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Multi-target pharmacological mechanisms of *Salvia miltiorrhiza* against oral submucous fibrosis: A network pharmacology approach

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ABSTRACT

Objectives: The herb *Salvia miltiorrhiza* is used to treat oral submucous fibrosis (OSF); however, the mechanism underlying its efficacy has not been elucidated. As such, a network pharmacology-based approach was applied to investigate the potential mechanisms of *Salvia miltiorrhiza* against OSF.

Materials and methods: Potential targets of *Salvia miltiorrhiza* were collected by Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine, and Swiss Target Prediction. Potential targets of OSF were collected from DisGeNET, GeneCards, and National Center for Biotechnology Information Gene database. *Salvia miltiorrhiza* against OSF targets protein-protein interaction and enrichment analyses network were constructed by Cytoscape and Metascape.

Results: Twelve active ingredients from *Salvia miltiorrhiza* and 57 potential OSF-related targets were identified. The constructed network predicted seven potential key targets of *Salvia miltiorrhiza* for the treatment of OSF. Functional enrichment analysis showed that biological processes such as cellular response to drugs and pathways such as bladder cancer were mainly regulated by the *Salvia miltiorrhiza* active ingredient targets. Furthermore, the protein-protein interaction network demonstrated that the molecular complex detection components were mainly related to the ErbB signaling pathway, cancer pathways and IL-17 signaling.

Conclusions: A network approach was employed to document how *Salvia miltiorrhiza* active ingredients change various pathways against OSF. *Salvia miltiorrhiza* active ingredient targets against OSF involved *CYP19A1*, *EGFR*, *PTPN11*, *ACHE*, *TERT*, *MAPK8* and *PGR* and were enriched in several signaling pathways.

1. Introduction

Oral submucous fibrosis (OSF) is a progressive chronic inflammatory disorder of the oral cavity. The clinical presentation of OSF includes a blanched mucosa, restricted mouth opening, a burning sensation, and fibrous bands in the oral cavity (Cai et al., 2019). It is diffuse oral precancerous condition, nowadays called oral potentially malignant disorder, and involves primarily buccal mucosa, palate and tongue. However, no oral site is immune to involvement by oral submucous fibrosis as disease progresses. OSF has become a global disease affecting the world, which used to be limited to southern China, India and other Southeast Asian regions with prevalence of 4.96 % and malignant of 4.2 % (Brennan & Arakeri, 2017; Kujan et al., 2020; Mello et al., 2018).

The pathogenesis of OSF is unknown; however, it is believed to have multifactorial origins. Factors in the pathogenesis of OSF include chewing of areca nut, genetic factors, immunologic processes, and deficiency in some nutrients (Gupta et al., 1998; Maher et al., 1994; Ray et al., 2019). Transforming growth factor-β signal induced by areca nut

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via muscarinic acid receptor, reactive oxygen species, and c-Jun-NH2--terminal kinase signals were playing important role in pathogenesis of OSF (Kondaiah et al., 2019). However, only a part of areca nut chewers developed diseases, indicating genetic background also take part in the development of OSF. Mutations of risk alleles and genotypes may change the transcriptional activity and the functions of proteins, leading to OSF (Cheng et al., 2020). In addition, local and systemic copper in pathogenesis of OSF was referred (Arakeri et al., 2017). Currently, there are available treatment options for patients with OSF, including physical therapy (hyperbaric oxygen therapy), drug therapy (glucocorticoids and colchicine), and natural compound remedies (herbs in traditional Chinese medicine [TCM]) (Shih et al., 2019). However, the effectiveness of these interventions for OSF treatment has not been verified. Additionally, assessing the efficacies of these interventions through comparisons and combinations using the available scientific methods remains a challenge (Ray et al., 2019; Sarode et al., 2020).

Salvia miltiorrhiza (*Danshen* in Chinese) has been used clinically for 1000 years to ease blood circulation and remove blood stasis. Also, it has multiple bioactivities, including antioxidant activity, antiplatelet activity, and anti-inflammatory properties (Chen et al., 2014). The cardiovascular protective effects and anti-cancer potential of *Salvia miltiorrhiza* have also been investigated (Chen et al., 2014; Zhou et al., 2005). *Salvia miltiorrhiza* is clinically used as a treatment for patients with OSF, but its mechanisms have not been fully explored (Xie et al., 2019).

Current pharmacology research investigating *Salvia miltiorrhiza* has identified ingredients with antioxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-atherogenic, anti-diabetic, and immunomodulatory activities. Examples include tanshinone IIA, cryptotanshinone, dihydrotanshinoneI, Danshenol A, and Danshenol B (Pang et al., 2016).

TCM network pharmacology is a systematic way of identifying the regulation of small molecules according to the interaction network of herbs, ingredients, targets, and diseases (Li et al., 2010; Li & Zhang, 2013). The method integrates bioinformatics, systems biology, and pharmacology. It elucidates the complex interactions of herbs versus diseases at a systematic level and meets the holistic and systematic view of the TCM theory (Hopkins, 2008; Li et al., 2014). Studies on human diseases used to mainly narrow on identifying genetic causes of disease. Indeed, epigenetic networks have increasingly been regarded as essential elements in numerous human diseases, which could also be the case in OSF (Ray et al., 2019). Herein, network pharmacology was employed to elucidate the underlying pharmacological mechanisms of *Salvia miltiorrhiza* against OSF.

In this study, a network pharmacology of *Salvia miltiorrhiza* against OSF was developed by computational tools and resources to predict the potential active ingredients and molecular targets, to identify potential targets and pathways by in silico method, explore the molecular mechanism of *Salvia miltiorrhiza* in the treatment of OSF and promote the follow-up experimental research and development of new drugs of TCM.

2. Materials and methods

Salvia miltiorrhiza was screened to identify its active ingredients and their pharmacological targets by in silico method. The potential target genes of OSF were also screened. The network was constructed by assessing the possible interactions between different target nodes. Also, enrichment and protein-protein interaction analyses were performed to characterize the potential mechanisms of *Salvia miltiorrhiza* in OSF treatment. The network pharmacology method was also used to identify candidate molecules and cellular pathways that *Salvia miltiorrhiza* therapy targets during OSF treatment. The potential mechanisms of *Salvia miltiorrhiza* in OSF treatment are described. A flowchart of the experimental procedures used herein is presented in Fig. 1.

2.1. Pharmacokinetic predictions

It is important to identify the absorption, distribution, metabolism, and excretion (ADME) parameters (Barton et al., 2006). Therefore, to explore the potential bioactive compounds contained in *Salvia miltiorrhiza*, two ADME-associated properties, that is, oral bioavailability and drug-likeness were chosen to guide screening. Herein, ingredients with oral bioavailability >45 % and drug-likeness >0.35 were utilized.

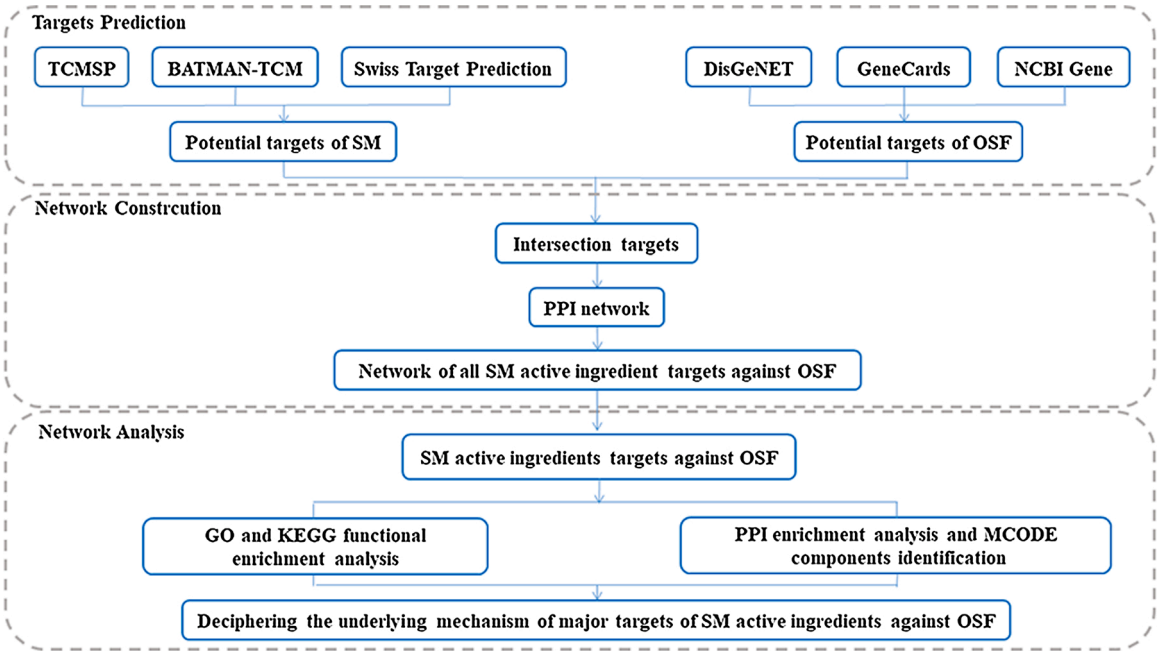


Fig. 1. The flowchart of multi-target pharmacological mechanisms of *Salvia miltiorrhiza* against oral submucous fibrosis based on a network pharmacology approach (TCMSP: Traditional Chinese medicine Systems Pharmacology Database, BATMAN-TCM: Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine, NCBI: National Center for Biotechnology Information, SM: *Salvia miltiorrhiza*, OSF: oral submucous fibrosis, PPI: protein-protein interaction, GO: Gene Ontology, KEGG: Kyoto Encyclopedia of Genes and Genomes, MCODE: molecular complex detection).

2.2. Retrieval of potential targets of Salvia miltiorrhiza

The following three databases were used to collect potential physiological targets of *Salvia miltiorrhiza*: (1) TCM Systems Pharmacology Database and Analysis Platform (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php) (Ru et al., 2014), (2) Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM, http://bionet.ncpsb.org/batman-tcm) (Liu et al., 2016), and (3) Swiss Target Prediction (http://www.swisstargetprediction.ch/) (Daina et al., 2019).

2.3. Retrieval of potential OSF targets

The following databases provided the possible OSF gene targets: (1) DisGeNET (http://www.disgenet.org/home/) (Piñero et al., 2015), (2) GeneCards (http://www.genecards.org/) (Safran et al., 2010), and (3) The NCBI (National Center for Biotechnology Information; https://www.ncbi.nlm.nih.gov/gene/). The protein ID type was converted to UniProt IDs for standardization.

2.4. Network construction

To examine and interpret the complex interactions between compounds, herbs, genes, and diseases (Li et al., 2014; Li & Zhang, 2013), a network analysis was carried out. The networks were generated using Cytoscape (version 3.7.2) (Otasek et al., 2019; Shannon et al., 2003). The "*Salvia miltiorrhiza* against OSF targets protein-protein interaction network" was established by linking the targets of the active ingredients in *Salvia miltiorrhiza* to the OSF targets that interacted with them.

2.5. Enrichment analysis

Metascape (http://metascape.org) is an applicable tool for performing gene annotation, as well as gene list enrichment analysis (Zhou et al., 2019). The program can assist in making informed decisions on the basis of functional gene annotation/protein provisional lists. Here, we employed Metascape to perform the pathway, as well as process enrichment analysis of similar genes that were targeted. Using the Metascape online tool, the GO (Gene Ontology) domains which include biological process, cellular components, and molecular function categories, and the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways were enriched. Terms that exhibited a minimum overlap of 3 (p-value cutoff 0.05) and a minimum enrichment of 3 were regarded as significant. The following databases were utilized to conduct protein-protein interaction enrichment analysis: InWeb_IM, OmniPath, and BioGrid. The network components that were closely connected were identified by the molecular complex detection algorithm.

3. Results

3.1. Identification of Salvia miltiorrhiza active ingredients

After ADME identification, we found 19 *Salvia miltiorrhiza* active ingredients, 7 of which were unrelated targets. Twelve active ingredients were identified as related targets of *Salvia miltiorrhiza* (Table 1). All the Mol IDs can be tracked in the TCMSP database.

3.2. Identification of Salvia miltiorrhiza active ingredient targets against OSF

After standardization of the microarray results, 559 *Salvia miltiorrhiza* targets and 480 OSF targets were identified. The overlap among the two datasets consisted of 57 targets.

Table 1
Active ingredients of *Salvia miltiorrhiza*.

Mol ID	Molecule Name	OB (%)	DL
MOL007050	2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	62.78	0.40
MOL007058	formyltanshinone	73.44	0.42
MOL007064	przewalskin b	110.32	0.44
MOL007069	przewaquinone c	55.74	0.40
MOL007081	Danshenol B	57.95	0.56
MOL007082	Danshenol A	56.97	0.52
MOL007088	cryptotanshinone	52.34	0.40
MOL007101	dihydrotanshinoneI	45.04	0.36
MOL007108	isocryptotanshi-none	54.98	0.39
MOL007132	(2R)-3-(3,4-dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl) acryloyl] oxy-propionic acid	109.38	0.35
MOL007154	tanshinone IIa	49.89	0.40
MOL007155	(6S)-6-(hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g] benzofuran-10,11-dione	65.26	0.45

Abbreviations: Mol: molecular; OB: oral bio-availability; DL: drug-likeness.

3.3. Network construction of Salvia miltiorrhiza active ingredient targets against OSF

To reveal the mechanisms underlying the efficacy of *Salvia miltiorrhiza* as OSF therapy, a protein-protein interaction network of the *Salvia miltiorrhiza* active ingredient targets against OSF was established. The *Salvia miltiorrhiza* active ingredient targets and OSF targets were connected in Cytoscape. This protein-protein interaction network comprised 69 nodes with a network diameter of five and an average degree of 5.014 (Fig. 2A). Also, seven target degrees were above average: aromatase (CYP19A1), epidermal growth factor receptor (EGFR), tyrosine-protein phosphatase non-receptor type 11 (PTPN11), acetylcholinesterase (ACHE), telomerase reverse transcriptase (TERT), mitogen-activated protein kinase 8 (MAPK8), and progesterone receptor (PGR). All 12 active ingredients of *Salvia miltiorrhiza* degrees were greater than the average.

The following 8 active ingredients had degrees greater than 13: 2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde, przewalskin b, Danshenol B, (2R)-3-(3,4-dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl)acryloyl]oxy-propionic acid, Danshenol A, formyltanshinone, tanshinone IIa, and dihydrotanshinone I. A protein-protein interaction network was constructed by linking the *Salvia miltiorrhiza* active ingredient targets and the seven target degrees that were above the average (Fig. 2B).

3.4. Functional enrichment analysis of Salvia miltiorrhiza active ingredient targets against OSF

Functional enrichment analysis of *Salvia miltiorrhiza* active ingredient targets against OSF was conducted by analyzing GO and KEGG in Metascape. We categorized the top 20 GO and KEGG enrichment items into two functional groups: GO biological process group (16 items) and KEGG pathways (4 items) (Fig. 3A, B and Table 2) (Bader & Hogue, 2003; Hochberg & Benjamini, 1990; Li et al., 2017; Stark et al., 2006). The *Salvia miltiorrhiza* active ingredient targets against OSF were mainly enriched in cellular response, cellular signaling pathways, developmental pathways (e.g., cellular response to drugs), response to metal ions, cytokine-mediated signaling pathways, apoptotic signaling, gland development, and heart development. The main KEGG pathways for the *Salvia miltiorrhiza* active ingredient targets against OSF were bladder cancer, cancer pathways, central carbon metabolism in cancer, and the interleukin (IL)-17 signaling pathway.

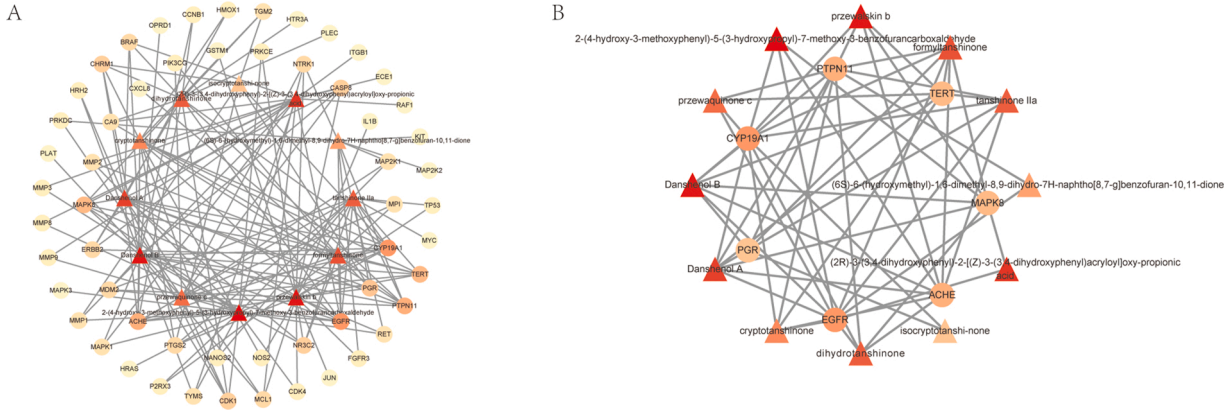


Fig. 2. *Salvia miltiorrhiza* ingredient–target network. Triangle represents the *Salvia miltiorrhiza* ingredients and ellipse represents the targets. The network of all *Salvia miltiorrhiza* active ingredients targets against OSF (A). The network of *Salvia miltiorrhiza* active ingredients targets and 7 targets degrees greater than the average (B).

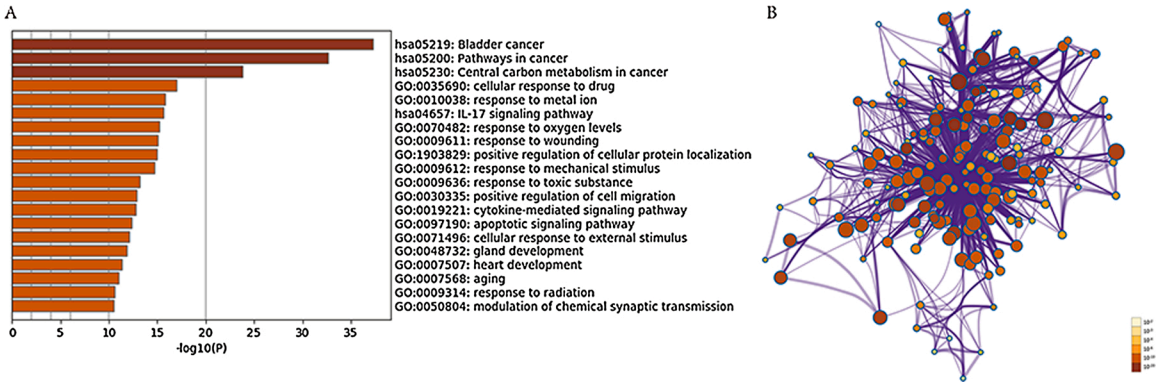


Fig. 3. Functional enrichment analysis of *Salvia miltiorrhiza* active ingredients targets against OSF. Heatmap of GO enriched terms colored by p-values (A). The network of GO enriched terms colored by p-values (B).

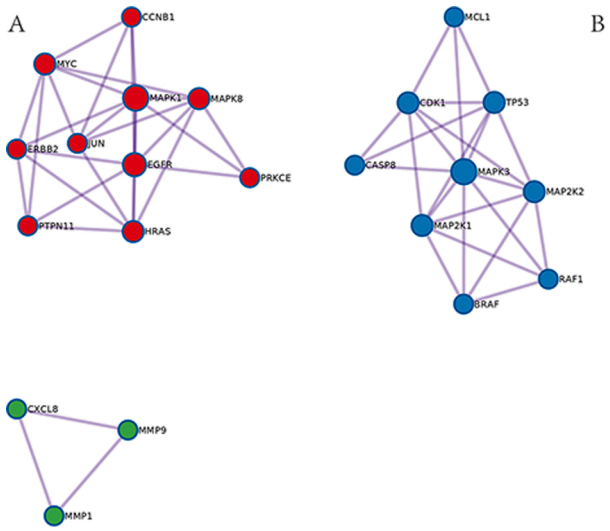
Table 2
The GO and KEGG function enrichment analysis of targets.

GO	Category	Description	Count	%	Log10(P)	Log10(q)
GO:0035690	GO Biological Processes	cellular response to drug	17	29.82	−16.99	−13.93
GO:0010038	GO Biological Processes	response to metal ion	16	28.07	−15.79	−12.88
GO:0070482	GO Biological Processes	response to oxygen levels	16	28.07	−15.23	−12.41
GO:0009611	GO Biological Processes	response to wounding	19	33.33	−15.07	−12.26
GO:1903829	GO Biological Processes	positive regulation of cellular protein localization	15	26.32	−14.97	−12.18
GO:0009612	GO Biological Processes	response to mechanical stimulus	13	22.81	−14.71	−11.95
GO:0009636	GO Biological Processes	response to toxic substance	16	28.07	−13.20	−10.61
GO:0030335	GO Biological Processes	positive regulation of cell migration	16	28.07	−12.87	−10.31
GO:0019221	GO Biological Processes	cytokine-mediated signaling pathway	18	31.58	−12.79	−10.24
GO:0097190	GO Biological Processes	apoptotic signaling pathway	16	28.07	−12.33	−9.81
GO:0071496	GO Biological Processes	cellular response to external stimulus	13	22.81	−12.09	−9.59
GO:0048732	GO Biological Processes	gland development	14	24.56	−11.84	−9.38
GO:0007507	GO Biological Processes	heart development	15	26.32	−11.34	−8.94
GO:0007568	GO Biological Processes	aging	12	21.05	−11.02	−8.67
GO:0009314	GO Biological Processes	response to radiation	13	22.81	−10.58	−8.26
GO:0050804	GO Biological Processes	modulation of chemical synaptic transmission	13	22.81	−10.50	−8.18
hsa05219	KEGG Pathway	Bladder cancer	18	31.58	−37.30	−32.94
hsa05200	KEGG Pathway	Pathways in cancer	27	47.37	−32.67	−28.61
hsa05230	KEGG Pathway	Central carbon metabolism in cancer	14	24.56	−23.79	−20.03
hsa04657	KEGG Pathway	IL-17 signaling pathway	11	19.30	−15.62	−12.73

Abbreviations: GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

3.5. Protein-protein interaction network analysis of Salvia miltiorrhiza active ingredient targets against OSF

To better understand the relationship of *Salvia miltiorrhiza* active ingredient targets against OSF, a Metascape protein-protein interaction enrichment analysis was performed. The protein-protein interaction network and the molecular complex detection components that were identified in the gene lists are presented in Fig. 4. The three most significant molecular complex detection components were extracted from the protein-protein interaction network. After independently applying the pathway and process enrichment analysis to each molecular complex detection component, we found that the KEGG pathway was largely



B

Color	MCODE	GO	Description	Log10(P)
	MCODE_1	hsa04012	ErbB signaling pathway	-15.2
	MCODE_1	hsa01522	Endocrine resistance	-12.2
	MCODE_1	hsa05219	Bladder cancer	-11.6
	MCODE_2	hsa05219	Bladder cancer	-14.9
	MCODE_2	hsa05213	Endometrial cancer	-14.3
	MCODE_2	hsa04210	Apoptosis	-14.2
	MCODE_3	hsa05219	Bladder cancer	-8.3
	MCODE_3	hsa04657	IL-17 signaling pathway	-7.3
	MCODE_3	hsa05200	Pathways in cancer	-5.4

Fig. 4. Protein-protein interaction network analysis of *Salvia miltiorrhiza* active ingredients targets against OSF. Molecular complex detection components form the protein-protein interaction network (A). Independent functional enrichment analysis of four molecular complex detection components (B).

associated with the ErbB signaling pathway, endocrine resistance, bladder cancer, endometrial cancer, apoptosis, IL-17 signaling, and cancer pathways.

4. Discussion

Although studies have confirmed that some *Salvia miltiorrhiza* active ingredients have inhibitory effects on OSF, the potential mechanisms have not been fully explored. To elucidate further the pharmacological mechanisms of *Salvia miltiorrhiza* as a therapy against OSF, a network pharmacology approach was applied. Network pharmacology integrates bioinformatics, pharmacology, and systems biology to interpret the complex interactions between herbs and diseases at a systematic level, while also conforming to the holistic and systematic view of the TCM theory. In this study, ingredient–target network construction, GO enrichment analysis, KEGG enrichment analysis, and protein-protein interaction network analysis were used. Oral bioavailability and drug-likeness were the chosen parameters to explore the potential bioactive ingredients of *Salvia miltiorrhiza*.

At present, the pharmacokinetic data on *Salvia miltiorrhiza* in OSF are insufficient. Dai et al. (2015) found that tanshinone IIa could inhibit abnormal collagen accumulation in mice oral mucosal fibroblasts, which were induced by areca nut extract, indicating that tanshinone IIa possesses anti-fibrotic activity *in vitro*. Zheng et al. (2018) also reported that tanshinone treatment can inhibit the arecoline-induced proliferation and epithelial-mesenchymal transformation of human oral mucosal fibroblasts. Furthermore, Wu et al. (2010) speculated that *Salvia miltiorrhiza* is effective in treating OSF by improving microcirculation and resisting fibrillation. They proposed that the possible mechanisms behind *Salvia miltiorrhiza* were: (1) removal of oxygen free radicals and restoration of morphology and function of damaged endothelial cells; (2) increase microvessel blood flow to increase oxygen supply; (3) reduce blood viscosity and inhibit blood platelet activation to reduce platelet adhesion and aggregation, which prevents blood clot formation; and (4) improve the spasmodic state of microvessels, increase the speed of blood flow, and increase the number of opening capillaries. Studies have shown that the up-regulation of hypoxia inducible factor-1α in OSF may promote the development of fibrosis and malignant transformation (Tilakaratne & Nissanka-Jayasuriya, 2011; Tilakaratne et al., 2008). Hypoxia is considered to be one of the main causes of malignant transformation of OSF, and *Salvia miltiorrhiza* may counteract this effect (Tilakaratne & Nissanka-Jayasuriya, 2011; Tilakaratne et al., 2008).

In total, 12 *Salvia miltiorrhiza* active ingredients and 57 potential OSF targets were identified. Together, the targets revealed a synergistic herb strategy that featured multi-ingredient, multi-target, and multi-pathway features. TERT mutations were identified in pulmonary fibrosis and affected survival (Borie et al., 2016; van der Vis et al., 2020). Seven of the key targets demonstrated EGFR and TERT over-expression in OSF and were involved in malignant transformation (Jyothi Meka et al., 2015; Raju et al., 2020). Down regulation of PTPN11 could lead to spontaneous pulmonary fibrosis and cardiac interstitial fibrosis (Schramm et al., 2012; Zhang et al., 2012). Studies have shown that MAPK8 signal promoted hepatic fibrosis, renal fibrosis and pulmonary fibrosis (Alcorn et al., 2009; Kodama et al., 2009; Ma et al., 2007). Though some targets involved in the fibrosis were identified, the roles of CYP19A1, PTPN11, ACHE, MAPK8, and PGR in OSF have not been studied and their molecular and signal mechanisms in the pathogenesis of OSF were still misty. Another mechanism that might contribute to the malignant transformation of OSF is the secretion of cytokines by fibroblasts stimulated by the areca nut (Illeperuma et al., 2015). As a result, cytokine-mediated reactive oxygen species generation and oxidative DNA damage occur. Studies have shown that senescent fibroblasts accumulate through a telomere independent mechanism involving reactive oxygen species (Pitiyage et al., 2011). Up-regulation of many inflammatory cytokines such as tissue inhibitors of metalloproteinases may be related to the early aging of fibroblasts in OSF (Pitiyage et al., 2012). And IL-17 signal was involved in systemic sclerosis, liver fibrosis, skin and lung fibrosis (Lei et al., 2016; Park et al., 2018; Wree et al., 2018). Arecoline also induced fibroblasts to produce inflammatory cytokines and change $CD4^+$ $IL\text{-}17^+$ helper T cells (Wang et al., 2020). These were consistent with the findings of GO enrichment analysis and KEGG enrichment analysis. The *Salvia miltiorrhiza* active ingredients might against OSF by targeting cytokine-mediated signaling pathways, apoptosis and IL-17 signaling.

This study utilized a network approach to document how *Salvia miltiorrhiza* active ingredients change various pathways against OSF; thus, it supplements other studies on OSF therapy. Moreover, it was revealed that *Salvia miltiorrhiza* considerably influenced many OSF-related targets. This is consistent with current research trends showing that OSF is associated with epigenetic networks.

These results will help promote the use of a network pharmacology approach to define potential mechanisms behind *Salvia miltiorrhiza* treatment in OSF. They also offer new insights into the understanding of the synergy of *Salvia miltiorrhiza* in the treatment of other complex diseases. However, there are limits to this study. Considering that this study was based on data analysis, there is a need to conduct additional

experiments to verify these findings. In addition, although published studies have rarely reported *Salvia miltiorrhiza* cytotoxicity, additional experiments should be performed to confirm its potential cytotoxicity. Meanwhile, there is a need to conduct more pharmacokinetic research to reveal the properties of *Salvia miltiorrhiza* against OSF.

5. Conclusions

In this study, twelve active ingredients were identified as related targets of *Salvia miltiorrhiza* against OSF. And 57 molecular targets were screened; seven of them were above average: *CYP19A1*, *EGFR*, *PTPN11*, *ACHE*, *TERT*, *MAPK8*, and *PGR*. The *Salvia miltiorrhiza* active ingredient targets against OSF were mainly enriched in cellular response, cellular signaling pathways, developmental pathways, response to metal ions, cytokine-mediated signaling pathways, apoptotic signaling, gland development, and heart development. The KEGG pathway was largely enriched in the ErbB signaling pathway, endocrine resistance, bladder cancer, endometrial cancer, apoptosis, IL-17 signaling, and cancer pathways.

Author contributions

Li Tiejun, Zhang Heyu and Cai Xinjia designed the study. Cai Xinjia analyzed the data, and wrote the draft of the manuscript. Li Tiejun and Zhang Heyu obtained copies of studies and revised the writing. All authors read and approved the submitted version.

Declaration of Competing Interest

All authors declared that there were no conflicts of interest with the contents of this article.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.archoralbio.2021.105131.

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