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Analysis of the potential biological mechanisms of geniposide on renal fibrosis by network pharmacology and experimental verification

Mengqian Liu[1,2†], Wenman Zhao[1,2†], Rui Shi[1,2], Zhijuan Wang[1,2], Xunliang Li[1,2] and Deguang Wang[1,2*]

Abstract

Background Renal fibrosis is crucial in the progression of chronic kidney disease (CKD) to end-stage renal failure. Geniposide, an iridoid glycoside, has shown therapeutic potential in acute kidney injury, diabetic nephropathy, and atherosclerosis. The aim of this study was to investigate the role of geniposide in renal fibrosis and its underlying mechanisms.

Methods The network pharmacology and molecular docking methods were used to identify potential targets and pathways of geniposide for treating renal fibrosis. In vivo, the unilateral ureteral obstruction (UUO) mouse model was treated with geniposide. In vitro, TGF-β1-stimulated human renal tubular epithelial (HK-2) cells were applied for validation. HE, PAS, Masson, and immunohistochemistry staining were performed to evaluate its effects on the kidneys of UUO mice. RT-qPCR and western blotting were used to detect the expression of hub genes and signaling pathways.

Results 101 overlapping genes were identified, with the top 10 including AKT1, MMP9, GAPDH, BCL2, TNF, CASP3, SRC, EGFR, IL-1β, and STAT1. GO analysis suggested that these key targets were mainly involved in cell proliferation and apoptosis. KEGG analysis revealed that the PI3K/AKT, MAPK, and Rap1 signaling pathways were associated with geniposide against renal fibrosis. Molecular docking suggested a strong binding affinity of geniposide to the hub genes. In vivo experiments showed that geniposide ameliorated kidney injury and fibrosis, and inhibited the mRNA levels of AKT1, MMP9, BCL2, and TNF. In addition, geniposide inhibited the activation of the PI3K/AKT signaling pathway, thereby suppressing renal fibrosis in UUO mice and TGF-β1-induced HK-2 cells.

Conclusions Geniposide can attenuate renal fibrosis by inhibiting the PI3K/AKT pathway, suggesting its potential as a therapeutic agent for renal fibrosis.

Keywords Geniposide, Renal fibrosis, Network pharmacology, Molecular docking, PI3K/AKT

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Introduction

Renal fibrosis is a key pathological feature in the progression of chronic kidney disease (CKD) toward end-stage renal failure [1]. It is characterized by renal tubular atrophy, chronic interstitial inflammation and fibrosis, glomerulosclerosis, and vascular rarefaction, manifesting at various stages of CKD. In the later stages, excessive collagen deposition and accumulation lead to sclerosis of the renal parenchyma, ultimately resulting in the loss of kidney function [2]. Early prevention and treatment of renal fibrosis is essential for delaying CKD progression. However, there is currently no effective treatment for renal fibrosis.

Increasing evidence suggests that Traditional Chinese Medicine (TCM) may offer promising therapeutic potential in treating renal fibrosis [3]. Geniposide, a bioactive compound found in plants such as *Gardenia jasminoides Ellis*, *Eucommia ulmoides*, and *Rehmannia Radix*. Modern studies have demonstrated that geniposide exhibits diverse pharmacological properties, including therapeutic effects in acute kidney injury (AKI) [4], diabetic nephropathy [5], cancer [6], atherosclerosis [7], and rheumatoid arthritis [8]. Additionally, geniposide has shown protective effects in CKD. In vivo, geniposide improves kidney function in AKI mice by upregulating peroxisome proliferator-activated receptor-gamma (PPARγ) and reducing apoptosis [9]. In addition, geniposide enhances autophagy and attenuates oxidative stress in diabetic nephropathy mice by activating the AMP-activated protein kinase (AMPK) pathway [10]. However, the role of geniposide in renal fibrosis remains unknown.

Network pharmacology has become an increasingly popular method for studying the therapeutic mechanisms of TCM in treating various diseases [11]. By combining systematic biology with network biology, this approach enables the visualization of drug-target-disease networks, providing a more comprehensive understanding of their interrelationships. This, in turn, aids in identifying key ingredients and potential mechanisms of action for drugs used to treat diseases [12]. In this study, we utilized a combination of network pharmacology and experimental verification to predict key targets and signaling pathways, explore its effects on renal fibrosis, and elucidate the mechanisms of geniposide.

Materials and methods

Network pharmacology analysis

Acquisition of related targets of geniposide

First, the SDF format of "geniposide" was obtained in the PubChem (https://pubchem.ncbi.nlm.nih.gov) database and then imported into PharmMapper (https://lilab-ecust.cn/pharmmapper/index.html), Swiss Target Prediction (http://www.swisstargetprediction.ch/), and TCMBank (https://tcmbank.cn) for prediction targets. Next, the targets were rectified and deduplicated using the Universal Protein database (UniProt, https://www.uniprot.org).

Searching potential treatment targets for renal fibrosis

Three databases, OMIM (https://www.omim.org), DisGenet (https://www.disgenet.org), and GeneCards (https://www.genecards.org), were used to compile the human genes linked to renal fibrosis. These databases provided useful targets when we searched for "renal fibrosis". Lastly, a Draw Venn Diagram was used to show the intersection targets between geniposide and renal fibrosis (http://bioinformatics.psb.ugent.be/webtools/Venn/).

Constructing a protein–protein interaction (PPI) network and acquisition of hub targets

In this study, the intersection targets of geniposide and renal fibrosis were submitted to the STRING 11.5 (https://cn.string-db.org) database, which can predict PPIs that gather data using bioinformatic techniques. Moreover, Multiple proteins and Homo sapiens were set to construct a protein interaction network to obtain the reflection network of geniposide treatment of renal fibrosis. Next, we applied twelve algorithms of the CytoHubba plugin, including MCC, MNC, DMNC, Degree, BottleNeck, Betweenness, Closeness, ClusteringCoefficient, EPC, EcCenTricity, Radiality and Stress, to obtain the hub targets. Among them, we mainly used Degree, MCC and DMNC algorithms and other algorithms for auxiliary validation.

Gene ontology (GO) and Kyoto encyclopaedia of genes and genomes (KEGG) pathway enrichment analysis

The results obtained from the mutual mapping of geniposide component targets and renal fibrosis targets were imported into the online software David6.8 (https://david.ncifcrf.gov), setting H. sapiens, selecting $P<0.05$, and the results obtained were subjected to GO and KEGG analysis by selecting GO Biological Processes(GBP), GO Molecular Functions(GMF), GO Cellular Components(GCC), and KEGG Pathway. The GO and KEGG pathway enrichment results were drawn into bar charts and bubble maps according to the number of genes for visual analysis.

Building a components-disease-target-pathway network

To create the pathway network diagram, import the component-disease-target-pathway network file into CytoHubba. More logical evidence has been shown for the multi-component and multi-target roles played by TCM's active ingredients in illness treatment.

Molecular docking

First, the 3D structures of geniposide were obtained from the PubChem and Traditional Chinese Medicine Systems

Pharmacology (TCMSP, https://test.tcmsp-e.com) databases. The small molecules were then converted to mol2 format using Open Babel 2.4.1 software. Next, the ligand molecules were optimized in Autodock Tools 1.5.6 software, with atom hydrogenation and charge loading applied to produce the optimized structure. The file was subsequently saved in "PDBQT" format. Finally, molecular docking with the target proteins was performed, and the interaction patterns were analyzed using PyMOL.

Chemicals and reagents

Geniposide (NY-N0009, purity 99.89%) was obtained from MedChemExpress (MCE, New Jersey, USA). PI3K/AKT inhibitor LY294002 was also provided by MCE (HY-10108). TGF-β1 was purchased from Abcam (ab50036, USA).

Animal experiments

Animal model construction

The unilateral ureteral obstruction (UUO) model was established by ligating the left ureter, and geniposide was administered via gavage at 50 mg/kg for 10 days. The C57BL/6J male mice were randomly assigned to four groups ($n = 5$ per group): Sham, UUO, UUO + GP (50 mg/kg), and Sham + GP (50 mg/kg). After 10 days, we euthanized the mice by intraperitoneal injection of sodium pentobarbital 50 mg/kg. The UUO model is widely recognized as a traditional model for studying renal fibrosis [13], making it an appropriate choice for our study. Based on previous research, geniposide was administered at a concentration of 50 mg/kg, a dose that has shown neuroprotective properties [14, 15] and no significant hepatic or renal toxicity with short-term administration [10, 16, 17]. Consequently, we adopted the same concentration for gavage in this study. C57BL/6J male mice (7 weeks old) were purchased from the Animal Center of Nanjing University (Nanjing, China) and housed in a pathogen-free environment at the Experimental Animal Center of the Second Affiliated Hospital of Anhui Medical University.

Histology and immunohistochemical staining

Kidney tissues were fixed overnight in 4% paraformaldehyde, dehydrated with gradient ethanol, cleared, embedded in paraffin, and sectioned at 4 μm thickness. The sections were deparaffinized and then stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and Masson's trichrome to assess histological changes. The extent of glomerular lesions and tubular damage were visualized by HE staining, with blue nuclei and red cytoplasm. The deeper the PAS staining, the higher the polysaccharide content. By observing the purplish-red complexes on the glomerular basement membrane and mesangium, we can understand the specific location and morphology of polysaccharides in the tissues and assess the degree of damage to the renal tissues. In Masson staining, collagen fibers usually appear blue or green. We can assess the degree of fibrosis by looking at the number and distribution of blue or green areas. The more severe the fibrosis, the more numerous and densely distributed the blue or green areas. The glomerular sclerosis score was calculated as follows: 0 (normal glomeruli); 1 point (mesangial expansion or sclerosis area < 20%); 2 points (20 – 50% sclerosis area); and 3 points (sclerosis area > 50%). A tubulointerstitial score was calculated based on the extent of the lesion (tubular atrophy, injury, tubulointerstitial fibrosis) on a scale of 0–3: 0 point (no tubulointerstitial injury); 1 point (lesion extent < 20%); 2 points (lesion extent 20 – 50%); 3 points (lesion extent > 50%) [18].

For immunohistochemistry (IHC) staining, the sections were firstly deparaffinized and blocked with 2.5% normal goat serum for 15 min and then incubated with collagen I (1:200, Abcam, ab34710, USA) overnight at 4 °C. After three washes with PBS (5 min each), the sections were incubated with a biotinylated secondary antibody at 37 °C for 40 min. The sections were then washed twice with PBS (5 min each), and nuclei were counterstained with hematoxylin. Finally, the sections were sealed for analysis. ImageJ software was used to quantify the degree of fibrosis.

Renal function analysis

Blood samples were centrifuged at 3000 rpm for 25 min and serum was extracted. Serum creatinine (Cr) and blood urea nitrogen (BUN) levels were determined using Nanjing jiancheng kits, and absorbance was recorded at 546 nm and 640 nm by spectrophotometer (BioTek Instruments, VT, USA).

RT- qPCR

RNA rapid extraction kit (ES Science) was used to extract RNA from kidney tissue. The HiScript III RT SuperMix for qPCR (+ gDNA wiper) reverse transcriptase kit (Vazyme) was used to synthetize the complementary DNA. AceQTM qPCR SYBR Green Master Mix (Vazyme) was used to RT-qPCR. Messenger mRNA expression in the corresponding samples was calibrated against β-actin. The sequences of the used primes for qRT- PCR are listed in Table 1.

Western blotting assay

Western blotting was performed to assess the expression of key molecules associated with renal fibrosis, including AKT, phosphorylated AKT (p-AKT), PI3K, phosphorylated PI3K (p-PI3K), and collagen I. Kidney tissues were homogenized, and proteins were extracted at 4 °C. Protein concentration was measured using an Enhanced

Table 1 Primer sequences for qRT-PCR applications

Gene	Forward (5′ to 3′)	Reverse (5′ to 3′)
AKT1	CCTTCTTGAGCAGCCCTGAA	TACGAGATGATGTGCGGTCG
MMP9	ATTCAGGGAGACGCCCATTT	CGGTCGTCGGTGTCGTAGTT
BCL2	GATAACGGAGGCTGGGATGC	TCACTTGTGGCCCAGATAGG
TNF-α	TGCACTTTGGAGTGATCGGC	ACTCGGGGTTCGAGAAGATG
Collagen I	TGACCTCAAGATGTGCCACT	ACCAGACATGCCTCTTGTCC
β-actin	CTGGAACGGTGAAGGTGACA	AAGGGACTTCCTGTAACA ATGCA

BCA Protein Assay Kit (Beyotime Institute of Biotechnology) according to the manufacturer's instructions. Proteins were separated on a 10% SDS-polyacrylamide gel (PAGE) and transferred onto PVDF membranes. The membranes were blocked with Protein-Free Rapid Blocking Buffer for 20 min and washed three times with Tris-buffered saline plus Tween-20 (TBST) for 6 min each. Primary antibodies were incubated with the membranes overnight at 4 °C. The next day, membranes were washed three times with TBST (7 min each) and incubated with the appropriate secondary antibodies for 1 h at room temperature. Following this, the membranes were washed three times with TBST (10 min each). The following antibodies were used: rabbit anti-collagen I (1:1000, Abcam, ab260043, USA), rabbit-anti-α-SMA (1:30000, Abacm, ab124964, USA), rabbit anti-AKT (1:1000, Affinity Biosciences, AF6261, OH, USA), rabbit anti-phospho-AKT (1:1000, Affinity Biosciences, AF0016, OH, USA), rabbit anti-PI3K (1:1000, Affinity Biosciences, AF6241, OH, USA), rabbit anti-phospho-PI3K (1:1000, Affinity Biosciences, AF3241, OH, USA), mouse anti-β-actin (1:20000, Proteintech, 66009-1-Ig, Wuhan, China), anti-rabbit IgG (1:15000, Proteintech, SA00001-2, Wuhan, China), and anti-mouse IgG (1:15000, Proteintech, SA00001-1, Wuhan, China). Electrochemiluminescence (ECL) was used to visualize the protein bands, with β-actin serving as the loading control to normalize protein expression levels.

Cell experiments

Cell culture and treatment

Human renal cortical proximal tubule epithelial (HK-2) cells were cultured in DMEM/F12 medium containing 10% fetal bovine serum and 1% antibiotics (streptomycin and penicillin) in a humidified incubator at 37℃, 5% CO_2. Previous studies have shown that TGF-β1 induces cellular fibrosis at 10 ng/ml [19], so we chose to use this concentration for validation in our cellular experiments. PI3K/AKT inhibitor LY294002 can significantly inhibit the PI3K/AKT pathway at a concentration of 10 μM, so we chose this concentration for intervention [20]. Protein levels were measured after TGF-β1 stimulation of HK-2 cells for 24 h, and geniposide and LY294002 were added 30 min before TGF-β1 stimulation. Cells were divided into four groups: Control, TGF-β1, TGF-β1 + GP, TGF-β1 + LY294002.

Cell viability analysis

The optimal concentration of geniposide was determined by measuring cell viability. HK-2 cells were inoculated in 96-well plates at a density of 1×10^4 cells per well, and then treated with different concentrations of geniposide (0, 25, 50, 100, 200, 300 μM) for 24 h. After treatment, CCK8 solution was added, and the cells were incubated in a humidified chamber containing 5% CO_2 at 37℃ for 2 h. Absorbance was measured at 450 nm using an enzyme marker (BioTek Instruments, VT, USA) to assess the cell viability.

Statistical analyses

Data are presented as the mean ± SEM. In the data processing stage, we normalized the data of the sham or control group in at least three batches of data according to different experimental data processing methods, and processed the data of other groups in the same way, and finally imported the results into GraphPad Prism 8.0 software for plotting and analysis. In this study, normality was tested by Shapiro-wilk test and one-way ANOVA test was used to analyze whether there were significant differences in biomarker expression levels under different models. $P < 0.05$ was considered as a difference.

Results

Data statistics

Potential target genes of geniposide for the treatment of renal fibrosis

The workflow of the network pharmacology analysis, molecular docking, and experimental validation of therapeutic effect of geniposide on renal fibrosis is illustrated in Fig. 1. The chemical formula of geniposide ($C_{17}H_{24}O_{10}$) was obtained from PubChem. To identify genes associated with renal fibrosis, a total of 3727 genes were found to be associated with renal fibrosis and 267 genes with geniposide. 101 overlapping genes were identified between geniposide and renal fibrosis by an online Venn diagram (Fig. 2A).

Bioinformatics analysis

PPI network of drug-disease targets

For analysis and visualization, the 101 target genes were imported into CytoHubba and the STRING database. The PPI network consisted of 101 nodes and 967 edges (Fig. 2B). Using the CytoHubba algorithm, the top 10 hub genes (AKT1, MMP9, GAPDH, TNF, BCL2, CASP3, EGFR, STAT1, IL1β, and SRC) were identified (Fig. 2C). The identified hub genes are primarily involved in

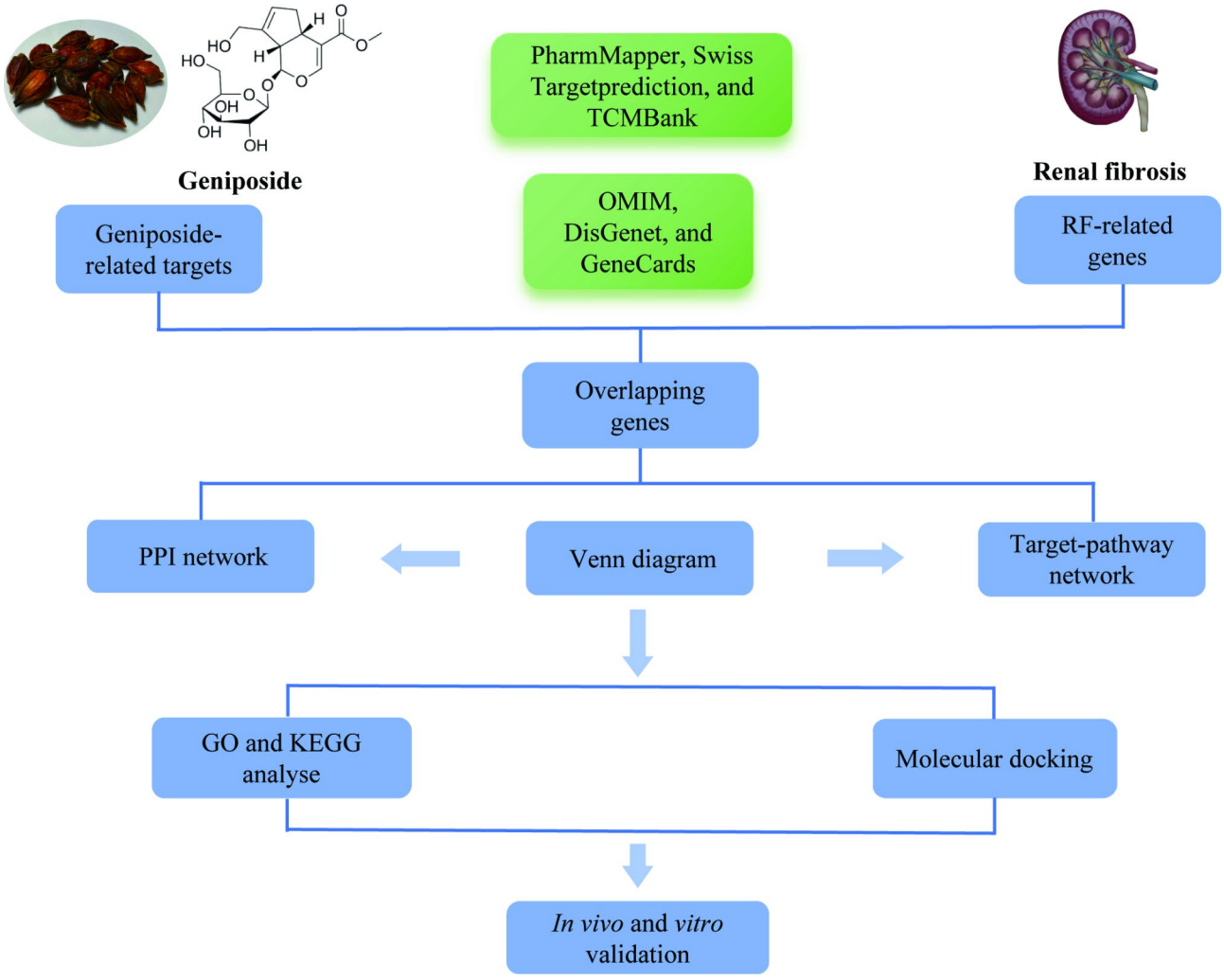

Fig. 1 Flowchart of network pharmacology analysis of geniposide against renal fibrosis

pathways related to cell proliferation, apoptosis, cancer, and inflammation.

GO and KEGG pathway enrichment

Enrichment analysis results identified 146 biological processes (BPs), 39 cellular components (CCs), 57 molecular functions (MFs), and 100 KEGG signaling pathways. The top 10 GO terms and the top 20 KEGG pathways were displayed as bubble plots and bar graphs, based on gene count. GO analysis revealed that geniposide is primarily involved in biological processes such as the negative regulation of apoptosis, positive regulation of protein kinase B (AKT) signaling, extracellular matrix disassembly, cellular response to lipopolysaccharide, positive regulation of MAP kinase activity, cell migration, and cell proliferation (Fig. 3A). KEGG pathway analysis showed that the enriched pathways included cancer, lipid metabolism, PI3K/AKT signaling, MAPK signaling, Ras-related protein 1 (Rap1) (Fig. 3B). These findings suggest that geniposide is closely associated with processes related to cell proliferation, differentiation, apoptosis, stress, and inflammation.

Construction of geniposide-renal fibrosis-target genes-pathways network

To demonstrate that the drug active ingredients has both multi-compotent and multi-target functions in the treatment of diseases, we constructed the geniposide-target-renal fibrosis-pathways network (Fig. 4).

Molecular docking assayes the binding ability of geniposide to the top 10 hub proteins

According to conventional criteria, a binding energy score with an absolute value greater than 5.0 kcal/mol is considered indicative of significant binding potential between a compound and a protein [11]. The top 10 hub proteins were selected to evaluate their binding energies with geniposide (Fig. 5). Molecular docking results

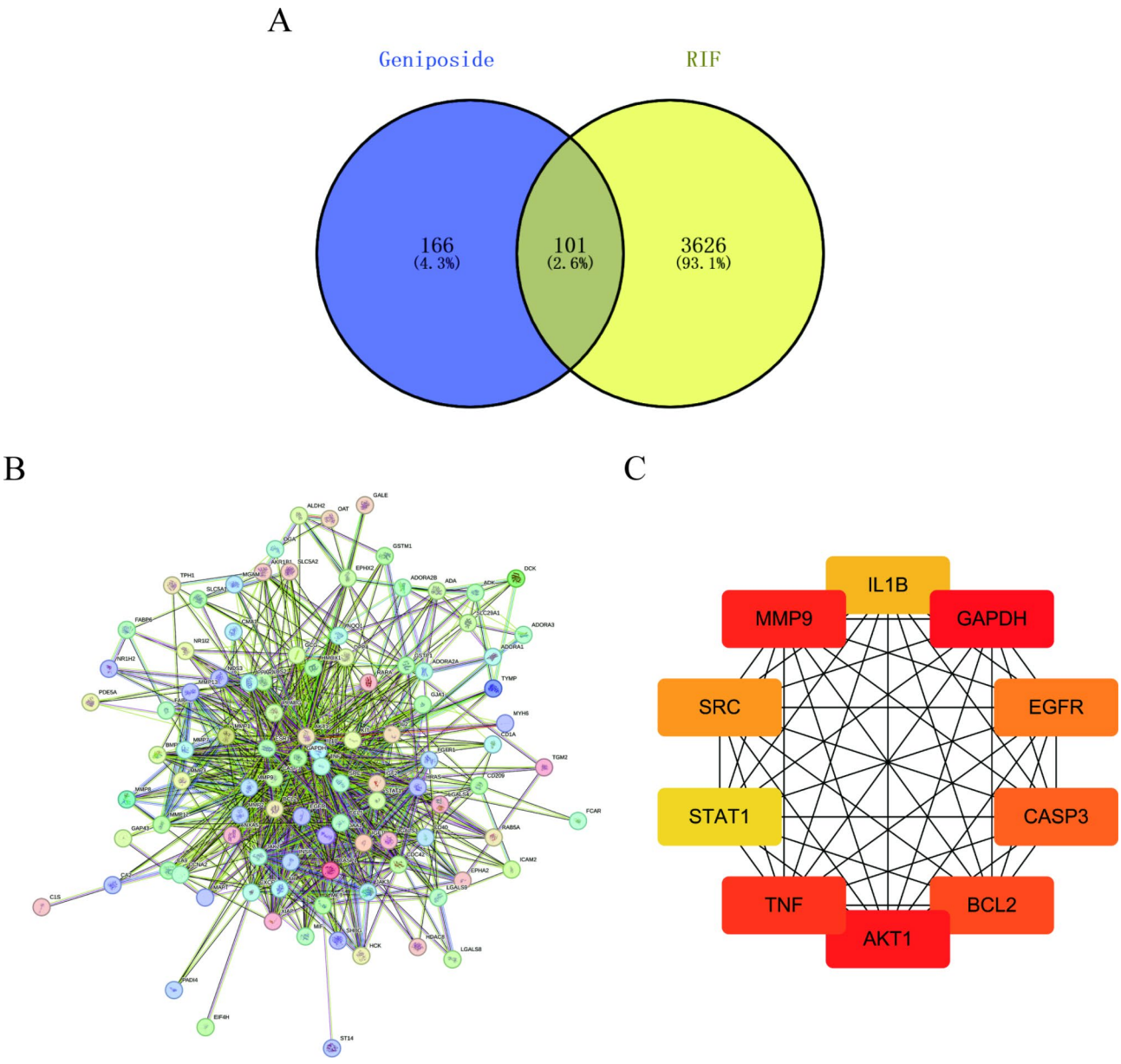

Fig. 2 Screening target genes for geniposide in the treatment of renal fibrosis. **A** Venn diagram of geniposide-related targets and renal fibrosis-related targets. The 101 overlapping genes between geniposide and renal fibrosis targets. **B** PPI network of 101 hub genes. **C** Ten high-freedom hub genes

revealed that geniposide had a strong binding affinity to the AKT1, TNF, BCL2, and MMP9 (Table 2).

Animal experiments

Geniposide improves UUO-induced renal injury and fibrosis in mice

The flow chart of geniposide intervention in UUO model mice was shown in Fig. 6A. We investigated the effects of geniposide on kidney injury and fibrosis in vivo. The results showed that serum Cr and BUN levels were significantly elevated in the UUO model group and that geniposide treatment reduced them (Fig. 6B). HE staining showed that geniposide attenuated renal interstitial inflammatory cell infiltration as well as tubular injury. PAS staining demonstrated that geniposide reduced tubular atrophy and epithelial cell necrosis. These results reflected that geniposide attenuated renal tubular injury. Masson staining results showed that geniposide reduced the area of renal tissue fibrosis in UUO-induced mice (Fig. 6C-D). Furthermore, geniposide significantly reduced the mRNA and protein expression levels of collagen I and α-SMA.(Fig. 6E-I).

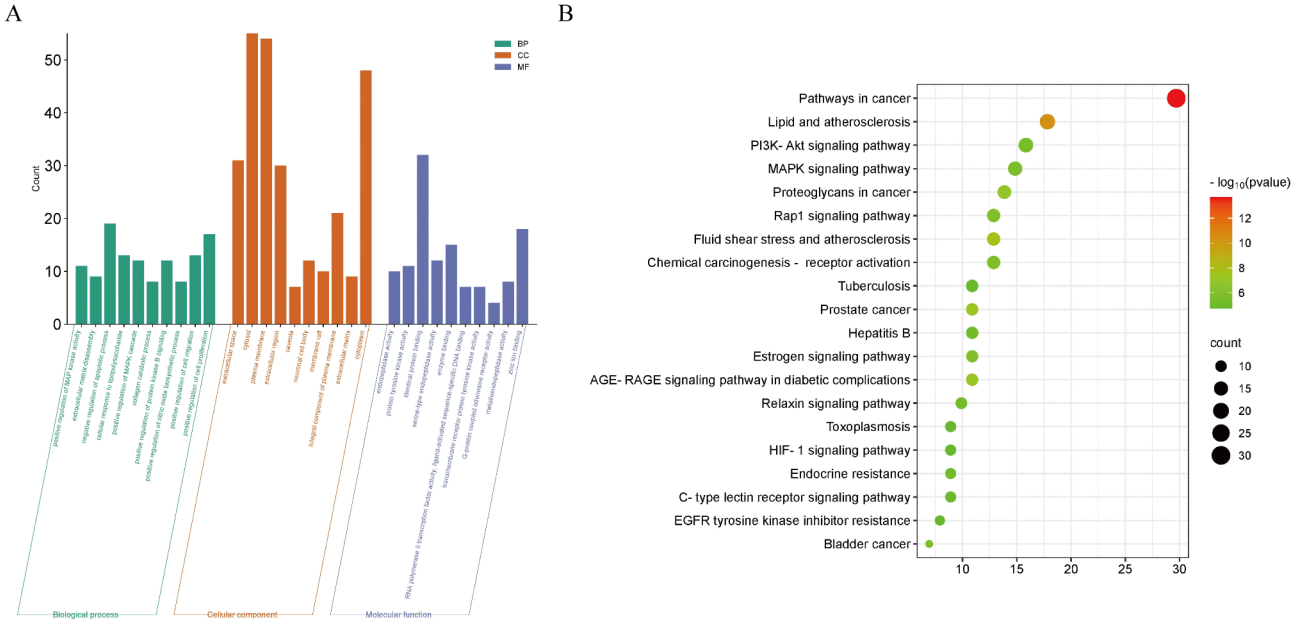

Fig. 3 GO (BP, CC, MF) and KEGG analyses of therapeutic target genes of geniposide for treatment of renal fibrosis. **A** Each bar represents a GO term on the horizontal axis. The number of genes enriched in each term is shown on the vertical axis. **B** Each bubble represents a KEGG pathway on the vertical axis. The gene ratio is shown on the horizontal axis. The size of each bubble indicates the number of genes enriched in each KEGG pathway. Larger bubbles indicate more genes involved in the pathway. The color of each bubble represents the adjusted *P*-value of each KEGG pathway, with redder color indicating smaller adjusted *P*-value

Geniposide inhibites the mRNA levels of hub genes predicted by network pharmacology in UUO-induced mice

Based on the PPI analysis and molecular docking results, the mRNA levels of the four hub genes (AKT1, MMP9, BCL2, and TNF-α) were detected. RT-qPCR results showed that the mRNA levels of AKT1, MMP9, and TNF-α significantly increased in the UUO group, and the mRNA level of BCL2 significantly decreased compared to the sham group. After geniposide intervention, the mRNA levels of AKT1, MMP9 and TNF-α were downregulated and the mRNA level of BCL2 was up-regulated in the UUO + GP group compared to the UUO group (Fig. 7A).

Geniposide regulates the PI3K/AKT1signaling pathway in UUO-induced mice

According to KEGG analysis results, it appears that the PI3K/AKT signaling pathway may be a major pathway in the treatment of renal fibrosis with geniposide. Therefore, we examined the expression of key proteins of the PI3K/AKT pathway in UUO mice. Western blotting analysis showed little change in the protein levels of total AKT and PI3K in the four groups, while the expression levels of p-AKT/AKT and p-PI3K/PI3K were higher in the UUO group than in the sham group and decreased after geniposide treatment (Fig. 7B-C). These results suggested that geniposide significantly inhibits the phosphorylation of AKT and PI3K in UUO-induced mice, thereby suppressing the PI3K/AKT signaling pathway.

Cell experiments

Geniposide alleviates renal fibrosis via regulation of the PI3K/AKT signaling pathway in TGF-β1-induced HK-2 cells

To further investigated the involvement of the PI3K/AKT signaling pathway in the process of renal fibrosis and whether it was the inhibition of its pathway that mitigated the progression of renal fibrosis after treatment with geniposide, we therefore performed the study on HK-2 cells in vitro. The CCK8 assay showed that geniposide significantly inhibited the cell viability at 300 μM, and we therefore chose a 200 μM concentration for treatment (Fig. 8A). In TGF-β1-induced HK-2 cells, the expression levels of p-PI3K and p-AKT were significantly elevated, as well as the fibrosis markers collagen I. However, these increases were reversed after geniposide treatment. In addition, the protein levels of p-PI3K and p-AKT as well as collagen I were downregulated after treatment with the PI3K/AKT inhibitor LY294002 (Fig. 8B-C). In summary, the protective effect of geniposide against renal fibrosis may be related to the inhibition of the PI3K/AKT signaling pathway.

Discussion

Renal fibrosis is a common pathophysiological condition in the later stages of CKD [21], At present, the drugs for clinical application and experimental treatment of renal fibrosis mainly include ACEI, ARB [2], SGLT2 inhibitors [22], which are effective in controlling blood pressure, reducing urinary protein, and delaying the progression

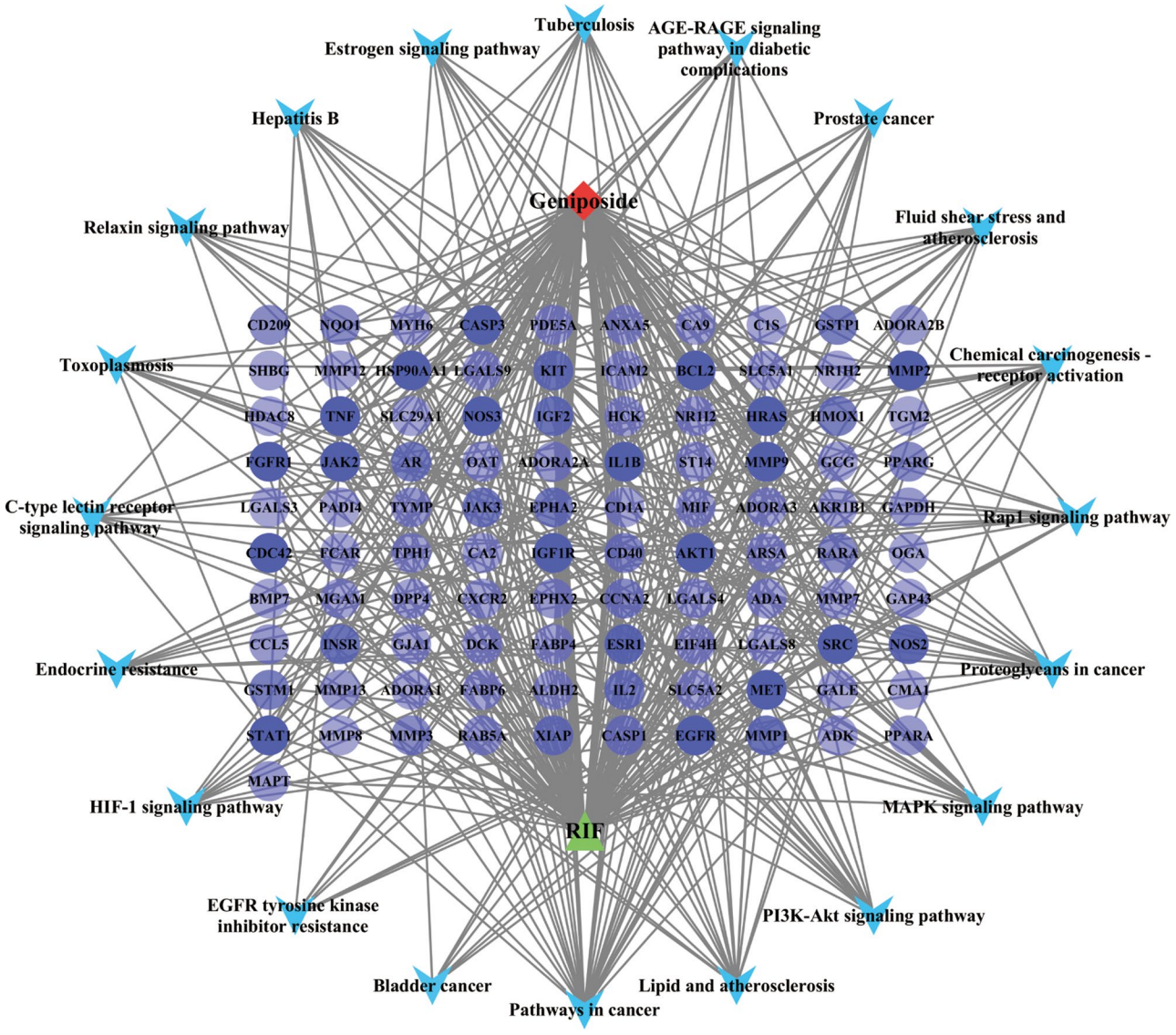

Fig. 4 Components-disease-target gene-pathway network. Red represents bioactive compounds. Green represents disease. Purple represents target genes, a node with higher degree would have darker colour. Blue represents signaling pathway

of CKD. However, there are currently no highly effective therapeutic drugs available. Therefore, identifying drug targets for the treatment of renal fibrosis is of great importance. Geniposide ameliorates AKI and diabetic nephropathy, as well as slows the progression of CKD [23, 24]. In our study, we employed network pharmacology analysis and molecular docking method to predict the potential targets and mechanisms of geniposide in treating renal fibrosis. AKT1, MMP9, GAPDH, BCL2, TNF, CASP3, SRC, EGFR, IL-1β, and STAT1 were the top 10 hub genes. The PI3K/AKT, MAPK, Rap1, and cancer-related signaling pathways were closely associated with the anti-fibrotic effects of geniposide. Our in vivo experiments indicated that geniposide inhibited the progression of renal fibrosis and suppressed the mRNA levels of hub genes including AKT, MMP9, BCL2, and TNF-α. Importantly, the results of in vivo and in vitro experiments showed that geniposide inhibited the phosphorylation of PI3K and AKT, suggesting that the alleviation of renal fibrosis by geniposide may be related to the inhibition of the activation of the PI3K/AKT signaling pathway.

AKT, also known as protein kinase B (PKB), is a serine/threonine protein kinase with a primary role in regulating cell growth [25] and apoptosis [26]. Among its three isoforms, AKT1 is particularly linked to renal fibrosis. When cells are stimulated by growth factors, cytokines, high-glucose conditions, or advanced glycation end-products, AKT1 is activated through phosphorylation [27]. It has been shown that AKT1 mediates tubular epithelial-mesenchymal transition during the transition from AKI to

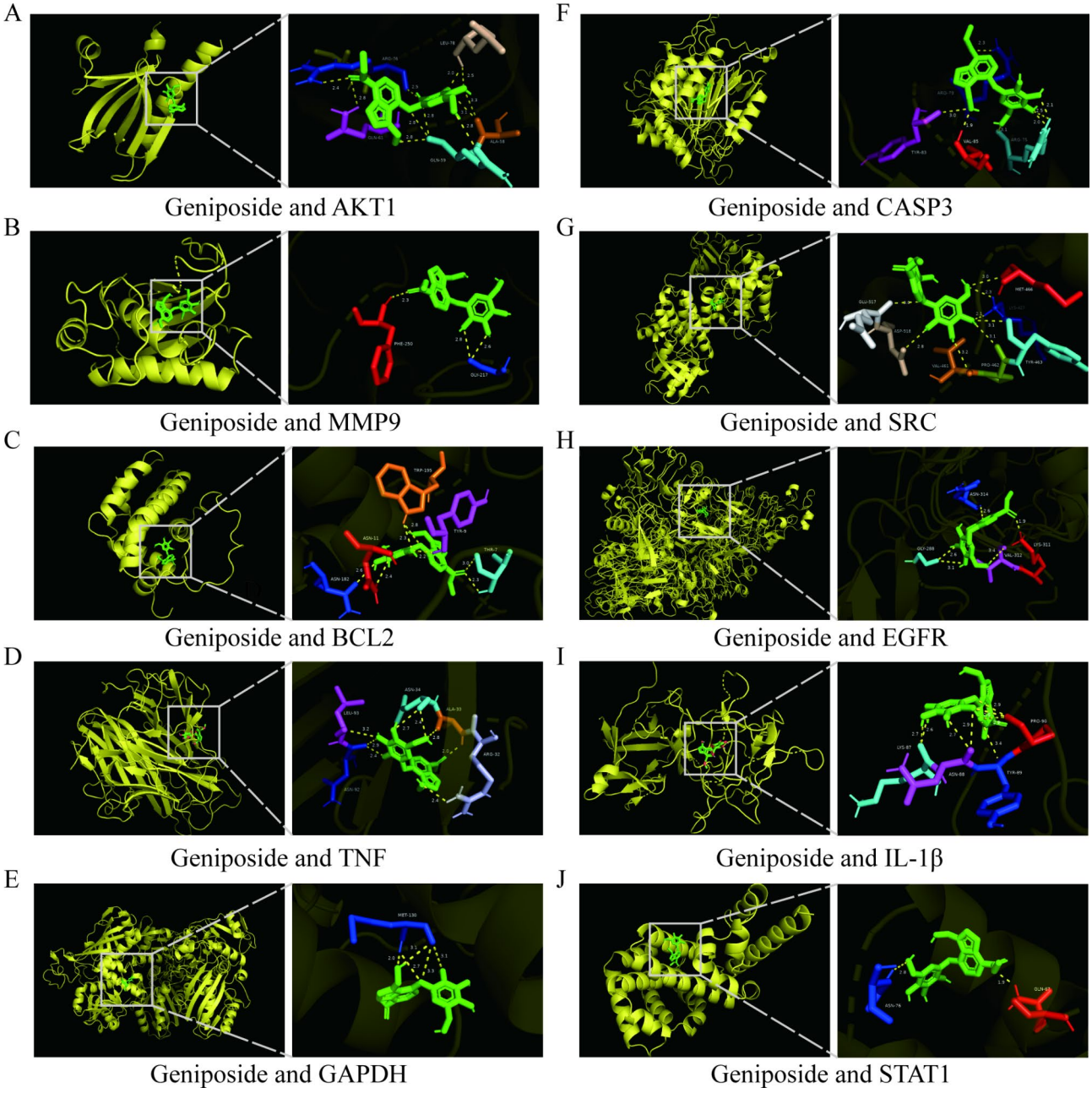

Fig. 5 Molecular docking pattern and mapping surface showing molecules occupying the active pocket of the top 10 hub proteins. (**A**) AKT1, (**B**) MMP9, (**C**) BCL2, (**D**) TNF, (**E**) GAPDH, (**F**) CASP3, (**G**) SRC, (**H**) EGFR, (**I**) IL-1β, (**J**) STAT1

CKD [27]. The PI3K/AKT pathway, in particular, plays a pivotal role in the progression of renal fibrosis [28]. Additionally, key targets identified in the PPI network, such as MMP9, BCL2, and TNF, are also closely related to pathways governing cell proliferation, apoptosis, and inflammation. And studies have shown their association with renal fibrosis [29, 30]. Matrix metalloproteinases (MMPs) belong to the zinc-dependent endoprotease family, which are involved in body growth, development and tissue repair, and have important biological regulatory capabilities. Their functions rely on the reconstruction and degradation of extracellular matrix protein components [31]. Research has indicated that macrophage-secreted MMPs, particularly MMP9, are involved in the initiation and progression of renal fibrosis by tubular cell epithelial-mesenchymal transition, endothelial-mesenchymal transition, activation of resident fibroblasts, and pericyte-myofibroblast transdifferentiation [32–34]. The BCL2 family of proteins plays a critical role in regulating the apoptosis process. It has been shown that the

Table 2 Details about the molecular docking of geniposide with the top 10 targets

Protein	Ligand	Binding capacity (Kcal / mol)	Geniposide Hydrogen bond interaction	
			No. of bonds	Residues involved
AKT1(1unq)	geniposide	-7.82	11	ARG-76, GLN-59, GLN-61, ALA-58, LEU-78
TNF(1a8m)	geniposide	-7.8	8	ARG-32, ALA-33, ASN-34, LEU-93, ASN-9
BCL2(1g5m)	geniposide	-7.4	7	ASN-182, ASN-11, TRP-195, THR-7, TYR-9
MMP9(6esm)	geniposide	-6.16	3	PHE-250, GLY-217
SRC(4u5j)	geniposide	-5.6	8	VAL-461, TYR-463, MET-466, LYS-427, PRO-462, GLU-517, ASP-518
GAPDH(6ade)	geniposide	-5.26	5	MET-130
IL-1β(4gai)	geniposide	-5.14	8	PRO-90, TYR-89, ASN-88, LYS-87
EGFR(5wb7)	geniposide	-5.12	5	ASN-314, GLY-288, VAL-312, LYS-311
CASP3(1cp3)	geniposide	-5.08	7	ARG-75, ARG-79, TYR-83, VAL-85
STAT1(3wwt)	geniposide	-4.19	2	GLN-67, ASN-76

mRNA levels of BCL2 are significantly reduced in UUO mice, and the apoptosis it causes is associated with tubular atrophy and renal fibrosis [35]. TNF-α regulates the progression of AKI and CKD [36] and is also involved in the progression of diabetic nephropathy [37]and obesity nephropathy [38]. In addition, TNF-α, as an important mediator of renal fibrosis, plays a significant role in causing renal fibrosis, especially promoting renal fibroblast proliferation and collagen production [30]. In addition, geniposide has anti-inflammatory properties and may ameliorate liver fibrosis by reducing TNF levels [39]. GO enrichment analysis further suggested that these targets are primarily involved in the positive regulation of cell proliferation, the negative regulation of apoptosis, and inflammation, contributing to the pathogenesis of renal fibrosis.

KEGG pathway enrichment analysis indicated that the overlapping genes are mainly associated with the PI3K/AKT, MAPK, and Rap1 signaling pathways. The PI3K/AKT pathway has been widely demonstrated to play a significant role in renal fibrosis and dysfunction by regulating various proteins [40]. However, some studies have shown that activation of the AKT-eNOS pathway has a protective effect on glomeruli [41], which seems inconsistent with our finding that activation of AKT aggravates tubulointerstitial fibrosis. This may be related to the fact that AKT overactivation mediates tubular epithelial-mesenchymal transition (EMT), which in turn aggravates renal fibrosis [27]. Moreover, it has been found that astragaloside IV inhibits AKT phosphorylation, blocks GSK-3β phosphorylation, and restores GSK-3β activity, which contributes to the degradation of β-catenin, thus preventing EMT [20]. And the phosphorylated AKT has an anti-apoptotic effect [42]. Studies have shown that inhibition of apoptosis can promote the activation of myofibroblasts, and activated myofibroblasts secrete cytokines, such as TGF-β, leading to a large number of myofibroblasts accumulation and excessive deposition of extracellular matrix, and ultimately the formation of irreversible renal interstitial fibrosis [43]. In this study, irreversible ureteral obstruction may cause overactivation of the PI3K/AKT pathway and thus lead to renal interstitial fibrosis. And geniposide can alleviate the progression of renal fibrosis by inhibiting the activation of the PI3K/AKT signaling pathway. For instance, Fufang Shenhua tablet prevents kidney fibrosis by inhibiting this pathway [44]. In addition, geniposide enhances lipophagy through inhibition of the PI3K/AKT signaling pathway for the treatment of atherosclerosis [45].

The MAPK family comprises three major subfamilies: extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38, which constitute the classical MAPK pathways [46]. The MAPK signaling pathway is implicated in a variety of physiological and pathological processes, including cell proliferation, differentiation, apoptosis, stress response, and inflammation. Furthermore, ERK, JNK, and MAPK pathways have been directly linked to renal fibrosis [47, 48]. Rap1, a Ras-associated protein, recruits numerous effector molecules when activated and regulates cell proliferation, migration, polarization, adhesion, and survival [49]. Some studies suggest that Rap1 activation exacerbates renal fibrosis [50]. MAPKs, as a group of evolutionarily conserved serine/threonine kinases, are closely related to the PI3K/AKT pathway [51]. Additionally, previous studies have identified Rap1 as a key regulator of PI3K activity, influencing cell proliferation and survival by activating the PI3K/AKT pathway [52]. B-Raf, an effector molecule of Rap1, mediates ERK activation, which in turn triggers the Rap1-MAPK signaling pathway to regulate cell proliferation and survival [53]. The PI3K/AKT, MAPK, and Rap1 signaling pathways are therefore intricately linked to the onset and progression of renal fibrosis, making the PI3K/AKT pathway particularly worthy of further investigation.

However, this study has some limitations. We only performed the validation of expression differences but not the functional validation. Secondly, the exploration of the downstream mechanism was also insufficient, and these need to be further investigated.

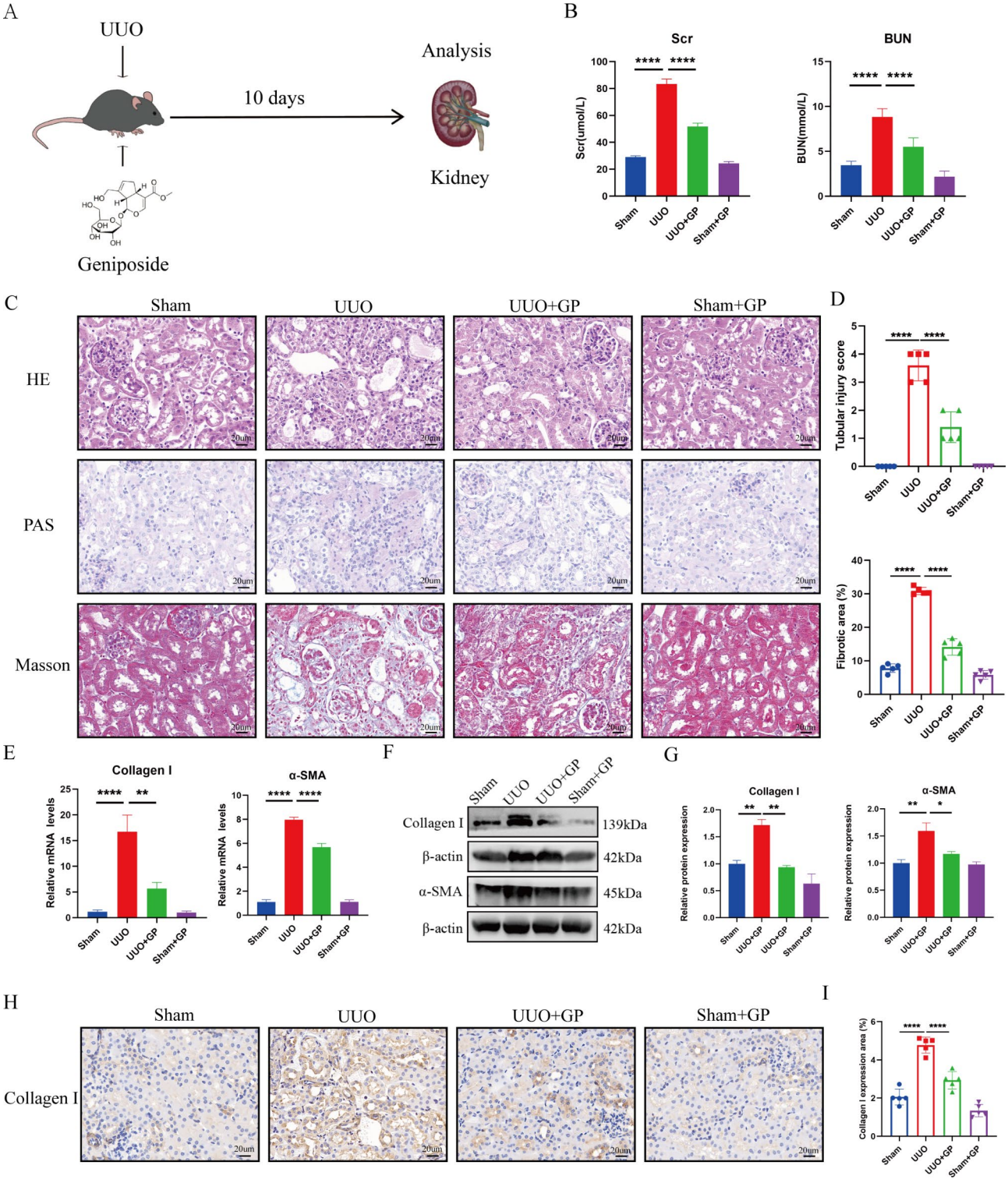

Fig. 6 Geniposide improves renal fibrosis in vivo. **A** Flow chart of geniposide intervention in UUO model mice. **B** Serum creatinine (Scr) and blood urea nitrogen (BUN) in different groups of mice. **C**-**D** HE, PAS, Masson and of kidney tissues of the four groups, scale bar = 20 μm. **E**-**G** The mRNA and protein levels of collagen I and α-SMA in mouse kidney tissues. **H**-**I** Immunohistochemical staining of collagen I and relative quantitative data in mice, scale bar = 20 μm. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

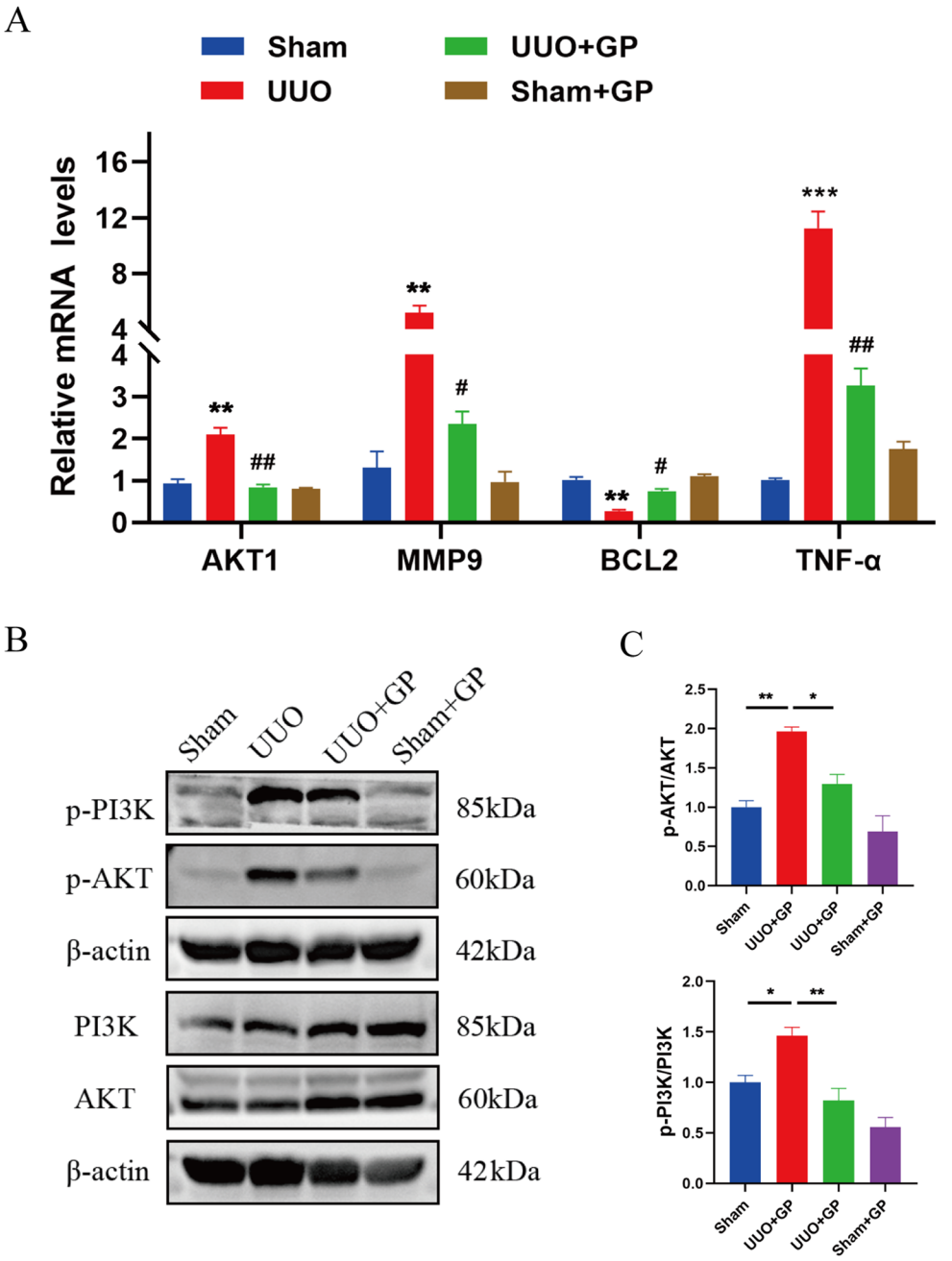

Fig. 7 Geniposide inhibits hub genes and the PI3K/AKT signaling pathway by network pharmacology in vivo. **A** The mRNA expression of AKT1, MMP9, BCL2 and TNF-α in UUO model mice, as determined by RT–qPCR. *$P < 0.05$ vs. the sham group; $^{\#}P < 0.05$ vs. the UUO group. **B**-**C** Western blotting analysis and relative quantitative analysis of p-AKT, AKT, p-PI3K and PI3K in mice. Quantitative histograms of western blotting analysis for the protein expression ($n = 3$). Other data represent the mean ± SEM for 5 mice. ****$p < 0.0001$, ***$p < 0.001$, ** $p < 0.01$, *$p < 0.05$

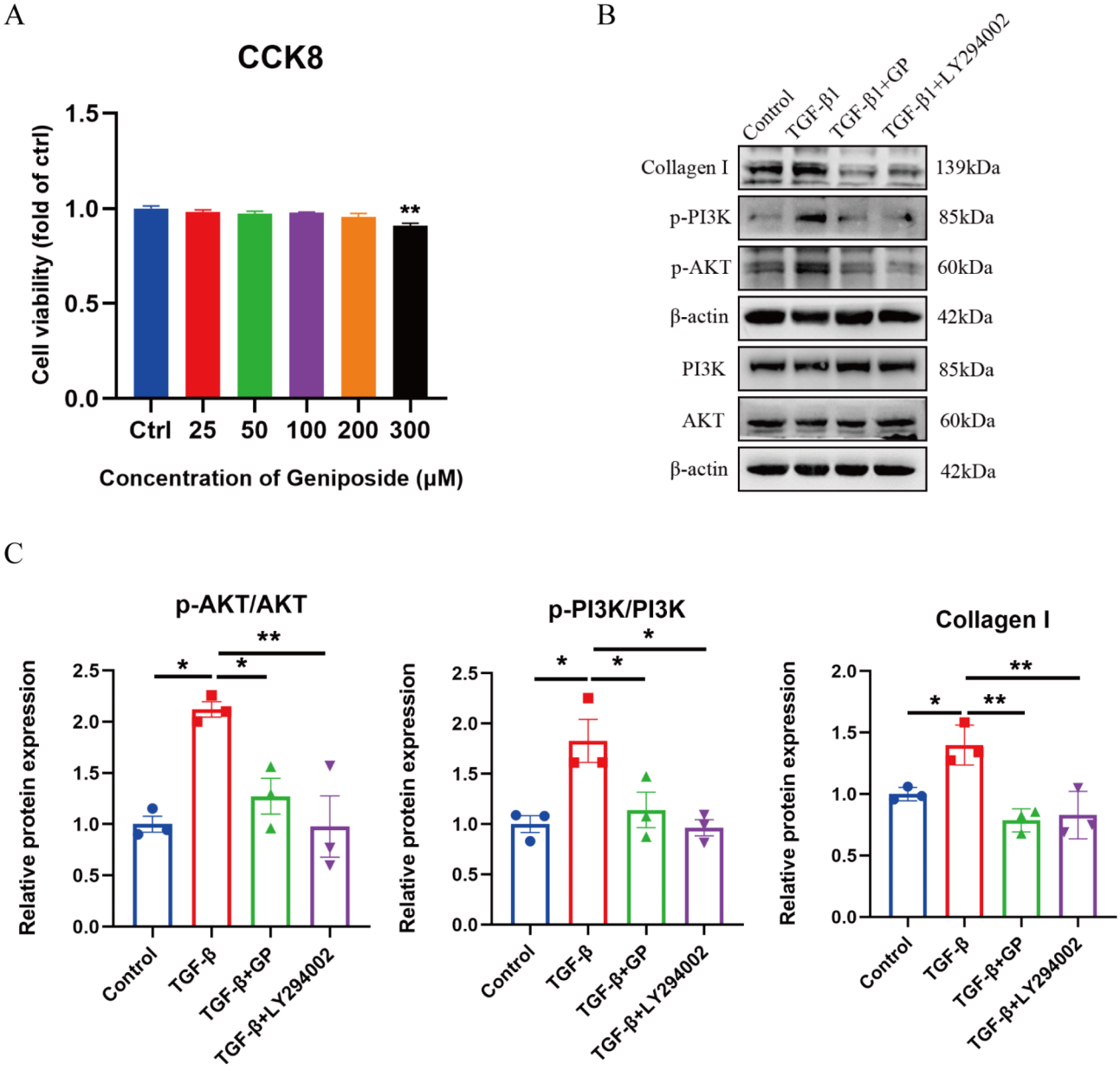

Fig. 8 Geniposide alleviates renal fibrosis through inhibiting PI3K/AKT signaling pathway in vitro. **A** The cytotoxic effect of geniposide (0, 25, 50, 100, 200, 300 μM) on HK-2 cells for 24 h determined by CCK-8 assay. $n = 3$, ** $p < 0.01$ vs. the control group. **B**-**C** Western blotting analysis and relative quantitative analysis of collagen I, α-SMA, p-AKT, AKT, p-PI3K and PI3K in HK-2 cells. Quantitative histograms of western blotting analysis for the protein expression ($n = 3$). ****$p < 0.0001$, ***$p < 0.001$, ** $p < 0.01$, *$p < 0.05$

Conclusion

This study confirmed that geniposide may alleviate renal fibrosis in mice by inhibiting the activation of the PI3K/AKT signaling pathway, as well as downregulating MMP9 and TNF-α, and upregulating the expression of BCL2. These findings provide a foundation for further exploration of geniposide as a potential treatment for renal fibrosis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40360-025-00855-w.

Supplementary Material 1

Supplementary Material 2

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Author contributions
Project design: Deguang Wang; Experimental verification and manuscript writing: Mengqian Liu; Animal experiment: Wenman Zhao and Rui Shi; Data analysis: Zhijuan Wang and Xunliang Li. All authors reviewed the manuscript.

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Data availability
All of the datasets analyzed in this study are as follows: PubChem (https://pubchem.ncbi.nlm.nih.gov), PharmMapper (https://lilab-ecust.cn/pharmmapper/index.html), Swiss Target Prediction (http://www.swisstargetprediction.ch/), TCMBank (https://tcmbank.cn), UniProt (https://www.uniprot.org), OMIM (https://www.omim.org), DisGenet (https://www.disgenet.org), GeneCards (https://www.genecards.org), STRING 11.5 (https://string-db.org), David6.8 (https://david.ncifcrf.gov), GO (http://www.geneontology.org), KEGG (https://www.kegg.jp/kegg/kegg1.html), and TCMSP (https://test.tcmsp-e.com).

Declarations

Ethical approval
The animal experiments in this study were approved by the Ethical Guidelines of the Anhui Medical University and the Guide to the Care and Use of Experimental Animals (approval number: LLSC20210354).

Competing interests
The authors declare no competing interests.

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