Original Article

The potential mechanism of *Astragali Radix* in the treatment of children with nephrotic syndrome

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Background: The molecular mechanism of *Astragali Radix* in the treatment of children with nephrotic syndrome (NS) is unclear. This study aimed to use network pharmacology to explore this potential mechanism.

Methods: The Traditional Chinese Medicine Systems Pharmacology (TCMSP) database was used to identify the main active ingredients of *Astragali Radix*. The PharmMapper, Online Mendelian Inheritance in Man (OMIM), and GeneCards databases were then used to identify the active ingredients of *Astragali Radix*. The String database and Cytoscape software were used to construct the protein-protein network. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using DAVID Database.

Results: In the TCMSP Database, a total of 20 chemical constituents of *Astragali Radix* were screened. After removing the duplicates and false positive genes, 394 targets of these active ingredients were obtained from PharmMapper. By comparing the NS-related genes in the GeneCards and OMIM Databases, a total of 39 potential NS-related targets were ultimately identified. The protein-protein-interaction network included 39 nodes and 366 edges. The top 5 proteins were albumin (ALB), serine/threonine kinase (AKT1), epidermal growth factor receptor (EGFR), mitogen-activated protein kinase (MAPK), and matrix metallopeptidase 9 (MMP9). The GO analysis showed that the target genes were mainly involved in biological processes (e.g., signal transduction, the positive regulation of cell proliferation, and the positive regulation of migration). The cellular components included a plasma membrane, extracellular exosome, and extracellular space. The molecular functions included protein binding, zinc-ion binding, protein tyrosine kinase activity, and enzyme binding. The KEGG analysis showed that the treatment of NS by *Astragali Radix* mainly involved pathways in cancer, proteoglycans in cancer, the phosphatidylinositol 3-kinase and protein kinase B (PI3K-Akt) signaling pathway, the rennin-angiotensin-system (Ras) signaling pathways, and Forkhead box protein O1 (FoxO) signaling pathways.

Conclusions: In the present study, the network pharmacology method was used to explore the potential targets and pathways of *Astragali Radix* in the treatment of NS. We also provided future research directions for the treatment of NS with a complex pathogenesis.

Keywords: Nephrotic syndrome (NS); *Astragali Radix*; network pharmacology

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Introduction

Nephrotic syndrome (NS), whose clinical features mainly include proteinuria, hypoproteinemia, hyperlipidemia, and systemic edema, is one of the most common renal diseases in children (1). The pathogenesis of NS is very complex, and is mainly related to podocyte injury, apoptosis, lipid metabolism disorder, and oxidative stress (2). At present, the treatment of NS is still dominated by chemical drugs; however, these drugs can cause serious toxic and side effects and complications (3). Conversely, traditional Chinese medicine considers overall regulation of the body and has less toxic and side effects than western medicine.

Studies have shown that Fangji Huangqi Decoction, Angelica Tonifying Blood Decoction, and Shenqi Dihuang Decoction all use *Astragali Radix* as the main drug, which has significant efficacy in the treatment of nephropathy (4,5). *Astragali Radix* originated from "Shennong's Herbal Classic" in the eastern Han Dynasty. As a perennial legume herb, it is based on Astragalus Mongol and Astragalus membranaceus. *Astragali Radix* has a sweet taste and warm nature, and is an important traditional Chinese medicine with a history of more than 2,000 years (6,7). Recent studies have found that astragalus can reduce urinary protein excretion and reduce kidney injury (8,9). Clinical studies have found (10) that the oxidative stress of NS patients is enhanced, and astragalus membranaceus has certain antioxidant effects. The efficacy of astragalus in NS intervention is relatively clear; however, its molecular mechanism is still unclear, and thus further research and exploration is needed. This study took common human disease targets as the research objects and explored the mechanism of *Astragali Radix* in the treatment of NS based on reverse molecular docking technology.

Due to the multi-component, multi-pathway, and multi-target characteristics of traditional Chinese medicine, it is difficult to clarify the substance basis and mechanism of action of traditional Chinese medicine. Many scholars have tried to explain the mechanism of action of traditional Chinese medicine with modern medicine. The concept of "network pharmacology" was first proposed by the British pharmacologist Hopkins in 2007. Based on the multidisciplinary theories, such as systems biology and multidimensional pharmacology, the molecular mechanism of drug intervention in diseases can be understood from a multidimensional perspective (11). Previous studies have shown that the study of mechanism of action of Chinese medicine through network pharmacology is in line with the overall action characteristics of Chinese medicine, and the method is accurate and reliable. For example, some scholars have used network pharmacological methods to study the mechanism of Naozhenning Granules' intervention for brain injury (12), the anti-depressive mechanism of Bubuurum (13), and the mechanism of Scolt Flower's in relieving cough and reducing phlegm (14). Based on the 20 components of *Astragali Radix*, this study used the network pharmacology method to construct a component-target-disease network, and explored the mechanism of action of the multiple components, multiple targets, and multiple pathways of *Astragali Radix* in the treatment of NS to pave the way for further research on the mechanism of action of *Astragali Radix* in the treatment of NS. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/tp-21-348).

Methods

Collection and screening of chemical constituents of Astragali Radix

The chemical composition of *Astragali Radix* was obtained from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (http://lsp.nwu.edu.cn/tcmsp.php) (15). We input the keyword "*Astragali Radix*" to obtain information on its active ingredients. In this study, oral bioavailability (OB) and drug-likeness (DL) were used to screen out the chemical components with an OB ≥30% and a DL ≥0.18.

Forecast potential targets

The PharmMapper Database (http://59.78.98.102/pharmmapper/index.php) was used to obtain the mol2 format file of the candidate components of *Astragali Radix*, and the calculation results were then downloaded. The Protein Data Bank identification (PDB ID), target name, and fit score were obtained after submission. The fit score refers to the matching degree between the molecule and target; the higher the score, the better the match. Target proteins with a fit score >3 were selected as the target proteins of the compound, and the PDB ID of the protein target was input into the Uniprot Database (http://www.uniprot.org/) to obtain the potential action targets of the main components of *Astragali Radix* using retrieval and transformation operation.

The keyword "Nephrotic syndrome" was input in the Online Mendelian Inheritance in Man (OMIM; https://

omim.org) and GeneCards (https://www.genecards.org/) databases to search for genes related to NS. Duplicate genes and false positive genes were removed, and the remaining genes were matched with the above targets to screen those related to the active components of *Astragali Radix*.

Construction and analysis of the protein-protein-interaction network

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (16) (https://string-db.org/) collects details of a large number of protein-interaction relationships. By importing the protein targets of *Astragali Radix* into the STRING Database and defining the species as human, a protein-protein-interaction analysis was carried out. Node1, node2, and the combined score information were imported from the exported file into Cytoscape Software. The node size was set to reflect the degree value, and the thickness of the edge was set to reflect the size of the combined score to produce the final protein-interaction network.

Analysis of biological processes and pathways

The action targets of *Astragali Radix* were then imported into the DAVID database, the select identifier was set as the "official gene symbol," the list type was set as the "gene list," and the species were defined as human. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed on *Astragali Radix* action targets, and the results were saved. A threshold of $P<0.05$ was set, and the number of targets involved was sorted according to the number of targets. The biological processes or pathways with the top rankings were screened and mapped with EXCEL. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

Most of the analyses were done automatically in the database. Graphics are made by Excel or automatically generated by database. All statistical analyses were two-sided, with a P value <0.05 considered statistically significant.

Results

Active components of Astragali Radix

In the TCMSP database, 20 chemical constituents were screened according to the OB and DL values. The basic information of the 20 components are summarized in *Table 1*. The target network diagram of the *Astragali Radix* single drug is shown in *Figure 1*.

Target prediction

All the targets obtained from the PharmMapper database were collected. After removing the duplicates and false positive genes, a total of 394 targets of active ingredients of *Astragali Radix* remained. By comparing the NS-related genes in the GeneCards and OMIM databases, a total of 39 potential NS-related targets were finally identified (see *Table 2*).

Protein-protein-interaction network

The selected targets were input into the String Database, the species were limited to human, and the results were imported into Cytoscape Software to obtain the interaction network among the targets (see *Figure 2*). The nodes represent proteins, and the edges represent associations between proteins. The network comprised a total of 39 nodes and 366 edges. The size of a node in *Figure 2* represents the size of the degree value; the larger the size of a node, the larger the degree. The thickness of the edge represents the combined score; the thicker the edge, the greater the combined score. The top 5 proteins were albumin (ALB), serine/threonine kinase (AKT1), epidermal growth factor receptor (EGFR), mitogen-activated protein kinase (MAPK), and matrix metallopeptidase 9 (MMP 9).

Gene function and pathway analysis

A GO analysis and a KEGG analysis were performed on the 39 action targets predicted by *Astragali Radix* active components by the DAVID database. As *Figure 3* shows, the GO rich-set analysis included biological processes, cellular components, and molecular functions. The biological processes included signal transduction,

Table 1 The main compounds of *Astragali Radix*

Serial number	MOLID	Relative molecular weight	Compound	OB/%	DL	AlgP
1	MOL000211	456.78	Mairin	55.37707338	0.7761	6.521
2	MOL000239	314.31	Jaranol	50.82881677	0.29148	2.087
3	MOL000296	414.79	Hederagenin	36.91390583	0.75072	8.084
4	MOL000033	428.82	(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	36.22847056	0.78288	8.54
5	MOL000354	316.28	Isorhamnetin	49.60437705	0.306	1.755
6	MOL000371	314.36	3,9-di-O-methylnissolin	53.74152673	0.47573	2.892
7	MOL000374	642.67	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	41.71766574	0.69251	–0.948
8	MOL000378	316.38	7-O-methylisomucronulatol	74.68613752	0.29792	3.379
9	MOL000379	462.49	9,10-dimethoxypterocarpan-3-O-13-dimethyl-1	36.73668801	0.9243	0.737
10	MOL000380	300.33	(6ar,11ar)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.25545452	0.42486	2.641
11	MOL000387	418.38	Bifendate	31.09782391	0.66553	2.563
12	MOL000392	268.28	Formononetin	69.67388061	0.21202	2.583
13	MOL000398	316.33	Isoflavanone	109.9866565	0.29572	2.415
14	MOL000417	284.28	Calycosin	47.75182783	0.24278	2.316
15	MOL000422	286.25	Kaempferol	41.88224954	0.24066	1.771
16	MOL000433	441.45	Fa	68.96043622	0.7057	0.007
17	MOL000438	302.35	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.66747949	0.26479	3.128
18	MOL000439	626.67	Isomucronulatol-7,2'-di-O-glucosiole	49.28105539	0.62065	–0.68
19	MOL000442	314.31	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.04541112	0.47943	3.113
20	MOL000098	302.25	Quercetin	46.43334812	0.27525	1.504

the positive regulation of cell proliferation, the positive regulation of migration, and the MAPK cascade. Cell composition included the plasma membrane, extracellular exosome, and extracellular space. The molecular functions included protein binding, zinc-ion binding, protein tyrosine kinase activity, and enzyme binding. The results of the pathway analysis are shown in *Figure 4*. The treatment of NS by *Astragali Radix* mainly involved pathways in cancer, proteoglycans in cancer, the phosphatidylinositol 3-kinase and protein kinase B (PI3K-Akt) signaling pathway, the rennin-angiotensin-system (Ras) signaling pathways, and Forkhead box protein O1 (FoxO) signaling pathways.

Discussion

Astragali Radix is an important drug in the treatment of NS, which has the effects of diuresis and detumescence. The compounds in which *Astragali Radix* is the main drug account for a vast proportion of NS treatments. Thus, this study on the treatment of NS with *Astragali Radix* is of great significance. There was a similar report about the mechanism of Astragali Radix in the treatment of children with nephrotic syndrome (17), however, our study used more data from official platforms, such as TCMSP, OMIM, and DAVID database, and so the results were relatively

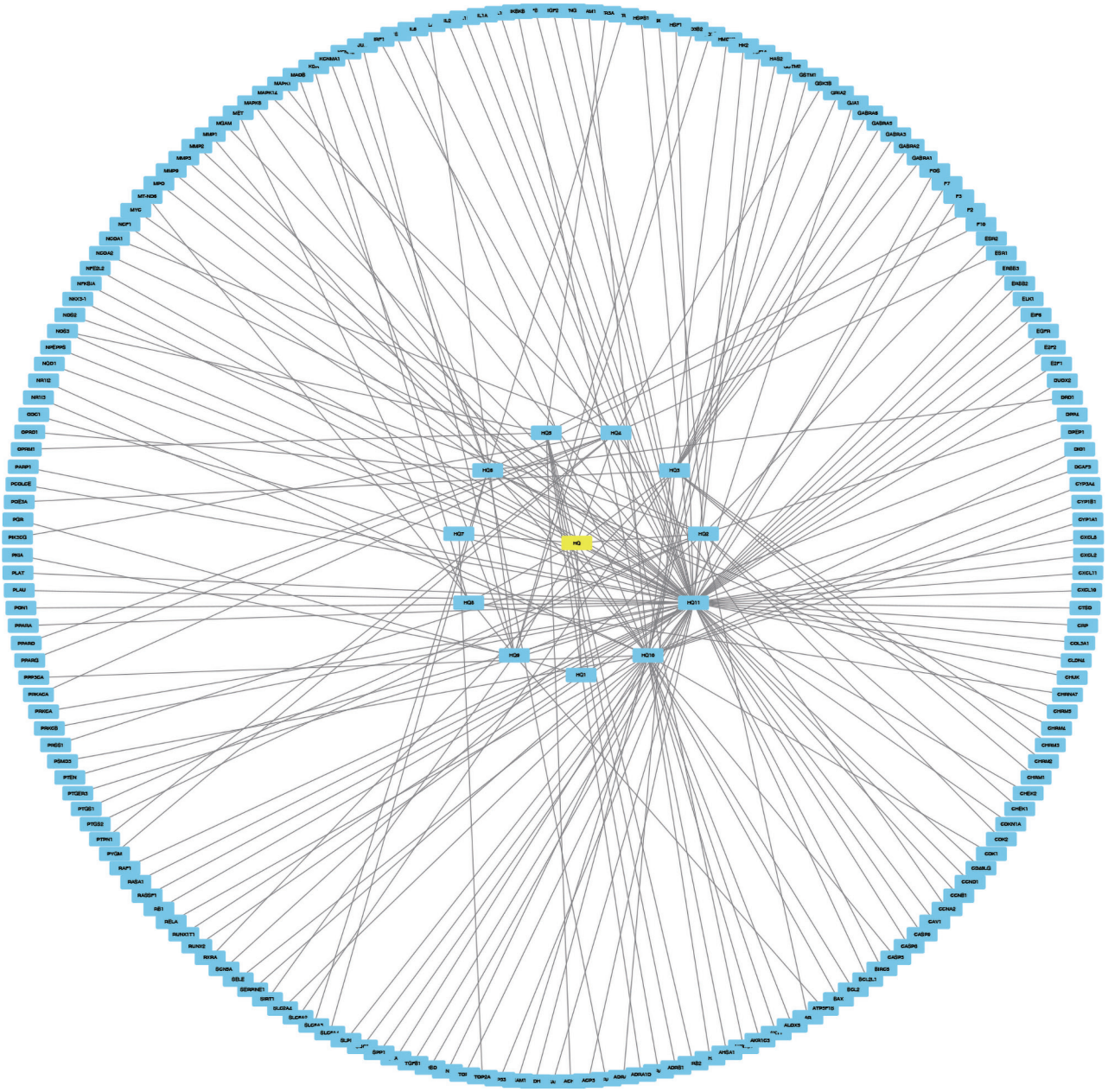

Figure 1 The target network of *Astragali Radix*.

Table 2 Potential targets of *Astragali Radix* for treating nephrotic syndrome

Serial number	Uniprot	Targets
1	Q06124	PTPN11
2	P15056	BRAF
3	P02768	ALB
4	P01112	HRAS
5	Q92793	CREBBP
6	P42768	WAS
7	P31749	AKT1
8	P06213	INSR
9	P12821	ACE
10	P61769	B2M
11	P02766	TTR
12	P00492	HPRT1
13	P01009	SERPINA1
14	P10721	KIT
15	P04150	NR3C1
16	P98170	XIAP
17	O60674	JAK2
18	P00533	EGFR
19	P37231	PPARG
20	P00734	F2
21	P02753	RBP4
22	P43403	ZAP70
23	P00797	REN
24	P07359	GP1BA
25	P60568	IL2
26	P05019	IGF1
27	P08253	MMP2
28	P28482	MAPK1
29	P35221	CTNNA1
30	Q00987	MDM2
31	P60953	CDC42
32	P13726	F3
33	P29474	NOS3
34	P09871	C1S
35	P61626	LYZ
36	P14780	MMP9
37	P08473	MME
38	P42224	STAT1
39	P04035	HMGCR

more reliable.

A reverse molecular docking method was used to predict the action targets of active ingredients, and combined with the relevant database, 39 NS-related action targets were identified. Among them, matrix metalloproteinases (MMP) are a specific group of enzymes that degrade the extracellular matrix through zinc-dependent proteolysis. MMP-9 plays a key role in podocyte injury, and podocyte migration and Adriamycin-induced cell injury may be related to the upregulation of MMP-9 expression. Sai *et al.* (18) found that the expression levels of MMP-2 and MMP-9 were decreased in a doxorubicin-induced podocyte injury group, while the use of *Astragali Radix* in the intervention group inhibited this decrease and prevented podocyte injury.

The GO analysis showed that target genes were mainly involved in biological processes, such as signal transduction, the positive regulation of cell proliferation, and the positive regulation of migration. The cellular components included the plasma membrane, extracellular exosome, and extracellular space. The molecular functions included protein binding, zinc-ion binding, protein tyrosine kinase activity, and enzyme binding. This suggests that *Astragali Radix* may regulate the generation and proliferation of podocytes and inhibit the apoptosis induced by oxidative stress in the treatment of NS.

The regulation of the cell membrane may be related to the regulation of lipids. Podocyte injury is considered one of the most critical factors in the development of proteinuria caused by glomerular filtration barrier dysfunction, and continuous injury may lead to severe apoptosis (19). *Astragali Radix* water extract (20) improves albuminuria in rats with Adriamycin nephropathy by inhibiting oxidative stress and endothelium-type nitric oxide synthase. Clinical manifestations of NS include high cholesterol, hyperlipidemia, and hypoproteinemia, which has been found

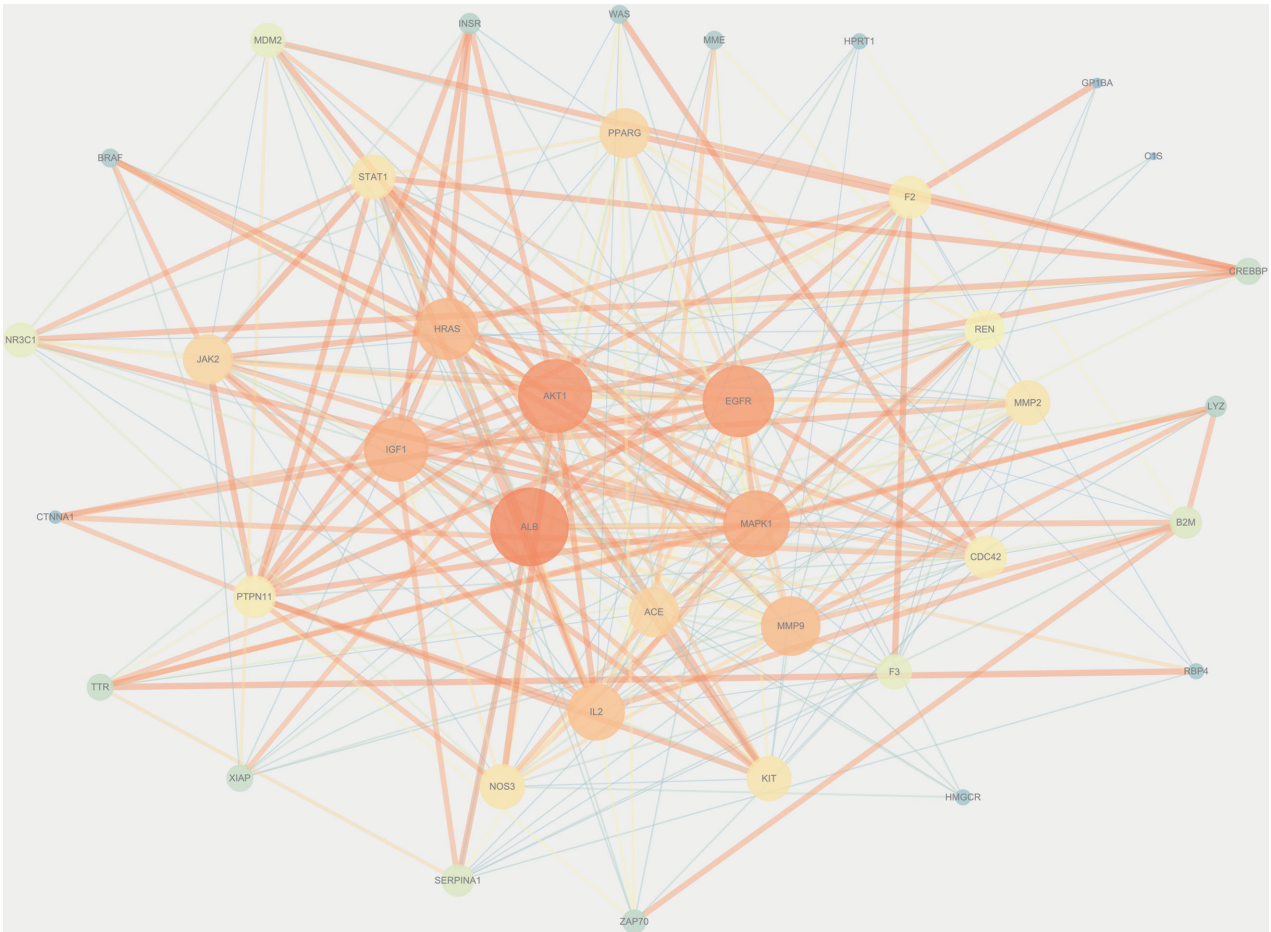

Figure 2 Interaction network of potential targets of *Astragali Radix* for treating nephrotic syndrome.

to be related to lipid metabolism disorders (21). Liu *et al.* (22) found that *Astragali Radix* regulates lipid metabolism and improves the ability of anti-lipid peroxidation. This is consistent with the results of the present study. Considering the role of *Astragali Radix* in pathways in cancer and in diabetic complications, the application of *Astragali Radix* in various cancers and diabetes is also worth researching in the future.

Conclusions

In the present study, we used the network pharmacology method to explore the potential targets and pathways of *Astragali Radix* in the treatment of NS, and provided future

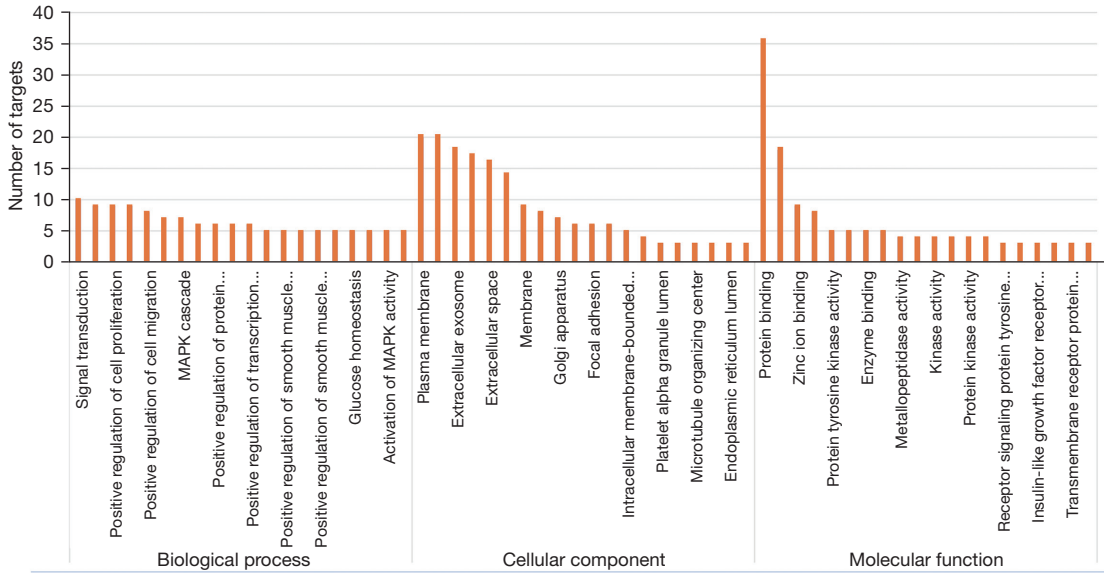

Figure 3 Biological function analysis of potential targets of *Astragali Radix* for treating nephrotic syndrome.

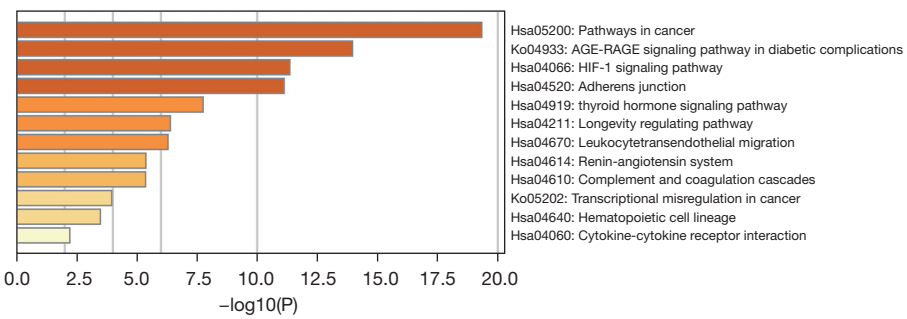

Figure 4 Enrich KEGG pathways analysis of potential targets of *Astragali Radix* for treating nephrotic syndrome.

research directions for the treatment of NS with a complex pathogenesis.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://dx.doi.org/10.21037/tp-21-348

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/tp-21-348). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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