Analysis of the potential biological mechanisms of diosmin against renal fibrosis based on network pharmacology and molecular docking approach

Wen-Man Zhao
The Second Hospital of Anhui Medical University
Zhi-Juan Wang
The Second Hospital of Anhui Medical University
Rui Shi
The Second Hospital of Anhui Medical University
Yvyv Zhu
The Second Hospital of Anhui Medical University
Xun-Liang Li
The Second Hospital of Anhui Medical University
De-Guang Wang (wangdeguang@ahmu.edu.cn)
The Second Hospital of Anhui Medical University

Research Article

Keywords: Diosmin, Molecular docking, Network pharmacology, Renal fibrosis, Therapeutic Targets

Posted Date: March 1st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2566837/v1

Abstract

Background Interstitial fibrosis is involved in the progression of various chronic kidney diseases and renal failure. Diosmin is a naturally occurring flavonoid glycoside that has antioxidant, anti-inflammatory, and antifibrotic activities. However, whether diosmin protects kidneys by inhibiting renal fibrosis is unknown.

Methods The molecular formula of diosmin was obtained, targets related to diosmin and renal fibrosis were screened, and interactions among overlapping genes were analyzed. Overlapping genes wereused for gene function and KEGG pathway enrichment analysis.TGF-β1 was used to induce fibrosis in HK-2 cells, and diosmin treatment was administered. The expression levels of relevant mRNA were then detected.

Results Network analysis identified 295 potential target genes for diosmin, 6828 for renal fibrosis, and 150 hub genes. Protein–protein interaction network results showed that CASP3, SRC, ANXA5, MMP9, HSP90AA1, IGF1, RHOA, ESR1, EGFR, and CDC42 were identified as key therapeutic targets. GO analysis revealed that these key targets may be involved in the negative regulation of apoptosis and protein phosphorylation. KEGG indicated that pathways in cancer, MAPK signaling pathway, Ras signaling pathway, PI3K-Akt signaling pathway, andHIF-1 signaling pathway were key pathways for renal fibrosis treatment. Molecular docking results showed that CASP3, ANXA5, MMP9, and HSP90AA1 stably bind to diosmin. Diosmin treatment inhibited the protein and mRNA levels of CASP3, MMP9, ANXA5, and HSP90AA1. Network pharmacology analysis and experimental results suggest that diosmin ameliorates renal fibrosis by decreasing the expression of CASP3, ANXA5, MMP9, and HSP90AA1.

Conclusions Diosmin has a potential multi-component, multi-target, and multi-pathway molecular mechanism of action in the treatment of renal fibrosis. CASP3, MMP9, ANXA5, and HSP90AA1 might be the most important direct targets of diosmin.

Introduction

The prevalence of chronic kidney disease (CKD) is estimated to be 8–16% worldwide [1], threatening human health and imposing a heavy economic burden on patients. Renal fibrosis is a pathological feature common to almost all CKD cases that progress to end-stage renal disease [2]. Renal fibrosis is a progressive pathophysiological change from healthy to injured kidney tissues, which are damaged until functional loss. In this process, the kidney is affected by various pathogenic factors such as infection, inflammation, blood circulation disorders, and immune response, resulting in damage and sclerosis of intrinsic cells. In later stages of development, an intense collagen deposition and accumulation occurs, resulting in gradual sclerosis of the renal parenchyma and scarring until the kidney completely loses its organ function [3]. Therefore, early prevention and treatment of renal fibrosis is of great significance to delay the progression of CKD. However, owing to the lack of treatments for renal fibrosis, the search for new therapeutic drugs is an important strategy to prevent renal fibrosis.

Diosmin is a glycosylated polyphenolic flavonoid with antioxidant, anti-inflammatory, and anti-apoptotic pharmacological activities [4]. In recent years, the anti-fibrotic effect of Diosmin has also received increasing attention. Diosmin has been shown to reduce paraquat-induced lung inflammation and fibrosis by increasing glutathione levels and catalase activity and by decreasing hydroxyproline and malondialdehyde levels. In addition, Gerges et al. [5] demonstrated that diosmin ameliorates inflammation, insulin resistance, and liver fibrosis in a rat model of nonalcoholic steatohepatitis. Recently, Geshnigani et al. [6] showed that diosmin ameliorates gentamicin-induced renal injury through antioxidant and anti-inflammatory activities in rats. However, whether diosmin protects against chronic kidney injury by inhibiting interstitial fibrosis remains unclear. This study combined network

pharmacology and molecular docking techniques to comprehensively reveal the mechanisms underlying the therapeutic effects of diosmin on renal fibrosis and to predict the key targets and signaling pathways involved.

Materials And Methods

Query of the targets of diosmin

'Diosmin' was used as a search term in PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [7]. The SDF format file of diosmin was obtained and imported into PharmMapper (https://www.lilab-ecust.cn/pharmmapper/) for prediction. This method is based on reverse pharmacophore matching of structural features. The molecular structures of the diosmin components were submitted to the PharmMapper database, and multiple conformations were generated by optimizing the compound structure. The standardized diosmin components of the protein targets were matched with all the human drug targets in the PharmMapper database in UniProt (https://www.uniprot.org/), and protein targets with a parameter matching score (fit) greater than 2 were selected as the component targets.

Screening the therapeutic targets for renal fibrosis

Human genes associated with renal fibrosis were gathered from three databases: OMIM (https://omim.org/) [8], GeneCards (https://www.genecards.org/) [9], and DisGenet (https://www.disgenet.org/) [10]. The search term 'renal fibrosis' was used to retrieve valuable targets from three databases. Finally, the intersection targets of diosmin and renal fibrosis were displayed using a Draw Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/) [11].

Protein–protein interaction (PPI)

PPIs of the therapeutic targets of diosmin in the treatment of renal fibrosis were gathered using STRING (https://string-db.org/) [18], a database of known and predicted PPIs that uses bioinformatic strategies to collect information. In this study, we limited the species to 'Homo sapiens', collected the PPIs with confidence scores > 0.4 and hid the disconnected nodes in the network.

Gene ontology (GO) and pathway enrichment analysis

To further explore the mechanisms of diosmin for renal fibrosis treatment, the intersection of targets of diosmin and the common proteins between renal fibrosis were additionally searched using GO (http://geneontology.org/) [12], enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) (www.kegg.jp/kegg/kegg1.html) [13], and pathway analysis via the online platform DAVID 6.8 (DAVID, https://david.ncifcrf.gov/) [14]. The results obtained by mapping the diosmin targets overlapping renal fibrosis targets were imported into the online software DAVID with H. sapiens set and $P < 0.05$ selected, and the results were analyzed by selecting GO Biological Processes (BP), GO Molecular Functions (MF), GO Cellular Components (CC), and KEGG Pathway for GO and KEGG analysis of the obtained results.

Molecular docking

The 2D structures of diosmin were downloaded from PubChem (https://pubchem.ncbi. nlm.nih.gov/) (https://pubchem.ncbi.nlm.nih.gov/) [7] and TCMSP database; they were then imported to Chemofce 2014 software for the SDF format switching to mol2 format (3D structure). The 3D structures of diosmin were obtained from the RCSB PDB database (http://www.rcsb.org/pdb/) [15] and were input to the PyMol software to separate the original ligand and remove the hydrone, phosphate radical, and other inactive ligands from the proteins [16]. The 3D structures of small molecules (ligands) and target proteins were imported into Auto Dock Vina software to acquire the

PDBQT format, and finally docking the molecules with the target proteins; the affinity and the hydrogen bond interaction was shown using PyMol [17].

Reagent

Diosmin (cat. no.: HY-N0178) was purchased from MedChemExpress (Monmouth Junction, NJ, USA). Human proximal tubular epithelial (HK-2) cells were obtained from American Type Culture Collection (Manassas, VA, USA). Fetal bovine serum was obtained from Millipore Sigma (Burlington, MA, USA). Transforming growth factor-β1 (TGF-β1) was purchased from Proteintech (Chicago, IL, USA). Reverse transcription kit and SYBR Green PCR Master Mix were purchased from Vazyme (Nanjing, China).

Cell culture and treatment

To assess cell viability, HK-2 cells were cultured at 1×10^4 cells/well in 96-well plates. After treatment with diosmin for 3 h, they were incubated with 0.5 mg/mL of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (100 μL/well) for 4 h. After washing with 1× PBS, dimethyl sulfoxide (150 μL/well) was added to dissolve purple crystals. The absorbance of the samples was measured at 570 nm using a microplate reader (BioTek Instruments, VT, USA). Furthermore, diosmin was administered to TGF-β1 (5 ng/mL)-stimulated HK-2 cells. The effects of diosmin on core genes were observed in HK-2 cells.

Real-time PCR

Total RNA was extracted from HK-2 cells or mouse kidney tissues using TRIzol® reagent. Complementary DNA was synthesized using Hiscript Q RT SuperMix and used with the qPCR (+gDNA wiper) reverse transcriptase kit (Vazyme). The sequences of the used primes for Real-time PCR are listed in **Table 1**. Messenger (m)RNA expression in the corresponding samples was normalized to GAPDH mRNA.

Statistical analyses

Values are presented as the mean ± SEM. Quantitative data were tested for normality. Two-tailed unpaired t-test and one-way ANOVA were used to compare differences between two groups and multiple groups, respectively. Prism 9.0 (GraphPad, San Diego, CA, USA) was used for the statistical analyses. $P < 0.05$ was designated significant.

Results

The targets of diosmin and therapeutic targets for renal fibrosis

Figure 1 shows the flow of the network pharmacological analysis of Diosmin against renal fibrosis. The chemical formula of the diosmin molecule $C_{28}H_{32}O_{15}$ was obtained from PubChem, and the targets related to diosmin were screened using the PharmMapper database and corrected using the UniProKB tool in the UniProt database. A total of 295 potential targets, including A1AT, CASP3, MAPK14, and MMP9, were screened after removing targets that were unrelated to humans and those without correspondence or duplication. A total of 266, 570, and 6546 target genes closely related to renal fibrosis were screened in the three commonly used databases of GeneCards, OMIM, and Dis Genet, respectively. A total of 6828 genes were identified after duplicates were eliminated. These results suggest that numerous factors cause renal fibrosis, and the pathogenesis is complex. Gene datasets obtained from the screening of renal fibrosis-related targets and diosmin component-related targets were imported into an online Venn diagram, and a total of 150 intersecting targets were obtained (Figure 2).

Drug-disease target PPI network

A total of 150 hub genes were screened by mapping the diosmin component targets to each other and to the renal fibrosis disease targets. The 150 targets were imported into the STRING database and imported into Cytoscape for visualization and analysis, and a network consisting of 112 nodes and 721 edges was obtained. The network density was 0.588, and the average node degree was 12.9. When the network density is greater than 0.5, and the average node degree is greater than 3, the network has a good correlation (Figure 3). In the network, the top ten targets with high degrees of freedom were caspase 3 (CASP3), SRC proto-oncogene, non-receptor tyrosine kinase (SRC), annexin A5 (ANXA5), matrix metallopeptidase 9 (MMP9), heat shock protein 90 alpha family class A member 1 (HSP90AA1), insulin-like growth factor 1 (IGF1), ras homolog family member A (RHOA), estrogen receptor 1 (ESR1), epidermal growth factor receptor (EGFR), and cell division cycle 42 (CDC42) (Figure 4). The related gene targets focused on apoptosis and inflammatory pathways.

GO and KEGG pathway enrichment

GO and KEGG pathway enrichment results were plotted as bar graphs and bubble plots by gene number for visual analysis. A total of 291 BP, 44 CC, 85 MF, and 98 KEGG signaling pathways were identified. GO analysis showed that diosmin mainly plays a role in the BPs of apoptosis negative regulation, protein phosphorylation, protein autophosphorylation, peptidyl-tyrosine phosphorylation, transmembrane receptor protein tyrosine kinase signaling pathway, response to foreign body stimulation, cellular response to insulin stimulation, and positive regulation of phosphatidylinositol 3-kinase signaling. Apoptosis was correlated with the development of renal fibrosis, while protein phosphorylation was associated with the regulation of protein activity. CC was mainly enriched in the cytoplasm and nucleoplasm. MF analysis showed an association between homologous protein binding and ATP binding (Figure 5). KEGG enriched 98 signaling pathways ($P < 0.05$), including cancer, lipids and atherosclerosis, proteoglycans in cancer, fluid shear stress and atherosclerosis, endocrine resistance, and Rap1, Ras, PI3K-Akt, chemokine, MAPK, FoxO, T-cell receptor, HIF-1, and estrogen signaling pathways (Figure 6). Among these, the MAPK, Ras, PI3K-Akt, FoxO, and HIF-1 signaling pathways were closely related to renal fibrosis.

Molecular docking

Diosmin and the top ten targets ranked by degree were selected for molecular docking (Table 2). The binding capacity score is an important indicator of the ability of the receptor and ligand to bind to each other; the lower the binding capacity score, the more stable the complex formed. Following the convention, a binding capacity between the tested molecules and proteins was assumed to exist when the binding energy score was greater than 4.25. Scores greater than 5.0 indicate relatively high binding affinity [49]. Hence, the selected diosmin was docked with MMP9, ANXA5, CASP3, and HSP90AA1 using AutoDock according to its binding capacity (Figure 7).

Effect of diosmin on the expression of target genes in HK-2

We further investigated the anti-fibrotic effect of diosmin on HK-2 cells. Diosmin treatment (0, 1, 5, 10, 25, 50, or 75 μM) of HK-2 cells did not significantly inhibit the viability (Figure 8). Cell viability was slightly inhibited at a concentration of 100 μM. Therefore, we treated the cells with 75 μM diosmin. Compared with the TGF-β1 group, the mRNA expression of ANXA5, CASP3, MMP9, and HSP90AA1 was significantly decreased in the diosmin group (Figure 9).

Discussion

Renal fibrosis is a common pathological feature in the terminal stages of various CKDs and is closely related to their prognosis [18]. Early prevention and treatment of renal fibrosis are of great significance in delaying the progression of chronic kidney disease. However, there is a lack of effective drugs for the treatment of renal fibrosis. Network pharmacology is an emerging discipline built on the disease and pharmacogenetic level. By predicting new drug targets, identifying modes of action, and exploring new drugs, network pharmacology opens a new research paradigm of complex web-like relationships between multiple targets and multiple diseases. In this study, we used a systematic network pharmacological approach to explore the potential molecular mechanisms of action of diazepine in renal fibrosis. Through PPI network and molecular docking module analyses, CASP3, MMP9, ANXA5, and HSP90AA1 were identified as major pivotal gene targets in renal fibrosis. Additionally, these four core genes were validated in HK-2 cells.

We found that core genes are mainly involved in the biological process of apoptosis. The protein encoded by CASP3 is a cysteine-aspartic acid protease that plays a central role in the execution phase of cell apoptosis. Caspase 3 is also a critical upstream regulator in the development of renal fibrosis [19]. Caspase 3 inhibitors have been shown to reduce renal interstitial fibrosis in diabetic nephropathy or obstructive nephropathy [20, 21]. Annexin 5 is a phospholipase A2 and protein kinase C inhibitory protein with calcium channel activity that has a potential role in cellular signal transduction, inflammation, growth, and differentiation [22]. Annexin V binds specifically to phosphatidylserine (PS) and is often used as a sensitive indicator of early apoptosis of cells. To date, no studies have proved a direct correlation between this protein and renal fibrosis. Matrix metalloproteinases (MMPs) belong to the family of zinc-dependent endoproteases. Their functions are based on remodeling and degradation of protein components of the extracellular matrix (ECM) [23]. Tan et al. suggested that MMP-9 directly contributes to the pathogenesis of renal fibrosis via induction of tubular cell epithelial-mesenchymal transition and osteopontin cleavage, which in turn recruits macrophages [24]. HSP90AA1 (heat shock protein 90 alpha family class A member 1) is a highly conserved molecular chaperone ubiquitously expressed in eukaryotic cells [25]. Research has suggested that myocardial fibroblasts from HSP90AA1 knock-out mice exhibited low collagen production [26]. Therefore, the core genes CASP3, ANXA5, MMP9, and HSP90AA1 may play key roles in the inhibition of the occurrence and development of renal fibrosis.

The core genes SRC, IGF1, RHOA, ESR1, EGFR and CDC42 may play a key role in the inhibition of the development and progression of renal fibrosis by diosgenin. This SRC gene may play a role in the regulation of embryonic development and cell growth. The protein encoded by this gene is a tyrosine-protein kinase whose activity can be inhibited by phosphorylation by c-SRC kinase. SRC kinase plays an important role in cell proliferation, differentiation, migration, and immune response, and it is a critical mediator of renal fibrosis [27, 28]. Activation of FXR has been found to attenuate renal fibrosis by inhibiting the phosphorylation of SRC, regulating the hippo pathway, and modulating the phosphorylation and localization of YAP [29]. Both IGF-1 and IGF-1 receptor levels are increased in glomeruli of diabetic rats, and this growth factor may be profibrotic [30, 31]. RhoA is one of the most studied Rho GTPases and is involved in a variety of cellular activities [32]. Research showed that extracellular vesicles produced by bone marrow mesenchymal stem cells attenuate renal fibrosis, in part by inhibiting the RhoA/ROCK pathway [33]. The ESR1 gene encodes the estrogen receptor. Research showed that Tamoxifen, a selective estrogen receptor modulator exhibits antifibrotic effects in the L-NAME model of hypertensive nephrosclerosis [34]. The protein encoded by the EGFR gene is a transmembrane glycoprotein that is a member of the protein kinase superfamily. In fibrotic disease induced by AKI or CKD, EGFR is frequently in a state of continuous activation in proximal tubule cells, which contributed to the progression of renal fibrosis in renal injury [35]. The protein encoded by the CDC42 gene is a small GTPase of the Rho-subfamily. Research indicated that ARAP1-AS2/ARAP1 may participate in cytoskeleton rearrangement and EMT processes in HK-2 cells through increased CDC42-GTP levels and induced renal Fibrosis [36].

According to the KEGG terms, the therapeutic targets of diosmin against renal fibrosis were mainly associated with the MAPK, Ras, PI3K-Akt, FoxO, and HIF-1 signaling pathways. MAPKs are a group of protein kinases containing three subfamilies: c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 [37]. MAPKs regulate many cellular functions, including proteasomal degradation [38]. ERK, p38, and JNK MAPK pathways are involved in kidney injury and fibrosis [39, 40]. Ras monomeric GTPases play a significant role in controlling proliferation, differentiation, and cell death. Research has shown that the CF ethanol extract may ameliorate renal fibrosis via the Wnt/β-catenin/RAS pathway [41]. An increasing number of reports have shown that the PI3K/AKT signaling pathway may play a crucial role in renal fibrosis and dysfunction by regulating various proteins [42]. Research has suggested that Chlorogenic Acid exerts protective effects against renal fibrosis by inhibiting PI3K/AKT signaling [43]. Forkhead homeobox type O (FoxO) transcription factors mediate cellular responses to oxidative stress and have been implicated in many ROS-regulated processes [44]. The PI3K–Akt–FoxO signaling pathway may play a role in ROS-mediated diseases, as shown by research in which Tempol attenuated renal fibrosis in mice with unilateral ureteral obstruction [45]. Hypoxia-inducible factors (HIFs), critical nuclear transcription factors, are involved in maintaining O_2 homeostasis [46]. Based on the difference in the α-subunits, HIFs are divided into three subtypes: HIF-1, HIF-2, and HIF-3. Reportedly, oxidative factors induced renal fibrosis by regulating the expression and activity of HIF-1 via PHD, ERK, and PI-3K/AKT pathways [47, 48]. Therefore, the MAPK, Ras, PI3K-Akt, FoxO, and HIF-1 signaling pathways are closely related to the occurrence and development of renal fibrosis.

We explored the potential molecular mechanism of action of diosmin in the treatment of renal fibrosis from a comprehensive and systematic perspective, and our results provide a theoretical basis for further experimental studies. Network pharmacology analysis and molecular docking technology were used to explore the potential mechanism of action of diosmin against renal fibrosis, and the key therapeutic targets of diosmin were identified as CASP3, MMP9, ANXA5, and HSP90AA1. The mechanism of action of diosmin in the treatment of renal fibrosis may be related to the regulation of biological pathways such as cell apoptosis and inflammation. This study provides a theoretical basis for the treatment of diosmin-induced renal fibrosis. A limitation of this study is the lack of additional experimental areas to validate our findings. However, the specific mechanisms involved in this process require further exploration.

Conclusion

Diosmin has a potential multi-component, multi-target, and multi-pathway molecular mechanism of action in the treatment of renal fibrosis. CASP3, MMP9, ANXA5, and HSP90AA1 might be the most important direct targets of diosmin. The mechanism of action may be related to the MAPK, Ras, PI3K-Akt, FoxO, and HIF-1 signaling pathways. This study provides a basis for further studies on diosmin in the treatment of renal fibrosis.

Declarations

Acknowledgements

We thank all authors for their contributions and support.

Authors' contributions

Conception and design: WM Zhao, ZJ Wang and DG Wang; Provision of study materials: XL Li; Collection and assembly of data: WM Zhao and ZJ Wang; Data analysis and interpretation: WM Zhao and R Shi; Manuscript writing: all authors; Final approval of the manuscript: all authors.

Funding

This work was supported by a grant from the Natural Science Foundation of the Anhui Province (number 2008085MH244).

Availability of data and materials

The data generated in this study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable to the present study.

Competing interests

The authors declare that there is no conflict of interest

References

1. Sharma S, Sarnak MJ: Epidemiology. The global burden of reduced GFR: ESRD, CVD and mortality. Nature reviews Nephrology 2017, 13(8):447-448.
2. Li L, Fu H, Liu Y. The fibrogenic niche in kidney fibrosis: components and mechanisms. Nature reviews Nephrology 2022, 18(9):545-557.
3. Lausecker F, Lennon R, Randles MJ. The kidney matrisome in health, aging, and disease. Kidney Int 2022, 102(5):1000-1012.
4. Gerges SH, Wahdan SA, Elsherbiny DA, El-Demerdash E. Pharmacology of Diosmin, a Citrus Flavone Glycoside: An Updated Review. European journal of drug metabolism and pharmacokinetics 2022, 47(1):1-18.
5. Gerges SH, Wahdan SA, Elsherbiny DA, El-Demerdash E. Diosmin ameliorates inflammation, insulin resistance, and fibrosis in an experimental model of non-alcoholic steatohepatitis in rats. Toxicology and applied pharmacology 2020, 401:115101.
6. Geshnigani SSH, Mahdavinia M, Kalantar M, Goudarzi M, Khorsandi L, Kalantar H. Diosmin prophylaxis reduces gentamicin-induced kidney damage in rats. Naunyn-Schmiedeberg's archives of pharmacology 2022.
7. Wang Y, Xiao J, Suzek TO, Zhang J, Wang J, Zhou Z, et al. PubChem's BioAssay Database. Nucleic acids research 2012, 40(Database issue):D400-412.
8. Amberger JS, Bocchini CA, Schiettecatte F, Scott AF, Hamosh A. OMIM.org: Online Mendelian Inheritance in Man (OMIM®), an online catalog of human genes and genetic disorders. Nucleic acids research 2015, 43(Database issue):D789-798.
9. Safran M, Dalah I, Alexander J, Rosen N, Iny Stein T, Shmoish M, et al. GeneCards Version 3: the human gene integrator. Database : the journal of biological databases and curation 2010, 2010:baq020.
10. Piñero J, Queralt-Rosinach N, Bravo À, Deu-Pons J, Bauer-Mehren A, Baron M,et al. DisGeNET: a discovery platform for the dynamical exploration of human diseases and their genes. Database : the journal of biological

databases and curation 2015, 2015:bav028.

11. Huang XF, Zhang JL, Huang DP, Huang AS, Huang HT, Liu Q, et al. A network pharmacology strategy to investigate the anti-inflammatory mechanism of luteolin combined with in vitro transcriptomics and proteomics. International immunopharmacology 2020, 86:106727.
12. Expansion of the Gene Ontology knowledgebase and resources. Nucleic acids research 2017, 45(D1):D331-d338.
13. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic acids research 2000, 28(1):27-30.
14. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature protocols 2009, 4(1):44-57.
15. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucleic acids research 2000, 28(1):235-242.
16. Seeliger D, de Groot BL. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. Journal of computer-aided molecular design 2010, 24(5):417-422.
17. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry 2010, 31(2):455-461.
18. Liu Y. Cellular and molecular mechanisms of renal fibrosis. Nature reviews Nephrology 2011, 7(12):684-696.
19. Yang B, Lan S, Dieudé M, Sabo-Vatasescu JP, Karakeussian-Rimbaud A, Turgeon J, Qi S, Gunaratnam L, et al. Caspase-3 Is a Pivotal Regulator of Microvascular Rarefaction and Renal Fibrosis after Ischemia-Reperfusion Injury. J Am Soc Nephrol 2018, 29(7):1900-1916.
20. Wen S, Wang ZH, Zhang CX, Yang Y, Fan QL. Caspase-3 Promotes Diabetic Kidney Disease Through Gasdermin E-Mediated Progression to Secondary Necrosis During Apoptosis. Diabetes, metabolic syndrome and obesity : targets and therapy 2020, 13:313-323.
21. Wu M, Xia W, Jin Q, Zhou A, Wang Q, Li S, et al. Gasdermin E Deletion Attenuates Ureteral Obstruction- and 5/6 Nephrectomy-Induced Renal Fibrosis and Kidney Dysfunction. Frontiers in cell and developmental biology 2021, 9:754134.
22. Gerke V, Creutz CE, Moss SE. Annexins: linking Ca2+ signalling to membrane dynamics. Nature reviews Molecular cell biology 2005, 6(6):449-461.
23. Cui N, Hu M, Khalil RA. Biochemical and Biological Attributes of Matrix Metalloproteinases. Progress in molecular biology and translational science 2017, 147:1-73.
24. Tan TK, Zheng G, Hsu TT, Lee SR, Zhang J, Zhao Y, et al. Matrix metalloproteinase-9 of tubular and macrophage origin contributes to the pathogenesis of renal fibrosis via macrophage recruitment through osteopontin cleavage. Laboratory investigation; a journal of technical methods and pathology 2013, 93(4):434-449.
25. Picard D. Heat-shock protein 90, a chaperone for folding and regulation. Cellular and molecular life sciences : CMLS 2002, 59(10):1640-1648.
26. García R, Merino D, Gómez JM, Nistal JF, Hurlé MA, Cortajarena AL, et al. Extracellular heat shock protein 90 binding to TGFβ receptor I participates in TGFβ-mediated collagen production in myocardial fibroblasts. Cellular signalling 2016, 28(10):1563-1579.
27. Yan Y, Ma L, Zhou X, Ponnusamy M, Tang J, Zhuang MA, et al. Src inhibition blocks renal interstitial fibroblast activation and ameliorates renal fibrosis. Kidney Int 2016, 89(1):68-81.
28. Chen J, Chen JK, Nagai K, Plieth D, Tan M, Lee TC, et al. EGFR signaling promotes TGFβ-dependent renal fibrosis. J Am Soc Nephrol 2012, 23(2):215-224.

29. Kim DH, Choi HI, Park JS, Kim CS, Bae EH, Ma SK, et al. Src-mediated crosstalk between FXR and YAP protects against renal fibrosis. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2019, 33(10):11109-11122.
30. Miyatake N, Shikata K, Wada J, Sugimoto H, Takahashi S, Makino H. Differential distribution of insulin-like growth factor-1 and insulin-like growth factor binding proteins in experimental diabetic rat kidney. Nephron 1999, 81(3):317-323.
31. Weiss O, Anner H, Nephesh I, Alayoff A, Bursztyn M, Raz I. Insulin-like growth factor-I (IGF-I) and IGF-I receptor gene expression in the kidney of the chronically hypoinsulinemic rat and hyperinsulinemic rat. Metabolism: clinical and experimental 1995, 44(8):982-986.
32. Nakamura M, Verboon JM, Parkhurst SM. Prepatterning by RhoGEFs governs Rho GTPase spatiotemporal dynamics during wound repair. The Journal of cell biology 2017, 216(12):3959-3969.
33. Shi Z, Wang Q, Zhang Y, Jiang D. Extracellular vesicles produced by bone marrow mesenchymal stem cells attenuate renal fibrosis, in part by inhibiting the RhoA/ROCK pathway, in a UUO rat model. Stem cell research & therapy 2020, 11(1):253.
34. Dellê H, Rocha JR, Cavaglieri RC, Vieira JM, Jr., Malheiros DM, Noronha IL. Antifibrotic effect of tamoxifen in a model of progressive renal disease. J Am Soc Nephrol 2012, 23(1):37-48.
35. Overstreet JM, Wang Y, Wang X, Niu A, Gewin LS, Yao B, et al. Selective activation of epidermal growth factor receptor in renal proximal tubule induces tubulointerstitial fibrosis. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2017, 31(10):4407-4421.
36. Li L, Xu L, Wen S, Yang Y, Li X, Fan Q. The effect of lncRNA-ARAP1-AS2/ARAP1 on high glucose-induced cytoskeleton rearrangement and epithelial-mesenchymal transition in human renal tubular epithelial cells. J Cell Physiol 2020, 235(7-8):5787-5795.
37. Johnson GL, Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science 2002, 298(5600):1911-1912.
38. Chaudhary P, Vishwanatha JK. c-Jun NH2-terminal kinase-induced proteasomal degradation of c-FLIPL/S and Bcl2 sensitize prostate cancer cells to Fas- and mitochondria-mediated apoptosis by tetrandrine. Biochem Pharmacol 2014, 91(4):457-473.
39. Grynberg K, Ma FY, Nikolic-Paterson DJ. The JNK Signaling Pathway in Renal Fibrosis. Front Physiol 2017, 8:829.
40. Feliers D, Kasinath BS. Erk in kidney diseases. Journal of signal transduction 2011, 2011:768512.
41. Cao B, Zeng M, Si Y, Zhang B, Wang Y, Xu R, et al. Extract of Corallodiscus flabellata attenuates renal fibrosis in SAMP8 mice via the Wnt/β-catenin/RAS signaling pathway. BMC complementary medicine and therapies 2022, 22(1):52.
42. Zhang Y, Jin D, Kang X, Zhou R, Sun Y, Lian F, et al. Signaling Pathways Involved in Diabetic Renal Fibrosis. Frontiers in cell and developmental biology 2021, 9:696542.
43. Zhou X, Zhang B, Zhao X, Lin Y, Zhuang Y, Guo J, et al. Chlorogenic Acid Prevents Hyperuricemia Nephropathy via Regulating TMAO-Related Gut Microbes and Inhibiting the PI3K/AKT/mTOR Pathway. Journal of agricultural and food chemistry 2022, 70(33):10182-10193.
44. Storz P. Forkhead homeobox type O transcription factors in the responses to oxidative stress. Antioxid Redox Signal 2011, 14(4):593-605.
45. Yoon HE, Kim SJ, Kim SJ, Chung S, Shin SJ. Tempol attenuates renal fibrosis in mice with unilateral ureteral obstruction: the role of PI3K-Akt-FoxO3a signaling. Journal of Korean medical science 2014, 29(2):230-237.
46. Choudhry H, Harris AL. Advances in Hypoxia-Inducible Factor Biology. Cell Metab 2018, 27(2):281-298.

47. Miao C, Zhu X, Wei X, Long M, Jiang L, Li C, et al. Pro- and anti-fibrotic effects of vascular endothelial growth factor in chronic kidney diseases. Ren Fail 2022, 44(1):881-892.
48. Wei X, Hou Y, Long M, Jiang L, Du Y. Molecular mechanisms underlying the role of hypoxia-inducible factor-1 α in metabolic reprogramming in renal fibrosis. Frontiers in endocrinology 2022, 13:927329.

Tables

Table 1 Primer sequences used for RT-qPCR

Gene	Forward (5’ to 3’)	Reverse (5’ to 3’)
HSP90AA1	CCAGTTCGGTGTTGGTTTTTAT	CAGTTTGGTCTTCTTTCAGGTG
Annexin 5	GTTCCATGGGCGCACAGGTTCTCAGAGGCA	TCCGCTCGAGTTAGCAGTCATCTTCTCCACAGAGCA
CASP3	CCAAAGATCATACATGGAAGCG	CTGAATGTTTCCCTGAGGTTTG
MMP9	CAGTACCGAGAGAAAGCCTATT	CAGGATGTCATAGGTCACGTAG
GAPDH	TGATGACATCAAGAAGGTGGTGAAG	TCCTTGGAGGCCATGTGGGCCAT

Table 2 Information on the molecular docking of diosmin with the top 10 targets

No.	Protein	Ligand	Binding capacity (Kcal / mol)	Protein	Ligand	Binding capacity (Kcal / mol)
1	ANXA5	diosmin	-5.17	CASP3	diosmin	-6.28
2	IGF1	diosmin	-4.14	RHOA	diosmin	-4.78
3	EGFR	diosmin	-2.67	MMP9	diosmin	-5.73
4	SRC	diosmin	-3.74	ESR1	diosmin	-2.45
5	HSP90AA1	diosmin	-5.76	CDC42	diosmin	-3.28

Figures

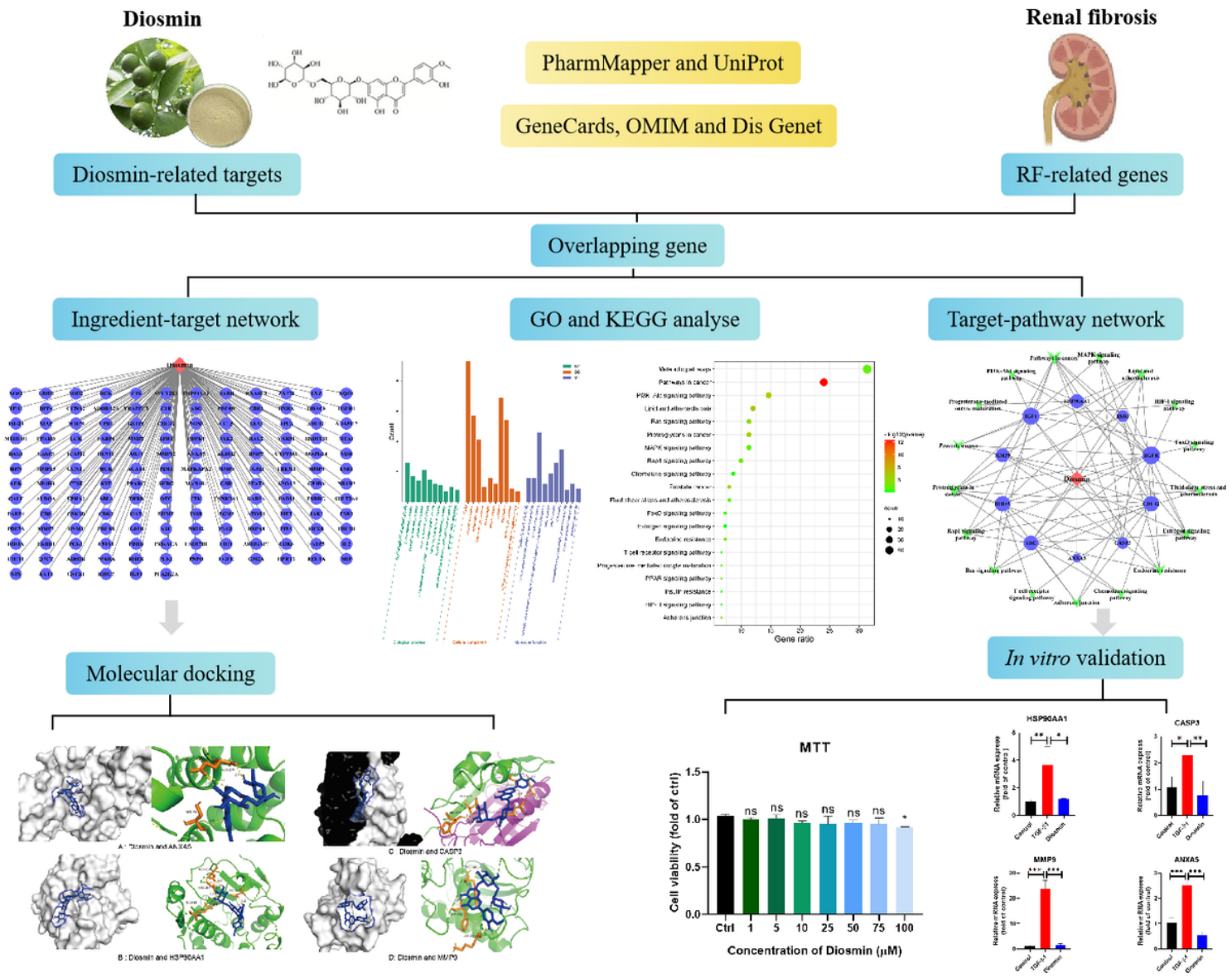

Figure 1

Flowchart of network pharmacology analysis of Diosmin against renal fibrosis.

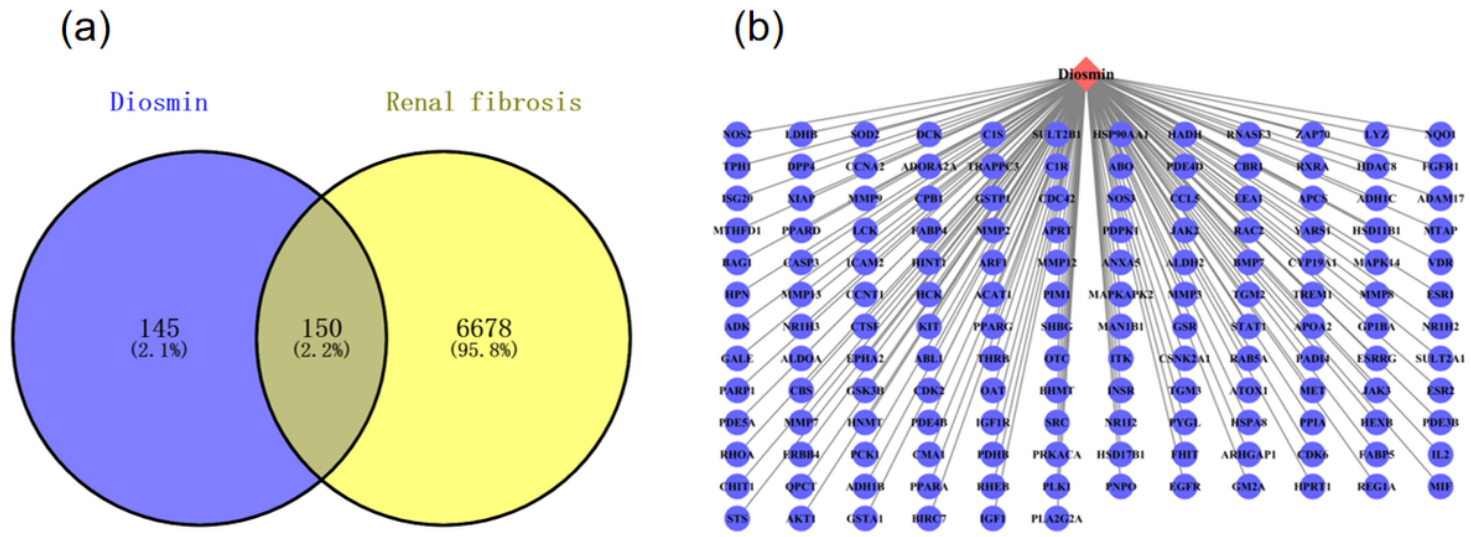

Figure 2

The 150 overlapping genes between diosmin and renal fibrosis targets.

(a) Venn diagram of Diosmin-related targets and renal fibrosis-related targets. (b) The ingredient-target network of Diosmin against renal fibrosis.

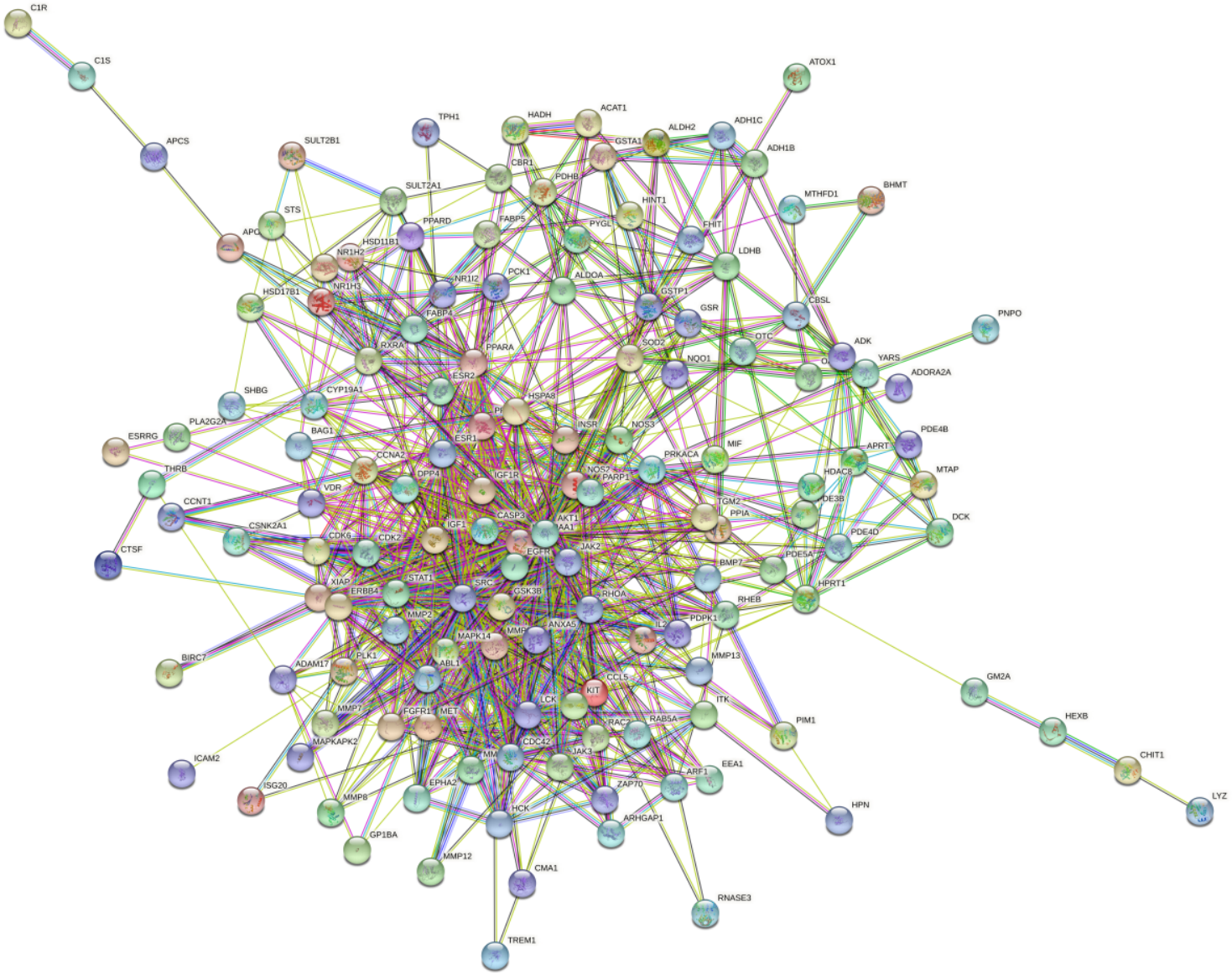

Figure 3

PPI network of intersection targets of 150 overlapping genes.

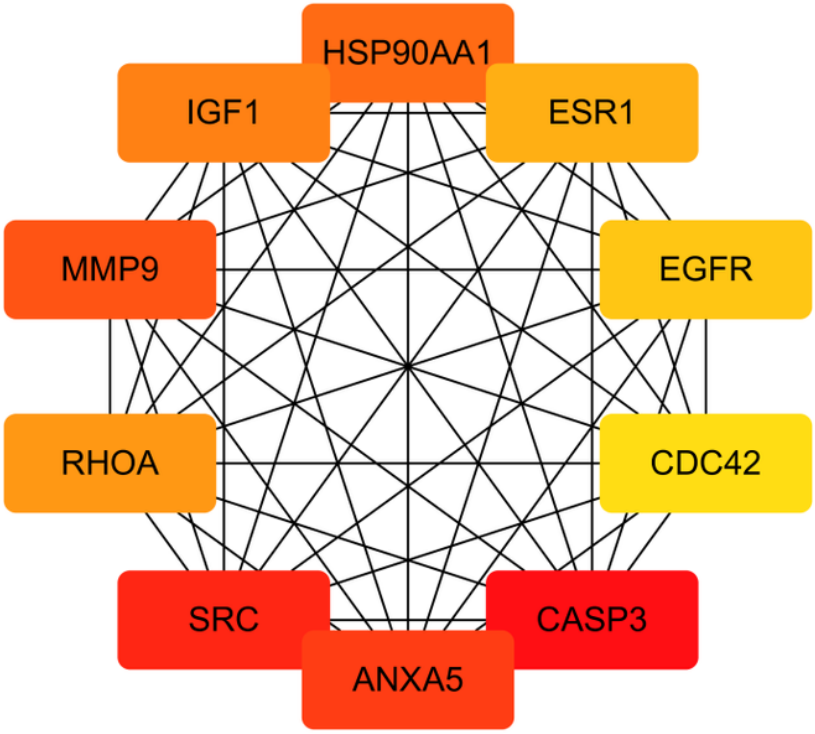

Figure 4

Ten high-freedom overlapping genes.

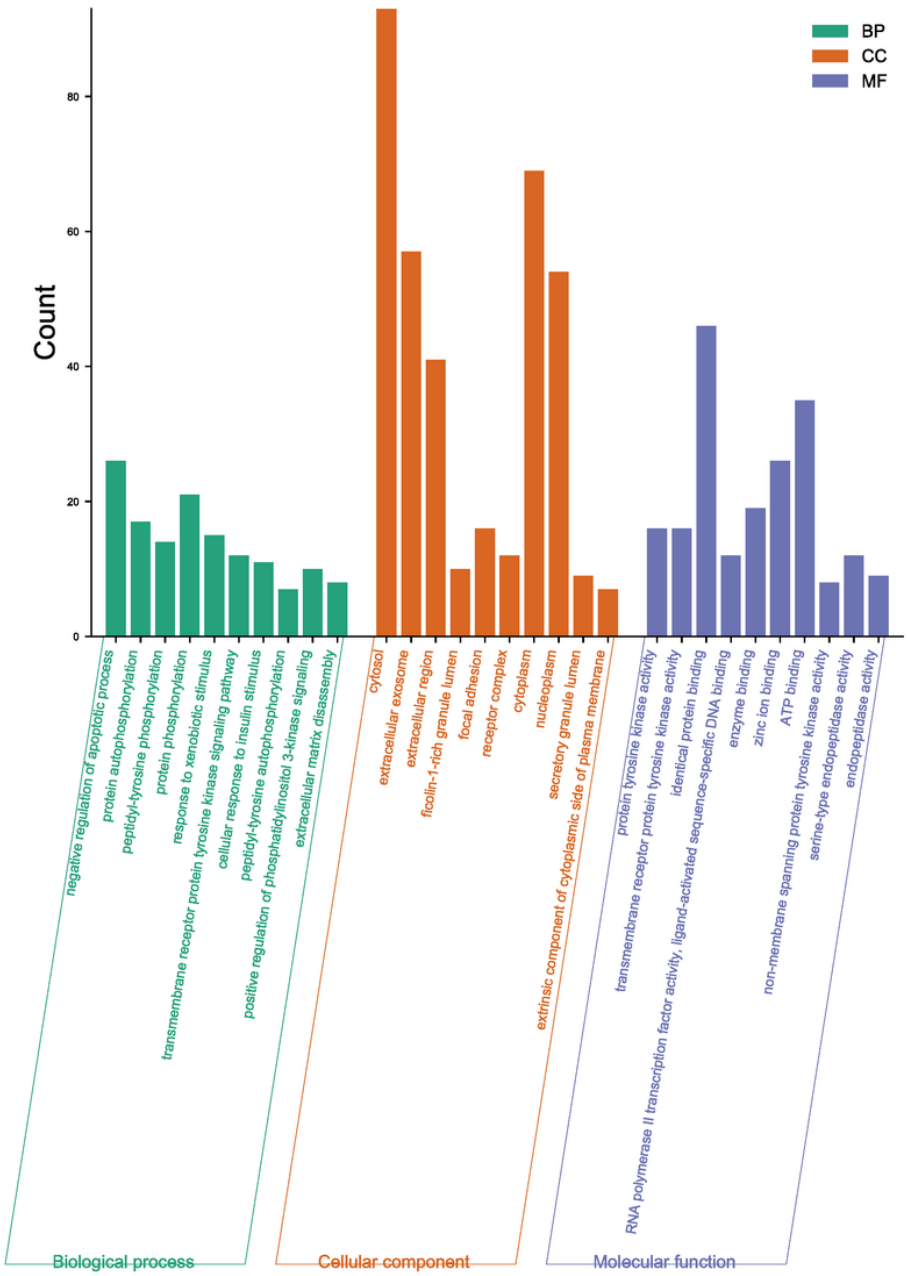

Figure 5

GO (BP, MF, CC) analyses of therapeutic target genes of diosmin for treatment of renal fibrosis. Each bar represents a GO term on the horizontal axis. The number of genes enriched in each term is shown on the vertical axis.

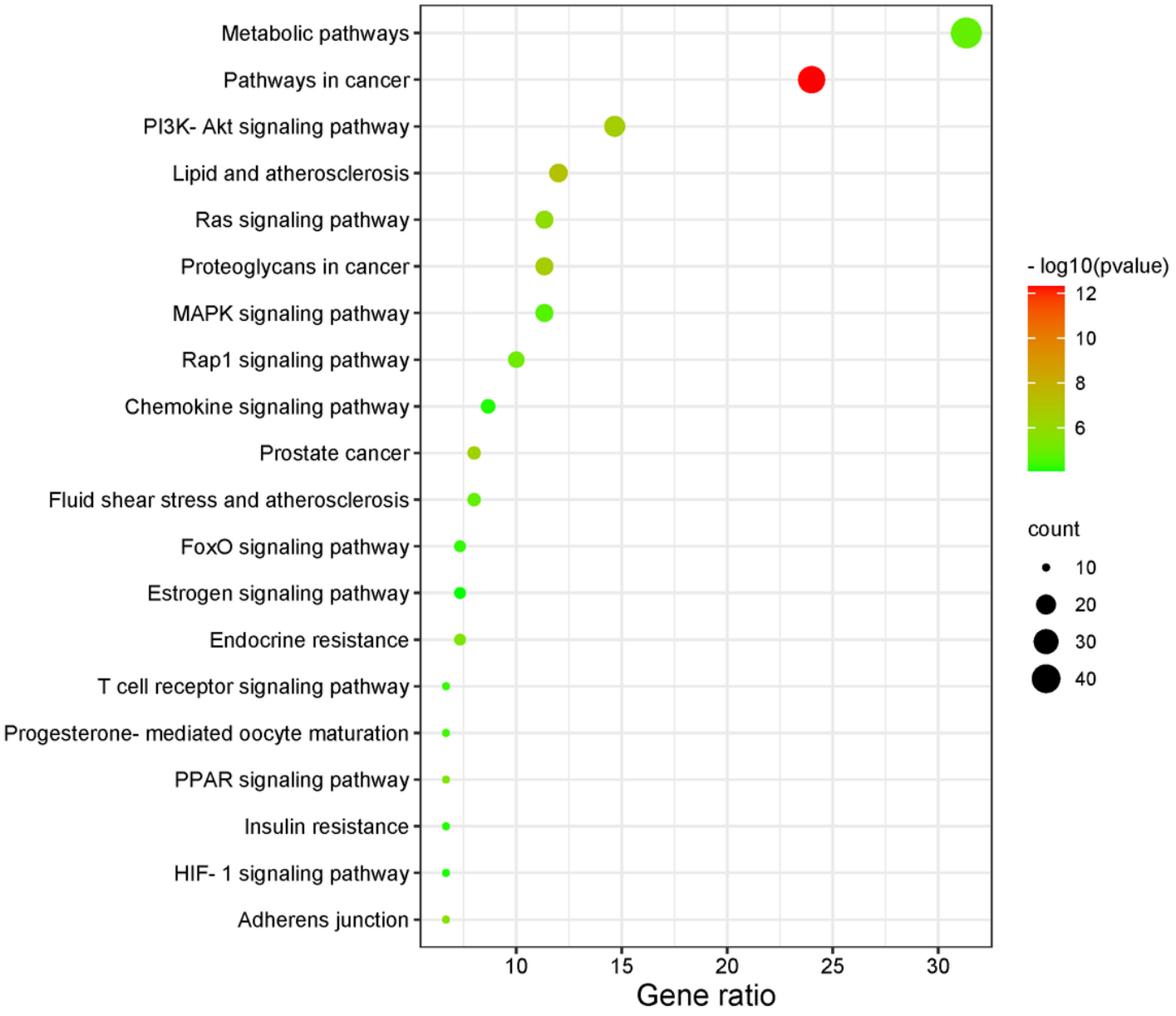

Figure 6

KEGG analyses of the therapeutic target genes of diosmin for treatment of renal fibrosis. 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.

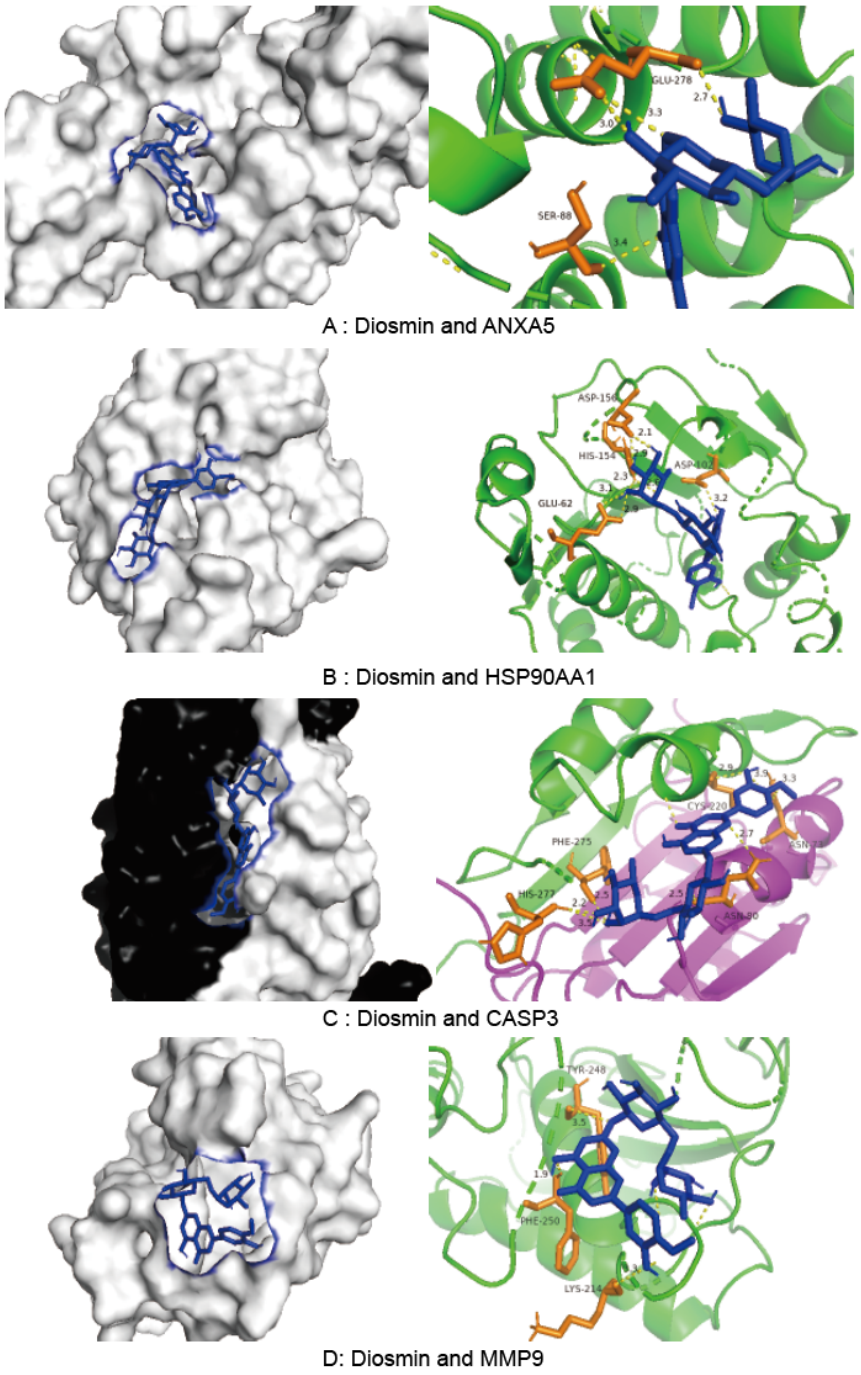

Figure 7

Molecular docking pattern and mapping surface showing molecules occupying the active pocket of proteins.

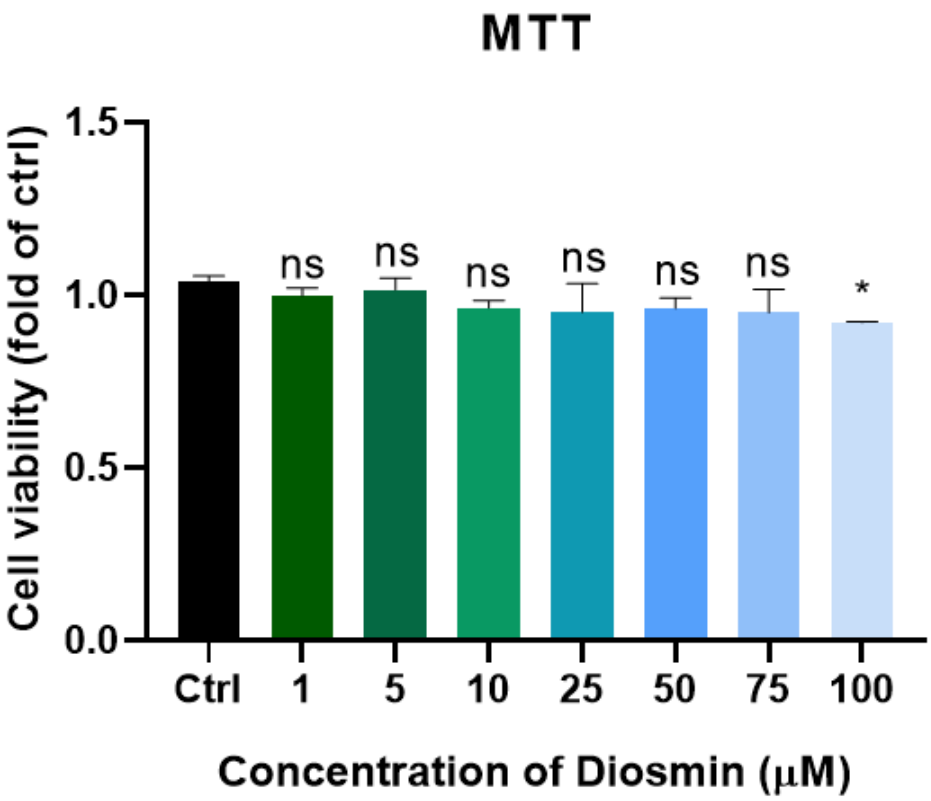

Figure 8

MTT assay detects the toxicity of diosmin on HK-2 cells.

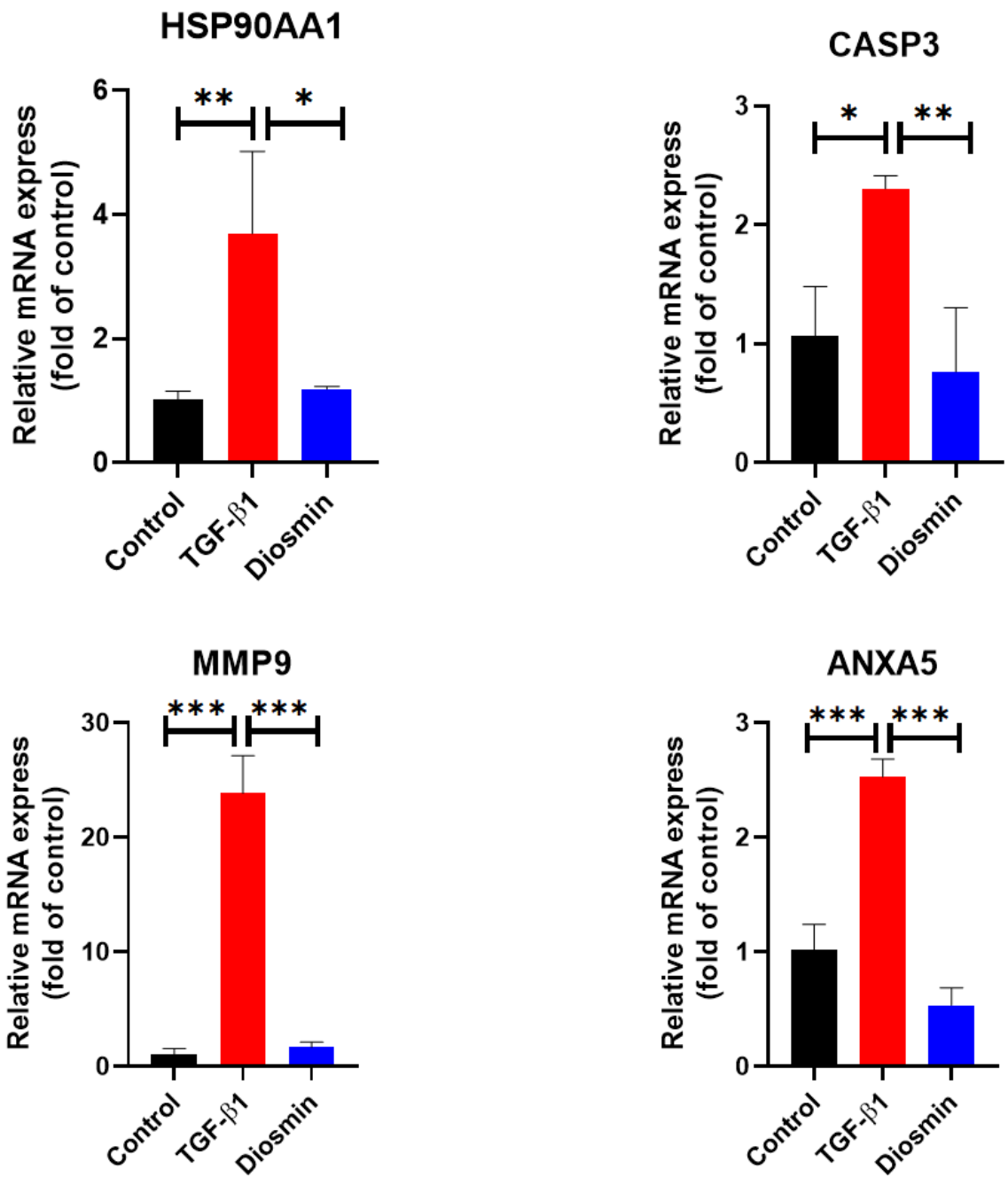

Figure 9

The mRNA expression levels of CASP3, MMP9, ANXA5, and HSP90AA1in different groups of HK-2 cells.