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Analysis of the mechanism of *Sophorae Flavescentis Radix* in the treatment of intractable itching based on network pharmacology and molecular docking

Y. XIAO[1], R.-J. WANG[1], H.-K. ZENG[1], J. XIE[1], Y.-L. SITU[1], S. KONG[1], T.-T. WANG[1], A. VERKHRATSKY[2,3,4], H. NIE[1]

[1]State Key Laboratory of Bioactive Molecules and Druggability Assessment, Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, International Cooperative Laboratory of Traditional Chinese Medicine Modernization and Innovative Drug Development of Chinese Ministry of Education (MOE), College of Pharmacy, Jinan University, Guangzhou, China
[2]Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK
[3]Achucarro Centre for Neuroscience, IKERBASQUE, Basque Foundation for Science, Bilbao, Spain
[4]Department of Stem Cell Biology, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania

Abstract. – OBJECTIVE: *Sophorae Flavescentis Radix (Kuh-seng, SFR)*, a Traditional Chinese Medicine (TCM), is widely used alone or within a TCM formula to treat pruritus, especially histamine-independent intractable itching. In the previous study, potential antipruritic active components of the SFR were screened based on cell membrane immobilized chromatography (CMIC), revealing oxymatrine (OMT) as an antipruritic agent. However, the low oral bioavailability (OB) of OMT cannot explain the antipruritic effect of SFR when administered orally in clinic. In this study, we investigated the antipruritic effects and underlying mechanisms of orally administered SFR.

MATERIALS AND METHODS: A network pharmacology and molecular docking were employed to screen the active components of SFR and predict their binding to disease-related target proteins, while the potential mechanisms were explored with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. The binding energy between components and target proteins was calculated by molecular docking.

RESULTS: The SFR-components-targets-intractable itching Protein-Protein Interactions (PPI) network was established, and 22 active components and 42 targets were screened. The GO enrichment analysis showed that the key target genes of SFR were related to nuclear receptors, transcription factors, and steroid hormone receptors. The results of the KEGG enrichment pathway analysis include Hepatitis B, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance, advanced glycation end product (AGE)-receptor for AGE (RAGE) signaling pathway in diabetic complications, etc. Molecular docking showed that three key target proteins in the network, the vascular endothelial growth factor A (*VEGFA*), epidermal growth factor receptor (*EGFR*) and caspase-3 (*CASP3*), have higher binding activities with inermine, phaseolin and kushenol O, respectively; the binding energy of each pair is stronger than that of the target protein-corresponding inhibitors.

CONCLUSIONS: The complexity of the SFR-components-targets-intractable itching network demonstrated the holistic treatment effect of SFR on intractable itching. The partial coherence between results screened by CMIC in the previous study and network pharmacology demonstrated the potential of network pharmacology in active component screening. Inermine screened from both CMIC and network pharmacology is a *VEGFA* inhibitor, which possibly accounts for the antipruritic effect of orally administered SFR.

Key Words:
Sophorae Flavescentis Radix, Intractable itching, Histamine-independent pruritus, Mechanism, Network pharmacology, Molecular docking.

Introduction

Pruritus is a pathological desire to scratch. Chronic pruritus is a persistent, intractable, and debilitating problem with limited treatments[1].

Corresponding Author: Hong Nie, MD; e-mail: tnieh@jnu.edu.cn

Chronic pruritus is associated with various skin diseases, neural diseases and systematic diseases, including eczema[2], atopic dermatitis[3], allergic contact dermatitis (ACD)[4], psoriasis[5], herpes zoster[6], neuromyelitis optica[7], uremia[8], hepatobiliary diseases[9] and some cancers[10-12]. The pathophysiology of chronic pruritus remains unclear, while incurability seriously affects patients' physical and mental health. Mechanistically, histamine-dependent and histamine-independent pruritus are distinguished. Most of the intractable itching belongs to the latter category and is resistant to antihistamine drugs. Corticosteroids are useful; however, after the cessation of therapy, pruritus often recurs, while long-term hormonal treatment causes serious adverse reactions.

Sophorae Flavescentis Radix (Kuh-seng, SFR), the root of *Sophora flavescens Aiton*, is a Traditional Chinese Medicine (TCM) first mentioned in *Shennong's Herbal Classic of Materia Medica*, the 2000 years old TCM medical book. SFR is widely used to treat diseases, including pruritus. It has a significant antipruritic effect in allergic contact dermatitis (ACD), psoriasis, and atopic dermatitis[13,14]. Although clinical and experimental studies[15,16] demonstrated that SFR is effective in treating intractable itching, the active components and underlying mechanisms are not fully understood.

Several chemical components were isolated and identified from SFR, including alkaloids, flavonoids, etc. In a previous study[17], five active components in SFR were isolated by cell membrane immobilized chromatography (CMIC), namely maackiain (inermine), calycosin, oxymatrine (OMT), oxysophocarpine and sophocarpine. OMT was investigated in depth and found to have a strong antipruritic effect in histamine-independent pruritus. However, the low oral bioavailability (OB) of OMT cannot explain the antipruritic effect of orally administered SFR. Here, we analyzed the SFR mode of action *in silico*, using a network pharmacology and molecular docking.

Materials and Methods

Acquisition of Active Components and Targets of SFR

All chemical components of SFR were obtained by searching the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://old.tcmsp-e.com/tcmsp.php), which provides pharmacokinetic information for each compound, including OB and Drug-likeness (DL). OB $\geq$ 30% reflects the high rate and degree of absorption of the drug into human circulation, while DL $\geq$ 0.18 has a high similarity to known drugs and is more likely to be the main active component. Therefore, active components in SFR were preliminarily screened under the conditions of OB $\geq$ 30% and DL $\geq$ 0.18[18]. Then, TCMSP was used to obtain all the potential targets of SFR, and ActivePerl and UniProt databases were used to add genes' names for the targets.

Acquisition of Common Target of SFR and Intractable Itching

Using the disease database GeneCards (https://www.genecards.org), the Online Mendelian Inheritance in Man (OMIM) database (http://www.ncbi.nlm.nih.gov/omim), the "intractable itching" keywords were screened to obtain relevant target genes. Data from two databases were merged, and duplicate or false positive genes were deleted; R version 3.5.3 (Vienna, Austria) was used to map the target related to the active components of SFR and the target of intractable itching to obtain the common target gene, i.e. the key target of SFR in the treatment of intractable itching and draw the Venn diagram.

Construction of Regulatory Network of SFR-Components-Intractable Itching-Targets

The gene relationship network of SFR-active components-intractable itching-targets data was derived from Cytoscape version 3.6.1 (http://www.cytoscape.org). Through this network, the active components of SFR, targeting intractable itching, were selected. The mechanisms of active components of SFR action in the treatment of intractable itching were explored by constructing the regulatory network.

Construction of Protein-Protein Interaction (PPI) Network of SFR-Intractable Itching and Screening of Key Targets

The PPI network of SFR-intractable itching data was derived from the STRING (https://string-db.org), the screening condition of species selection person, the minimum required interaction score of 0.4[19], and the results were saved. The core target map of the protein interaction network was obtained by R language calculation.

Gene Ontology (GO) Functional and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

GO functional enrichment analysis and KEGG pathway enrichment analysis were obtained by using R version 3.5.3 to calculate the SFR-intractable itching targets. $p < 0.05$ was considered statistically significant.

Molecular Docking of Key Target Proteins and Active Components

The target proteins in the top three of the PPI network of SFR-intractable itching were selected for molecular docking with the corresponding active components of SFR. The corresponding protein structure was downloaded from the Protein Data Bank (PDB) (https://www.rcsb.org), while the protein complexes with ligands were selected to ensure the docking accuracy. Subsequently, the 3D structures of the corresponding active components of SFR were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov). The Autodock Vina 1.1.2 (FL, USA) was used for molecular docking. Finally, the conformation with the lowest Vina score was selected and analyzed by the PyMOL 2.5.2 (NY, USA) and the Discovery Studio 4.5.0 (Paris, France). The corresponding inhibitor was also used as a control for molecular docking with the target protein.

Results

Screening of Active Components in SFR and Target Prediction

By screening all active components and related targets of SFR in TCMSP, 113 active components and 963 related targets were identified. According to the OB and DL values, the active components of SFR were screened again. Finally, a total of 45 active components were revealed (Table I).

Prediction of Therapeutic Target of SFR on Intractable Itching

The R software generated a Venn diagram depicting the intersection of SFR targets and targets related to intractable itching (Figure 1).

Construction of Regulatory Network of SFR-Active Components-Intractable Itching-Target

22 active components of SFR mainly act on 42 targets related to intractable itching, which may affect its occurrence and development, as shown on the regulatory network of SFR-active components and intractable itching targets (Figure 2).

Construction of Protein-Protein Interaction (PPI) network of SFR-Intractable Itching and Screening of Key Targets

According to the number of adjacent nodes, the order of the top 20 targets of SFR-intractable itching was *VEGFA*, *CASP3*, *EGFR*, *IL6*, *ESR1*, *MYC*, *FOS*, *AR*, *PPARG*, *MDM2*, *MCL1*, *CASP9*, *HIF1A*, *NOS3*, *CD44*, *RB1*, *AHR*, *NFE2L2*, *PARP1*, *PGR*. The higher the number of adjacent nodes, the greater the probability of becoming a key target (Figure 3).

GO Functional Enrichment Analysis and KEGG Pathway Enrichment Analysis

GO functional analysis of the core gene of SFR revealed multiple biological processes, such as RNA polymerase II transcription factor binding, nuclear receptor activity, transcription factor activity, direct ligand regulated sequence-specific DNA binding, nuclear hormone receptor binding, activating transcription factor binding, steroid hormone receptor binding, estrogen receptor binding, hormone receptor binding, cysteine-type endopeptidase activity involved in apoptotic process, E-box binding, DNA-binding transcription activator activity, RNA polymerase II-specific, steroid hormone receptor activity, RNA polymerase II basal transcription factor binding, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen (**Supplementary Figure 1**).

KEGG pathway of the key genes of SFR in the treatment of itching mainly involves hepatitis B, colorectal cancer, prostate cancer, fluid shear stress and atherosclerosis, advanced glycation end product (AGE)-receptor for AGE (RAGE) signaling pathway in diabetic complications, Kaposi sarcoma-associated herpesvirus infection, Human cytomegalovirus infection, apoptosis, bladder cancer, proteoglycans in cancer, platinum drug resistance, Phosphatidylinositol-4,5-bisphosphate 3-kinase-protein kinase B (PI3K-Akt) signaling pathway, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance, hepatocellular carcinoma, thyroid hormone signaling pathway, MicroRNAs in cancer, apoptosis-multiple species, endocrine resistance, Epstein-Barr virus infection, breast cancer, etc. (**Supplementary Figure 2**).

Table I. The active components of SFR were screened by the TCMSP.

MOL ID	MOL Name	OB (%)	DL
MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	42.36	0.21
MOL001484	Inermine	75.18	0.54
MOL003542	8-Isopentenyl-kaempferol	38.04	0.39
MOL003627	Sophocarpine	64.26	0.25
MOL003648	Inermin	65.83	0.54
MOL003673	Wighteone	42.8	0.36
MOL003676	Sophoramine	42.16	0.25
MOL003680	Sophoridine	60.07	0.25
MOL000392	Formononetin	69.67	0.21
MOL004580	Cis-Dihydroquercetin	66.44	0.27
MOL004941	(2R)-7-hydroxy-2-(4-hydroxyphenyl)chroman-4-one	71.12	0.18
MOL005100	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one	47.74	0.27
MOL005944	Matrine	63.77	0.25
MOL000006	Luteolin	36.16	0.25
MOL006561	(+)-14alpha-hydroxymatrine	35.73	0.29
MOL006562	(+)-7,11-dehydromatrine,(leontalbinine)	62.08	0.25
MOL006563	(+)-9alpha-hydroxymatrine	32.04	0.29
MOL006564	(+)-allomatrine	58.87	0.25
MOL006565	AIDS211310	68.68	0.25
MOL006566	(+)-lehmannine	58.34	0.25
MOL006568	Isosophocarpine	61.57	0.25
MOL006569	(-)-14beta-hydroxymatrine	37.26	0.29
MOL006570	(-)-9alpha-hydroxysophoramine	35.23	0.29
MOL006571	Anagyrine	62.01	0.24
MOL006572	1,4-diazaindan-type,alkaloid,flavascensine	34.64	0.24
MOL006573	13,14-dehydrosophoridine	65.34	0.25
MOL006582	5α,9α-dihydroxymatrine	40.93	0.32
MOL006583	7,11-dehydromatrine	44.43	0.25
MOL006596	Glyceollin	97.27	0.76
MOL003347	Hyperforin	44.03	0.6
MOL006604	(2S)-7-hydroxy-2-(4-hydroxyphenyl)-5-methoxy-8-(3-methylbut-2-enyl)chroman-4-one	48.09	0.39
MOL006613	Kushenin	47.62	0.38
MOL006619	Kushenol J	51.39	0.74
MOL006620	Kushenol J_qt	50.86	0.24
MOL006622	Kushenol O	42.41	0.76
MOL006623	Kushenol,t	51.28	0.64
MOL006626	Leachianone,g	60.97	0.4
MOL006627	Lehmanine	62.23	0.25
MOL006628	(+)-Lupanine	52.71	0.24
MOL006630	Norartocarpetin	54.93	0.24
MOL000456	Phaseolin	78.2	0.73
MOL006649	Sophranol	55.42	0.28
MOL006650	(-)-Maackiain-3-O-glucosyl-6'-O-malonate	48.69	0.52
MOL006652	Trifolrhizin	48.53	0.74
MOL000098	Quercetin	46.43	0.28

Drug-likeness (DL), oral bioavailability (OB).

Molecular Docking of the Selected Key Target Proteins with the Active Components of SFR

In order to further verify the results of network pharmacology, *VEGFA* (PDB ID 3QTK), *CASP3* (PDB ID 3KJF) and *EGFR* (PDB ID 5XGM), which were the top 3 target proteins in the PPI network of SFR-intractable itching targets, were selected to conduct molecular docking with the active components of SFR to find the components with the optimal Vina value. Vina value is the value of the complex obtained by molecular docking of receptor and ligand with the corresponding pocket parameters by the Vina program, i.e., the calculated ΔG value. The binding energy was less than 0, which indicates that the

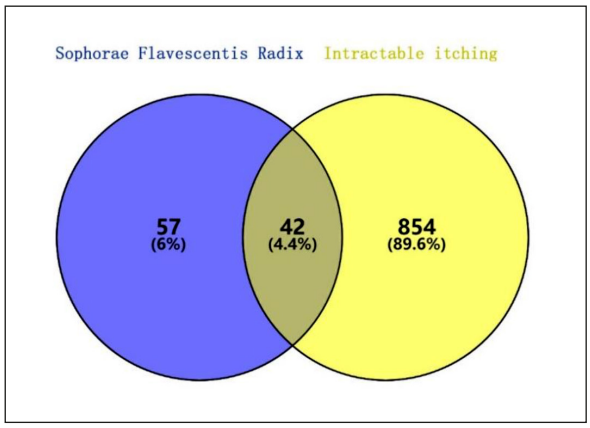

Figure 1. Prediction of therapeutic target of SFR on intractable itching.

ligand and the receptor can spontaneously bind, and the smaller the value, the higher the binding energy, and the easier the active component can bind to the receptor. The results of molecular docking showed that the Vina value of inermine for *VEGFA* was the lowest, at -4.7 kcal · mol^{-1}, and for the inhibitor cedrinaib the Vina value was -3.1 kcal · mol^{-1} (**Supplementary Figure 3A**). The Vina value of kushenol O for *CASP3* was the lowest, at -9.6 kcal · mol^{-1}, while for its inhibitor Z-DEVD-FMK, the Vina value was -7.1 kcal · mol^{-1} (**Supplementary Figure 3B**). The Vina value for phaseolin for *EGFR* was the lowest, at -7.3 kcal · mol^{-1}, and for its inhibitor gefitinib the Vina value was -6.5 kcal · mol^{-1} (**Supplementary Figure 3C**).

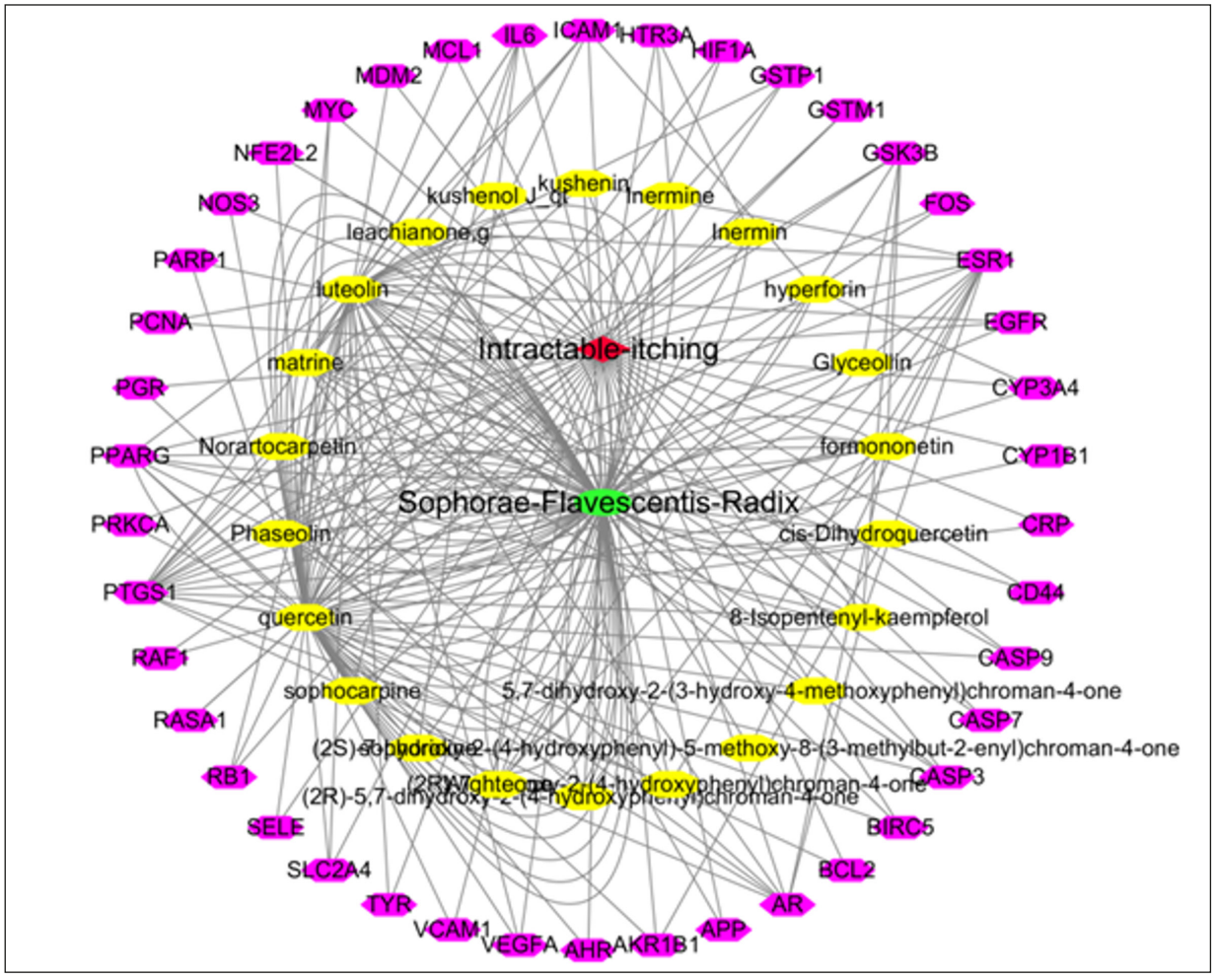

Figure 2. The regulatory network of SFR-active components-intractable itching-target. The red stands for intractable itching, green represents SFR, yellow represents the active components of SFR, and purple represents the common target of SFR-intractable itching.

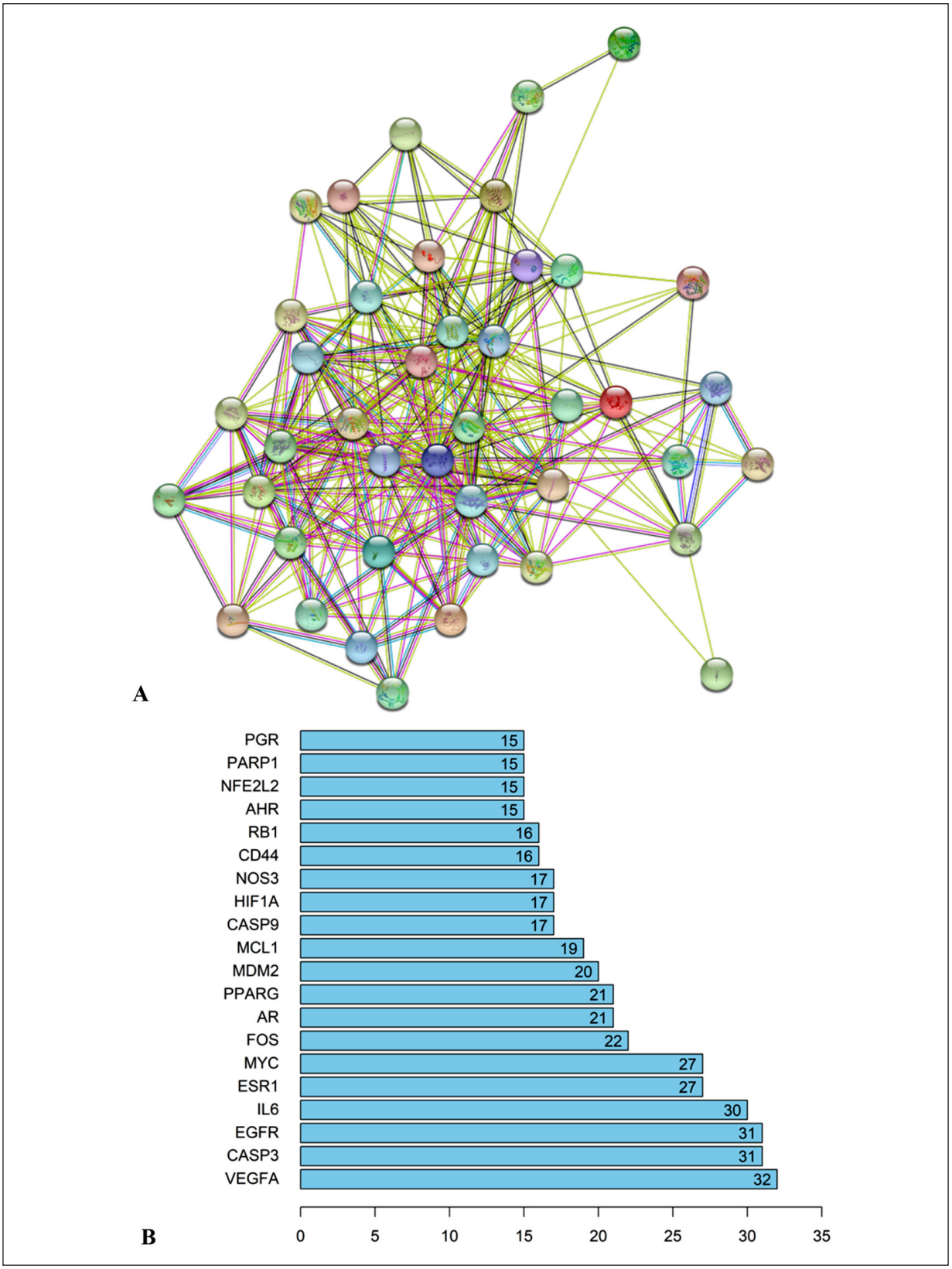

Figure 3. Protein-protein interaction (PPI) network of SFR-intractable itching targets and key targets. **A**, The PPI network of SFR-intractable itching; **B**, Top 20 targets for the number of adjacent nodes in the PPI network.

Discussion

SFR is widely used for the treatment of histamine-independent intractable itching. In our previous study[17] we used dorsal root ganglion (DRG) sensory neurons involved in transmitting itch to construct a novel screening strategy, namely cell membrane immobilized chromatography (CMIC). This strategy is based on bio-specific affinity adsorption of bioactive substances to receptors or channels on cells; active compounds are 'fished' from a complex sample of natural products in a rapid, high-throughput, and applicable way. We thus identified five active components of SFR, namely calycosin, inermine, OMT, oxysophocarpine, and sophocarpine. Subsequently, we showed that OMT can significantly reduce skin inflammation and pruritus and have a significant inhibitory effect on histamine-independent pruritus[17,20,21]. OMT, however, has a low OB and thus cannot account for the antipruritic effect of orally administered SFR. In the present study, network pharmacology was used to analyze the mechanism of action of SFR active components with OB greater than 30% in the treatment of intractable itching. We identified 22 active components acting on 42 related targets of intractable itching. These active compounds and intractable itching targets were used to construct the regulation network of SFR-active components-intractable itching targets, as well as the PPI network of SFR-intractable itching. Using this approach, we identified vascular endothelial growth factor A (*VEGFA*), epidermal growth factor receptor (*EGFR*), and caspase-3 (*CASP3*) as key targets. Serum *VEGFA* levels reflect itching severity in Sézary syndrome and mycosis fungoides[22]. Upregulation of *VEGFA* in the imiquimod-induced psoriasiform dermatitis in mice promotes epidermal hyperinnervation[23]. *VEGFA* contributes to the pathophysiology of pruritus in psoriasis and may be a potential new therapeutic target for cases of intractable pruritic psoriasis. *VEGF* levels were significantly increased in the serum of patients with prurigo, while substantial improvement was reported[24] for a patient with prurigo simplex treated with bevacizumab, a monoclonal *VEGF* antibody. Another key target, *EGFR*, regulates cell proliferation and survival. Keratin defects trigger the itch-inducing cytokine thymic stromal lymphopoietin through amphiregulin-EGFR signaling[25]. Pruritus is a major *EGFR* inhibitor-associated dermatological adverse event with an impact on health-related quality of life[26]. The involvement of mast cells in *EGFR* inhibitor-induced pruritus was also reported[27]. A five-year clinical study[28] demonstrated that the epidermis displayed increased BCL2-Associated X protein (Bax) and decreased B cell lymphoma/leukemia-2 (Bcl-2) expression in the basal and intermediate epidermal layers, as well as the presence of *CASP3* positive apoptotic cells both in the superficial and intermediate epidermal layers in dialysis patients. This study suggested that these changes in epidermal cells could possibly affect the intra-epidermal nerve endings, thus leading to pruritus.

The GO enrichment analysis revealed key target genes of the active components of SFR. The Pregnane X receptor (PXR) is a member of the nuclear receptor superfamily. PXR activators are used to treat pruritus in chronic inflammatory liver diseases, while PXR activation is an anti-inflammatory in the liver[29]. Central bile acid sensor Farnesoid X receptor (FXR) was also shown[30] to be a mediator of pruritus in humans. Functional variants of the FXR were identified in intrahepatic cholestasis of pregnancy, characterized by liver impairment, pruritus, and elevated maternal serum bile acids[31]. *Lactobacillus plantarum* K-1, isolated from kimchi, may improve allergic diseases and pruritus by inhibiting the expression of interleukin (IL)-4, IL-1β and tumor necrosis factor-alpha (TNF)-α through nuclear factor kappa B (NF-κB) and AP-1 signaling pathways[32]. BSASM (a mixture of several plant extracts) inhibited lipopolysaccharide (LPS)-induced activation of NF-κB promoter, and has anti-inflammatory and atopic dermatitis-mitigating effects, and relieves itching[33].

Based on the KEGG pathway analyses, we found that compounds of SFR may regulate multiple signal pathways in various diseases, including hepatitis B, colorectal cancer, prostate cancer, AGE-RAGE signaling pathway in diabetic complications, apoptosis, PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, hepatocellular carcinoma, etc. Clinical studies[34-36] suggested a close relationship between hepatitis B and intractable itching. *EGFR* tyrosine kinase inhibitor resistance is also closely related to pruritus. Activation of the *EGFR* pathway is essential for tumor proliferation and metastasis. Overexpression of the *EGFR* is found[37,38] in many cancers, and *EGFR* inhibitors are used in anti-cancer therapy, with pruritus being the most common side effect. Similarly, diabetes is associated with generalized pruritus[39,40]. All of these pathways of KEGG enrichment analysis may become potential targets of SFR in the treatment of intractable itching.

Molecular docking prediction suggested that the top three target proteins in the PPI network of SFR-intractable itching targets, *VEGFA*, *CASP3*, and *EGFR*, had the best binding activities with inermine, kushenol O and phaseolin, respectively. In particular, inermine, the *VEGFA* inhibitor, has an OB of 75.18%. We expanded the target proteins for molecular docking to the top five and found that inermine not only had the best docking result with *VEGFA* but also interacted with *CASP3* (PDB ID 3KJF, ΔG, -7.6 kcal · mol^{-1}), *EGFR* (PDB ID 5XGM, ΔG, -6.7 kcal · mol^{-1}), *IL-6* (PDB ID 1ALU, ΔG, -7.4 kcal · mol^{-1}), *ERα* (PDB ID 7UJO, ΔG, -9.6 kcal · mol^{-1}). The binding of inermine is higher than that of the corresponding target protein inhibitors (*CASP3* inhibitor Z-DE-VD-FMK, ΔG, -7.1 kcal · mol^{-1}, *EGFR* inhibitor Gefitinib, ΔG, -6.5 kcal · mol^{-1}, *IL-6* inhibitor Hydrocortisone hemisuccinate, ΔG, -6.9 kcal · mol^{-1}, *ERα* inhibitor amcenestrant, ΔG, -7.5 kcal · mol^{-1}). Inermine is currently relatively poorly researched. Inermine (also known as maackiain) is a potential anti-angiogenic compound that may interfere with VEGF-induced cancer malignancy[41]. Inermine has antiallergic properties and alleviates nasal symptoms in toluene diisocyanate (TDI)-sensitized allergy model rats through the inhibition of *IL-4* and *H1R* gene expression[42]. Intermine suppression of *H1R* gene expression is mediated by the inhibition of PKCδ activation. In addition, inermine inhibits the Hsp90 pathway through PMA-induced up-regulation of *H1R* gene expression and Hsp90 inhibition of PKCδ activation through the disruption of Hsp90-PKCδ interaction[43]. For the remaining two active components, phaseolin and kushenol O, there are not many reports about their anti-itching properties. Phaseolin shows some estrogenic activities[44], whereas kushenol O is a flavonoid compound extracted from SFR[45].

Conclusions

In conclusion, the complexity of the "TCM-components-target-disease" network demonstrated the holistic treatment effect of SFR on intractable itching, reflecting the characteristic of the TCM multi-component action on multiple therapeutic targets and multiple signaling pathways. Inermine previously screened by CMIC is also screened by network pharmacology, which means that network pharmacology could be used for drug screening. Compared with oxymatrine, which is low in OB but antipruritic, inermine with high OB may be the active antipruritic component of SFR when it is orally administrated. Further research is needed to confirm the antipruritic effect of inermine and verify its possible high-affinity interaction with *VEGFA*.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Authors' Contribution

H. Nie, A. Verkhratsky, and Y. Xiao designed the project; J. Xie and Y.-L. Situ performed network pharmacology; Y. Xiao, J. Xie, Y.-L. Situ and R.-J. Wang performed the molecular docking; Y. Xiao, S. Kong, R.-J. Wang analyzed the data; Y. Xiao and H.K. Zeng wrote the manuscript; H. Nie, A. Verkhratsky, Y. Xiao, H.-K. Zeng, S. Kong, R.-J. Wang, T.-T. Wang revised the manuscript.

Ethics Approval and Informed Consent

Not applicable.

ORCID ID

H. Nie: 0000000274094260

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