Chinese Herbal Medicines 11 (2019) 281–291

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Original Article

Mechanisms of Chinese medical formula Fangji Huangqi Decoction as an effective treatment of nephrotic syndrome based on systems pharmacology

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ARTICLE INFO

Article history:
Received 28 June 2018
Revised 30 November 2018
Accepted 12 December 2018
Available online 30 May 2019

Keywords:
Fangji Huangqi Decoction
material basis
molecular mechanism
nephrotic syndrome
system pharmacology

ABSTRACT

Objective: With the development of the society, the number of people who catch the nephrotic syndrome (NS) is going up roughly. As we all know, traditional Chinese medicine (TCM), especially Fangji Huangqi Decoction (FHD), has a long history with good curative effects on NS. However, the mechanism of FHD treating NS has not been clearly elucidated.

Methods: In this study, TCMSP platform was employed to screen active compounds of each herb of Fangji Huangqi Decoction combined with literatures. Furthermore, PharmMapper and SEA were used to predict and screen the active targets of FHD, and the HOME-NCBI-GENE, GeneCards and OMIM database were used to screen the active targets of NS. The GO and KEGG pathways involved in the targets were analyzed by ClueGO. Finally, contribution index was used to screen the active ingredients of FHD in the treatment of NS.

Results: After drug-likeness (DL) and bioavailability (OB) filtering, 43 compounds were selected from FHD, interacting with 85 NS-related targets. Systematic analysis of the constructed networks revealed that it was mainly involved in PI3K-Akt signaling pathway, MAPK signaling pathway, T cell receptor signaling pathway and TNF signaling pathway. The contribution index of every active ingredient also indicated five compounds, including astragaloside IV, quercetin, glycyrrhizic acid, glycyrrhizin and fangchinoline.

Conclusions: These results have successfully predicted the pharmacodynamic material basis and the mechanism efficiency of FHD in the treatment of NS.

1. Introduction

Nephrotic syndrome (NS) is a chronic metabolic disorder influenced by both environmental and genetic factors (Orth & Ritz, 1998). NS is a syndrome, manifested as the change of glomerular filtration membrane permeability due to various causes, leding to hypertension, hyperlipidemia and a large amount of urinary proteins as its main characteristics and incentives (Kim, Lim, & Jeon, 2011). Despite the great progress in the treatment of NS, the therapies have not been changed significantly and the molecular mechanisms remain unclear. Hence, it is necessary to develop a useful method to uncover the mechanism of NS.

Traditional Chinese medicine (TCM) has particular therapeutic effect in treating various diseases, as it has many advantages, including little side effect, low cost and low recurring rate. Fangji Huangqi Decoction (FHD), a classical phytotherapeutic formula from China, was first recorded in *Golden Chamber Synopsis*, an ancient Chinese medical classic. FHD has been the most prevalent prescription for treating edema and dysuria in the traditional Chinese medical system for more than 1800 years. FHD consists of four herbs: *Stephaniae Tetrandrae Radix* (Fangji in Chinese, FJ), *Astragali Radix* (Huangqi in Chinese, HQ), *Atractylodis Macrocephalae Rhizoma* (Baizhu in Chinese, BZ), and *Glycyrrhizae Radix* et *Rhizoma* (Gancao in Chinese, GC). Many studies have demonstrated that FHD could deal with various clinical edemas (Feng, Liu, & Feng, 2009; Wang et al., 2016; Wang, Chen, & Fu, 2014). Both *Astragali Radix* and *Stephaniae Tetrandrae Radix* are regarded as monarch drug in the FHD, and the main components of *Stephania Tetrandrae Radix* have a wide range of pharmacological activities in the anti-inflammatory, anti-pathogenic microorganisms, anti-hypertensive, anti-arrhythmic, anti-myocardial ischemia, anti-fibrosis, anti-silicosis and other aspects (Wang, Ma, & Liu, 2017). In addition, previous study (Chen et al., 2007) found that *Astragali Radix* had anti-nephrotic syndrome effects in some aspects of increasing kidney blood perfusion and glomerular filtration rate,

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https://doi.org/10.1016/j.chmed.2019.05.005
1674-6384/

reducing podocytes injury and urinary protein, as well as delaying glomerular sclerosis. In our previous research (Li, 2017), FHD reduced the level of urinary protein clearly, alleviated hyperlipidaemia, hyperlipemia symptoms, and improved the kidney injure scores in nephrotic syndrome model rats induced by adriamycin.

System pharmacology, a new subject in pharmacology, could provide a new perspective for the complicated interactions between ingredients and targets, so it is more suitable for researching the mechanism and material basis of TCM (Barabási, Gulbahce, & Loscalzo, 2011; Hopkins, 2007). As an emerging area of network pharmacology, system pharmacology emphasizes the concept of "network target, multicomponent therapeutics" and highlights a holistic thought which is also shared by TCM. With high throughput operation, combining oral bioavailability (OB) and drug likeness (DL) to screen active ingredients of TCM, the targeted characteristics and pharmacological effects can be predicted, and the interactions among drugs, targets, diseases and pathways can be deciphered (Arrell & Terzic, 2010). Moreover, it is prospective that the topological properties of the biological network may enrich our knowledge about the therapeutic mechanisms of the multiple actions of herbs.

Therefore, in the present study, we tried to establish the compound-target-pathway and compound-organ network by using Cytoscape software. Subsequently, we tried to analyze the GO and KEGG pathways by ClueGO software, and acquire the tissue distribution of targets by the BioGPS database, so as to uncover the underlying mechanisms of FHD in treating NS. Finally, the contribution index results can reveal the principal components of FHD in the treatment of NS.

2. Materials and methods

2.1. Chemical ingredients database building

All the constituent data of FHD were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database (http://lsp.nwu.edu.cn/tcmsp.php,TCMSP), Traditional Chinese Medicines Integrated Database (http://www.megabionet.org/tcmid/,TCMID), and the previous results of the chromatogram peak identification (Chen, Li, Li, & Qin, 2017), and manually supplemented through a text-mining method (Cui et al., 2007; Liang et al., 2012).

2.2. Active ingredients screening

TCM is mostly used in decoction, and the processes of absorption, distribution, metabolism and excretion are inevitably to produce effects, that is, the absorption, distribution, metabolism and excretion (ADME) processes. ADME prediction methods are mainly simulated by the computer including log octanol/water partition coefficient (AlogP), OB, DL, Caco-2 permeability prediction (Caco-2) and blood brain barrier (BBB). To ensure the reliability of screening model, some major specific descriptors of molecular properties including OB and DL should be employed as important screening parameters. OB value is governed by the dissolution of drug in the gastrointestinal tract, the intestinal and hepatic first-pass metabolism, as well as the intestinal membrane permeation, which makes it one of the major pharmacokinetic profiles in drug development (Xu, Zhang, & Huang, 2012). And only assuring molecules with a high DL index can increase the possibility of therapeutic success, and the higher DL value a molecule has, the larger possibility that it possesses certain biological properties (Ma, Wang, & Xie, 2011). Thus, those molecules with proper OB value and DL value were selected for subsequent research. Therefore, a robust in silico model OB oavail 1.1 that integrated the Cytochrome P450 proteins and P-glycoprotein transport information was employed to calculate the OB values of all herbal ingredients (Xu, Zhang & Huang, 2012). Those ingredients with OB greater than or equal to 30% were selected. An optional DL model based on 6511 molecules in the DrugBank database was constructed to prescreen potential active ingredients. Also, those compounds with low OB or DL value, having profound pharmacological effects and strong identifiability, were also selected for further research.

2.3. Target identification

Indisputably, TCM exerts their therapeutic effects through the synergistic effects of multiple compounds and targets. Thus, besides exploring active ingredients, the therapeutic targets exploration is also necessary. In order to identify the targets of FHD active ingredients, we used the PharmMapper Server (Liu, Ouyang, & Yu, 2010) (http://59.78.98.102/pharmmapper/submit_file.php) and the Similarity Ensemble Approach Database (Bindea, Mlecnik, & Hackl, 2009) (http://sea.bkslab.org/,SEA) to hunt for targets. PharmMapper server is a freely accessed web server designed to identify potential target candidates for the given small molecules using pharmacophore mapping approach. PharmMapper hosts a large, in-house repertoire of pharmacophore database (namely PharmTargetDB) annotated from all the targets information in TargetBank, BindingDB, DrugBank and potential drug target database, including over 7000 receptor-based pharmacophore models (Liu et al., 2010). SEA, the identification of protein function based on biological information is an area of intense research. They began with 65 000 ligands annotated into sets for hundreds of drug targets. The similarity score between each set was calculated using ligand topology (Keiser, Roth, & Armbruster, 2007).

First of all, by uploading mol2. file format of FHD active components' chemical structure, we can obtain the top score 300 targets for each active component by the PharmMapper server. Then, SMILES files of active components were uploaded, the target points corresponding to each active ingredient can be acquired by the SEA Database. What's more, only the targets from "Homo sapiens" were employed for the further research. Finally, all targets were sent to UniProtKB Database (http://www.uniprot.org/uniprot/) to obtain the UniProt number.

In addition, the related disease targets were searched and analyzed by inputting the keywords "Nephrotic Syndrome" into GeneCards Database (http://www.genecards.org/), HOME-NCBI-GENE Database (https://www.ncbi.nlm.nih.gov/gene), OMIM Database (http://omim.org/) Drugbank database (https://www.drugbank.ca/), and Genetic Association database (https://geneticassociationdb.nih.gov/). Then, the duplication and false positive targets were deleted and integrated.

Finally, the targets of FHD and the targets of NS were intersected to obtain the potential targets for the treatment of NS by FHD, that is, FHD-targets-NS.

2.4. Gene Ontology (GO) and pathway analysis

The ClueGO is an important plug-in for Cytoscape visualization software. ClueGO analysis technology enables using the same descriptors from the functions of target product in different Databases, and the targets were classified and analyzed from three levels of Biological Process (BP), Molecular Function (MF) and Cellular Component (CC). What's more, DAVID Bioinformatics Resources 6.8 (The Database for Annotation, Visualization and Integrated Discovery, http://david.abcc.ncifcrf.gov) was employed to perform the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis.

2.5. Network construction

In order to deeply interpret the complex relationships among active compounds, targets and pathways, the networks were constructed.

(1) **Target-target network (T-T network)** String Database is a known and predicted protein-protein interaction (PPI) database. It can predict the significance of target and its connection with other targets.

(2) **Target-organ network (T-O network)** BioGPS Database (Wu, Orozco, & Boyer, 2009) (http://biogps.org/#goto=welcome) was used to obtain the main location of each target distribution, which can get a target-organ network (T-O network). T-O network was constructed to further reveal the mechanism of FHD in the treatment of NS.

(3) **Compound-target-pathway network (C-T-P network)** Active ingredients of FHD in treating NS generated the compound-target network (C-T network). The pathways were extracted from the Database of KEGG to probe into the major signaling pathways of FHD in the treatment of NS, resulting in a target-pathway network (T-P network). Then, we use the "merge" function of Cytoscape software to link the C-T network and T-P network to get a compound-target-pathway network.

All the visualized networks were constructed by Cytoscape 3.2.1 software, an open software for analyzing, integrating, visualizing and validating the networks.

2.6. Pathway construction and analysis

To probe into the biological effects of cellular targets affecting the diseases through modulating specific pathways, an incorporated "NS pathway" was integrated in light of present cognition of NS pathology. In brief, the obtained target proteins were mapped to KEGG to distribute them to several pathways.

2.7. Contribution indexes calculation

In order to evaluate the contribution of chemical components of FHD to NS, Yue first proposed the effective components of Contribution index (CI) and Network based efficacy (NE). Eqs. (1) and (2) were as follows (Yue, Liu, & Feng, 2017):

$$NE(j) = \sum_{i=1}^{n} d_i \tag{1}$$

$$CI(j) = \frac{c_j \times NE(j)}{\sum_{i=1}^{m} c_i \times NE(i)} \times 100\% \tag{2}$$

Where n was the number of targets connected with ingredient j, di was the degree of target i connected with ingredient j; NE was ingredient j connected with all targets' degree sum. Ci was the number of NS-associated literature-mining way, the following keywords were used for NS terms: Nephrotic Syndrome and the common names of active ingredients were also used as keywords. The numbers of papers having keywords in the title/abstract from 1997 to 2017 were obtained from the CNKI and PubMed Database. If the sum of CIs for the top n ingredients was more than 85%, these relevant n ingredients were regarded to contributing best as the pharmacodynamic material basis.

3. Results and discussion

3.1. Active components identification

A total of 469 major compounds were collected from TCMSP Database, TCMID Database, the previous experiments and literatures. According to the OB and DL values, we obtained 45 effective compounds, including 22, 7, 11 and 5 from *Stephaniae Tetrandrae Radix* (FJ), *Astragali Radix* (HQ), *Atractylodes macrocephalae Rhizoma* (BZ) and *Liquiritiae Radix* et *Rhizoma* (GC), respectively, which were presented in Table 1.

3.2. Target fishing and interaction

With the aid of PharmMapper server and SEA Database, a total of 846 predicted targets of active compounds were obtained. A total of 745 NS disease targets were predicted using Genecards Database, HOME-NCBI-GENE Database and OMIM Database. Finally, the compounds targets of FHD and disease targets of NS were intersected to obtain 85 potential targets. Specific target information was shown in Table 2.

The interaction of the potential targets was constructed using the String server. Fig. 1 showed that the network contains 85 nodes, 545 kinds of interaction relationship, and the average node degree was 12.8, combined with the average local clustering coefficient of 0.63. Moreover, the thicker the lines were, the stronger the interaction was; the larger the circle was, the greater the effect was. We can see that the targets such as ALB (serum albumin), INS (insulin), AKT1 (RAC serine/Threonine protein kinase), VEGFA (vascular endothelial growth factor A), and HSP90AA1 (heat shock protein HSP) played significant roles in this network.

ALB in the urine usually denotes the presence of kidney disease, and it functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones in the blood and could stabilize extracellular fluid volume by contributing to oncotic pressure of plasma. Survival factors can suppress apoptosis in a transcription-independent manner by AKT1, which then phosphorylates and inactivates components of the apoptotic machinery (Cohen, 2014). VEGFA is a glycosylated mitogen that specifically acts on endothelial cells and has various effects, including mediating increased vascular permeability, inducing angiogenesis, vasculogenesis and endothelial cell growth, promoting cell migration, and inhibiting apoptosis (Martynova et al., 2016). This suggested that the mechanism of FHD treating NS is related with regulation of plasma colloid osmotic pressure stability and inhibition of apoptosis.

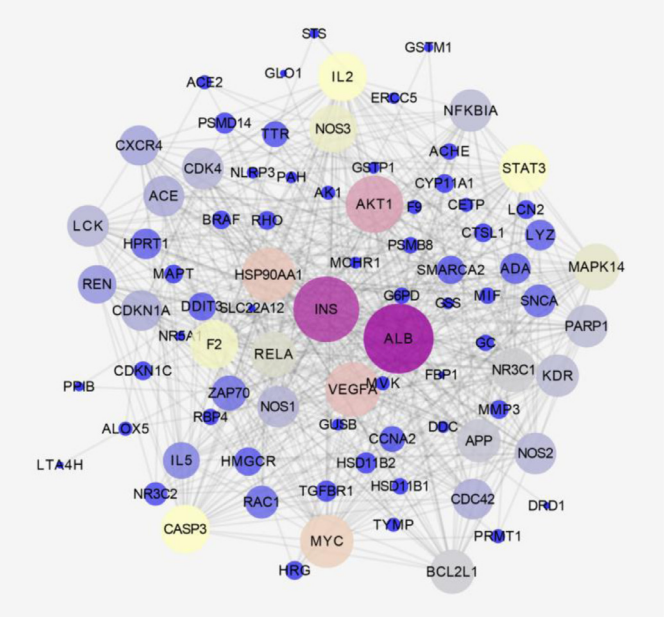

Fig. 1. Targets-targets network built by target interaction.

Table 1
Active compounds in *Stephaniae Tetrandrae Radix, Astragali Radix, Atractylodis Macrocephalae Rhizoma* and *Liquiritiae Radix* with corresponding pharmacokinetic parameters and chemical constituents and topological attributes of FHD.

No.	Molecule names	OB /%	DL	Degree	Betweenness
FJ1 #	β-sitosterol	36.91	0.75	12	0.00429918
FJ2 #	Cyclanoline	2.64	0.57	20	0.05358245
FJ3 #	Hesperetin	70.31	0.27	6	0.00950394
FJ4 #	N-Methylflindersine	32.36	0.18	14	0.01605971
FJ5 *#	Tetraneurin A	35.39	0.31	13	0.03647162
FJ6 *	Hanfangichin B, Fangchinoline	–	–	31	0.08538367
FJ7 *	Corydine	–	–	9	0.0178696
HQ1 #	(3R)-3-(2–hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.67	0.26	17	0.03158523
HQ2 #	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78	15	0.0057581
HQ3 #	3,9-di-*O*-methylnissolin	53.74	0.48	12	0.00812505
HQ4 #	5′-hydroxyiso-muronulatol-2′,5′-di-*O*-glucoside	41.72	0.69	11	0.02102894
HQ5 #	7-*O*-methylisomucronulatol	74.69	0.3	13	0.02104848
HQ6 #	9,10-dimethoxypterocarpan-3-*O*-β-*D*-glucoside	36.74	0.92	9	0.01303694
HQ7 #	Bifendate	31.1	0.67	8	0.01706221
HQ8 #	FA	68.96	0.71	4	0.00485561
HQ9 #	Formononetin	69.67	0.21	13	0.02837696
HQ10 #	Hederagenin	36.91	0.75	12	0.00324548
HQ11 *#	Isoflavanone	109.99	0.3	13	0.03569691
HQ12 #	Isomucronulatol-7,2′-di-*O*-glucosiole	49.28	0.62	8	0.0018419
HQ13 #	Isorhamnetin	49.6	0.31	7	0.0067138
HQ14 *#	Jaranol	50.83	0.29	8	0.02077885
HQ15 #	Kaempferol	41.88	0.24	5	0.00683869
HQ16 #	Mairin	55.38	0.78	14	0.00673553
HQ17 *#	Quercetin	46.43	0.28	7	0.02100403
HQ18 *	Astragaloside IV	–	–	25	0.0187987
HQ19 #	Ononin	11.52	0.78	10	0.02268433
HQ20 *	Calycosin-7-glucoside	–	–	3	6.15E-04
HQ21 *	Pterocarpan	–	–	22	0.07670719
HQ22 #	Calycosin	47.75	0.24	10	0.01473383
BZ1 #	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78	16	0.00543536
BZ2 #	12-senecioyl-2E,8E,10E-atractylentriol	62.4	0.22	26	0.01858789
BZ3 #	14-acetyl-12-senecioyl-2*E*,8*E*,10*E*-atractylentriol	60.31	0.31	24	0.0124213
BZ4 #	14-acetyl-12-senecioyl-2*E*,8*Z*,10*E*-atractylentriol	63.37	0.3	25	0.01580938
BZ5 #	3β-acetoxyatractylone	54.07	0.22	24	0.01203367
BZ6 #	8β-ethoxy atractylenolide III	35.95	0.21	25	0.01519559
BZ7 #	Atractylenolide I	37.37	0.15	12	0.01608551
BZ8 #	Atractylenolide III	68.11	0.17	14	0.02832768
BZ9 #	Atractylenolide II	47.5	0.15	12	0.01114457
BZ10 #	α-Amyrin	39.51	0.76	22	0.00973033
BZ11 *	Atractyline	–	–	13	0.02145701
GC1 *#	Licoricone	63.58	0.47	20	0.11161029
GC2 #	Schaftoside	7.88	0.75	1	0
GC3 #	18β-glycyrrhetinic acid	22.06	0.74	25	0.02329035
GC4 *#	Glucuronic acid	46.18	0.06	23	0.04527532
GC5 *#	Glycyrrhizin	9.06	0.11	24	0.02174275

(FJ, *Stephaniae Tetrandrae Radix*; HQ, *Astragali Radix*; BZ, *Atractylodes Macrocephalae Rhizoma*; GC, *Liquiritiae Radix*; OB, oral bioavailability; DL, druglikeness. "*" represents chemical constituents of FHD by identification of chromatograms and literatures, "#" represents chemical constituents of FHD by TCMSP and TCMID database.).

3.3. ClueGo analysis and pathway analysis

Totally, 85 potential targets were analyzed from three levels of biological processes, molecular functions and cellular components using ClueGO plugin. The KEGG analysis was conducted by DAVID server.

3.3.1. Biological process

As shown in Fig. 2A, there were many targets involved in the regulation of hydrogen peroxide metabolic process, the negative regulation of ATP metabolic process and the positive regulation of B cell proliferation were associated with metabolic processes, redox processes and so on. The changes of Katp channel structure and function in the process of oxidized ATP metabolism are closely related to renal function, and the metabolites advanced oxidized protein products can induce apoptosis, renal tubular epithelial injury, kidney membrane cell proliferation and differentiation (Zhao, 2012), suggesting that above-mentioned biological processes were closely related to pathogenesis of NS.

3.3.2. Molecular function

The 85 targets were mainly related to these molecular functions as shown in Fig. 2B, including nitric-oxide synthase activity, tetrahydrobiopterin binding and NADPH-reductase activity. Nitric oxide synthase can catalyze *L*-arginine to produce nitric oxide (NO), and NO has a strong vasodilator effect, which can reduce the average arterial blood pressure and regulate the resting tension of the blood vessels of the body, acting as an important factor in renal hemodynamics (Liu, 2014). In addition, tetrahydrobiopterin is of necessary in NO biosynthesis, implying the intervention effects of FHD on NS were associated with regulating early glomerular hyperperfusion and high filtration-induced hemodynamic abnormalities (Xie et al., 2002).

3.3.3. Cellular component

Analysis of cell composition data were shown in Fig. 2C, a number of genes were associated with serine/threonine protein kinase complex, cyclin-dependent protein kinase holoenzyme complex and vesicle lumen. The location of these cellular components

Table 2
Target information of FHD.

No.	Genes	Uniprot	Protein names	Degree	Betweenness
1	ACE	P12821	Angiotensin-converting enzyme	20	0.007723
2	ACE2	Q9BYF1	Angiotensin-converting enzyme 2	4	4.78E-05
3	ACHE	P22303	Acetylcholinesterase	5	0
4	ADA	P00813	Adenosine deaminase	11	0.009846
5	AK1	P00568	Adenylate kinase isoenzyme 1	4	0.02381
6	AKT1	P31749	RAC-alpha serine/threonine-protein kinase	40	0.046207
7	ALB	P02768	Serum albumin	55	0.242143
8	ALOX5	P09917	Arachidonate 5-lipoxygenase	4	0.02381
9	APP	P05067	Amyloid beta A4 protein	23	0.020422
10	BCL2L1	Q07817	Bcl-2-like protein 1	24	0.004661
11	BRAF	P15056	Serine/threonine-protein kinase B-raf	6	0.00051
12	CASP3	P42574	Caspase-3	30	0.008162
13	CCNA2	P20248	Cyclin-A2	9	0.002527
14	CDC42	P60953	Cell division control protein 42 homolog	20	0.006174
15	CDK4	P11802	Cyclin-dependent kinase 4	21	0.017057
16	CDKN1A	P38936	Cyclin-dependent kinase inhibitor 1	20	0.02166
17	CDKN1C	P49918	Cyclin-dependent kinase inhibitor 1C	6	0.000464
18	CETP	P11597	Cholesteryl ester transfer protein	4	0
19	CTSL1	P07711	Cathepsin L1	6	0.00106
20	CXCR4	P61073	C-X-C chemokine receptor type 4	19	0.008404
21	CYP11A1	P14137	Cholesterol side-chain cleavage enzyme, mitochondrial	6	0.006812
22	DDC	P20711	Aromatic-*L*-amino-acid decarboxylase	3	0.02381
23	DDIT3	P35638	DNA damage-inducible transcript 3 protein	10	0.011911
24	DRD1	P21728	D(1A) dopamine receptor	1	0
25	ERCC5	P28715	DNA repair protein complementing XP-G cells	3	7.17E-05
26	F2	P00734	Prothrombin	29	0.035831
27	F9	P00740	Coagulation factor IX	4	0.000111
28	FBP1	P09467	Fructose-1,6-bisphosphatase 1	1	0
29	G6PD	P11413	Glucose-6-phosphate 1-dehydrogenase	5	0.02381
30	GC	P02774	Vitamin D-binding protein	5	0.000387
31	GLO1	Q04760	Lactoylglutathione lyase	1	0
32	GSS	P48637	Glutathione synthetase	3	0.001673
33	GSTM1	P09488	Glutathione S-transferase Mu 1	2	0
34	GSTP1	P09211	Glutathione S-transferase P	3	0.034695
35	GUSB	P08236	Beta-glucuronidase	3	5.74E-05
36	HMGCR	P04035	3–hydroxy-3-methylglutaryl-coenzyme A reductase	10	0.006672
37	HPRT1	P00492	Hypoxanthine-guanine phosphoribosyltransferase	11	0.007407
38	HRG	P04196	Histidine-rich glycoprotein	6	0.00048
39	HSD11B1	P28845	Corticosteroid 11-beta-dehydrogenase isozyme 1	5	0.000709
40	HSD11B2	P80365	Corticosteroid 11-beta-dehydrogenase isozyme 2	7	0.001187
41	HSP90AA1	P07900	Heat shock protein HSP 90-alpha	36	0.088943
42	IL2	P60568	Interleukin-2	30	0.011244
43	IL5	P05113	Interleukin-5	15	0.000696
44	INS	P01308	Insulin	50	0.18376
45	KDR	P35968	Vascular endothelial growth factor receptor 2	22	0.010784
46	LCK	P06239	Tyrosine-protein kinase Lck	21	0.00446
47	LCN2	P80188	Neutrophil gelatinase-associated lipocalin	6	0.000464
48	LTA4H	P09960	Leukotriene A-4 hydrolase	1	0
49	LYZ	P61626	Lysozyme C	11	0.00398
50	MAPK14	Q16539	Mitogen-activated protein kinase 14	27	0.013492
51	MAPT	P10636	Microtubule-associated protein tau	6	0
52	MCHR1	Q99705	Melanin-concentrating hormone receptor 1	3	0
53	MIF	P14174	Macrophage migration inhibitory factor	5	0.001024
54	MMP3	P08254	Stromelysin-1	6	2.21E-05
55	MVK	Q03426	Mevalonate kinase	4	0.012081
56	MYC	P01106	Myc proto-oncogene protein	35	0.030322
57	NFKBIA	P25963	NF-kappa-B inhibitor alpha	22	0.012138
58	NLRP3	Q96P20	NACHT, LRR and PYD domains-containing protein 3	3	0.001769
59	NOS1	P29475	Nitric oxide synthase, brain	21	0.031455
60	NOS2	P35228	Nitric oxide synthase, inducible	21	0.012085
61	NOS3	P29474	Nitric oxide synthase, endothelial	28	0.025978
62	NR3C1	P04150	Glucocorticoid receptor	24	0.018987
63	NR3C2	P08235	Mineralocorticoid receptor	8	0.001099
64	NR5A1	Q13285	Steroidogenic factor 1	2	0
65	PAH	P00439	Phenylalanine-4-hydroxylase	3	0.00043
66	PARP1	P09874	Poly [ADP-ribose] polymerase 1	22	0.019099
67	PPIB	P23284	Peptidyl-prolyl cis-trans isomerase B	2	7.17E-05
68	PRMT1	Q99873	Protein arginine N-methyltransferase 1	4	0.000187
69	PSMB8	P28062	Proteasome subunit beta type-8	5	0.000102
70	PSMD14	O00487	26S proteasome non-ATPase regulatory subunit 14	7	0.001713

(*continued on next page*)

Table 2 (*continued*)

No.	Genes	Uniprot	Protein names	Degree	Betweenness
71	RAC1	P63000	Ras-related C3 botulinum toxin substrate 1	12	0.001806
72	RBP4	P02753	Retinol-binding protein 4	5	0.000697
73	RELA	Q04206	Transcription factor p65	26	0.034383
74	REN	P00797	Renin	17	0.005672
75	RHO	P61586	Transforming protein RhoA	6	0.000626
76	SLC22A12	Q96S37	Solute carrier family 22 member 12	1	0
77	SMARCA2	P51531	Probable global transcription activator SNF2L2	10	0.004377
78	SNCA	P37840	Alpha-synuclein	12	0.00167
79	STAT3	P40763	Signal transducer and activator of transcription 3	30	0.010458
80	STS	P08842	Steryl-sulfatase	2	0.000179
81	TGFBR1	P36897	TGF-beta receptor type-1	7	0.000118
82	TTR	P02766	Transthyretin	9	0.018578
83	TYMP	P19971	Thymidine phosphorylase	4	0.00013
84	VEGFA	P15692	Vascular endothelial growth factor A	37	0.055239
85	ZAP70	P43403	Tyrosine-protein kinase ZAP-70	13	0.012881

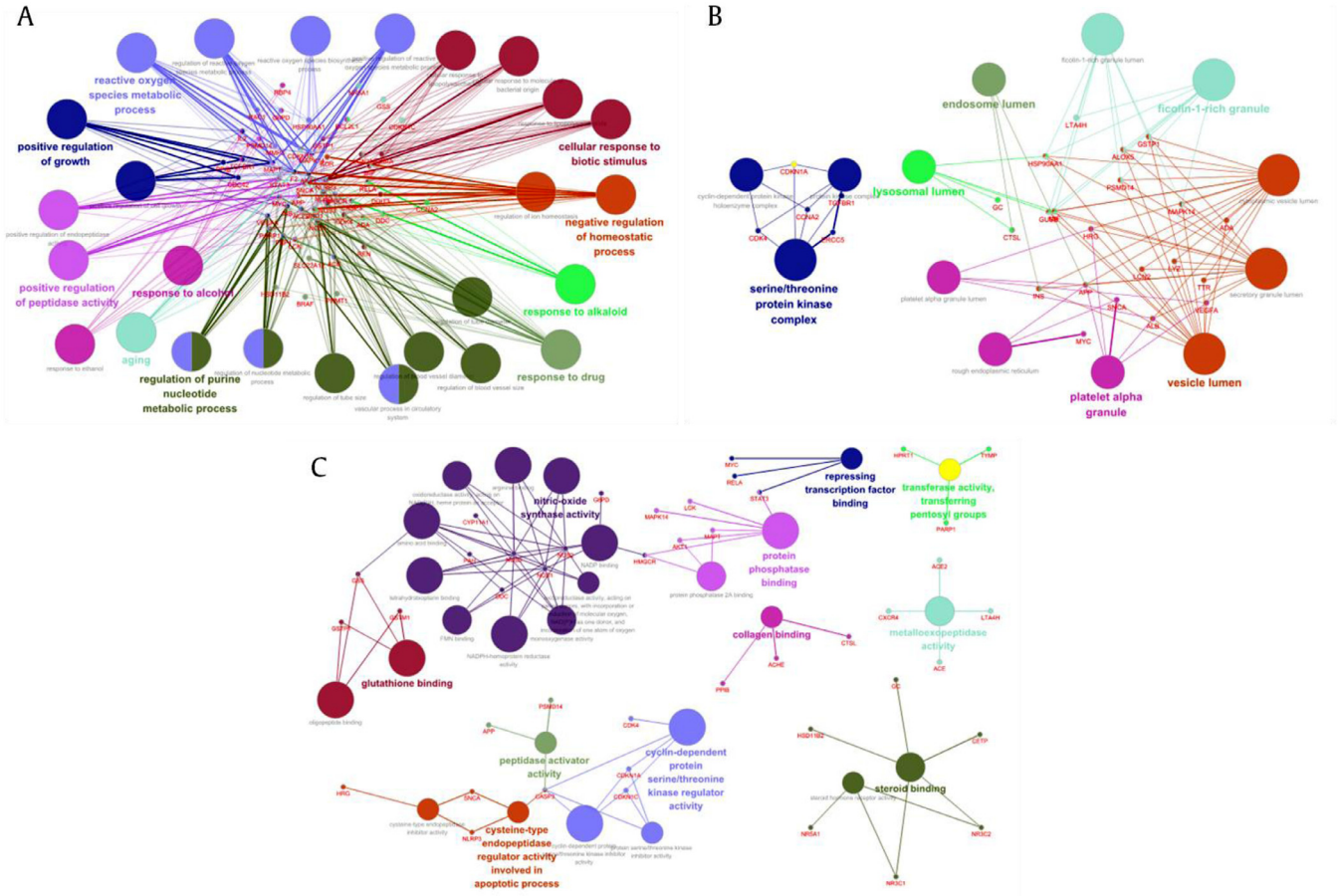

Fig. 2. Network of biological process-targets (A), molecular function-targets (B) and cellular component-target (C).

involved redox reactions and cell cycle processes, and the cyclin-dependent protein kinase is reported to be inhibited to reduce glomerular mesangial cell proliferation induced by certain factors and late proteinuria, suggesting that cells cyclin-dependent protein kinases may be involved in the physiological and pathological of NS (Yu, 2012).

3.3.4. KEGG analysis

As shown in Fig. 3, it can be concluded that PI3K-Akt signaling pathway, MAPK signaling pathway, T cell receptor signaling pathway, TNF signaling pathway, Renal cell carcinoma, NF-κB receptor and B cell receptor signaling pathway were significantly associated with these targets.

In this section, pathways directly related to NS were assembled into the "NS pathway" based on the present cognition of NS pathology. These targets exhibited incredibly functional connection closeness to other targets linked with NS pathway. As shown in Fig. 4, this NS-associated pathway can be decomposed into several functional modules such as angiogenesis, cell cycle, DNA repair, apoptosis, remodeling of extracellular matrix and differentiation immune response.

The PI3K/Akt signaling pathway, associated with 13 targets, was predicted to play a major role in NS. Moreover, as one of the

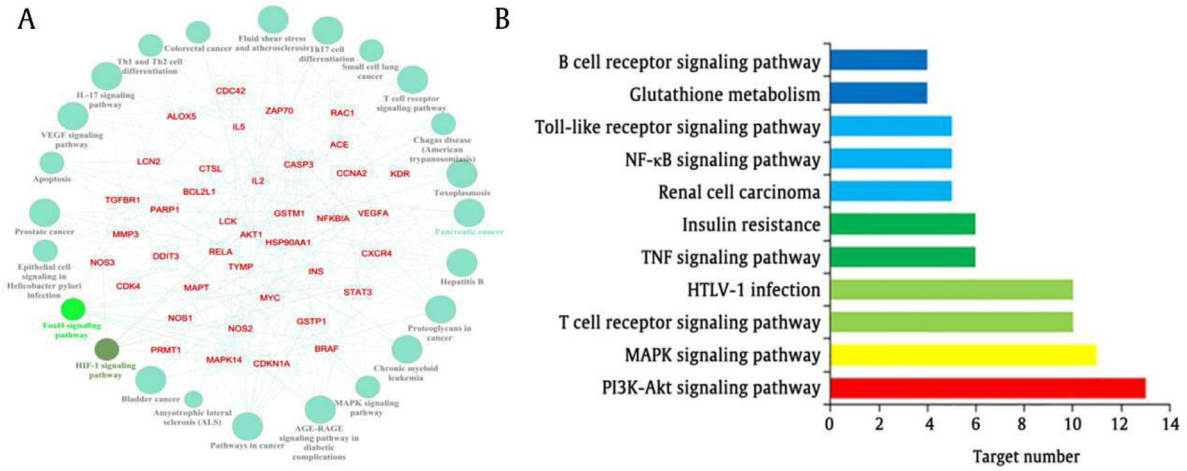

Fig. 3. Network of KEGG pathways-targets (A) and GO-KEGG enrichment analysis (B).

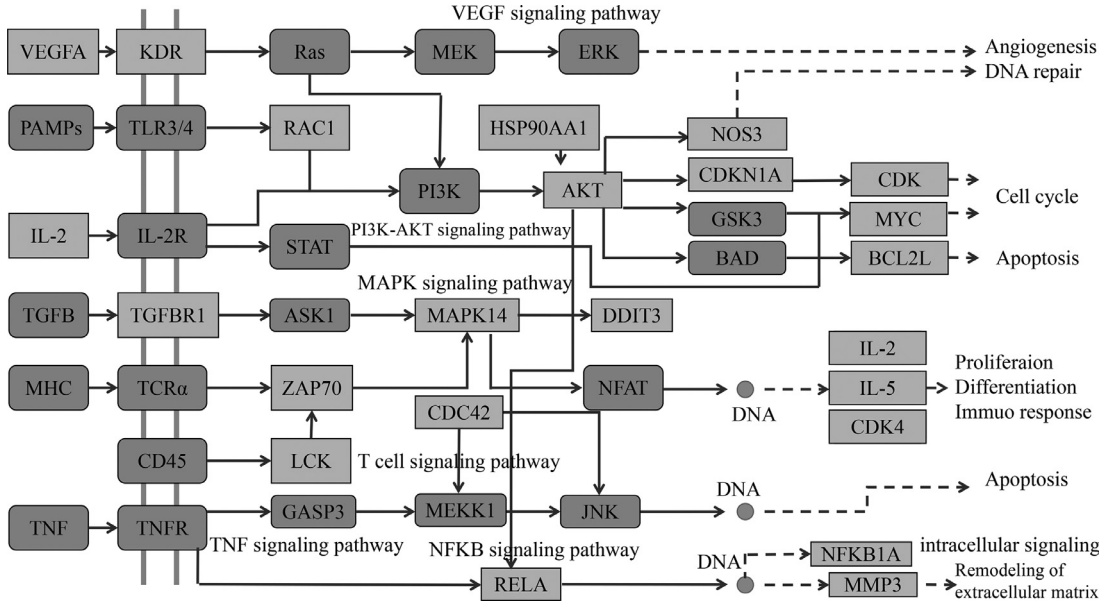

Fig. 4. Anti-nephrotic syndrome pathways of potential targets from main active ingredients of FHD (Squares represent anti-nephrotic syndrome targets of FHD, ellipse represent targets in pathway).

intracellular signal transduction pathways, PI3K/Akt signaling pathway involves in promoting cell metabolism, proliferation, survival and growth. This pathway is mediated by the serine and/or threonine phosphorylation of a series of downstream substrates, in which phosphatidylinositol 3-kinase (PI3K) and Akt/protein kinase B (Akt/PKB) play an important role. Previous results showed that Kangxianling had effects on suppressing PI3K/AKT/m TOR signaling pathway, indicating that Kangxianling reducing the level of urinary protein and delaying renal fibrosis to protect the kidneys may be related to the inhibition of PI3K/AKT/mTOR signaling pathway (Zhong, Ma, Yu, & He, 2015).

It has been reported that MAPK play key roles in cell signaling networks and could be activated by extracellular stimuli such as cytokines and neurotransmitters. And the MAPK includes four subfamilies: ERK, JNK/SAPK, p38 and ERK5, among which p38 is mainly responsible for mediating inflammation and apoptosis. It is well known that NF-κB is able to participate in the early immune response in the body and regulate various stages of the inflammatory response. Some studies showed that inhibition of p38MAPK signaling pathway is essential to alleviate podocyte injury (Zheng, Deng, & Chen, 2012). The p38MAPK immune mechanism was reviewed based on experimental autoimmune encephalomyelitis mice, and the involvement of p38MAPK in immune-inflammatory injury was elucidated in a variety of adaptive and innate immune cells (Tong, Deng, Liu, Wang, & Guo, 2017). It was also reported that total flavonoids (TFA)-containing serum can inhibit mesangial cell proliferation, and down-regulate inflammatory cytokines IL-1β and TNF-α, which implied that promoting the recovery of renal function might through anti-oxidation and regulation of the p38MAPK/NF-κB signaling pathway (Nan et al., 2017). A similar study has also claimed that Liuwei Dihuang Pill can inhibit renal tissue oxidative stress and reduce the expression of p38MAPK and NF-κB p65 protein, suggesting that renal injury is closely related to the p38MAPK and NF-κB signal pathway (Zhang et al., 2017).

Toll-like receptors (TLRs), one of the important proteins involving in innate immunity, can recognize pathogenic microorganisms and activate the body's immune response. By activating the immune cells and providing costimulatory signals in the adaptive immune system, series of inflammatory responses were eventually

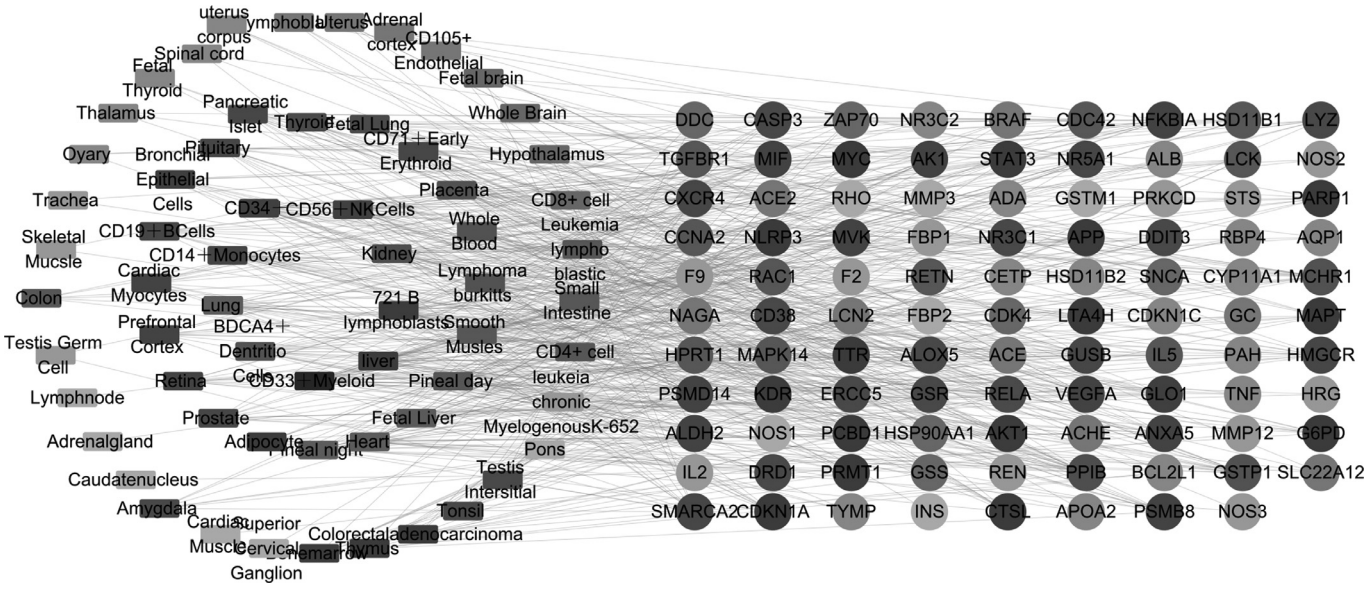

Fig. 5. Target-organ network of FHD. (Circular nodes represent targets and rectangle nodes represent organs, edgs are interactions between targets and organs).

induced. The immunohistochemical analyze was used to determine the expression of Toll-like receptor 4 and Toll-like receptor 7 in renal tissue of children with primary nephrotic syndrome (PNS). The result showed that compared with the normal group, the expression of Toll-like receptor was higher in the patients with PNS, and the expression of Toll-like receptor was different among the various pathological types of patients, suggesting that the pathological mechanism is related to Toll-like receptor expression (Zhang, Han, & Zhao, 2014). In the present study, the pharmacological mechanism of Fufang Shenhua Tablet on ischemia-reperfusion injury in rats was explored, and the results showed that the protective effects of this formula were mainly related to the MyD88-dependent TLR2/TLR4 signaling pathway inhibition mediated by the reduced release of inflammatory cytokines such as IL-6, IL-12 and TNF-γ (Zheng, 2013).

3.4. Network construction

3.4.1. Target-organ network

The tissue distribution network (Fig. 5) of the 85 targets was mapped using the Cytoscape software. Most targets acted in two or more tissues, suggesting that these tissues were closely correlated. Specially, high expression of 30 targets in $CD33^+$ myeloid indicted that they were potential effective targets for the treatment of NS based on autoimmune. Moreover, 23 targets acted in $CD14^+$ monocytes, as a part of the vertebrate innate immune system monocytes, and they also influenced the process of adaptive immunity. Twenty targets were over expressed in $BDCA4^+$ Dentritic Cells, whose main function is to process antigen material and present $BDCA4^+$ on the cell surface to the T cells of the immune system. $BDCA4^+$ Dentritio Cells act as messengers between the innate and the adaptive immune systems. It was reported (Wang, 2014) that complement system is an important mediator of renal immune injury in the course of nephrotic syndrome, there are abnormal T lymphocyte number or function and imbalance of lymphocyte subsets. It was predicted based on all these data that FHD treated NS by improving immune. There is no question that most targets acted in kidney and liver simultaneously, consistent with the fact that NS can increase the risk of liver disease.

3.4.2. Compound-target-pathway network

The "merge" function of Cytoscape software was used to construct the network (Fig. 6) of the active ingredients of FHD, potential targets and pathways. There were 192 nodes in the figure, and among these nodes, purple, red, green and yellow represented FJ, HQ, BZ and GC, respectively; blue nodes represent the potential targets, and the deeper the color was, the stronger the effect was; neon yellow nodes represent pathways. There were 805 sides, each side represents the interaction between the active ingredient and the potential target, or between the potential target and the pathway.

The results showed that the correlation between ACHE, GSTM1, HSD11B1, LCK, RBP4, F2, ACE and active components was strong, and these targets had high degree values. Furthermore, ACHE was associated with 32 active ingredients; GSTM1 was associated with 26 active ingredients; HSD11B1, LCK and RBP4 were all associated with 24 active ingredients; F2 was associated with 22 active ingredients; ACE was associated with 20 active ingredients. During neurotransmission, acetylcholine (ACh) is released from the presynaptic neuron into the synaptic cleft and binds to ACh receptors on the post-synaptic membrane to deliver the signal from the nerve. GSTM1 functions were in detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress, by conjugation with glutathione. FHD may play the role of renal protection through facilitating vasodilatation, regulating oxidative stress and detoxification. It can be concluded that different active ingredients in FHD can be applied to the same target, and same component can also act on different targets, which fully reflects the multi-components and multi-targets synergy therapy of NS.

Surprisingly, FJ6 (degree = 31), BZ2 (degree = 26), HQ18, BZ4, BZ6 and GC3 (degree = 25), BZ3, BZ5 and GC5 (degree = 24), GC4 (degree = 23), HQ21 and BZ10 (degree = 22) were probably the crucial compounds in the FHD due to their important positions in this network.

Integrating the two networks mentioned above, a CI of every active ingredient was proposed based on NE weighted by literatures (Fig. 7). Five compounds emerged from the active ingredients, including astragaloside IV (HQ18), quercetin (HQ17), fangchinoline (FJ6), glycyrrhizic acid (GC4) and glycyrrhizin (GC5), and they made great contributions to FHD treating NS with a sum

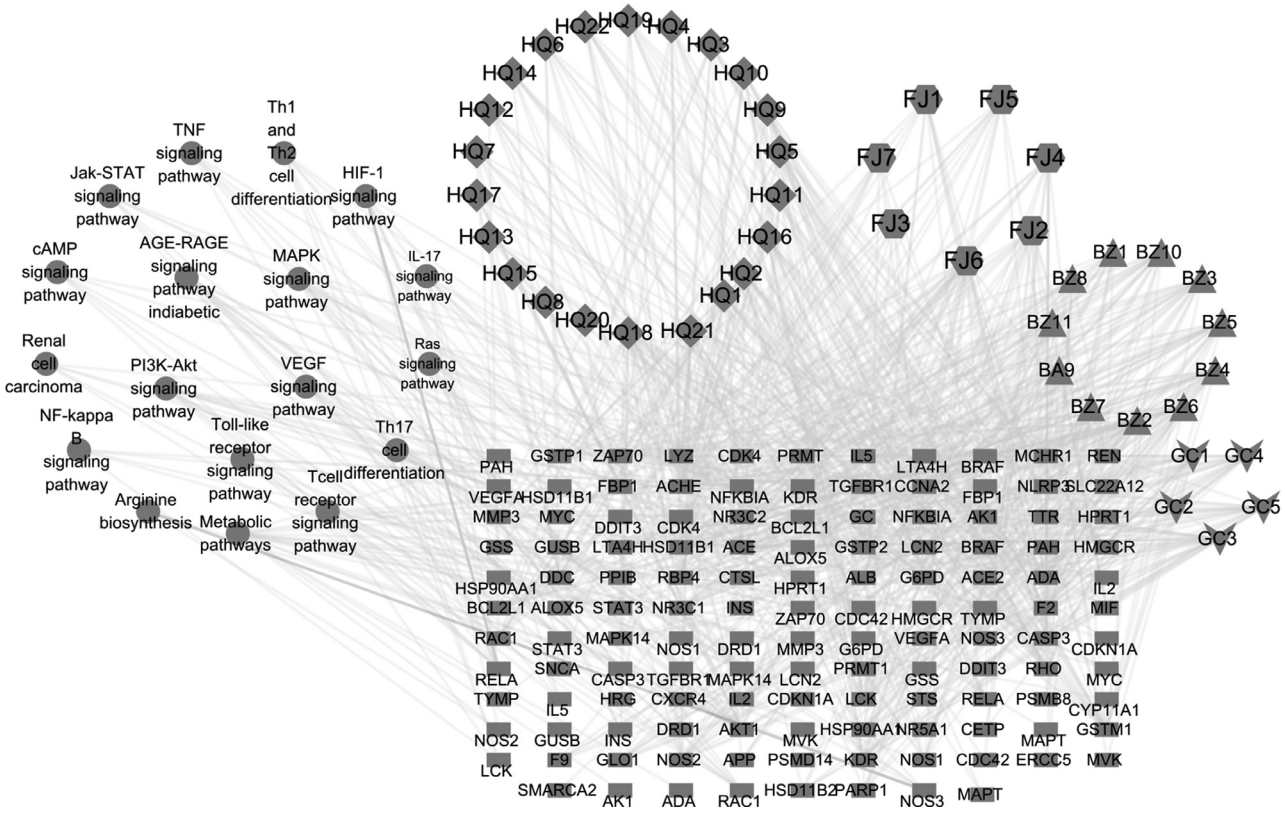

Fig. 6. Compound-target-pathway network of TFA. (Compound-target-pathway network was built by compounds, potential targets and pathways. 85 target protein (circular) were connected to 45 compounds (diamond, hexagon, triangle and V), and connected to 18 pathways (rectangle)).

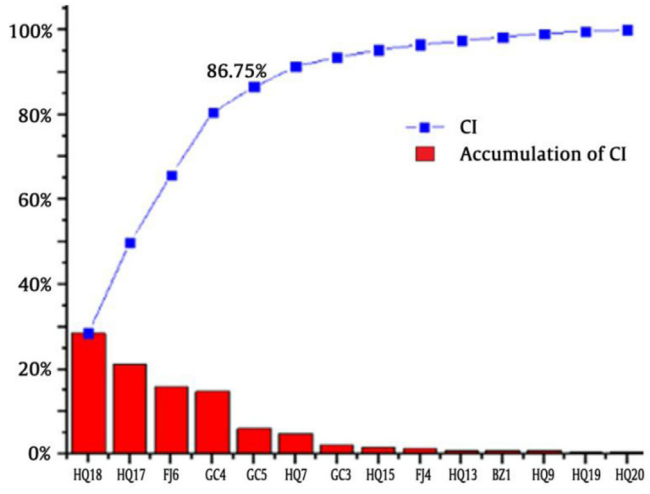

Fig. 7. CI and accumulative CI of active ingredients in FHD. Sum of CIs for top five ingredients, including HQ18 (astragaloside IV), HQ17 (quercetin), FJ6 (fangchinoline), GC4 (glycyrrhizic acid), and GC5 (glycyrrhizin), was more than 85%.

of CIs of 86.57%. Therefore, the discussion above may fully clarify why each drug of FHD could produce complementary effects.

Previous studies have shown that astragaloside IV can inhibit the expression of NRK-25E cells induced by high glucose and the activity of TGF- beta 1/Smad signaling pathway, thereby delaying renal interstitial fibrosis (Wang, 2017). It can be predicted based on previous results that astragaloside IV could alleviate the oxidative stress injury of glomerular mesangial cells by decreasing the production of intracellular ROS and inhibiting the P38/MAPK signaling pathway, suggesting protective effects of astragaloside IV on diabetic nephropathy (Cao, Li, & Si, 2013). By observing the morphology of podocytes and identifying the markers of podocytes, it was concluded that quercetin had protective effects on podocytes (Zhang, 2016). It could also be concluded that quercetin protect renal tissue mainly through effectively regulating immune reaction, achieving immune tolerance, and reducing the occurrence of chronic renal injury (Yang et al., 2017). Previous studies indicated that fangchinoline can reduce glomerular extracellular matrix sedimentation and balloon adhesion in nephrotic syndrome rats to improve glomerular sclerosis (Dong et al., 2000). It was also reported that fangchinoline combined with prednisone could significantly improve the rat nephrotic syndrome (Bai et al., 2016)). Fangchinoline could not only suppress the expression of CTGF, increase MMP13D activity in the matrix metalloproteinase system and block calcium channels, but also alleviate the adverse reactions of prednisone. It has been reported that glycyrrhizic acid could reduce the production of ROS and increase the expression of Mn-SOD protein to alleviate the oxidative stress injury of Mesangial cells (Hou, Zhang, & Yuan, 2017). A variety of previous reports proposed the protective effects of glycyrrhizic acid on renal injury (Zheng, Wei, & Shi, 2012). It was demonstrated via immunohistochemical method and qPCR to propose that glycyrrhizin can not only reduce proteinuria and blood lipid, but also inhibit the expression of cytokines such as TGF-β1, CTGF and mRNA, indicating that glycyrrhizin plays an early protective role on glomerulosclerosis in rats (Yu, Zhang, & Hao, 2010). It was also reported that glycyrrhizin can directly or indirectly inhibit the activation of transcription factor NF-κB associated with inflammation, reduce the accumulation of extracellular matrix in glomeruli, and delay the progression of glomerulosclerosis (Wang, Zhou, Zhong, Yang, & Yu, 2007).

4. Conclusion

In our study, an integrated system pharmacology approach was used to select active compounds, predict targets, construct networks, and illuminate the molecular synergy of FHD on NS. Forty-five active ingredients with oral bioactivities and drug likeness were selected from 846 compounds of FHD by ADME filtering. Meanwhile, 85 targets by these 45 compounds were identified as the potential anti-nephrotic syndrome targets of FHD. Finally, systematic analysis revealed that the C-T-P network was mainly involved in PI3K-Akt signaling pathway and MAPK signaling pathway. The T-O network revealed that these targets were mainly located in $CD34^+$, $CD56^+$NK Cells, $CD33^+$Myeloid, kidney and liver. The CI of every active ingredient also indicated five compounds, including astragaloside IV, quercetin, fangchinoline, glycyrrhizic acid and glycyrrhizin, as the principal components of FHD. These results successfully predicted the mechanisms underlying the efficiency of FHD for NS treatment, and probed into the potential novel therapeutic strategies for NS in TCM. All of these results are expected to identify novel curative efficacy and take full clinical advantage of FHD.

In summary, a system pharmacology-based method involving system biology approaches and networks-construction, could be used to study drugs, targets, pathways and effects. The systems pharmacology not only introduces a novel way for developing new drugs from medicinal herbs and discovering the principal compounds of herbs, but also provides a research platform for deciphering the molecular mechanism of herbs. However, active compounds may have different binding affinities towards their related targets, and these targets can be recognized and connected by other targets depending on target engagement. Thus, further experiments are necessary to support our intriguing findings.

Conflict of interest

The authors declare that there are no conflicts of interest in relation to this work.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (No. 31570346 and No. 31770362), and Research Project Supported by Shanxi Scholarship Council of China (2017-017), Key Laboratory of Effective Substances Research and Utilization in TCM of Shanxi Province (201605D111004), and Science and Technology Innovation Team of Shanxi Province (201605D131045-18). We also thank Scientific Instrument Center of Shanxi University for the NMR analysis.

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