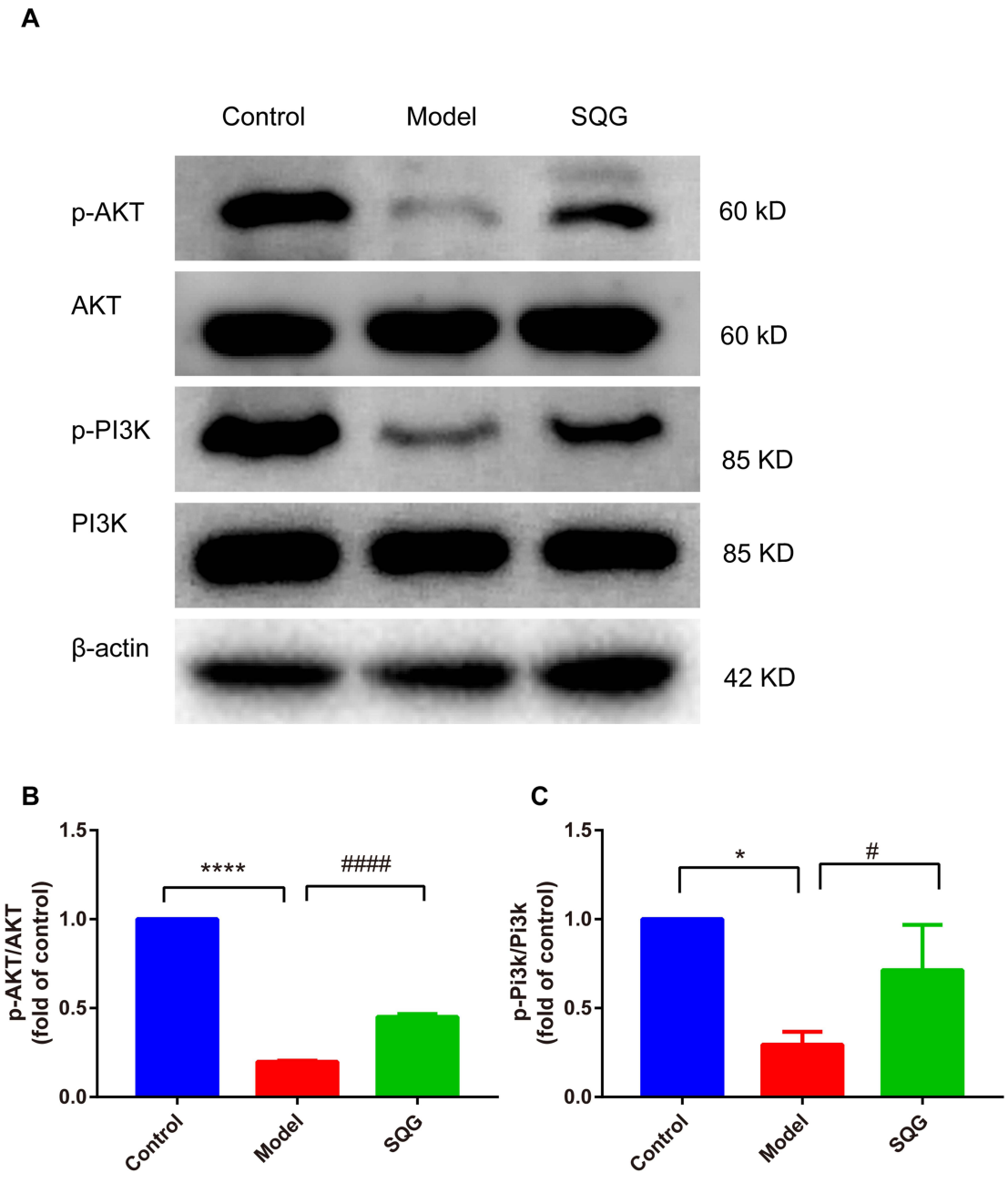

Figure 7 SQG activated PI3K/AKT signaling pathway against NS. (**A**) Expressions of protein AKT, p-AKT, PI3K and p-PI3K were determined by Western blotting. (**B**) Quantitative analysis of p-AKT/AKT expression ratio. (**C**) Quantitative analysis of p-PI3K/PI3K ratio. Data are shown as mean ± s.d. $^{*}p < 0.05$, $^{****}p < 0.0001$, vs Control group. $^{\#}p < 0.05$, $^{\#\#\#\#}p < 0.0001$ vs Model group. n=3 samples per group.

Discussion

Nephrotic syndrome has a high prevalence globally, which often initiates various complications despite marked advances in its treatment.[10] It is urgent to develop a promising drug for NS. In this study, we validated SQG efficacy for alleviating albuminuria and its protective role on podocyte in vivo experiment. Combined with network pharmacology method, in-depth study exploration proposed that SQG could inhibit podocyte apoptosis through the PI3K/AKT signaling pathway in NS rats.

A classical adriamycin-induced nephropathy (AN) rat model was adopted, which is characterized by resembling phenotypes to clinical nephrotic syndrome including severe proteinuria and histopathological damages.[11,12] AN rat model was built by a single adriamycin tail vein injection and applied extensively in nephrology studies due to its stability and similarity to human nephrotic syndrome.[13–15] After modeling, the rats developed heavy proteinuria, impaired renal function, and notable morphological changes, such as tubular lumen dilatation, leukocytic cell infiltration, and vacuolar degeneration in our study the model of NS rats was successfully established. Proteinuria is a hallmark of NS. The albuminuria highly represents podocyte damage and glomerular filtration dysfunction.[16,17] Studies have demonstrated that proteinuria has a direct nephrotoxicity on tubular cells, which initiates interstitial inflammation and fibrosis, ultimately contributing to the onset of irreversible kidney injury.[18,19] A rapid reduction of proteinuria is essential for containing progression to end-stage renal failure. Thus, one of the main objectives in the treatment of NS is to attenuate or eliminate proteinuria rapidly. In our study, SQG administration appreciably relieves proteinuria.

Podocytes are highly specialized cells in the glomerulus with limited proliferation and division capacity.[20] It, as the glomerular filtration barrier, together with silt diaphragms are highly associated with the product of proteinuria.[21] Once podocyte is damaged or dead, the protein leaks past the glomerular basement membrane into urine.[22] Therefore, protecting podocyte against injury can attenuate proteinuria and delaying disease progression.[23] The results of our study indicated that the expression of nephrin and podocin, marker of podocyte injury, were restored after SQG intervention, compared with NS rats.

With disease progression, renal function may worsen, followed by concentrations elevation of urea nitrogen and creatinine in blood. Hyperlipidemia is another clinical manifestation of NS with an increase in low-density lipoprotein and cholesterol.[21] It was revealed that due to deposition of lipoprotein on glomeruli and tubules, renal cells are prone to apoptosis and renal insufficiency develops ultimately.[24] Apoptosis of kidney cell is possibly associated with proteinuria and aberrant lipid metabolism.[25,26] In this study, after modeling, the levels of BUN, Cr, TG, TC, and LDL-C in NS rats were higher than in control group. However, treatment with SQG improved renal functions and ameliorated lipid metabolism disorder in NS rats.

Moreover, apoptosis, which plays a significant role in the genesis and progression of NS, is also discovered to be remarkably increased in the renal cells of NS models.[27,28] The present TUNEL staining indicated that compared with control group, a large amount of apoptosis occurred in glomeruli and tubules, when rats were injected with ADR, consistent with previous study. However, in SQG treatment group, the apoptosis was alleviated dramatically. Simultaneously, we detected the expression of a cluster of key apoptosis-associated proteins, including Bax, Bcl-2 by Western blot analysis to measure cell survival. The data showed that SQG treatment downregulated the Bax/Bcl-2 ratio treated with ADR. This implied that SQG could suppress apoptosis in the impaired kidney to exert reno-protective effect.

To elucidate the upstream modulation mechanism of apoptosis, a KEGG analysis was performed. Several signaling pathways are highly enriched, particularly PI3K/AKT pathway. The PI3K/AKT pathway serves as an important anti-apoptotic signaling pathway, which plays an important role in promoting apoptosis and autophagy.[29] AKT is activated when PI3K-dependent generation of PIP3 binds to AKT PH domain at the plasma membrane.[30,31] Activated AKT can phosphorylate Forkhead box O (FOXO) proteins, leading to growth inhibition and apoptosis.[32,33] Furthermore, the Thr23 site phosphorylation of κB kinase is inhibited by activation of AKT, which is involved in the gene regulation of apoptosis.[34] In addition, p-Akt phosphorylates Bcl-2-associated protein, enhancing the antiapoptotic effects of Bcl-2.[35] The PI3K/Akt pathway is crucial for treatment in nephrology. Reportedly, Pulsed Focused Ultrasound alone has a beneficial therapeutic effect, improving renal histological injury, diminishing inflammation and apoptosis in the kidney, through PI3K/Akt signaling.[36]

In the network of components-target, the major active components of SQG are quercetin, beta-sitosterol, stigmasterol, baicalein, and naringenin ranked by degree. These active constituents have never been investigated in MN. However, Reportedly, stigmasterol could trigger PI3K/AkT, AkT/mTOR, JAK/STAT, and VEGFR-2 signaling pathways.[37] Naringenin could activate the PI3K/Akt pathway and increase the expression of Bcl-xL and Bcl-2, inhibiting neuroapoptosis and ameliorating cognitive impairment in rats.[38] Intriguingly, quercetin suppressed the PI3KR1 gene, which activated the PI3K/AKT signaling pathway, thus modulating lipid metabolism.[39] The results in our study indicated that the p-PI3K and p-AKT expression with SQG intervention was higher than that without SQG intervention. The SQG treatment could activate PI3K/AKT signaling pathway. In this way, SQG treatment may exert anti-apoptotic effect to attenuate NS in ADR-induced rats. Yet, more detailed mechanisms focusing on pharmacology still remain unelucidated. In summary, the results of this study demonstrated that at least to some extent, SQG could suppress apoptosis and alleviate proteinuria via the PI3K/AKT signaling pathway in rats with NS.

Conclusion

In conclusion, this work demonstrated that SQG has the potential to alleviate proteinuria, protect podocytes from injury and inhibit kidney apoptosis through the PI3K/AKT pathway. Furthermore, the network pharmacology-based framework explained in our study offers novel insights into the treatment of NS with TCM.

Data Sharing Statement

The original contributions presented in this study are included in this article and Supplementary Materials, further inquiries can be directed to the corresponding authors.

Ethics Statement

The Ethics Committee of Guangdong Provincial Hospital of Chinese Medicine granted exemption from review for available data from public databases involving in our research. And in vivo experiment was conducted after approval by the Research Institute of the Animal Protection and Use Committee of Guangdong Provincial Hospital of Chinese Medicine (No. 20180691). We adhered strictly to the guidelines for ethical review of animal welfare (GB_T 35892-2018).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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