The effect of *Astragali Radix-Radix Angelica Sinensis* on acute kidney injury: a network pharmacology and molecular docking study

Qin Wang[1,2], Wenrui Zhen[3], Giuseppe Lippi[4], Qi Liu[1,2]

[1]Suzhou Medical College of Soochow University, Suzhou, China; [2]Department of Nephrology, Affiliated Hospital of Zunyi Medical University, Zunyi, China; [3]Department of Intervention Therapy, The Second Affiliated Hospital of Zunyi Medical University, Zunyi, China; [4]Section of Clinical Biochemistry and School of Medicine, University of Verona, Verona, Italy

Contributions: (I) Conception and design: Q Wang, Q Liu, W Zhen; (II) Administrative support: Q Liu, W Zhen; (III) Provision of study materials or patients: Q Wang, Q Liu; (IV) Collection and assembly of data: Q Wang, W Zhen; (V) Data analysis and interpretation: Q Wang, Q Liu, W Zhen; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Qi Liu, MD. Suzhou Medical College of Soochow University, Suzhou, China; Department of Nephrology, Affiliated Hospital of Zunyi Medical University, 149 Dalian Rd., Zunyi 563000, China. Email: liuqi1964@hotmail.com.

Background: Acute kidney injury (AKI) is a devastating clinical syndrome with high mortality rate attributed to lack of effective treatment. The herbal pair of *Astragali Radix* (AR) and *Radix Angelica Sinensis* (RAS) is a commonly prescribed herbal formula or is added to other traditional Chinese medicine (TCM) prescriptions for the treatment of kidney diseases. AR-RAS has certain protective effects on AKI in experiments, but the relevant mechanisms have yet to be clear. So this study aims to explore the mechanism of action of AR-RAS in AKI by combining network pharmacology and molecular docking methods.

Methods: In Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), the major AR-RAS chemical components and associated action targets were found and screened. The DrugBank and GeneCards databases were used to find AKI-related targets. The targets that are in close relationship with AKI were obtained from Therapeutic Target database (TTD), Online Mendelian Inheritance in Man (OMIM), and PharmGKB databases. The "herb-active ingredient-target" network was drawn by Cytoscape 3.8.0 software. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database was used to build the protein-protein interaction network. Bioconductor/R was used to examine Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. AR-RAS components and critical targets were docked using the AutoDock Vina program.

Results: A compound-target network, built by screening and analyzing the results, allowed to identify 19 active components and 101 possible therapeutic targets for AKI. The main ingredients were quercetin, kaempferol, 7-o-methylisocronulatol, formononetin and isorhamnetin. The key targets included AKT serine/threonine kinase 1 (AKT1), nuclear receptor coactivator 1 (NCOA1), JUN, estrogen receptor alpha (ESR1) and mitogen-activated protein kinase 8 (MAPK8). These molecules are targeted by pathways such as the calcium signaling route, the tumor necrosis factor (TNF) signaling pathway and the interleukin-17 (IL-17) signaling pathway, as well as the development of T helper 17 cells. Molecular docking demonstrated that AR-active RAS components exhibited strong binding activities to probable targets of AKI.

Conclusions: We described here the potential active ingredients, possible targets responsible for the efficacy of AR-RAS in AKI treatment, providing a theoretical basis for further research.

Keywords: *Astragali Radix-Radix Angelica Sinensis* (AR-RAS); acute kidney injury (AKI); network pharmacology; molecular docking

Introduction

Acute kidney injury (AKI), which is also known as acute renal failure (ARF), is a common and serious condition caused by a variety of factors, such as nephrotoxic medications, ischemia, trauma and urinary tract blockage (1). It is an independent risk factor for death, with mortality rates of 25% overall (2). The pathogenesis of AKI involves vasoconstriction, oxidative stress, apoptosis, inflammation, and hypoxia (3-5). At present, except for intermittent hemodialysis and continuous renal replacement therapy, there are no effective drugs or treatment measures that can significantly contribute to AKI prevention. Therefore, new therapies with better efficacy and safety in treatment of AKI are urgently needed. In recent years, the research on drug treatment of AKI has subsequently intensified within this field.

Astragali Radix (AR)-*Radix Angelica Sinensis* (RAS) is made up of two specific herbs. AR can dilate blood vessels, reduce blood pressure, protect red blood cells, increase renal blood flow, and protect the kidney (6). Renowned for its active components, RAS has a wide range of pharmacological effects (7), including reducing serum creatinine concentration, increasing the activity of renal

ATPase, and reducing renal ischemia-reperfusion injury (8). The AR-RAS combination has been shown to reduce the level of serum creatinine and renal tissue injury caused by acute ischemia-reperfusion in rats (9). Modern clinical data have effectively confirmed that AR-RAS are safe and effective herbs, which has significant effects on AKI and can profoundly promote recovery of renal function (10,11). However, the molecular mechanism of AR-RAS has yet to be fully understood, and research concerning AR-RAS in AKI treatment is limited. Therefore, this study aimed to deduce the bioactive AR-RAS components and their AKI-linked activity.

In recent years, computer technology, network pharmacology, and systems biology have developed vigorously. Network pharmacology, based on theoretical system biology, is an emerging field (12). Through the integration of multidisciplinary technology and content, a disease-drug-target network can be constructed to investigate the relationship between illnesses and medicines, which is consistent with the overall syndrome differentiation theory of traditional Chinese medicine (TCM) (13). This tool can be used to provide deeper insight into the true potential of certain medicines (14,15).

Using network pharmacology and molecular docking technologies, we set out to identify the active ingredients and action targets of AR-RAS. We present this article in accordance with the STREGA reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-23-562/rc).

Highlight box

Key findings
- We investigated the active ingredients and action targets of *Astragali Radix* (AR)-*Radix Angelica Sinensis* (RAS).

What is known and what is new?
- AR can dilate blood vessels, reduce blood pressure, protect red blood cells, increase renal blood flow, and protect the kidney. RAS has a wide range of pharmacological effects, including reducing serum creatinine concentration, increasing the activity of renal ATPase, and reducing renal ischemia-reperfusion injury.
- This study used a network pharmacology approach to investigate the connection among multicomponents, multitargets, and the regulation of multiple signal pathways related to the effect of AR-RAS on acute kidney injury (AKI).

What is the implication, and what should change now?
- Our study not only provides important information for further understanding the interaction mechanism of active components, targets and disease, but provides also research ideas for treatment of AKI with traditional Chinese medicine. However, based on data analysis, this study only provides a theoretical reference for revealing the pharmacological mechanism, and thus further experiments *in vitro* and *in vivo* are still needed to verify our findings.

Methods

Screening active components of AR-RAS

The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com) was used to find the active chemicals of AR-RAS and their target proteins, and to discern the connections between illnesses, targets, and medicines (16). Oral bioavailability (OB) and drug-likeness (DL) of >30% and >0.18, respectively, were the screening criteria (17). Furthermore, in the UniProt (https://www.uniprot.org/) database, the appropriate targets of the aforementioned chemical components were identified, and the target gene annotation was completed by entering the species name "*Homo sapiens*".

AKI-related target screening

The AKI-related targets were gathered from the five well-

known disease databases: (I) GeneCards (18) (http://www.genecards.org); (II) Online Mendelian Inheritance in Man [OMIM (19); http://www.omim.org/ (III) PharmGkb (20) (https://www.pharmgkb.org/); (IV) TTD (21) (http://db.idrblab.net/ttd/; and (V) DrugBank (22) (https://www.drugbank.ca/). We used the term "acute renal damage" and the species name "*Homo sapiens*" to search the five databases. A Venn diagram was created using the associated genes of AKI.

Prediction of potential targets of AR-RAS in the treatment of AKI

Using the R programming language (The R Foundation of Statistical Computing) to match the annotated chemical targets to the summarized AKI illness targets, we identified the AR-RAS targets and the AKI targets that intersected on the Venn diagram, and these intersectional targets were deemed promising AR-RAS therapeutic targets in AKI.

Construction and analysis of a protein-protein interaction network

Using Cytoscape 3.8.0, the network diagram was created by selecting the points of intersection between AR-RAS and AKI. The common potential therapeutic targets in the Venn diagram were input into The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (23), which is capable of predicting interactions between proteins. Then, to determine the interaction connection between the objectives, the species name "*Homo sapiens*" was used. The TSV format file, which was downloaded from the STRING database, was imported into the Cytoscape 3.8.0 for analysis. This created a more complex protein interaction network diagram, where the node color and size were determined by the degree value, while the combined score determined the edge thickness.

Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment

GO is a global standard framework for categorizing gene activities. GO databases are classified into three types: cellular component (CC), molecular function (MF), and biological process (BP) (24). The KEGG is a collection of artificially created route maps that depict molecular interactions and reaction networks. In this process, P values of <0.01 were used with the GO keywords.

Molecular docking

Molecular docking, considered to be the key technology of structure-based drug discovery (25), facilitates the analysis of the binding site, molecular conformation, and macromolecular target affinity. First, the compound's 2-dimensional (2D) structure diagrams were collected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) and input into Chem3D software for constructing 3-dimensional (3D) structural diagrams of energy optimization, which were subsequently saved to mol2 format. Core target protein PDB data were acquired from the Protein Data Bank (PDB; http://www.rcsb.org/pdb/home/home.do) and entered into the PyMOL program to extract data on heteromolecules and water molecules. In order to manage the data set of the aforementioned protein receptors and ligands, AutoDock Tools 1.5.6 software was used, as it can routinely save data into a PDBQT file and scan the active pockets. AutoDock Vina was used for molecular docking and bioinformatics research. PyMOL software-based visualization analysis revealed the optimum affinity configuration. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Results

Active AR-RAS component screening

Review of TCMSP and the relevant literature yielded 87 AR components and 125 RAS components. A final number of 19 AR-RAS compounds with OB ≥30% and DL ≥0.18 were ultimately selected (*Table 1*).

A total of 7,197 AKI disease targets were gathered from the disease-related databases [see table online (available at https://cdn.amegroups.cn/static/public/tau-23-562-1.pdf)], including OMIM and GeneCards (see *Figure 1*). Through the comparison of AR-RAS with the targets linked to AKI, a final number of 101 genes were identified as possible AR-RAS targets in AKI treatment [see *Figure 2*; see table online (available at https://cdn.amegroups.cn/static/public/tau-23-562-2.pdf)].

Construction of the active component-target network and protein-protein interaction network

The 193 node and 430 edge-containing regulatory networks of "herb-active ingredient-target" were created in Cytoscape 3.8.0 (see *Figure 3*). The 17 purple circular

Table 1 Main chemical components of AR-RAS

Herb name	Mol ID	Molecule name	OB (%)	DL
Astragali Radix	MOL000211	Mairin	55.3770733787	0.7761
	MOL000239	Jaranol	50.8288167701	0.29148
	MOL000296	Hederagenin	36.9139058327	0.75072
	MOL000033	(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	36.2284705584	0.78288
	MOL000354	Isorhamnetin	49.604377053	0.306
	MOL000371	3,9-di-O-methylnissolin	53.7415267252	0.47573
	MOL000378	7-O-methylisomucronulatol	74.6861375238	0.29792
	MOL000379	9,10-dimethoxypterocarpan-3-O-β-D-glucoside	36.736688011	0.9243
	MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-Dihydro-6H-benzofurano(3,2-c)chromen-3-ol	64.2554545232	0.42486
	MOL000387	Bifendate	31.0978239059	0.66553
	MOL000392	Formononetin	69.6738806088	0.21202
	MOL000417	Calycosin	47.7518278266	0.24278
	MOL000422	Kaempferol	41.8822495352	0.24066
	MOL000433	FA	68.9604362164	0.7057
	MOL000439	Isomucronulatol-7,2'-di-O-glucosiole	49.281055391	0.62065
	MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.0454111203	0.47943
	MOL000098	Quercetin	46.4333481195	0.27525
Radix Angelica Sinensis	MOL000358	Beta-sitosterol	36.9139058327	0.75123
	MOL000449	Stigmasterol	43.8298515785	0.75665

AR-RAS, *Astragali Radix-Radix Angelica Sinensis*; OB, bioavailability; DL, drug-likeness.
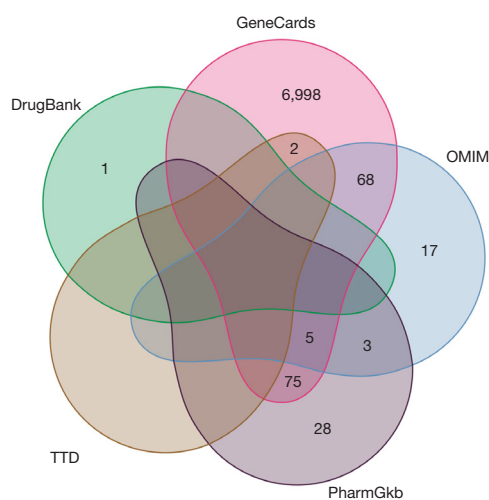

Figure 1 Venn diagram of the AKI disease targets. TTD, Therapeutic Target database; OMIM, Online Mendelian Inheritance in Man; AKI, acute kidney injury.

Figure 2 Venn of compounds' screening.

nodes indicate AR's active ingredients, whereas the two red circular nodes represent RAS's active ingredients. The target genes are represented by the 174 blue square nodes.

In order to investigate the interplay of core aims, we used the STRING database. The 101 AR-RAS and AKI
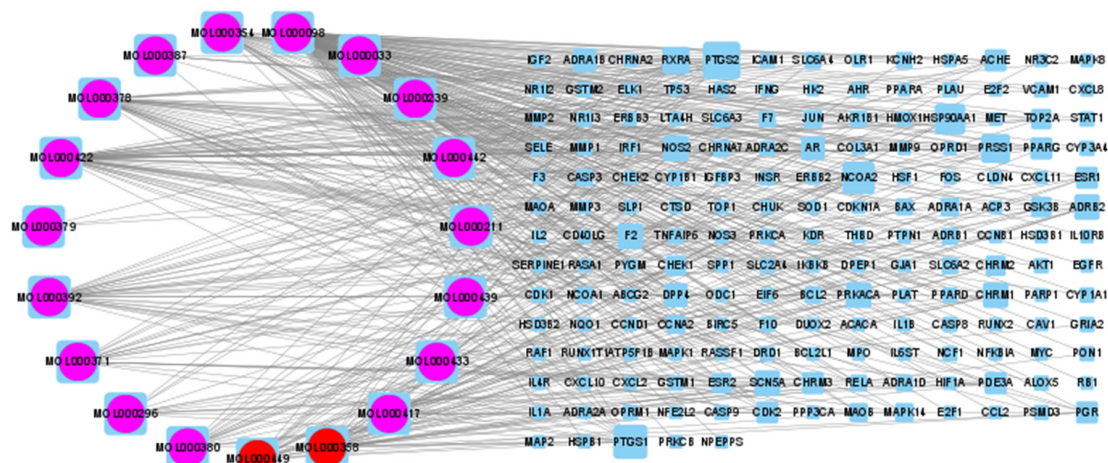

Figure 3 Interaction network of AR-RAS compound-AKI-targets. AR-RAS, *Astragali Radix-Radix Angelica Sinensis*; AKI, acute kidney injury.

common targets were used to construct protein interaction diagrams with STRING and Cytoscape 3.8.0, and the protein-protein interaction findings were produced. The network diagram, as illustrated in *Figure 4*, has 87 nodes and 195 interactive lines. In terms of degree value, the top 5 nodes in the network were *AKT* serine/threonine kinase 1 (*AKT1*) (17), nuclear receptor coactivator 1 (NCOA1) (14), JUN (13), estrogen receptor alpha (ESR1) (13), and mitogen-activated protein kinase 8 (MAPK8) (12). These nodes are the potential primary targets of AR-RAS; the bigger the node's degree value is, the larger the target's role in the network (see *Figure 4*).

GO function and KEGG pathway enrichment analysis

AR-RAS targets were imported into The Database for Annotation, Visualization and Integrated Discovery (DAVID) for GO analysis and KEGG pathway analysis for further investigation. A threshold (P<0.05) was used to filter the findings. The GO functional enrichment analysis produced 1,642 GO terms (P<0.05), which included 1,418 BP terms, 74 CC terms, and 150 MF terms. *Figure 5* depicts the top 10 most substantially enriched GO terms. In regard to blood pressure, the targets were mostly connected to the cellular response to medication, the vascular process in the circulatory system, xenobiotic stimulus, antibiotics, steroid hormones, and lipopolysaccharides. For CC, the majority of the targets were involved with the integral component of the presynaptic membrane, along with the membrane's microdomains, regions, plasma membrane

rafts, and other rafts. Meanwhile, the most common MF targets were adrenergic receptor activity, nuclear receptor activity, G protein-coupled amine receptor activity, direct ligand regulated sequence-specific DNA binding steroid hormone receptor activity, and transcription factor activity (see *Figure 5*).

KEGG pathway enrichment yielded a total of 159 enriched pathways, the top 30 of which are displayed in *Figure 6*. The name of the route is shown on the Y-axis, while the fraction of genes is shown on the X-axis. The size of the bubble indicates the number of genes concentrated in the changed route in the bubble diagram, and the color of the point represents the enrichment's importance. The enriched pathways mainly included the interleukin-17 (IL-17) signaling pathway, tumor necrosis factor (TNF) signaling pathway, T helper 17 (Th17) cell differentiation, calcium signaling pathway, chemical carcinogenesis-receptor activation, and lipid and atherosclerosis (see *Figure 6*).

Molecular docking verification

In the protein-protein interaction analysis, the greater the degree value a node has, the greater its importance in the whole network. AKT1 and NCOA1 had higher degree values in the protein-protein interaction network and were thus considered the main protein receptors in this investigation.

In general, affinity <–7.00 kcal/mol indicates adequate binding strength, affinity <–5.00 kcal/mol indicates good binding strength, whilst affinity <–4.25 kcal/mol indicates the

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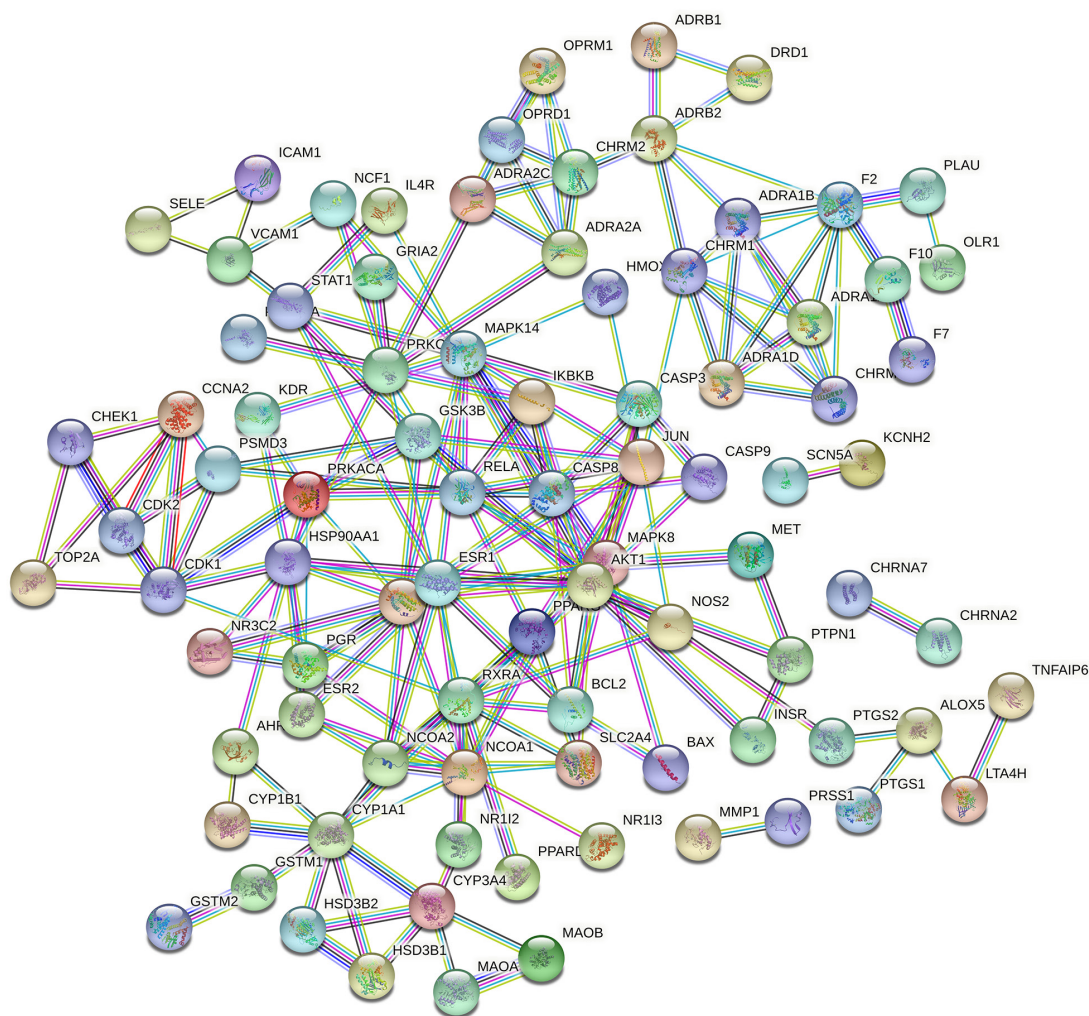

Figure 4 Protein-protein interaction network.

likelihood of ligand-receptor interaction, respectively (24-26).

Stigmasterol and beta-sitosterol showed the highest binding affinity for AKT1, with affinities of –11.1 and –10.1 kcal/mol, respectively. Kaempferol and quercetin showed the highest binding for NCOA1, with affinities of –8.2 kcal/mol. These findings suggest that the key active components of AR-RAS have a high affinity for the primary targets (see *Figure 7* and *Table 2*).

Discussion

TCM contains numerous components, multiple targets outcomes with a synergistic impact, and has long been used to treat a variety of illnesses. Network pharmacology, as an essential pharmacological research approach, can

better illustrate the connection between components, drugs and targets. TCM network pharmacology offers a fresh approach for discovering active herbal components for certain illnesses or pathological processes. In addition, molecular docking is based on network pharmacological prediction and analysis. High-precision docking simulation and molecular pathway diagrams enable to show the selectivity of ligands, clarify the relationship between ligands and molecular networks, and evaluate the binding potential of protein ligands (27).

The approach of network pharmacology was used in this study for clarifying the pharmacological mechanism of AR-RAS in AKI treatment. TCMSP was examined for 19 active compounds with OB ≥30% and DL ≥0.18. There were 101 possible therapeutic targets found, and 6 main targets.
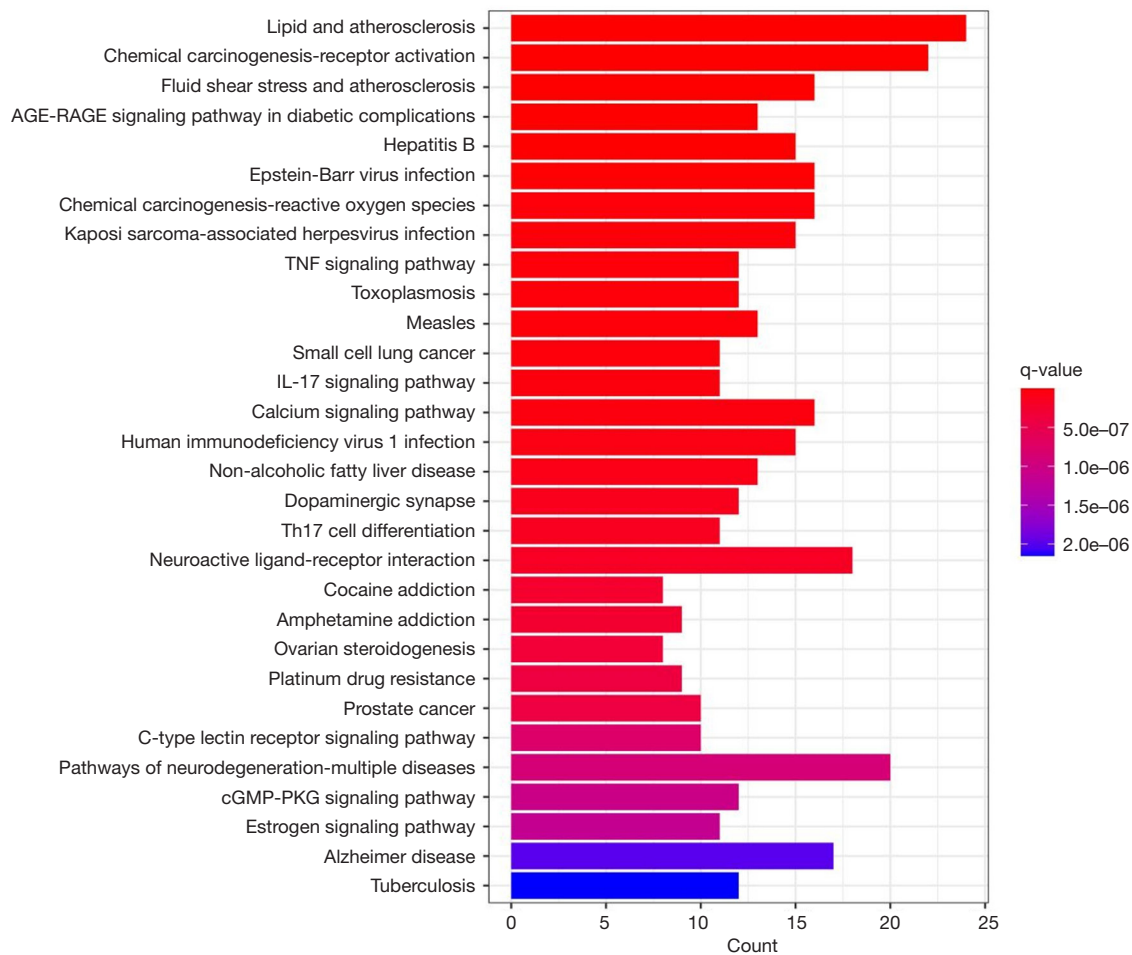
Figure 5 GO function analysis. AGE, advanced glycation end products; RAGE, receptor for AGE; TNF, tumor necrosis factor; IL-17, interleukin 17; Th17, T helper 17; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; GO, Gene Ontology.

Additionally, 1,642 enriched GO functional items and 160 KEGG pathways relevant to AKI illness were identified, showing that AR-RAS contained multiple components, multiple targets, and multiple pathways in treatment of AKI. Moreover, 19 components of AR-RAS were identified that had potential effects on AKI, including, among others, quercetin, kaempferol, 7-O-methylisomucronulatol, formononetin, and isorhamnetin.

Quercetin, a natural flavonoid present in many fruits and vegetables, exhibits a wide range of pharmacological properties such as anticancer effects (e.g., antioxidant, anti-inflammatory and antisenescence effects) along with antiviral activity (28-30). Quercetin can be used for treating AKI by inhibiting ferroptosis of renal tubular epithelial cells (31). Kaempferol has anticancer, anti-inflammatory, antioxidant, antibacterial, and antiviral properties. It can

be used in the cure of different diseases, such as diabetes and osteoporosis (32-35). An interesting study has recently found that kaempferol can attenuate calcium oxalate crystal-induced renal injury through repressing oxidative stress and inflammation in the kidney (36). Formononetin is a natural and bioactive isoflavone isolated from herbal medicines (37), displaying various potential pharmacological and biological properties, such as anti-inflammatory (38), antioxidative (39), and anti-apoptotic effects (40). One study found that formononetin can protect cisplatin-induced AKI rats and cells by inhibiting oxidative stress, apoptosis, and inflammatory pathways (41). Isorhamnetin, a flavonoid, has potent anti-inflammatory (42), antioxidant (43,44), and ameliorative effects on diabetes-induced renal damage (45).

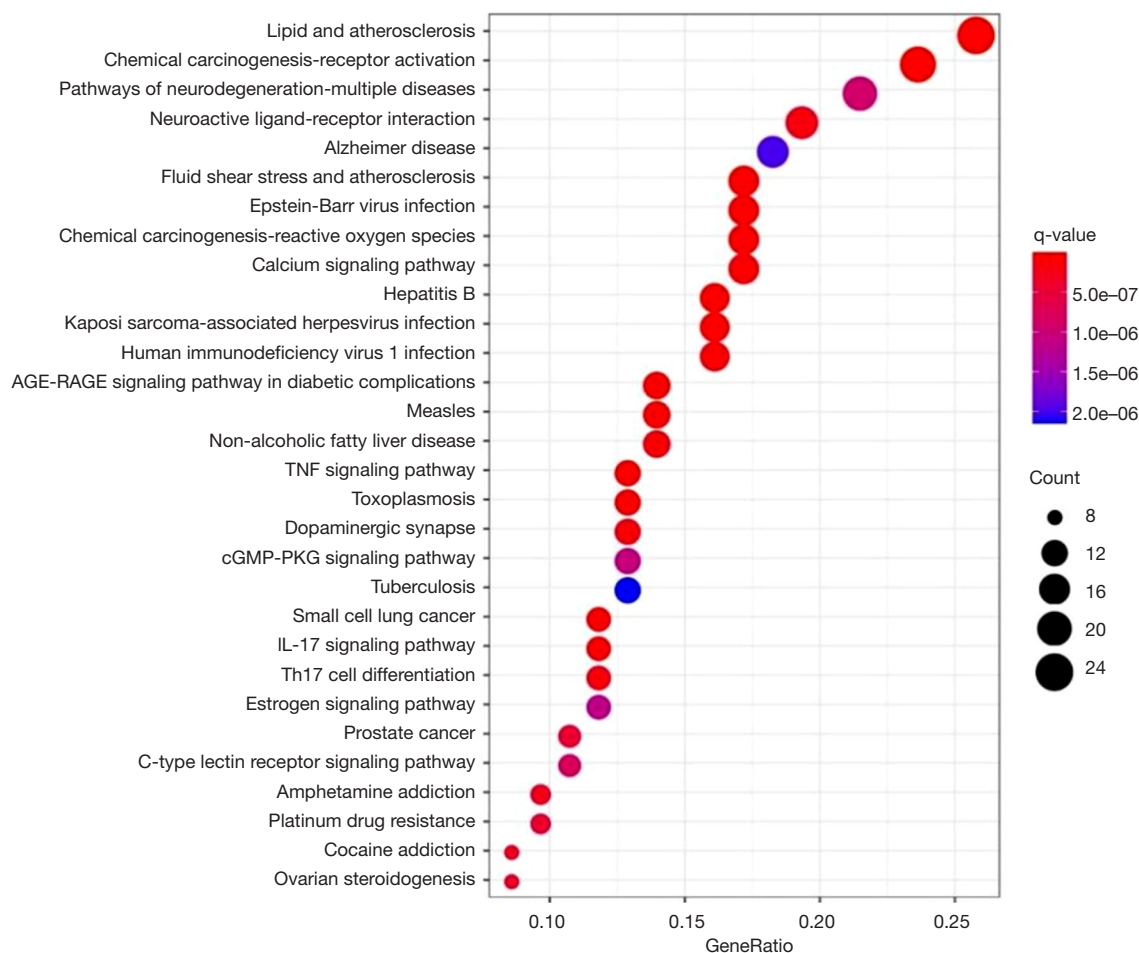AR's multicomponent and RAS's and multitarget properties were represented in a component-target

Figure 6 KEGG pathway analysis. AGE, advanced glycation end products; RAGE, receptor for AGE; TNF, tumor necrosis factor; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; IL-17, interleukin 17; Th17, T helper 17; KEGG, Kyoto Encyclopedia of Genes and Genomes.

network. The protein-protein interaction network showed an interaction between the targets of AR-RAS. AKT1 (17), NCOA1 (14), JUN (13), ESR1 (13), and MAPK8 (12) were the five nodes with the highest degree value. We speculated that these potential core target genes might play a key role in the development of AKI. The protein-protein network, which shows protein-protein interaction, was created to investigate essential targets in AR-RAS for AKI, indicating that MAPK8, ESR1, JUN, NCOA1, AKT1, particularly JUN NCOA1, and AKT1 might be the core targets in AKI.

Akt/protein kinase B (PKB) is a serine/threonine kinase that can be involved in tubular apoptosis and inflammatory response during renal ischemia-reperfusion injury (46,47). The transcription factor hypoxia-inducible factor-1α (HIF-1α) (48) has been associated to glomerulosclerosis and

renal interstitial fibrosis (49). NCOAs include coactivators and corepressors and significantly boost nuclear receptor transcriptional activity. A study has found that NCOA1, NCOA2, and NCOA3 are associated with HIF-1α in chronic kidney disease (50). The gene proto-oncogene JUN (c-Jun) is essential to the inflammatory response. When exposed to external stimuli, cellular c-Jun expression increases substantially in cells, enabling their ready adaptation to changes in their environment (51-54). These findings complement our previous findings regarding the significance of AKT1, NCOA1, and JUN in AR-RAS for AKI.

Through GO function enrichment and KEGG pathway analysis, we observed that the AR-RAS targets in AKI treatment mostly concentrated on the Th17 cell
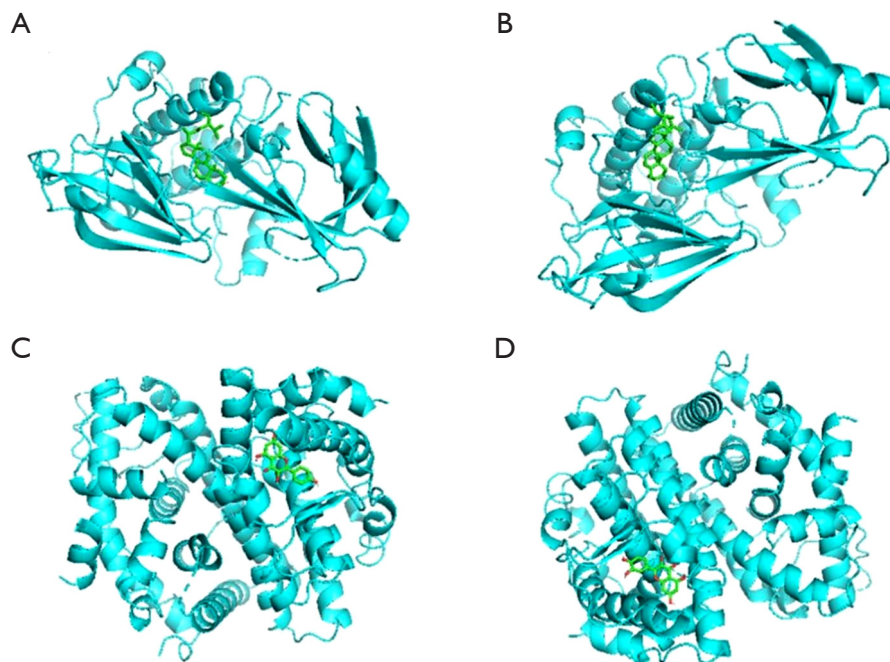
Figure 7 Molecular docking. Molecular models of the respective binding of stigmasterol, beta-sitosterol, kaempferol, and quercetin with AKT1 (A,B) and NCOA1 (C,D).

Table 2 Molecular docking scores of major active compound-main target molecular docking (unit: kcal/mol)

Molecule name	Target name	
	AKT1 (5KCV)	NCOA1 (4TV1)
Jaranol	−9.2	−6.9
Hederagenin	−8.8	−7.2
Isorhamnetin	−9.5	−7.5
3,9-di-O-methylnissolin	−9.3	−6.8
7-O-methylisomucronulatol	−8.7	−6.7
Formononetin	−9.7	−7.5
Calycosin	−9.9	−7
Kaempferol	−9.5	−8.2
Quercetin	−9.8	−8.2
Beta-sitosterol	−10.1	−7.2
Stigmasterol	−11.1	−7.4

differentiation IL-17 signaling pathway, TNF signaling pathway and calcium signaling pathway.

The calcium signaling pathway is involved in nearly every physiological function of the normal cell cycle, including cell growth, proliferation, and differentiation (55). As calcium signaling is important for AKI, this process could be manipulated therapeutically to improve AKI (56). Moreover, IL-17 can promote renal tissue injury in crescentic glomerulonephritis (57), and IL-17 secretion participates in the pathogenesis of AKI-induced fibrosis, possibly via the recruitment of neutrophils (58). In addition, IL-17 signaling pathway is involved in the innate immune inflammation associated with kidney ischemia-reperfusion injury (59). Th17 cells were shown to be important mediators of renal injury associated with autoimmune-mediated nephritis induced via an experiment which injected sheep serum into mice (60). Th17 cells can induce an overall inflammatory response that leads to the recruitment of effectors cells or that precipitates vascular congestion and hypoxia, resulting in renal injury (61). Tissue fibrosis, AKI-to-chronic kidney disease, and ischemia-reperfusion-induced AKI are exacerbated by a strong Th17 inflammatory response (62). Taken together, the above results showed that the core components and key targets of AR-RAS play an important role in treatment of AKI.

The most frequently used method for assessing component-target interactions is molecular docking. From our docking results, it was found that the AKT1-stigmasterol, AKT1-beta-

sitosterol, AKT1-calycosin, NCOA1-kaempferol, NCOA1-quercetin, and NCOA1-stigmasterol pairs had the lower binding energies. From these molecular docking findings, we clarified the effect of AR-RAS in the treatment of AKI. The molecular docking and network pharmacology results were consistent with each other, demonstrating network pharmacology's dependability in target prediction.

Finally, this study used the network pharmacology approach to investigate the connection among multicomponents, multitargets, the regulation of multiple signal pathways, and AKI in AR-RAS. Our results can aid in clarifying the active component-target-disease interaction mechanism and informing further research into the treatment of AKI with TCM. However, as this study mainly used data analysis, we have only provided a theoretical reference for revealing the pharmacological mechanism, and thus further experiments *in vitro* and *in vivo* with these compounds would be needed to verify our findings.

Conclusions

In this study, network pharmacology identified 19 main active compounds in AR-RAS and 101 genes targeted by AKI. Through integrating network pharmacology, five core targets (AKT1, NCOA1, JUN, ESR1, and MAPK8) from complex networks were predicted to be the key targets for AR-RAS in AKI treatment. These results clarify the potential effective mechanism of AR-RAS in the treatment of AKI at multiple targets and pathways, and provide a basis for understanding the molecular mechanism of AR-RAS in the treatment of AKI.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-23-562/rc

Peer Review File: Available at https://tau.amegroups.com/article/view/10.21037/tau-23-562/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-23-562/coif). 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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