Tohoku J. Exp. Med., 2023, **259**, 37-47

Unraveling the Mechanism of Zhibaidihuang Decoction against IgA Nephropathy Using Network Pharmacology and Molecular Docking Analyses

Xiaoqi Deng,[1, 2,*] Yu Luo,[2,*] Meiqi Lu,[1,*] Tianjun Guan,[1] Yu Li[1] and Xiaodan Guo[1]

[1]Department of Nephrology, Zhongshan Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, China
[2]School of Medicine, Xiamen University, Xiamen, China

Zhibaidihuang Decoction (ZBDHD) is a traditional Chinese medicine with immense potential to treat IgA nephropathy. However, its core ingredients and representative mechanism remain unclear. In this study, we uncovered the key component and underlying mechanisms of ZBDHD for IgA nephropathy by applying network pharmacology and molecular docking approaches. This was done by first identifying the active ingredients and, subsequently, their corresponding gene targets in ZBDHD with the help of the Traditional Chinese Medicine Systems Pharmacology and analysis platform (TCMSP) database, thereby constructing the drug-compound-target network. The IgA nephropathy-associated genes were then identified using GeneCards, Drugbank, and OMIM databases. The overlapped targets were later obtained to establish Protein-Protein Interaction (PPI) networks, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Finally, we performed molecular docking among active compounds and hub genes, and thereby verified the key compound of ZBDHD. The drug-compound-gene network consisted of 289 nodes and 1,113 edges. The top four active ingredients were beta-sitosterol, kaempferol, quercetin and stigmasterol. The top five hub genes in the PPI network were *AKT1*, *ILB1*, *IL-6*, *TNF*, and *TP53*. Molecular docking results could demonstrate that there was high affinity among active compounds and the core targets, while quercetin may possibly be the key compound of ZBDHD. We first identified the positive compound and the candidate molecular mechanisms of ZBDHD in an IgA nephropathy treatment and discovered that quercetin might be the core compound of ZBDHD in the treatment of IgA nephropathy.

Keywords: IgA nephropathy; molecular docking; network pharmacology; quercetin; Zhibaidihuang Decoction (ZBDHD).
Tohoku J. Exp. Med., 2023 January, **259** (1), 37-47.
doi: 10.1620/tjem.2022.J088

Introduction

IgA nephropathy, first described as Berger's disease by the Parisian pathologist Jean Berger in 1968, is the most popular primary glomerular disease across the globe (Feehally and Cameron 2011). The prevalence of IgA nephropathy is highest in Asia, accounting for approximately 40-50 percent of primary glomerulonephritis worldwide (Lai et al. 2016). Due to the lack of a specific treatment, approximately 50% of IgA nephropathy patients rapidly progress into end-stage renal disease (Mestecky et al. 2016). According to the 2021 Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guidelines for Glomerulonephritis (Kidney Disease: Improving Global Outcomes (KDIGO) Glomerular Diseases Work Group 2021), the main management of IgA nephropathy lies in supportive care. For patients with a high risk of progressing into chronic kidney diseases after maximal supportive care, glucocorticoid and immunosuppressive drugs could be considered. However, glucocorticoid and immunosuppressive drugs are mostly accompanied by various adverse effects like infection, immune defi-

Received September 27, 2022; revised and accepted October 13, 2022; J-STAGE Advance online publication October 27, 2022
*These three authors contributed equally to this work.
Correspondence: Xiaodan Guo, Department of Nephrology, Zhongshan Hospital of Xiamen University, School of Medicine, Xiamen University, No. 201-209, Hubin South Road, Siming District, Xiamen 361000, China.
e-mail: guoxiaodanzz@163.com

ciency, and so on. Therefore, it is crucial to discover other effective drugs for the treatment of IgA nephropathy.

Zhibaidihuang Decoction (ZBDHD) originates from *the Golden Book of Medicine* (*Yizong Jinjian* in Chinese) (Mingjuan et al. 2022). ZBDHD consists of eight traditional Chinese drugs, namely Cortex Moutan, Phellodendri Chinensis Cortex, Poria Cocos, Rhizoma Dioscoreae, Rehmanniae Radix Praeparata, Cornus Officinalis, Alisma Orientale and Anemarrhenae Rhizoma (Yousheng et al. 2016). It is known to have the ability to relieve fire and reduce heat, as well as nourish the kidneys (Mingjuan et al. 2022; Tiannan et al. 2022). Modern pharmacological research has also shown that ZBDHD has various benefits, such as enhancing immunity, lowering blood sugar, and regulating neuroendocrine, anti-tumor, antioxidant, and anti-fatigue mechanisms (Ling 2016; Wu et al. 2020; Bin et al. 2021). In the Chinese population, ZBDHD has been used to treat IgA nephropathy (Yanli et al. 2010), nephrotic syndrome (Wei 1992), diabetic nephropathy (Mingjuan et al. 2022), and acute kidney injury (Liu et al. 2022). However, its active compounds and potential mechanism, as previously mentioned, is currently unknown.

Network pharmacology is a recent method for understanding herbal formulas at the molecular level, which aids in the translation of Traditional Chinese Medicine (TCM) from a purely experience-based system to an evidence-based medicine system (Li and Zhang 2013). Molecular docking is very widely used to discover drugs and predict ligand-receptor interactions at the molecular level (Pinzi and Rastelli 2019). Recently, many studies have been investigating the potential mechanism of Chinese Medicine against diseases using network pharmacology and molecular docking analyses, such as the Lianhua Qingwen capsule against Coronavirus disease 2019 (Xia et al. 2020), the Salvia miltiorrhiza against diabetic nephropathy (Zhang et al. 2021) and the Huanglian Jiedu Decoction against sepsis (Li et al. 2022). In this study, we could ascertain the targets of ZBDHD and IgA nephropathy by network pharmacology methods, using which drug-compound-target networks were constructed to identify the key active compounds and Protein-Protein Interaction (PPI) networks were established to identify the hub genes. We later performed KEGG and GO enrichment analyses to explore the signaling pathways and biological functions of the common targets. Ultimately, molecular docking was performed to verify the relationship between the core active compounds and the hub genes. The core compound of ZBDHD for treating IgA nephropathy was also identified in the meantime. Our results may substantiate the application of ZBDHD in IgA nephropathy and provide new avenues for further research on ZBDHD with respect to IgA nephropathy.

Materials and Methods

Identifying the active compounds and targets in ZBDHD

The active compounds and their targets in ZBDHD were identified on the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP: https://old.tcmsp-e.com/tcmsp.php) (Ru et al. 2014). TCMSP contains information on 499 Chinese herbs, 837 associated diseases, 3,311 targets, and 29,384 ingredients, which provides a comprehensive platform for users to construct compound-target-disease networks and further understand the potential mechanism of the drug (Ru et al. 2014). In this study, drug similarity (DL) $\geq$ 0.18 and oral bioavailability (OB) $\geq$ 30% were set as the threshold values (Li et al. 2022).

Construction of the drug-compound-target network

The visual drug-compound-target network was constructed on Cytoscape 3.9.1, based on the aforementioned datasets, to show the relationship between drug, compound, and their corresponding targets (Otasek et al. 2019). A node in the drug-compound-target network represents a compound, drug, or target, while an edge represents the association between the drug, compound, and target.

Identification of IgA nephropathy-associated targets

OMIM (https://omim.org/) (Amberger et al. 2015), GeneCards (https://www.genecards.org/) (Safran et al. 2010) and Drugbank (https://go.drugbank.com/) (Wishart et al. 2018) were the resource databases used to collect the potential targets of IgA nephropathy. The keyword "IgA nephropathy" was fed into these three databases to acquire the IgA nephropathy-associated targets. The overlapped targets of ZBDHD and IgA nephropathy were gathered as the potential therapeutic targets of ZBDHD in IgA nephropathy treatment using the Venn diagram webtool (https://bioinformatics.psb.ugent.be/webtools/Venn/) (Jia et al. 2021).

Construction of the PPI network

The intersection targets between ZBDHD and IgA nephropathy were input into the STRING (https://string-db.org/) (Szklarczyk et al. 2021) database to gather information on the protein interaction network. The data was then imported into Cytoscape 3.9.1 (Otasek et al. 2019) to construct the visual PPI network. Based on the resultant network, the top five protein targets were identified by the Cytoscape plugin.

The enrichment analysis of GO and KEGG pathways

GO and KEGG pathway enrichment analyses were carried out using the "clusterProfiler" R package (Yu et al. 2012). GO enrichment analysis included three aspects, namely biological processes (BP), molecular functions (MF), and cellular components (CC). The threshold value of the P-value was set at 0.05 for this course.

Molecular docking of active compound and target

The target structures were retrieved using the RCSB PDB database (https://www.rcsb.org/) (Quinn et al. 2015), while the structures of active compounds were acquired from the PubChem database (https://pubchem.ncbi.nlm.nih.

gov/) (Kim et al. 2021). The targets and active compounds were considered as receptors and ligands, respectively, in the AutoDock Tools 1.5.7 software (Goodsell et al. 2021). A total of 50 conformations were gathered in each molecular dock process, and the best conformation was screened out as the eventual docking result, which was put into Pymol 2.5.3 (Seeliger and de Groot 2010) for visual analysis.

Identification of the key compound of ZBDHD for IgA nephropathy

Compound-associated genes and therapeutic targets of ZBDHD were intersected to further identify the core ingredient of ZBDHD, which was set as the ingredient with the most intersection targets. Further bioinformatics analyses were also performed on the core ingredient targets.

Results

Active compounds in ZBDHD

All 126 active compounds in ZBDHD were searched on the TCMSP database. They consisted of 11 compounds in Cortex Moutan, 15 compounds in Poria Cocos, 37 compounds in Phellodendri Chinensis Cortex, 16 compounds in Rhizoma Dioscoreae, 20 compounds in Cornus Officinalis, 2 compounds in Rehmanniae Radix Praeparata, 10 compounds in Alisma Orientale and 15 compounds in Anemarrhenae Rhizoma. Some active components are as given in Table 1.

Construction of drug-compound-target network

The drug-compound-target network contained 289 nodes (including 8 drugs, 213 targets, and 68 compounds) and 1,113 edges (Fig. 1) after removing compounds without any targets and integrating the common compounds. The size of the node changes with the degree score. Therefore, a larger node would mean a higher degree score, which also connotes a higher significance. According to the degree score, we screened out the top four compounds: beta-sitosterol, kaempferol, quercetin, and stigmasterol. The corresponding information is displayed in Table 2.

Identification of disease-associated targets and establishment of PPI network

A total of 732 genes in IgA nephropathy were retrieved from OMIM, GeneCards, and Drugbank databases. There were 79 overlapped targets between ZBDHD and IgA nephropathy (Fig. 2), which could possibly be the therapeutic targets of ZBDHD with respect to IgA nephropathy. Based on the overlapped targets, we established the PPI network and identified the hub genes using the Cytoscape plugin (Fig. 3). The five hub targets were *IL-6*, *TP53*, *TNF*, *AKT1*, and *IL1B*, whose detailed information is given in Table 3.

GO and KEGG pathway enrichment analyses

GO enrichment analysis is widely used in exploring gene products and their corresponding characteristics (Wan et al. 2020). In this study, we applied the R package "clusterProfiler" to explore the GO enrichment analysis (Fig. 4). In the case of biological processes, targets were mainly enriched in response to molecules of bacterial origin, lipopolysaccharides, and nutrient levels. With regards to cellular components, membrane raft, membrane microdomain, and caveolae were enriched. In molecular function, receptor-ligand activity, cytokine receptor binding, and cytokine activity were the top three factors.

Table 1. The information of some active components in ZBDHD.

Mol ID	Molecule name	OB%	DL	Herb
MOL000211	Mairin	55.38	0.78	*Cortex Moutan*
MOL000492	(+)-Catechin	54.83	0.24	
MOL000282	Ergosta-7,22E-dien-3beta-ol	43.51	0.72	*Poria Cocos*
MOL000283	Ergosterol peroxide	40.36	0.81	
MOL000785	Palmatine	64.6	0.65	*Phellodendri Chinrnsis Cortex*
MOL000622	Magnograndiolide	63.71	0.19	
MOL000546	Diosgenin	80.88	0.81	*Rhizoma Dioscoreae*
MOL001736	(-)-Taxifolin	60.51	0.27	
MOL005531	Telocinobufagin	69.99	0.79	*Cornus Officinalis*
MOL001495	Ethyl linolenate	46.1	0.2	
MOL000359	Sitosterol	36.91	0.75	*Rehmanniae Radix Praeparata*
MOL000449	Stigmasterol	43.83	0.76	
MOL000359	Sitosterol	36.91	0.75	*Alisma Orientale*
MOL002464	1-Monolinolein	37.18	0.3	
MOL000631	Coumaroyltyramine	112.9	0.2	*Anemarrhenae Rhizoma*
MOL000546	Diosgenin	80.88	0.81	

OB%, oral bioavailability%; DL, drug-likeness.

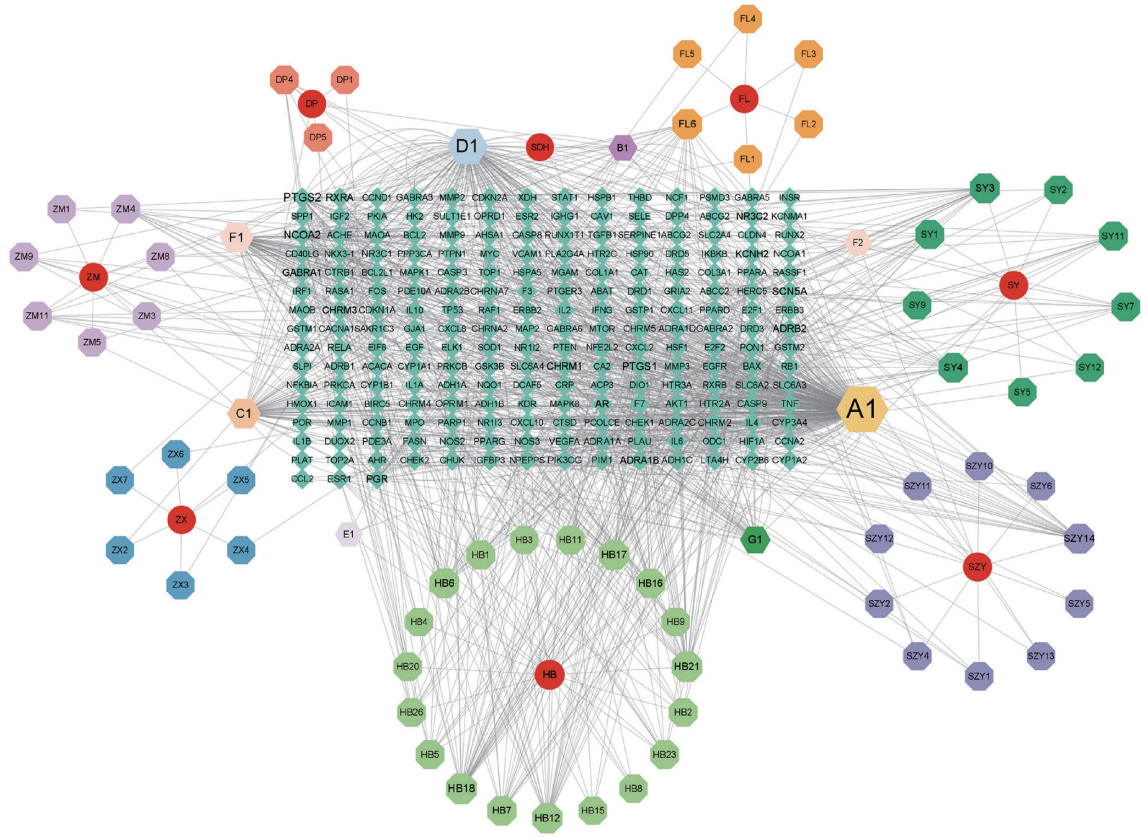

Fig. 1. The drug-compound-target network of Zhibaidihuang Decoction (ZBDHD). The circle nodes represent the drugs of ZBDHD. The octagon nodes are the active compounds surrounding the corresponding drugs. The hexagon nodes are the common compounds among drugs. The diamond nodes represent the targets of ZBDHD.

Table 2. The top four active components in the Herb-compound-target network.

Number	Mol ID	Molecule name	OB%	DL	Degree	Herb
F1	MOL000358	beta-Sitosterol	36.91	0.75	70	*Phellodendri Chinrnsis Cortex, Cornus Officinalis Sieb.*
C1	MOL000422	Kaempferol	41.88	0.24	67	*Cortex Moutan, Anemarrhenae Rhizoma*
A1	MOL000098	Quercetin	46.43	0.28	281	*Cortex Moutan, Phellodendri Chinrnsis Cortex*
D1	MOL000449	Stigmasterol	43.83	0.76	150	*Phellodendri Chinrnsis Cortex, Rhizoma Dioscoreae, Cornus Officinalis Sieb. Et Zucc. Rehmanniae Radix Praeparata, Anemarrhenae Rhizoma*

OB%, oral bioavailability%; DL, drug-likeness.

KEGG signal pathway analysis was performed to discern the metabolic pathways associated with ZBDHD (Wan et al. 2019). The top 20 pathways were as shown in Fig. 5. The major pathway contained the AGE-RAGE signaling pathway in diabetic complications, along with HIF-1, TNF, and IL-17 signaling pathways.

Molecular docking analysis

According to the network pharmacology analysis results, we verified the top four core compounds as ligands (beta-sitosterol, kaempferol, quercetin, and stigmasterol) and five hub genes as receptors (*IL-6*, *TNF*, *TP53*, *AKT1*, and *IL1B*). We performed molecular docking to countenance the effect of core compounds in IgA nephropathy. The results demonstrated that there was a strong affinity between the ligands and receptors, and the whole binding energy was less than –5 kcal/mol. The binding between stigmasterol and IL1B was the most stable, with a binding energy of –8.64 kcal/mol. At the same time, quercetin displayed a tight binding pattern vis-a-vis the active pocket of IL6 protein. It formed up to seven hydrogen bonds with the IL6 proteins, leading to a very steady complex formation (Fig. 6). A comprehensive account of the docking energy information is given in Table 4.

Identification of the key compound of ZBDHD for IgA nephropathy

Using the overlapped targets of each active compound

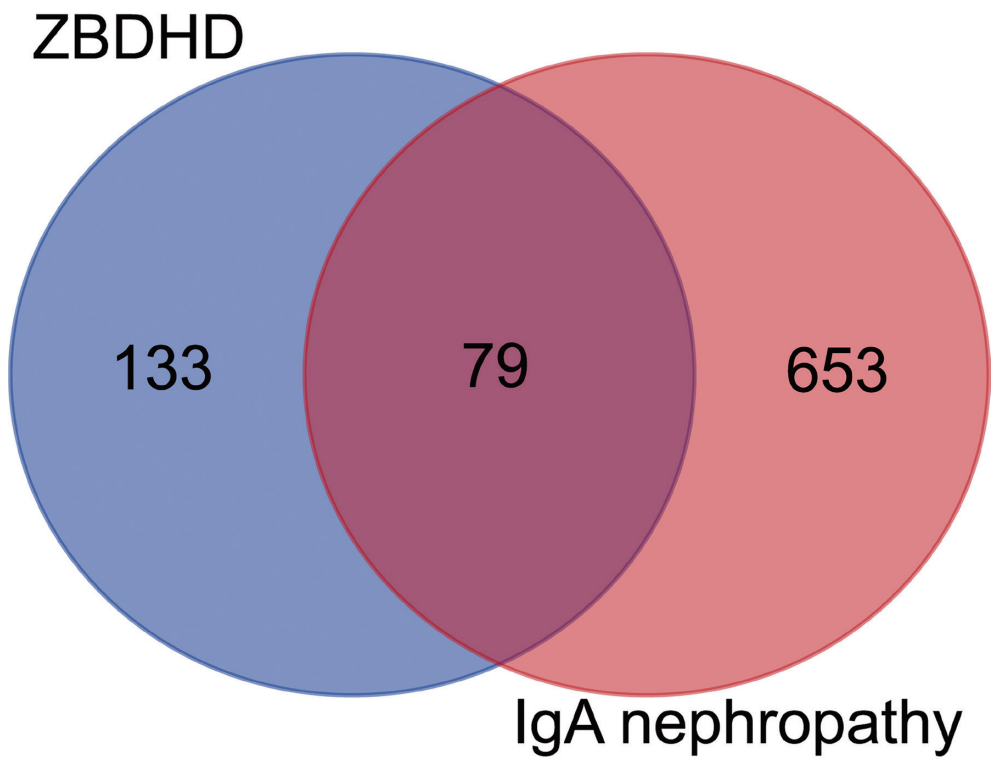

Fig. 2. The Venn diagram of overlapped targets.
There are 212 genes and 732 genes in ZBDHD and IgA nephropathy respectively. 79 overlapped targets were identified between ZBDHD and IgA nephropathy.

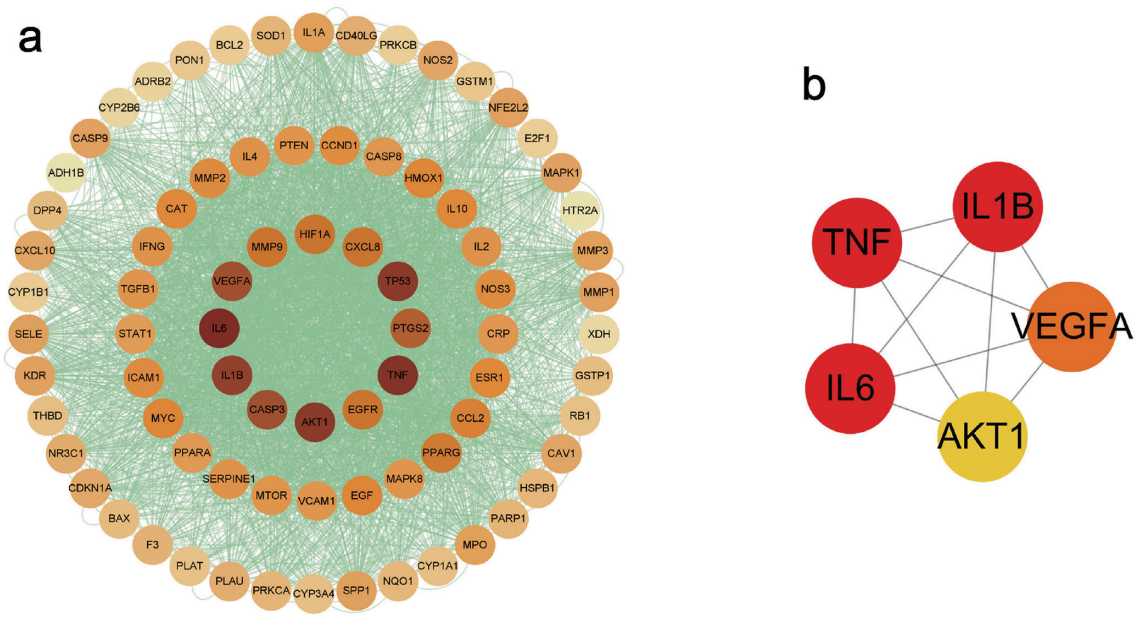

Fig. 3. The Protein-Protein Interaction (PPI) networks of overlapped targets (a) and the top five hub genes (b).
(a) The color changes according to the value of the degree. The darker the color, the greater the value of the degree, and the more important the gene. (b) The top five hub genes are *TP53*, *IL-6*, *TNF*, *AKT1* and *IL1B*.

with their potential therapeutic targets of ZBDHD, we finally identified quercetin as the key ingredient of ZBDHD. There were 68 overlapped genes in total, as shown in Fig. 7a. We could also discover from the PPI network that the top five hub genes were the same as those in ZBDHD (Fig. 7b). Meanwhile, the results of GO and KEGG analyses were nearly identical to the ZBDHD results (Fig. 7c, d).

Table 3. The top five genes in Protein-Protein Interaction (PPI) networks.

Gene symbol	Degree	Betweenness centrality	Closeness centrality
IL6	138	0.02928794164971598	41.44927536231884
TNF	136	0.026790681623938488	41.80882352941177
TP53	134	0.026309260790582205	42.04477611940298
AKT1	134	0.02660231822255318	41.88059701492537
IL1B	132	0.020675674714858823	42.60606060606061

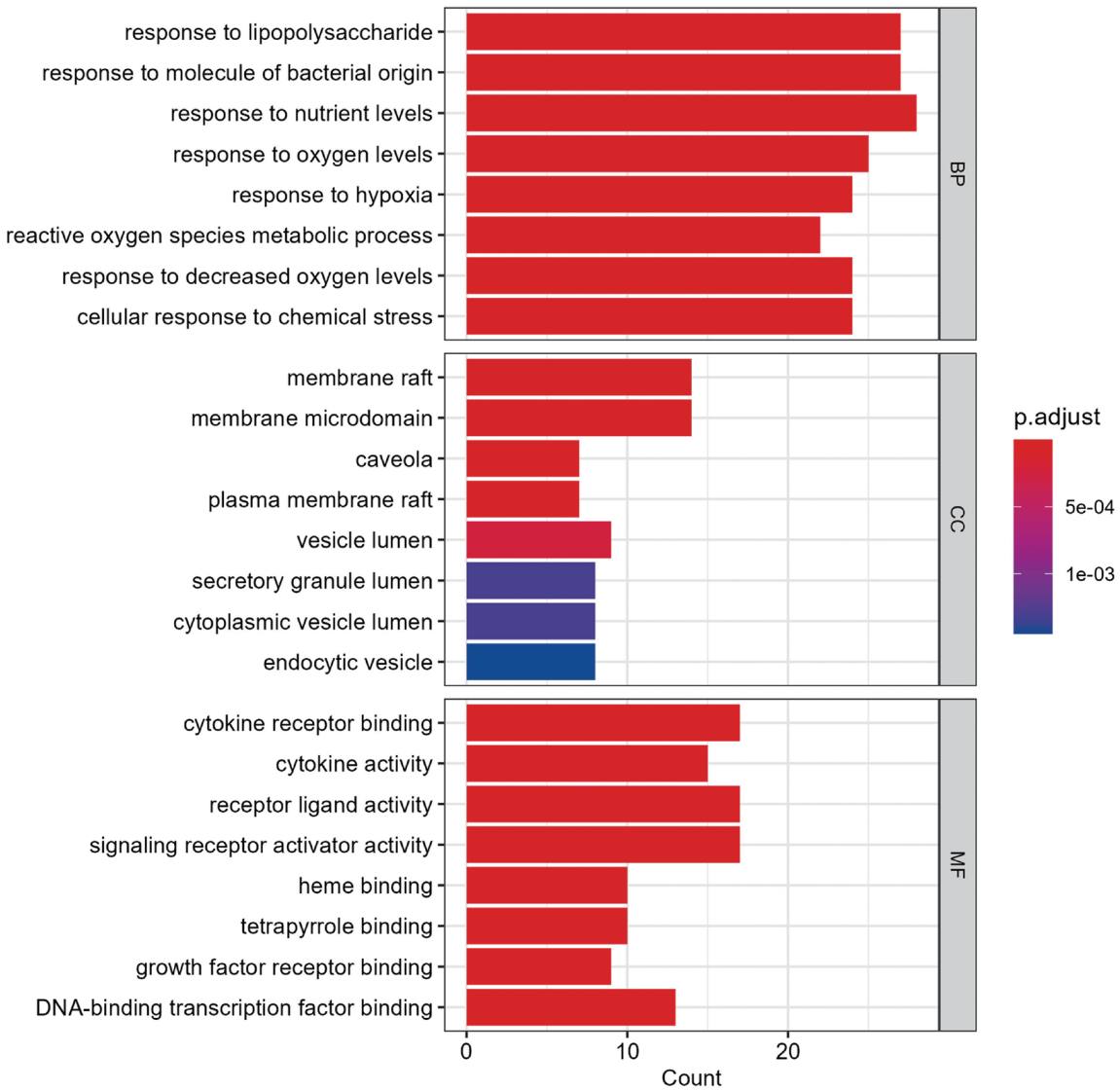

Fig. 4. The top eight terms of Gene Ontology (GO) analysis.
BP, biological process; CC, cellular component; MF, molecular function.

Discussion

IgA nephropathy is the most common primary glomerulonephritis around the world (Sallustio et al. 2019). Patients with IgA nephropathy usually advance into end-stage kidney disease within 20-30 years post diagnosis (Lai et al. 2016). In patients with end-stage kidney disease, a variety of comorbidities manifest, such as infections, cardiovascular disease, and hyperparathyroidism, which in turn harbor a high mortality rate. So, it is necessary to develop effective drugs to treat IgA nephropathy. ZBDHD has been demonstrated to show benefits such as nourishing the kidneys, antioxidant functions, and enhancing immunity, which points to its latent efficiency in treating IgA

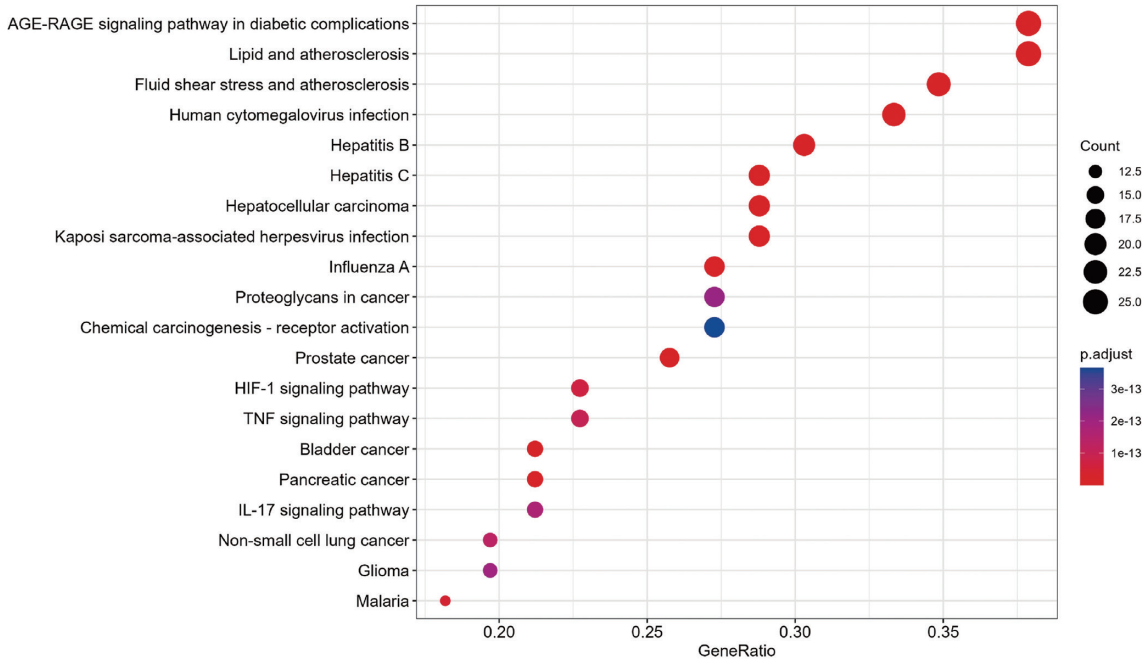

Fig. 5. The top 20 terms of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.
The redder the color means the smaller the P-value, and the larger the circle means the more genes are enriched.

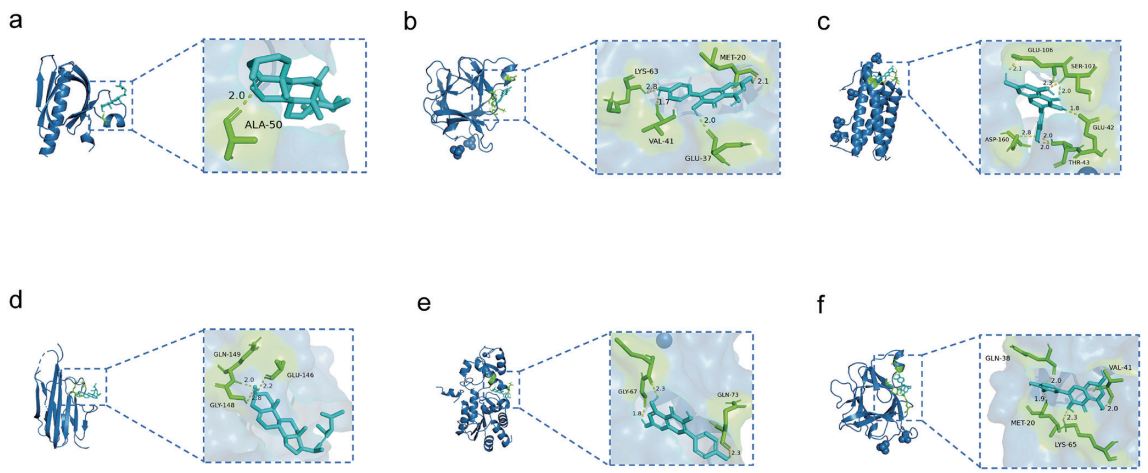

Fig. 6. The binding energy results between core compounds and targets.
Green represents amino acid residues, blue represents active compounds, and dashed lines represent hydrogen bonds. (a) AKT1-beta-sitosterol; (b) ILB1-kaempferol; (c) IL6-quercetin; (d) TNF-Stigmasterol; (e) TP53-kaempferol; (f) ILB1-quercetin.

Table 4. The binding energy of core active compounds and top five genes.

Gene	PDB ID	Binding energy (kcal/mol)			
		beta-Sitosterol	Kaempferol	Quercetin	Stigmasterol
IL6	1ALU	−7.33	−6.15	−6.1	−8.0
TNF	5UUI	−6.9	−6.53	−6.38	−7.4
TP53	1YC5	−7.45	−5.94	−5.78	−8.4
AKT1	1H10	−7.04	−6.76	−5.89	−7.45
IL1B	2NVH	−8.15	−6.47	−6.49	−8.64

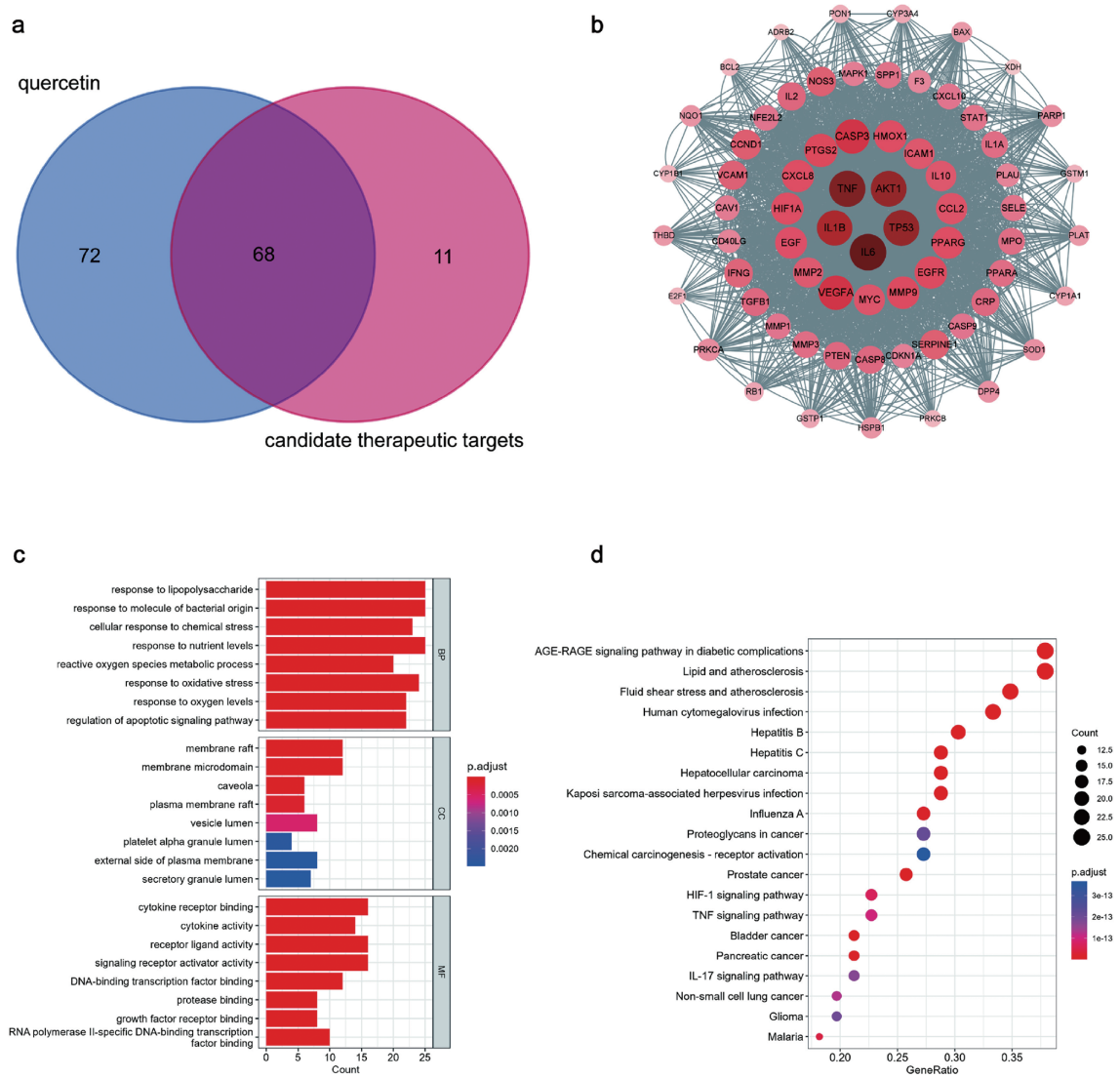

Fig. 7. The bioinformatic analysis results of quercetin associated therapeutic targets.
(a) There are 68 shared targets between quercetin-associated therapeutic targets and ZBDHD-associated therapeutic targets. (b) The PPI network of shared genes. (c) The top eight GO terms. (d) The top 20 terms of KEGG pathway analysis.

nephropathy. However, its underlying mechanism and key compound have remained an enigma so far. In this work, we aimed to uncover the latent mechanisms of ZBDHD against IgA nephropathy through network pharmacology and molecular docking analyses.

In this study, we collected chemical components of ZBDHD from the TCMSP database and subsequently identified 126 compounds, of which the main compounds involved were beta-sitosterol, kaempferol, quercetin, and stigmasterol. Meanwhile, we further identified the targets of ZBDHD, based on the above compounds, and constructed a drug-compound-target network, according to which the main compounds could interact at various genes with other components. Among the predicted compounds, kaempferol, quercetin, and other compounds are known to be beneficial for kidney diseases. Quercetin, the main compound in flavonoids, has been demonstrated to have multiple functions, such as anti-inflammatory, antioxidant, decreasing blood pressure, and anticancer mechanisms (Hosseini et al. 2021). Quercetin is known to relieve diabetic nephropathy by inhibiting inflammatory and oxidative stress inflammatory pathways (Hu et al. 2021). Meanwhile, some studies have also demonstrated that quercetin exerted a nephroprotective effect by regulating macrophage polarization (Lu et al. 2018). Kaempferol as well has been demonstrated to have a nephroprotective effect through its anti-inflammatory and antioxidant action (Wang et al. 2020; Hu et al. 2021). According to these findings, we speculate that these ingredients could assume a major role in ZBDHD in the treatment of IgA nephropathy. However, the putative mechanism remains unknown.

In the present study, we gathered ZBDHD targets and IgA nephropathy-associated targets from certain databases and obtained 79 shared targets, which were considered as

the potential therapeutic targets of ZBDHD for IgA nephropathy treatment. For further exploring the common targets, we constructed the PPI network and identified the top five hub genes, namely *TP53*, *IL-6*, *TNF*, *AKT1*, and *IL1B*. The levels of IL-6 and TNF have been detected to be higher in the serum of IgA nephropathy patients when compared with healthy persons (Wada et al. 2003), which elicited that both of them may possibly be involved in the pathogenesis of IgA nephropathy. In a study on mice, IL-6-mediated pathways were demonstrated to promote aberrant glycosylated IgA production, which was considered as the critical compound in the development of IgA nephropathy (Makita et al. 2020).

We performed GO and KEGG enrichment analyses to further explore the potential mechanism of ZBDHD in the management of IgA nephropathy. The results of the GO analysis confirmed that the targets were primarily enriched in response to nutrient levels, lipopolysaccharides, molecules of bacterial origin, and oxidative stress in the biological functions regard. Meanwhile, the enrichment result of the KEGG pathway verified that there were various pathways involved in the treatment of ZBDHD for IgA nephropathy, including the AGE-RAGE signaling pathway in diabetic complications, HIF-1, TNF, and IL-17 signaling pathways. The AGE-RAGE signaling pathway could induce oxidative stress and promote the production of pro-inflammatory cytokines (Shen et al. 2020; Wu et al. 2021). Besides, this pathway could also activate chronic inflammation and oxidative stress in kidney tissues which damage kidney cells and the subsequent loss of physiological functions (Sanajou et al. 2018). The TNF signaling pathway has an important role in a series of pathophysiological processes, such as inflammation induction, immune reactions regulation, cell proliferation, and so on, as well, as it acts as an important pathogenic signaling pathway in the development of IgA nephropathy (Leung et al. 2008; Szondy and Pallai 2017; Tang et al. 2021).

To explore the molecular mechanism of ZBDHD against IgA nephropathy, we screened out four key ingredients and five hub genes to carry out molecular docking to further confirm the prediction result of our network pharmacology. The docking result showed that the binding between the compounds and targets was tight, and the whole binding energy was less than –5.0 kcal/mol. Quercetin forms a very stable structure with the active pocket of IL6 protein because they form up to seven hydrogen bonds, which further confirms that quercetin is an important ingredient in ZBDHD.

To identify the core compound in ZBDHD for the treatment of IgA nephropathy, we intersected all the compound targets with the therapeutic targets of ZBDHD. The results elicited that quercetin may be the key compound since it has 68 overlapped genes with ZBDHD. We also constructed the PPI network and carried out the GO and KEGG enrichment analyses. The results showed that the top five hub genes in the PPI network and the enrichment results were both similar to the results for therapeutic targets in ZBDHD. In *in vitro* and *in vivo* studies, quercetin has been shown to inhibit Hedgehog signaling activation and finally remit renal fibrosis and the epithelial to mesenchymal transition (EMT) (Liu et al. 2019). Meanwhile, quercetin has been demonstrated to block PI3k/Akt signaling pathways for relieving chronic renal failure (Tu et al. 2021). Hence, we speculate that quercetin may be the main compound of ZBDHD against IgA nephropathy.

This study, however, has certain limitations. First of all, only network pharmacology and molecular docking approaches were applied to explore the effect of ZBDHD in IgA nephropathy. Hence, we need other methodologies to further verify this case. Secondly, more comprehensive TCM, OMIM, GeneCards, and Drugbank databases are required to make the results more reliable. Finally, we need more accurate ways to explore the therapeutic mechanism of ZBDHD in IgA nephropathy apart from molecular docking and network pharmacology approaches.

In conclusion, in this study, we conducted molecular docking and network pharmacology methods to untangle the potential mechanism of ZBDHD against IgA nephropathy and ultimately discovered that quercetin may possibly be the main compound of ZBDHD.

Acknowledgments

We are thankful to all of patients who donated their blood samples and the GEO database which provided the platform for us to obtain databases. This study was supported by Xiamen Medical and Health Guidance Project (Grant No. 3502Z2019173).

Conflict of Interest

The authors declare no conflict of interest.

References

Amberger, J.S., Bocchini, C.A., Schiettecatte, F., Scott, A.F. & Hamosh, A. (2015) OMIM.org: Online Mendelian Inheritance in Man (OMIM®), an online catalog of human genes and genetic disorders. *Nucleic Acids Res.*, **43**, D789-798.

Bin, D.H., Zhang, S.Y., Zhan, M., Li, L., Li, Y.Q., Zhou, X., Lu, F.G., Zhou, Q. & He, Q.H. (2021) Exploring the mechanism of Zhibai Dihuang Decoction in the treatment of ureaplasma urealyticum-induced orchitis based on integrated pharmacology. *Front. Pharmacol.*, **12**, 602543.

Feehally, J. & Cameron, J.S. (2011) IgA nephropathy: progress before and since Berger. *Am. J. Kidney Dis.*, **58**, 310-319.

Goodsell, D.S., Sanner, M.F., Olson, A.J. & Forli, S. (2021) The AutoDock suite at 30. *Protein Sci.*, **30**, 31-43.

Hosseini, A., Razavi, B.M., Banach, M. & Hosseinzadeh, H. (2021) Quercetin and metabolic syndrome: a review. *Phytother. Res.*, **35**, 5352-5364.

Hu, Q., Qu, C., Xiao, X., Zhang, W., Jiang, Y., Wu, Z., Song, D., Peng, X., Ma, X. & Zhao, Y. (2021) Flavonoids on diabetic nephropathy: advances and therapeutic opportunities. *Chin. Med.*, **16**, 74.

Jia, A., Xu, L. & Wang, Y. (2021) Venn diagrams in bioinformatics. *Brief. Bioinform.*, **22**, bbab108.

Kidney Disease: Improving Global Outcomes Glomerular Diseases Work Group (2021) KDIGO 2021 clinical practice guideline

for the management of glomerular diseases. *Kidney Int.*, **100**, S1-S276.

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J. & Bolton, E.E. (2021) PubChem in 2021: new data content and improved web interfaces. *Nucleic Acids Res.*, **49**, D1388-D1395.

Lai, K.N., Tang, S.C., Schena, F.P., Novak, J., Tomino, Y., Fogo, A.B. & Glassock, R.J. (2016) IgA nephropathy. *Nat. Rev. Dis. Primers*, **2**, 16001.

Leung, J.C., Tang, S.C., Chan, L.Y., Chan, W.L. & Lai, K.N. (2008) Synthesis of TNF-alpha by mesangial cells cultured with polymeric anionic IgA--role of MAPK and NF-kappaB. *Nephrol. Dial. Transplant.*, **23**, 72-81.

Li, S. & Zhang, B. (2013) Traditional Chinese medicine network pharmacology: theory, methodology and application. *Chin. J. Nat. Med.*, **11**, 110-120.

Li, X., Wei, S., Niu, S., Ma, X., Li, H., Jing, M. & Zhao, Y. (2022) Network pharmacology prediction and molecular docking-based strategy to explore the potential mechanism of Huanglian Jiedu Decoction against sepsis. *Comput. Biol. Med.*, **144**, 105389.

Ling, Z. (2016) Effects of Zhi Bai Di Huang Tang on the internal environment of elderly patients with vaginitis. *Journal of Sichuan of Traditional Chinese Medicine*, **34**, 163-165 (in Chinese).

Liu, X., Sun, N., Mo, N., Lu, S., Song, E., Ren, C. & Li, Z. (2019) Quercetin inhibits kidney fibrosis and the epithelial to mesenchymal transition of the renal tubular system involving suppression of the Sonic Hedgehog signaling pathway. *Food Funct.*, **10**, 3782-3797.

Liu, Z., Xu, Y., Bai, X., Guo, L., Li, X., Gao, J., Teng, Y. & Yu, P. (2022) Prediction of the mechanisms of action of Zhibai Dihaung Granule in cisplatin-induced acute kidney injury: a network pharmacology study and experimental validation. *J. Ethnopharmacol.*, **292**, 115241.

Lu, H., Wu, L., Liu, L., Ruan, Q., Zhang, X., Hong, W., Wu, S., Jin, G. & Bai, Y. (2018) Quercetin ameliorates kidney injury and fibrosis by modulating M1/M2 macrophage polarization. *Biochem. Pharmacol.*, **154**, 203-212.

Makita, Y., Suzuki, H., Kano, T., Takahata, A., Julian, B.A., Novak, J. & Suzuki, Y. (2020) TLR9 activation induces aberrant IgA glycosylation via APRIL- and IL-6-mediated pathways in IgA nephropathy. *Kidney Int.*, **97**, 340-349.

Mestecky, J., Novak, J., Moldoveanu, Z. & Raska, M. (2016) IgA nephropathy enigma. *Clin. Immunol.*, **172**, 72-77.

Mingjuan, T., Xiuying, W., Zhexue, X., Rui, X. & Ningxu, L. (2022) Study on the mechanism of the protective effect of Zhibai Dihuang Decoction on kidney of diabetic rats. *Guiding Journal of Traditional Chinese Medicine and Pharmacy*, **28**, 19-24 (in Chinese).

Otasek, D., Morris, J.H., Boucas, J., Pico, A.R. & Demchak, B. (2019) Cytoscape Automation: empowering workflow-based network analysis. *Genome Biol.*, **20**, 185.

Pinzi, L. & Rastelli, G. (2019) Molecular docking: shifting paradigms in drug discovery. *Int. J. Mol. Sci.*, **20**, 4331.

Quinn, G.B., Bi, C., Christie, C.H., Pang, K., Prlic, A., Nakane, T., Zardecki, C., Voigt, M., Berman, H.M., Bourne, P.E. & Rose, P.W. (2015) RCSB PDB Mobile: iOS and Android mobile apps to provide data access and visualization to the RCSB Protein Data Bank. *Bioinformatics*, **31**, 126-127.

Ru, J., Li, P., Wang, J., Zhou, W., Li, B., Huang, C., Li, P., Guo, Z., Tao, W., Yang, Y., Xu, X., Li, Y., Wang, Y. & Yang, L. (2014) TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J. Cheminform.*, **6**, 13.

Safran, M., Dalah, I., Alexander, J., Rosen, N., Iny Stein, T., Shmoish, M., Nativ, N., Bahir, I., Doniger, T., Krug, H., Sirota-Madi, A., Olender, T., Golan, Y., Stelzer, G., Harel, A., et al. (2010) GeneCards Version 3: the human gene integrator. *Database* (*Oxford*), **2010**, baq020.

Sallustio, F., Curci, C., Di Leo, V., Gallone, A., Pesce, F. & Gesualdo, L. (2019) A new vision of IgA nephropathy: the missing link. *Int. J. Mol. Sci.*, **21**, 189.

Sanajou, D., Ghorbani Haghjo, A., Argani, H. & Aslani, S. (2018) AGE-RAGE axis blockade in diabetic nephropathy: current status and future directions. *Eur. J. Pharmacol.*, **833**, 158-164.

Seeliger, D. & de Groot, B.L. (2010) Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *J. Comput. Aided Mol. Des.*, **24**, 417-422.

Shen, C.Y., Lu, C.H., Wu, C.H., Li, K.J., Kuo, Y.M., Hsieh, S.C. & Yu, C.L. (2020) The development of maillard reaction, and Advanced Glycation End product (AGE)-Receptor for AGE (RAGE) signaling inhibitors as novel therapeutic strategies for patients with AGE-related diseases. *Molecules*, **25**, 5591.

Szklarczyk, D., Gable, A.L., Nastou, K.C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N.T., Legeay, M., Fang, T., Bork, P., Jensen, L.J. & von Mering, C. (2021) The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.*, **49**, D605-D612.

Szondy, Z. & Pallai, A. (2017) Transmembrane TNF-alpha reverse signaling leading to TGF-beta production is selectively activated by TNF targeting molecules: therapeutic implications. *Pharmacol. Res.*, **115**, 124-132.

Tang, R., Meng, T., Lin, W., Shen, C., Ooi, J.D., Eggenhuizen, P.J., Jin, P., Ding, X., Chen, J., Tang, Y., Xiao, Z., Ao, X., Peng, W., Zhou, Q., Xiao, P., et al. (2021) A partial picture of the single-cell transcriptomics of human IgA nephropathy. *Front. Immunol.*, **12**, 645988.

Tiannan, Z., Zeyuan, X., Lingang, Z., Wen-ming, G. & Yan, L. (2022) Study of molecular mechanisms of ZhibaiDihuang Decoction in treatment of bladder cancer based on network pharmacology and molecular docking method. *Journal of Southwest University for Nationality* (*Natural Science Edition*), **48**, 275-282 (in Chinese).

Tu, H., Ma, D., Luo, Y., Tang, S., Li, Y., Chen, G., Wang, L., Hou, Z., Shen, C., Lu, H., Zhuang, X. & Zhang, L. (2021) Quercetin alleviates chronic renal failure by targeting the PI3k/Akt pathway. *Bioengineered*, **12**, 6538-6558.

Wada, J., Sugiyama, H. & Makino, H. (2003) Pathogenesis of IgA nephropathy. *Semin. Nephrol.*, **23**, 556-563.

Wan, Z., Zhang, X., Luo, Y. & Zhao, B. (2019) Identification of hepatocellular carcinoma-related potential genes and pathways through bioinformatic-based analyses. *Genet. Test. Mol. Biomarkers*, **23**, 766-777.

Wan, Z., Zhao, B., Zhang, X. & Zhao, Y. (2020) Drug discovery in cardiovascular disease identified by text mining and data analysis. *Ann. Palliat. Med.*, **9**, 3089-3099.

Wang, Y., Liu, T., Ma, F., Lu, X., Mao, H., Zhou, W., Yang, L., Li, P. & Zhan, Y. (2020) A network pharmacology-based strategy for unveiling the mechanisms of Tripterygium Wilfordii Hook F against diabetic kidney disease. *J. Diabetes Res.*, **2020**, 2421631.

Wei, J. (1992) Treatment of 100 children with infantile nephrotic syndrome by integrated traditional Chinese medicine and Western medicine. *Zhongguo Zhong Xi Yi Jie He Za Zhi*, **12**, 465-468 (in Chinese).

Wishart, D.S., Feunang, Y.D., Guo, A.C., Lo, E.J., Marcu, A., Grant, J.R., Sajed, T., Johnson, D., Li, C., Sayeeda, Z., Assempour, N., Iynkkaran, I., Liu, Y., Maciejewski, A., Gale, N., et al. (2018) DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res.*, **46**, D1074-D1082.

Wu, P., Ding, B., Ye, L., Huang, Y., Ji, J., Fan, Y. & Xu, L. (2020) Zhibaidihuang Decoction ameliorates cell oxidative stress by regulating the Keap1-Nrf2-ARE signalling pathway. *Evid. Based Complement. Alternat. Med.*, **2020**, 9294605.

Wu, X.Q., Zhang, D.D., Wang, Y.N., Tan, Y.Q., Yu, X.Y. & Zhao, Y.Y. (2021) AGE/RAGE in diabetic kidney disease and

ageing kidney. *Free Radic. Biol. Med.*, **171**, 260-271.
Xia, Q.D., Xun, Y., Lu, J.L., Lu, Y.C., Yang, Y.Y., Zhou, P., Hu, J., Li, C. & Wang, S.G. (2020) Network pharmacology and molecular docking analyses on Lianhua Qingwen capsule indicate Akt1 is a potential target to treat and prevent COVID-19. *Cell Prolif.*, **53**, e12949.
Yanli, S., Binxiang, X. & Aiguo, D. (2010) Clinical observation of 50 cases of IgA nephropathy treated with Zhi Bai Di Huang Tang plus flavor and western medicine. *Heilongjiang Medicine and Pharmacy*, **33**, 67 (in Chinese).
Yousheng, L., Shuiyu, L., Dongping, L. & Lingling, C. (2016) Efficacy enhancing and toxicity reducing effect of Zhibai Dihuang Decoction on membranous nephropathy rats treating with a large dose of glucocorticoids. *Journal of East China University of Science and Technology* (*Natural Scinence Edition*), **42**, 187-193 (in Chinese).
Yu, G., Wang, L.G., Han, Y. & He, Q.Y. (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*, **16**, 284-287.
Zhang, L., Han, L., Wang, X., Wei, Y., Zheng, J., Zhao, L. & Tong, X. (2021) Exploring the mechanisms underlying the therapeutic effect of Salvia miltiorrhiza in diabetic nephropathy using network pharmacology and molecular docking. *Biosci. Rep.*, **41**, BSR20203520.