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ORIGINAL ARTICLE

Targets of total glucosides of paeony in the treatment of Sjogren syndrome: A network pharmacology study

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Abstract

Background: We aimed to explore the underlying mechanism of the total glucoside of peony (TGP) in treating Sjogren syndrome (SS) using the network pharmacology approach.

Methods: The protein targets of TGP and SS were identified by database search. Then, the intersection of the two groups was studied. The drug–target network between TGP and the overlapping genes was constructed, visualized, and analyzed by Cytoscape software. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment were performed to analyze these genes. Finally, the predictions of potential targets were evaluated by docking study.

Results: Forty-six overlapping genes were discovered. The results suggested that TGP used in the treatment of SS is associated with cellular tumor antigen p53, neurotrophic tyrosine kinase receptor type 1, and epidermal growth factor receptor, as well as their related 3372 protein networks, which regulate intrinsic apoptotic signaling pathway, cellular response to oxidative stress, rhythmic process, and other processes. Molecular docking analysis proved that hydrogen bonding is the main form of interaction.

Conclusion: Our research provided the protein targets affected by TGP in SS treatment. The key targets (caspase 3, vascular endothelial growth factor A, glyceraldehyde-3-phosphate dehydrogenase, etc.), which involve 3372 proteins, are the multitarget mechanism of TGP in SS treatment.

Keywords: CASP3; Network pharmacology; Sjogren syndrome; Total glucosides of peony

1. INTRODUCTION

Sjogren syndrome (SS) is a systemic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands.[1] Recent epidemiological studies reported that SS is the second most common rheumatic disease in China with a mean annual incidence rate of 7.7 cases per 100,000 adults.[2] The classical clinical manifestations of SS are dry mouth and dry eyes, as well as high levels of the ribonucleoproteins, Ro/SSA, and/or La/SSB.[3] Current therapeutic regimens are focused on symptom relief and broad-spectrum immunosuppression. Recent advancements in medicine and pharmacology have led to the development of more comprehensive, multistage SS therapies, and alternative medicine has become the patient's choice. Traditional Chinese medicine (TCM) is the most frequently used alternative medicine in the prevention and control of SS in China because of its good therapeutic effect and low toxic side effect.

Total glucoside of peony (TGP) is extracted from Radix Paeoniae Alba, which is considered an effective TCM for several diseases. TGP consists of several active ingredients, including paeoniflorin, albiflorin, and benzoylpaeoniflorin; among which, paeoniflorin accounts for more than 90%.[4,5] TGP was approved as a disease-modifying oral drug for rheumatoid arthritis (RA) in 1998 by the Chinese Food and Drug Administration and has been widely used for the treatment of systemic lupus erythematosus and SS.[4,6] Previous studies demonstrated that TGP affects Th1/Th2 cytokine balance, decreases the expression levels of numerous cytokines, and reduces the pathological damage of submandibular glands.[7–9] Therefore, TGP has been a novel therapeutic agent for SS treatment in this modern age. Although researches on SS therapy with TGP have continued, a systematic and comprehensive understanding of the relationships between the targets and pathways involved in SS treatment is still limited.

The rapid progress of bioinformatics, systematic biology, and polypharmacology has facilitated the development of network-based pharmacology as a novel and promising drug development approach. It introduces a paradigm shift from the current "one research-based target, one drug" strategy to a novel version of the "network multitarget" strategy.[10,11] Network-based pharmacology has been universally applied in many drug discoveries because of its holistic and efficient characteristics for the systematic study of the interrelationship among drugs, targets, pathways, and diseases. The holistic theory of network pharmacology is also shared by TCM and has long been central to TCM treatments.[10–12] Therefore, it has become an increasingly valuable technology for the exploration of TCM-related issues.

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In this study, network pharmacology was used to elucidate the underlying mechanism of TGP in SS treatment. First, the potential molecular targets of TGP were predicted. Then, the intersection of these targets with SS-related proteins was analyzed. Furthermore, a protein–protein interaction (PPI) network was constructed to enlarge the number of proteins that are closely related to the mutual genes. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment were performed. Finally, docking studies were conducted to verify the chemical force that allowed TGP to bind to their predicted targets and drew the possible binding sites.

2. METHODS

2.1. Predicted target proteins of TGP

The chemical structures (Simplified Molecular Input Line Entry System) of the active ingredients in TGP were searched in PubChem and subjected to target prediction in different databases (SwissTargetPrediction and PharmMapper) according to the results of chemical structures.[13,14] The species was limited to "*Homo sapiens*."

2.2. Collection of SS-related genes

SS-related target genes were identified by searching public databases (GeneCards, http://www.genecards.org/) using the keywords, "Sjogren syndrome" and "*Homo sapiens*."

2.3. Identification of overlapping genes

The overlapping genes between the targets of TGP and SS were identified and visualized by Venn diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/). The symbols of the overlapping genes and the names of compounds were uploaded into the Cytoscape software (3.8.0).[15] A network was constructed to show the relationship between the compounds and targeted genes.

2.4. Screening, GO enrichment, and KEGG analysis of pivotal target proteins

The plug-in "Bisogenet" in Cytoscape (3.8.0) software was used to construct the PPI network of the mutual targets between TGP and SS. Pivotal targets were screened according to seven key parameters, namely, closeness, eccentricity, radiality, bottleneck, stress, betweenness, and edge percolated component.[15]

R packages (clusterProfile and ggplot2) were used to perform the GO enrichment and KEGG pathway analysis.[16] The clusterProfile and ggplot2 packages were applied to analyze and visualize the results, respectively.

2.5. PPI network analysis and hub gene identification

A PPI network for the overlapping genes was constructed by STRING database (http://www.string-db.org/) to further investigate the hub genes in SS treatment by TGP (cutoff standard: combined score > 0.4). Then, the Cytoscape software was used to visualize the result. Cytohubba, a Cytoscape plug-in, was employed to study the essential nodes in the network. The nodes with high degrees of interaction were considered hub genes.[17]

2.6. Molecular docking

The 3D crystal structures of the potential target proteins of TGP and the chemical structures of TGP were searched from the RCSB Protein Data Bank (PDB, http://www1.rcsb.org/). The AutoDock 4.2 software was used to modify the structure and perform molecular docking.[18] The binding energy and binding sites calculated by AutoDock were recorded, and the predicted models were saved in PDB format. The PyMOL 3.6 software was used to visualize the models.

3. RESULTS

3.1. Predicted targets of TGP and their network

The potential targets of TGP were predicted by database search according to the 2D and 3D chemical structures of TGP (Fig. 1A).

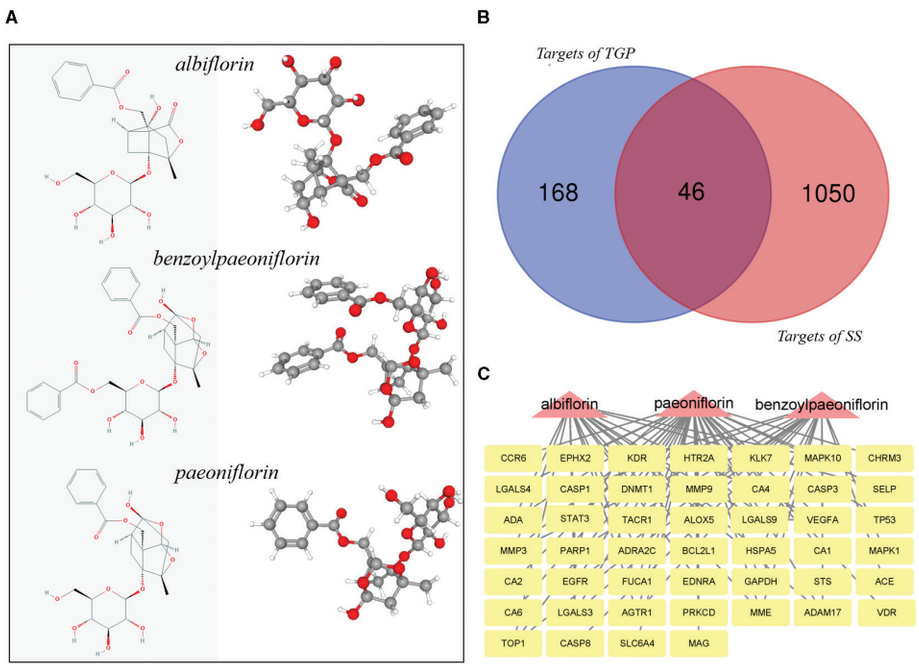

Fig. 1 Structures of different compounds in TGP and the 46 intersecting genes between TGP and SS. A, 2D and 3D structures of TGP. B, Venn diagram of overlapping genes between TGP and SS. C, Predicted target genes of TGP. SS = Sjogren syndrome; TGP = total glucoside of peony.

A total of 1096 human genes associated with SS were collected from the databases. Among these genes, 46 were predicted as the targets of TGP (Fig. 1B and Supplementary Table 1, http://links.lww.com/JCMA/A178). The drug–target network constructed by Cytoscape software is shown in Fig. 1C.

3.2. Topological network analysis of the overlapping genes

The PPI network of the 46 intersecting genes was constructed by Cytoscape software through the "Bisogenet" plug-in. A total of 81,547 edges (interactions) from the 3372 nodes (targets) are shown in Fig. 2A. The index of degree > 54 (twice the median) was used as a criterion to screen the nodes preliminarily and show the most important nodes. A total of 904 nodes with 38,729 edges were returned (Fig. 2B). Furthermore, the following indexes were used in the second screening: betweenness > 5667.19 (median), closeness > 0.45 (median), local average connectivity > 16.65 (median), and degree > 96 (median). The returned 251 related proteins and their 8772 interrelationships, which may play important roles in SS treatment by TGP, are shown in Fig. 2C. Finally, the 10 core targets in the topological analysis, namely, cellular tumor antigen p53 (TP53), neurotrophic tyrosine kinase receptor type 1, epidermal growth factor receptor (EGFR), amyloid-beta A4 protein, cullin-3, estrogen receptor 1, DNA replication licensing factor mini-chromosome maintenance 2, exportin 1, polyubiquitin-C, and fibronectin, were screened out by Cytohubba (Table 1).

3.3. GO and KEGG enrichment of the 251 related genes screened by topological network analysis

The 251 human genes screened by "Bisogenet," which may play a relatively important role in the mechanism of TGP in SS treatment, were subjected to GO and KEGG enrichment (Fig. 3). According to GO enrichment, the biological process of TGP acted on intrinsic apoptotic signaling pathway, cellular response to oxidative stress, and rhythmic process. These proteins were located in the cell–substrate junction, cell–substrate adherens junction, and focal adhesion. In terms of molecular functions, these proteins took part in histone deacetylase binding, ubiquitin-like protein ligase binding, and ubiquitin protein ligase binding. The results of KEGG pathway analysis showed that these proteins were involved in the hepatitis B, viral carcinogenesis, and chronic myeloid leukemia pathways.

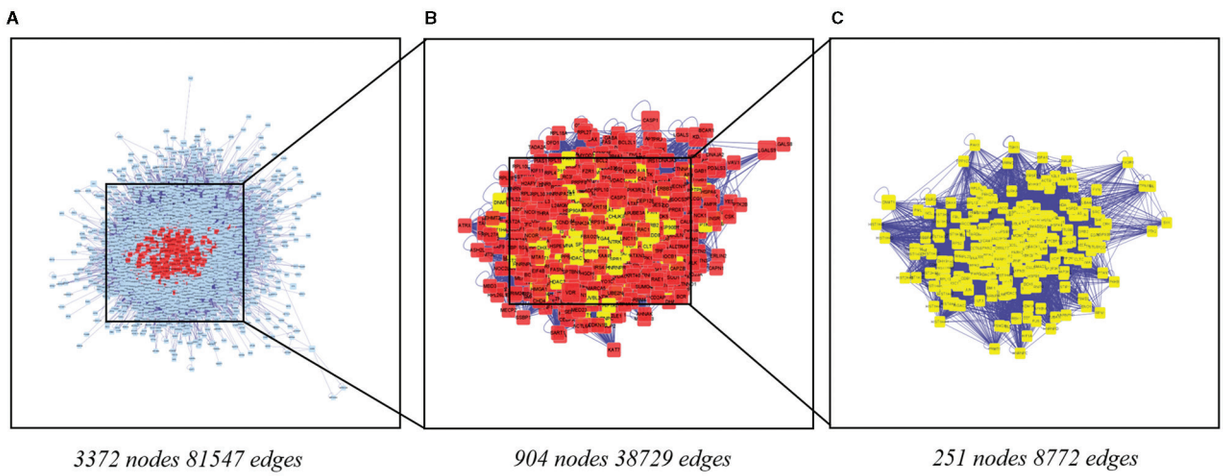

Fig. 2 Topological network analysis of the 46 intersecting genes of SS and TGP's targets. A, PPI network of the enlarged 3372 proteins. B, The 904 nodes after the first screening. C, The 251 nodes after the second screening. PPI = protein–protein interaction; SS = Sjogren syndrome; TGP = total glucoside of peony.

Table 1

Top 10 potential targets associated with TGP in SS treatment from the topology analysis

Gene symbol	Protein name	Degree	Betweenness
TP53	Cellular tumor antigen p53	947	959286.5
NTRK1	Neurotrophic tyrosine kinase receptor type 1	941	624200.8
EGFR	Epidermal growth factor receptor	934	1152536
APP	Amyloid-beta A4 protein	595	495970.9
CUL3	Cullin-3	593	178378.2
ESR1	Estrogen receptor 1	501	158589.9
MCM2	DNA replication licensing factor mini-chromosome maintenance 2	494	130740.8
XPO1	Exportin 1	484	194117.2
UBC	Polyubiquitin-C	477	191634.1
FN1	Fibronectin	464	163019.8

SS = Sjogren syndrome; TGP = total glucoside of peony.

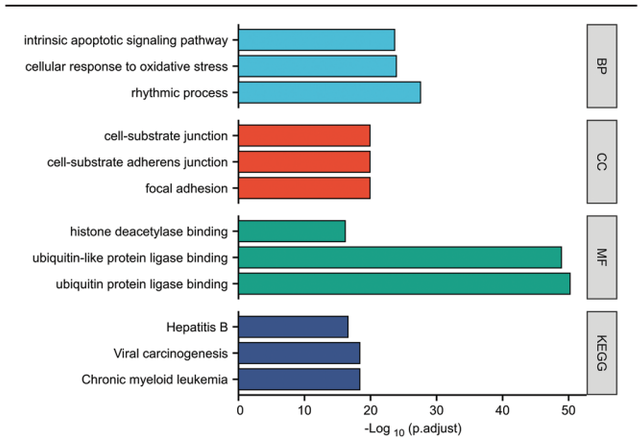

Fig. 3 GO enrichment and KEGG analysis of the 251 genes. BP = biological process; CC = cellular components; GO = Gene Ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes; MF = molecular function.

3.4. Molecular docking

The PPI network of the 46 overlapping genes is shown in Fig. 4A. The top 10 hub genes are listed in Table 2, and the interactions are demonstrated in Fig. 4B. The top 10 candidate targets of TGP were caspase 3 (CASP3), vascular endothelial growth factor A (VEGFA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), TP53, EGFR, signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein kinase 1, Bcl-2–like protein 1, matrix metalloproteinase 9, and caspase 1 (CASP1) as shown in Table 2.

The top 10 hub genes were analyzed by molecular docking to provide a visual explanation of the interaction between the active ingredients of TGP and its potential targets associated with SS. The binding energy and binding sites of the identified hub genes are listed in Table 2. The predicted models with binding energies less than −5 kcal/mol are shown in Fig. 4C.

4. DISCUSSION

TGP is widely used in China as an antirheumatic drug. It is a mixture of the various active compounds of Radix Paeoniae Alba and has a variety of pharmacological effects, such as anti-inflammation and immune regulation. Previous studies demonstrated that TGP can alleviate the symptoms of xerostomia and xerophthalmia in patients with SS.[19] However, the exact pharmacological mechanism is still unclear.

According to the results of network pharmacology, the 10 key targets, including CASP3, VEGFA, and GAPDH, play central roles in SS treatment by TGP. Furthermore, pathway analysis suggested that TGP may exert therapeutic effects by regulating the intrinsic apoptotic signaling pathway, cellular response to oxidative stress, and rhythmic process.

The apoptotic death of epithelial cells in SS seems to result from the release of perforin and granzyme B by activated cytotoxic T lymphocytes, as well as the subsequent activation of the caspase cascade. In the past few years, apoptosis has emerged as a possible mechanism for the damaged salivary and lachrymal glands of patients with SS, which result in the impairment of their secretory function.[20] Previous randomized controlled trial demonstrated that TGP appears to improve the glandular secreting function and decrease the level of apoptotic cytokines in patients with SS.[7] In addition, a systematic review and meta-analysis concluded that TGP can be considered a potentially valid and safe drug for the clinical treatment of SS.[21] Therefore,

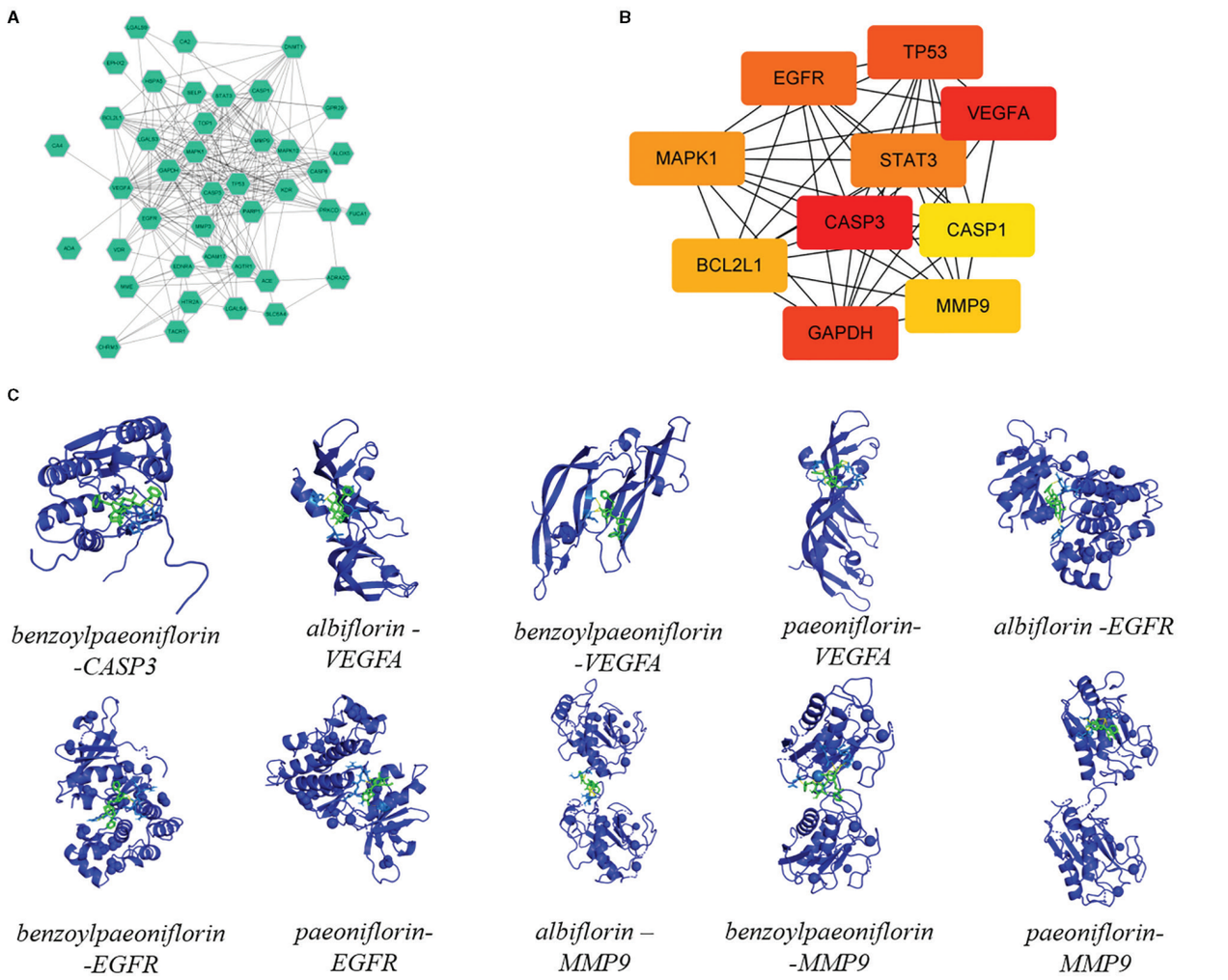

Fig. 4 PPI network and top 10 hub gene web of the 46 intersecting genes with the molecular models. A, PPI network of the 46 intersecting genes. B, Network of the top 10 hub genes from the PPI network. C, Molecular models of TGP binding to its predicted protein targets. The blue ones represent proteins, and the green ones represent the active ingredients of TGP. PPI = protein–protein interaction; TGP = total glucoside of peony.

Table 2

Top 10 hub gene targets of TGP in SS treatment

No.	Target	Protein name	Compounds	Binding energy (kcal/mol)	Amino acid in the bond(s)
1	CASP3	Caspase 3	Benzoylpaeoniflorin	–6.15	ASP192
2	VEGFA	Vascular endothelial growth factor A	Albiflorin	–5.75	SER50
			Benzoylpaeoniflorin	–6.0	LEU32, CYS68
			Paeoniflorin	–7.13	CYS60, GLU64, SER50
3	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Benzoylpaeoniflorin	–4.44	ILE14
			Paeoniflorin	–3.39	PRO252, ASP257
4	TP53	Cellular tumor antigen p53	Paeoniflorin	-	-
5	EGFR	Epidermal growth factor receptor	Albiflorin	–5.48	ARG832, EDO1018
			Benzoylpaeoniflorin	–5.81	VAL834
			Paeoniflorin	–5.54	ARG832
6	STAT3	Signal transducer and activator of transcription 3	Paeoniflorin	–3.26	LYS658
7	MAPK1	Mitogen-activated protein kinase 1	Benzoylpaeoniflorin	–3.47	LYS270, ARG277
8	BCL2L1	Bcl-2–like protein 1	Paeoniflorin	–2.97	ASN172
9	MMP9	Matrix metalloproteinase 9	Albiflorin	–5.42	-
			Benzoylpaeoniflorin	–5.9	GLN126, HIS203
			Paeoniflorin	–5.71	PRO254
10	CASP1	Caspase 1	Benzoylpaeoniflorin	–2.98	THR125, LEU135

SS = Sjogren syndrome; TGP = total glucoside of peony.

apoptotic signals may be useful therapeutic targets, and the caspase family may work as disease markers in primary SS.[22] According to our findings, the role of TGP in SS treatment may affect two core targets in the apoptosis signaling pathway (CASP1 and CASP3), which may be the evidence for its pharmacological effect. VEGF, an important factor in a variety of human pathological situations that are associated with aberrant endothelial proliferation and neovascularization, has been detected to contribute to the pathogenesis and exacerbation of SS.[23] A relation between VEGFA production and SS antibodies has been established by several previous studies, which demonstrated that anti-Ro/SSA antibody increases VEGFA expression.[24] Considerable amounts of VEGFA were discovered in the glandular epithelium and inflammatory cells of chronically inflamed glands of patients with SS compared with healthy controls. The angiogenic effect of VEGFA may be responsible for the increased number of blood vessels observed in the salivary glands of patients with SS.[23,25] Hence, on the basis of the result of network pharmacology, the binding of TGP with VEGF may be one of the multitarget mechanisms in SS treatment. EGFR, a transmembrane protein with cytoplasmic kinase activity, plays an important role in the regulation of cell proliferation, differentiation, migration, and apoptosis.[26] EGFR signaling drives the inflammatory epithelial response in SS.[27] Therefore, drugs targeting EGFR may give rise to new therapeutic intervention to control SS progression. STAT3, which was first described as a molecule with DNA-binding activity, functions as a component of the interleukin-6–activated acute-phase response factor complex.[28] Previous studies revealed the role of STAT3 in SS. One study reported that peripheral T cells in SS are characterized by abnormal STAT3 activation.[29] Okuma et al[30] found the exact pathogenic mechanism, namely, the dysfunction of epithelial cells caused by STAT3 disruption in patients with SS. Our study determined that TGP has the potency to inhibit STAT3; therefore, TGP may be a useful drug for SS. Additionally, according to our result, GAPDH was also a treatment target for TGP. GAPDH has generally been identified as a moonlighting protein based on its ability to perform mechanistically distinct functions. Due to its pivotal role in the glycolysis, GAPDH represents a rate-limiting enzyme in those cells that mostly or exclusively rely on this pathway for energy production.[31,32] Targeting glycolysis is an attractive approach for the treatment of a wide range of pathologies, SS included.[31,33] Therefore, GAPDH inhibition can be a valuable approach for the treatment of SS. Our result demonstrated that TGP was able to target GAPDH in SS treatment, making it a promising drug for SS.

Cellular response to oxidative stress is also involved in the TGP treatment process. TGP markedly suppresses lipopolysaccharide (LPS)-induced nitric oxide production and inducible nitric oxide synthase expression in RA.[34] In addition, the production of reactive oxygen species from LPS-stimulated macrophages is inhibited by high TGP concentrations.[35] Kim et al[36] demonstrated that TGP is able to protect cells from the harmful effects of oxidative stress. Another study found that oxidative stress is increased in diabetic rat kidneys, but TGP can prevent diabetes-associated renal damage against oxidative stress.[37] These previous results were in accordance with our result that oxidative stress was involved in SS treatment by TGP.

Collectively, the 10 key targets involving 3372 proteins become the multitarget mechanism of TGP in SS treatment according to our research. They are enriched in the intrinsic apoptotic signaling pathway, cellular response to oxidative stress, and rhythmic process. These data illustrate the application of network pharmacology in clinical treatment. In addition, this study could also provide guidance for drug development and further scientific drug research.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://links.lww.com/JCMA/A178.

REFERENCES

1. Brito-Zerón P, Baldini C, Bootsma H, Bowman SJ, Jonsson R, Mariette X, et al. Sjögren syndrome. *Nat Rev Dis Primers* 2016;2:16047.

2. Sambataro D, Sambataro G, Dal Bosco Y, Polosa R. Present and future of biologic drugs in primary Sjögren's syndrome. *Expert Opin Biol Ther* 2017;**17**:63–75.
3. Ramos-Casals M, Brito-Zerón P, Sisó-Almirall A, Bosch X. Primary Sjogren syndrome. *BMJ* 2012;**344**:e3821.
4. Xiang N, Li XM, Zhang MJ, Zhao DB, Zhu P, Zuo XX, et al. Total glucosides of paeony can reduce the hepatotoxicity caused by methotrexate and leflunomide combination treatment of active rheumatoid arthritis. *Int Immunopharmacol* 2015;**28**:802–7.
5. Huang Y, Wang H, Chen Z, Wang Y, Qin K, Huang Y, et al. Efficacy and safety of total glucosides of paeony combined with methotrexate and leflunomide for active rheumatoid arthritis: a meta-analysis. *Drug Des Devel Ther* 2019;**13**:1969–84.
6. Zhang W, Dai SM. Mechanisms involved in the therapeutic effects of paeonia lactiflora pallas in rheumatoid arthritis. *Int Immunopharmacol* 2012;**14**:27–31.
7. Zhou Y, Jin L, Kong F, Zhang H, Fang X, Chen Z, et al. Clinical and immunological consequences of total glucosides of paeony treatment in Sjögren's syndrome: a randomized controlled pilot trial. *Int Immunopharmacol* 2016;**39**:314–9.
8. Wu GL, Pu XH, Yu GY, Li TY. Effects of total glucosides of peony on AQP-5 and its mRNA expression in submandibular glands of NOD mice with Sjogren's syndrome. *Eur Rev Med Pharmacol Sci* 2015;**19**:173–8.
9. Wu G, Wu N, Li T, Lu W, Yu G. Total glucosides of peony ameliorates Sjögren's syndrome by affecting Th1/Th2 cytokine balance. *Exp Ther Med* 2016;**11**:1135–41.
10. Ma C, Xu T, Sun X, Zhang S, Liu S, Fan S, et al. Network pharmacology and bioinformatics approach reveals the therapeutic mechanism of action of baicalein in hepatocellular carcinoma. *Evid Based Complement Alternat Med* 2019;**2019**:7518374.
11. Luo TT, Lu Y, Yan SK, Xiao X, Rong XL, Guo J. Network pharmacology in research of Chinese medicine formula: methodology, application and prospective. *Chin J Integr Med* 2020;**26**:72–80.
12. Ding M, Ma W, Wang X, Chen S, Zou S, Wei J, et al. A network pharmacology integrated pharmacokinetics strategy for uncovering pharmacological mechanism of compounds absorbed into the blood of Dan-Lou tablet on coronary heart disease. *J Ethnopharmacol* 2019;**242**:112055.
13. Wang X, Shen Y, Wang S, Li S, Zhang W, Liu X, et al. PharmMapper 2017 update: a web server for potential drug target identification with a comprehensive target pharmacophore database. *Nucleic Acids Res* 2017;**45**:W356–60.
14. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res* 2018;**46**:D1074–82.
15. Otasek D, Morris JH, Bouças J, Pico AR, Demchak B. Cytoscape automation: empowering workflow-based network analysis. *Genome Biol* 2019;**20**:185.
16. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics* 2012;**16**:284–7.
17. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol* 2014;**8**(Suppl 4):S11.
18. Rizvi SM, Shakil S, Haneef M. A simple click by click protocol to perform docking: AutoDock 4.2 made easy for non-bioinformaticians. *Excli J* 2013;**12**:831–57.
19. Li H, Cao XY, Dang WZ, Jiang B, Zou J, Shen XY. Total glucosides of paeony protects against collagen-induced mouse arthritis via inhibiting follicular helper T cell differentiation. *Phytomedicine* 2019;**65**:153091.
20. Sisto M, Lorusso L, Lisi S. X-linked ectodermal dysplasia receptor (XEDAR) gene silencing prevents caspase-3-mediated apoptosis in Sjögren's syndrome. *Clin Exp Med* 2017;**17**:111–9.
21. Feng Z, Zhang BQ, Zhu YM, Yu BB, Fu L, Zhou LL, et al. The effectiveness and safety of total glucosides of paeony in primary Sjögren's syndrome: a systematic review and meta-analysis. *Front Pharmacol* 2019;**10**:550.
22. Wang Y, Virji AS, Howard P, Sayani Y, Zhang J, Achu P, et al. Detection of cleaved alpha-fodrin autoantigen in Sjögren's syndrome: apoptosis and co-localisation of cleaved alpha-fodrin with activated caspase-3 and cleaved poly(ADP-ribose) polymerase (PARP) in labial salivary glands. *Arch Oral Biol* 2006;**51**:558–66.
23. Sisto M, Lisi S, Lofrumento DD, D'Amore M, Frassanito MA, Ribatti D. Sjögren's syndrome pathological neovascularization is regulated by VEGF-A-stimulated TACE-dependent crosstalk between VEGFR2 and NF-κB. *Genes Immun* 2012;**13**:411–20.
24. Szyszko EA, Brokstad KA, Oijordsbakken G, Jonsson MV, Jonsson R, Skarstein K. Salivary glands of primary Sjögren's syndrome patients express factors vital for plasma cell survival. *Arthritis Res Ther* 2011;**13**:R2.
25. Manzo A, Bugatti S, Caporali R, Prevo R, Jackson DG, Uguccioni M, et al. CCL21 expression pattern of human secondary lymphoid organ stroma is conserved in inflammatory lesions with lymphoid neogenesis. *Am J Pathol* 2007;**171**:1549–62.
26. Saint-Jean A, Sainz de la Maza M, Morral M, Torras J, Quintana R, Molina JJ, et al. Ocular adverse events of systemic inhibitors of the epidermal growth factor receptor: report of 5 cases. *Ophthalmology* 2012;**119**:1798–802.
27. Sisto M, Lisi S, D'Amore M, Lofrumento DD. The metalloproteinase ADAM17 and the epidermal growth factor receptor (EGFR) signaling drive the inflammatory epithelial response in Sjögren's syndrome. *Clin Exp Med* 2015;**15**:215–25.
28. Zou S, Tong Q, Liu B, Huang W, Tian Y, Fu X. Targeting STAT3 in cancer immunotherapy. *Mol Cancer* 2020;**19**:145.
29. Ramos HL, Valencia-Pacheco G, Alcocer-Varela J. Constitutive STAT3 activation in peripheral CD3(+) cells from patients with primary Sjögren's syndrome. *Scand J Rheumatol* 2008;**37**:35–9.
30. Okuma A, Hoshino K, Ohba T, Fukushi S, Aiba S, Akira S, et al. Enhanced apoptosis by disruption of the STAT3-IκB-ζ signaling pathway in epithelial cells induces Sjögren's syndrome-like autoimmune disease. *Immunity* 2013;**38**:450–60.
31. Galbiati A, Zana A, Conti P. Covalent inhibitors of GAPDH: from unspecific warheads to selective compounds. *Eur J Med Chem* 2020;**207**:112740.
32. Hawley D, Aluri H, Armaos H, Kim G, Kublin C, Zoukhri D. Human postmortem lacrimal and submandibular glands stored in RNAlater are suitable for molecular, biochemical, and cell biological studies. *Mol Vis* 2016;**22**:1221–28.
33. Pucino V, Bombardieri M, Pitzalis C, Mauro C. Lactate at the crossroads of metabolism, inflammation, and autoimmunity. *Eur J Immunol* 2017;**47**:14–21.
34. GBD 2016 Disease and Injury Incidence and Prevalence Collaborator. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016:a systematic analysis for the global burden of disease study 2016. *Lancet* 2017;**390**:1211–59.
35. Luo C, Wang H, Chen X, Cui Y, Li H, Long J, et al. Protection of H9c2 rat cardiomyoblasts against oxidative insults by total paeony glucosides from radix paeoniae rubrae. *Phytomedicine* 2013;**21**:20–4.
36. Kim SH, Lee MK, Lee KY, Sung SH, Kim J, Kim YC. Chemical constituents isolated from paeonia lactiflora roots and their neuroprotective activity against oxidative stress in vitro. *J Enzyme Inhib Med Chem* 2009;**24**:1138–40.
37. Su J, Zhang P, Zhang JJ, Qi XM, Wu YG, Shen JJ. Effects of total glucosides of paeony on oxidative stress in the kidney from diabetic rats. *Phytomedicine* 2010;**17**:254–60.