Understanding the molecular mechanism of the effect of ginkgo folium on the treatment of IgA nephropathy using network pharmacology and molecular docking

Xue Ru, Yi Zhang, Yan Gao and Huaikun Wang

ABSTRACT
This work focused on identifying the molecular target of Ginkgo Folium (GF) for treating IgA nephropathy and underlying mechanism through network pharmacology (NP). The active components and targets of GF and targets associated with IgAN were obtained by TCMSP database, DrugBank etc. The key targets of GF against IgAN were searched by network topology. The drug-disease intersection targets were performed GO functional annotation as well as KEGG pathway analysis, and molecular docking (MD) was conducted to verify the degree of combination of the target and ligand. Three core compounds and seven key targets were found by topological analysis. GO and KEGG results suggested that GF effect on IgAN was strongly associated with OS cellular response and AGE-RAGE pathways. Molecular docking of the three core components with AKT1 indicated that they had good binding activity. Ginkgo biloba had multicomponent, multitarget, and multi-pathway effects on IgAN.

ARTICLE HISTORY
Received 16 August 2021
Accepted 2 December 2021

KEYWORDS
network pharmacology; IgA nephropathy; traditional Chinese medicine; molecular docking

CONTACT Xue Ru, E-mail: 421584275@qq.com

Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2021.2018433.
1. Introduction

IgA nephropathy (IgAN) is generally featured by IgA deposition within glomerular mesangium (Álvarez and Sánchez). It is a global medical challenge due to high incidence, complicated and incompletely understood pathogenesis, and the absence of practical treatment methods (Li et al. 2018). Traditional Chinese herbal medicine from *Ginkgo Folium* (GF) has been utilized in China for a range of chronic diseases, including asthma, bronchitis, and heart dysfunction, for at least 5,000 years (Yeh et al. 2009; Brondino et al. 2013). Also, GF is suggested to show pharmacological activity for chronic kidney diseases (CKD) by improving the endothelium-dependent vascular dilation, lowering blood lipid levels and proteins in the urine, and preventing renal fibrosis (Chen et al. 2020; Hz et al. 2020). However, the pharmacological basis and therapeutic mechanism by which GF treats IgAN is not completely clear. Thus, a comprehensive and systematic evaluation of the molecular mechanism of the effect of GF on IgAN is indispensable. Network pharmacology can be used to explore the physical basis of drug treatment of diseases and the underlying molecular mechanisms, based on the theory and network analysis of biological systems (Ming et al. 2013). This work focused on determining the pharmacological mechanism by which the GF effective components managed IgAN progression through bioinformatics and NP analyses. Findings in this work provide certain foundation for better exploring the “multicomponent, multitarget, multi-function, and multi-channel” pharmacological mechanism of GF in treating IgAN.
2. Results and discussion

2.1. Active compounds and potential targets of ginkgo folium

In the TCMSP database, the active chemical components of GF had 27 total active compounds (Table S1). Meanwhile, a total of 204 predicted targets with activity fractions were collected after deleting duplicates.

2.2. IgAN-Related targets

The keyword "IgA Nephropathy" was used to obtain 1020 relevant targets from the GeneCards database with a relevance score of ≥ 1. The targets associated with IgAN were further identified by the OMIM, PharmGKB, and the DrugBank databases. At last, altogether 1,119 targets associated with IgAN treatment were acquired after merging and removing duplicates (Fig S1).

2.3. Network construction for protein interaction

The core active target components of GF were matched with the disease targets of IgAN, and 86 composite targets of GF and IgAN were selected (Fig S2). A component-target network was built to visualize the associations of GF components with candidate targets (Fig S3). We found that the key compounds of GF in the treatment of IgAN included quercetin, luteolin, and kaempferol. Quercetin regulates oxidative stress and the iNOS/p38 MAPK pathway and can significantly reduce glomerular and renal interstitial parenchymal cell damage (Chang et al. 2017). Reports suggest that luteolin can remove ROS, provide feedback inhibition in the NF-κB pathway, protect the kidney mesangial cells, and delay the progress of renal function (Park et al. 2012). Kaempferol has an inhibitory effect on oxidative stress and apoptosis of advanced glycation end products and can induce mesangial cells (Jiang et al. 2018). In Fig S4, three sub-networks were identified. Finally, seven critical nodes, or "key targets", were further screened according to two topological analyses, including TP53, MYC, AKT1, MAPK1, JUN, FOS, and CCND1; these seven targets might potentially be important targets of GF for the treatment of IgAN and require further investigation.

2.4. Go and KEGG enrichment of related targets

Altogether 86 critical targets of GF for treating IgAN were subjected to GO and KEGG pathway enrichment analysis. A total of 2,240 GO enrichment analysis items were obtained, including 2,064 BP, 39 CC, and 137 MF. A histogram was plotted for visual representation by taking the first 10 items of each enrichment process (Fig S5). The biological processes mainly focused on cellular response to chemical stress, OS cellular response, and ROS metabolic processes. This indirectly demonstrated the complexity of the pathogenesis of IgAN, and GF might be able to regulate these biological processes, and thus, help in the treatment of IgAN.

A total of 167 KEGG pathways were obtained, and bubble diagrams of the top 30 significantly enriched pathways were determined for visual analysis (Fig S6). Among
them, three main signaling pathways were identified, which included AGE-RAGE, IL-17, TNF signal transduction pathways. Tubular aging and renal tubular damage have been suggested to be related to activation of receptor for AGE (RAGE)-AGE pathway. On the other hand, RAGE and the corresponding ligands are involved in inducing OS and chronic renal inflammation, leading to a loss of kidney function (Inagi 2016). IL-17 can inhibit myofibroblast transformation and expression of α-SMA, fibronectin, and type 1 collagen induced by TGF-β through the inhibition of the phosphorylation of the non-classical pro-fibrosis signaling molecules, AKT and p38 MAPK, thereby inhibiting renal fibrosis (Sun et al. 2018). TNF-α is a key target of the TNF signaling pathway. In IgAN, TNF-α originating from glomerular mesangium represents a key mediator related to the degradation of glomerular basilar membrane components as well as glomerulotubular communication during interstitial injury. In the case of nephropathy, infiltrating macrophages can enhance the apoptosis of podocytes through the TNF-α-mediated p38 MAPK pathway (Lai et al. 2008). These signaling pathways are directly or indirectly associated with inflammatory response, immune response, apoptosis, and oxidative stress, and ultimately affect the outcome of IgAN. The KEGG signaling pathway enrichment results were incorporated to Cytoscape 3.7.0 for obtaining KEGG enrichment pathway network diagram of GF targets in IgAN treatment (Fig S7). Among them, AKT1 was the gene related to most pathways (degree = 17), suggested that AKT1 was the most important target for the treatment of IgAN using GF.

2.5. Molecular Docking

The top three key active compounds, kaempferol, luteolin, and quercetin, were molecularly docked with seven key targets, MYC, FOS, JUN, CCND1, AKT1, TP53, and MAPK1 (Table S2). It was confirmed that the core active components of GF had a high binding activity with the key targets, among which binding with AKT1 was the strongest. More details about MD local structures are presented in Fig S8. Thus, it was speculated that AKT1 might be an essential target of GF in the treatment of IgAN, which is consistent with the result above. The activation of AKT causes the polymeric IgA (PIgA) to bind to the FcαRI receptor on monocytes, forming a circulating complex, which is deposited in the mesangial region of the glomerulus to form IgAN (Lai et al. 2011).

3. Conclusion

In summary, through NP and MD, we confirmed that our results were reliable and consistent with previously reported results; besides, GF showed remarkable efficacy in treating IgAN, with multicomponent, multitarget, and multi-pathway effects. This study preliminarily verified the pharmacological mechanism of the effects of GF in treating IgAN and provided a foundation for follow-up experiments. However, subsequent experiments in vitro and in vivo should be conducted to confirm relevant targets and determine whether these targets are involved in IgAN. This study can be considered an initial step for further experiments, as well as the treatment of IgAN.
Disclosure statement
No potential competing interest was reported by the authors.

Funding
This research was supported by Special Science Research Fundation of Sichuan Administration of Traditional Chinese Medicine of China (NO.2021MS373).

Abbreviations
MD  molecular docking  
NP  network pharmacology  
IgAN  IgA nephropathy  
PPI  protein-protein interaction  
OS  oxidative stress  
ROS  reactive oxygen species  
TCM  Traditional Chinese medicine  
GF  Ginkgo Folium  
CKD  chronic kidney diseases  
TCMSP  the traditional Chinese medicine system pharmacology database  
DL  drug-likeness  
OB  oral bioavailability  
OMIM  the Online Mendelian Inheritance in Man  
PharmGKB  the Pharmacogenomics Knowledgebase  
BC  Betweenness Centrality  
CC  Closeness Centrality  
DC  Degree Centrality  
EC  Eigenvector Centrality  
LAC  Local Average connectivity  
NC  Network Centrality  
GO  Gene Ontology  
KEGG  Kyoto Encyclopedia of Genes and Genomes  
BP  biological process  
CC  cellular component  
MF  molecular function

ORCID
Xue Ru  http://orcid.org/0000-0002-2201-7853

References
Brondino N, Silvestri AD, Re S, Lanati N, Thiemann P, Verna A, Emanuele E, Politi P. 2013. A Systematic Review and Meta-Analysis of Ginkgo biloba in Neuropsychiatric Disorders: From Ancient Tradition to Modern-Day Medicine. Evid Based Complement Alternat Med. 2013:915691.
Chang XY, Lei C, Wang XZ, Zhang L, Zhu D, Zhou XR, Hao LR. 2017. Quercetin Attenuates Vascular Calcification through Suppressed Oxidative Stress in Adenine-Induced Chronic Renal Failure Rats. BioMed Res Int. 2017:1–7.
Chen H, Deng Q, Wang W, Tao H, Gao Y. 2020. Identification of an autophagy-related gene signature for survival prediction in patients with cervical cancer. J Ovarian Res. 13(1):131.
Hz A, Kh B, Dg A, Tc A, Ry A, Lh A, Xc A, Zj A, Yan WA, Yg A. 2020. Inhibitory effect of Ginkgo biloba extract on vascular calcification in rats with chronic kidney disease by ROS-NF-κB signaling pathway. J King Saud Univ - Sci. 32(2):1306–1311.

Inagi R. 2016. RAGE and glyoxalase in kidney disease. Glycoconjugate J. 33(4):1–8.

Jiang W, Wang R, Liu D, Zuo M, Zhao C, Zhang T, Li W. 2018. Protective Effects of Kaempferitrin on Advanced Glycation End Products Induce Mesangial Cell Apoptosis and Oxidative Stress. IJMS. 19(11):3334.

Lai K, Leung J, Chan L, Saleem M, Mathieson P, Lai F, Tang S. 2008. Activation of podocytes by mesangial-derived TNF-alpha: glomerulo-podocytic communication in IgA nephropathy. Am J Physiol Renal Physiol. 294(4):F945–F955.

Lai K, Tang S, Leung J. 2011. Recent advances in IgA nephropathy—the glomerulopodocytic-tubular communication. Adv Oto-Rhino-Laryngol. 72(72):40–44.

Li Z, Gao L. 2018. Pathogenesis and Clinical Treatment Progress of IgA Nephropathy. Chin Foreign Med Res. 16(11):191–194.

Ming Y, Chen JL, Xu LW, Ji G. 2013. Navigating Traditional Chinese Medicine Network Pharmacology and Computational Tools. Evid-Based Compl Alt Med. 2013(12):731969.

Park CM, Jin K-S, Cho CW, Lee Y-W, Huh G-H, Cha Y-S, Song YS. 2012. Luteolin inhibits inflammatory responses by downregulating the JNK, NF-κB, and AP-1 pathways in TNF-α activated HepG2 cells. Food Sci Biotechnol. 21(1):279–283.

Sun B, Wang H, Zhang L, Yang X, Zhang M, Zhu X, Ji X, Wang H. 2018. Role of interleukin 17 in TGF-β signaling-mediated renal interstitial fibrosis. Cytokine. 106:80–88.

Yeh Y-C, Liu T-J, Wang L-C, Lee H-W, Ting C-T, Lee W-L, Hung C-J, Wang K-Y, Lai H-C, Lai H-C. 2009. A standardized extract of Ginkgo biloba suppresses doxorubicin-induced oxidative stress and p53-mediated mitochondrial apoptosis in rat testes. Brit J Pharmacol. 156(1):48–61.