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Prediction of active ingredients and mechanism of Siwei Jianbu decoction in the treatment of atherosclerosis by network pharmacology

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Abstract. – OBJECTIVE: Siwei Jianbu Decoction (SJD) has been shown to be effective in treating atherosclerosis (AS). However, its mechanism is still unclear.

MATERIALS AND METHODS: The active compounds and targets of SJD were identified from the Traditional Chinese Medicine System Pharmacology (TCMSP) database. The target genes of AS were obtained from the Online Mendelian Inheritance in Man (OMIM), GeneCards, DrugBank, and Therapeutic Target (TTD) databases. Interactions between drug and disease targets were analyzed to obtain common targets. Subsequently, "herb-compound-target" and protein-protein interaction (PPI) networks were constructed and analyzed using the Cytoscape software. Thereafter, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed by DAVID online database. Then, the results were visualized by R software. Finally, molecular docking was performed using AutoDockTools and PyMOL software.

RESULTS: A total of 61 active compounds and 377 target genes were identified for SJD, as well as 726 target genes for AS. Interactive analyses revealed 126 common genes between SJD and AS. Quercetin, ellagic acid, baicalein, and kaempferol were the 4 key compounds in SJD. Moreover, eight key targets, namely TNF, SRC, RELA, AKT1, STAT3, JUN, MAPK1 and FOS were found. Results from enrichment analysis indicated that the MAPK pathway may play an important role. The analysis of molecular docking revealed that the key compounds formed strong bonds with their corresponding key targets.

CONCLUSIONS: These findings indicate that SJD could prevent AS by inhibiting the expression of genes associated with MAPK pathway such as MAPK1, RELA, and FOS.

Key Words:
Atherosclerosis, MAPK signaling pathway, Network pharmacology, Siwei Jianbu Decoction, Traditional Chinese medicine.

Introduction

Atherosclerosis (AS) is a multifactorial disease characterized by elevated lipid accumulation, proliferation of smooth muscle cells, and local inflammation[1]. Clinically, it may manifest as ischemic heart disease (IHD), ischemic stroke, and peripheral arterial disease (PAD)[2]. Several research has been conducted on AS because it is a major risk of cardiovascular disease (CVD), which is a leading cause of morbidity and mortality[3]. Several studies[4,5] have shown that hypoglycemic and lipid lowering agents may play an important role in the prevention and treatment of AS. The disease has a complex pathogenesis mechanism which makes it difficult to develop effective therapy. Traditional Chinese medicines (TCMs), which contain multiple compounds and targets, have been used to treat diseases[6]. Consequently, TCMs are used in the management of diseases in China[7,8].

A previous study[9] has shown that network pharmacology, which integrates system-level network analysis and pharmacology, can reveal the complex mechanism of Chinese medicinal formula in various diseases. In recent years, it has become a powerful tool for identifying the function and behavior of a biological system. It is used to explore the pharmacological mechanism of TCMs[10]. This study aimed to elucidate the mech-

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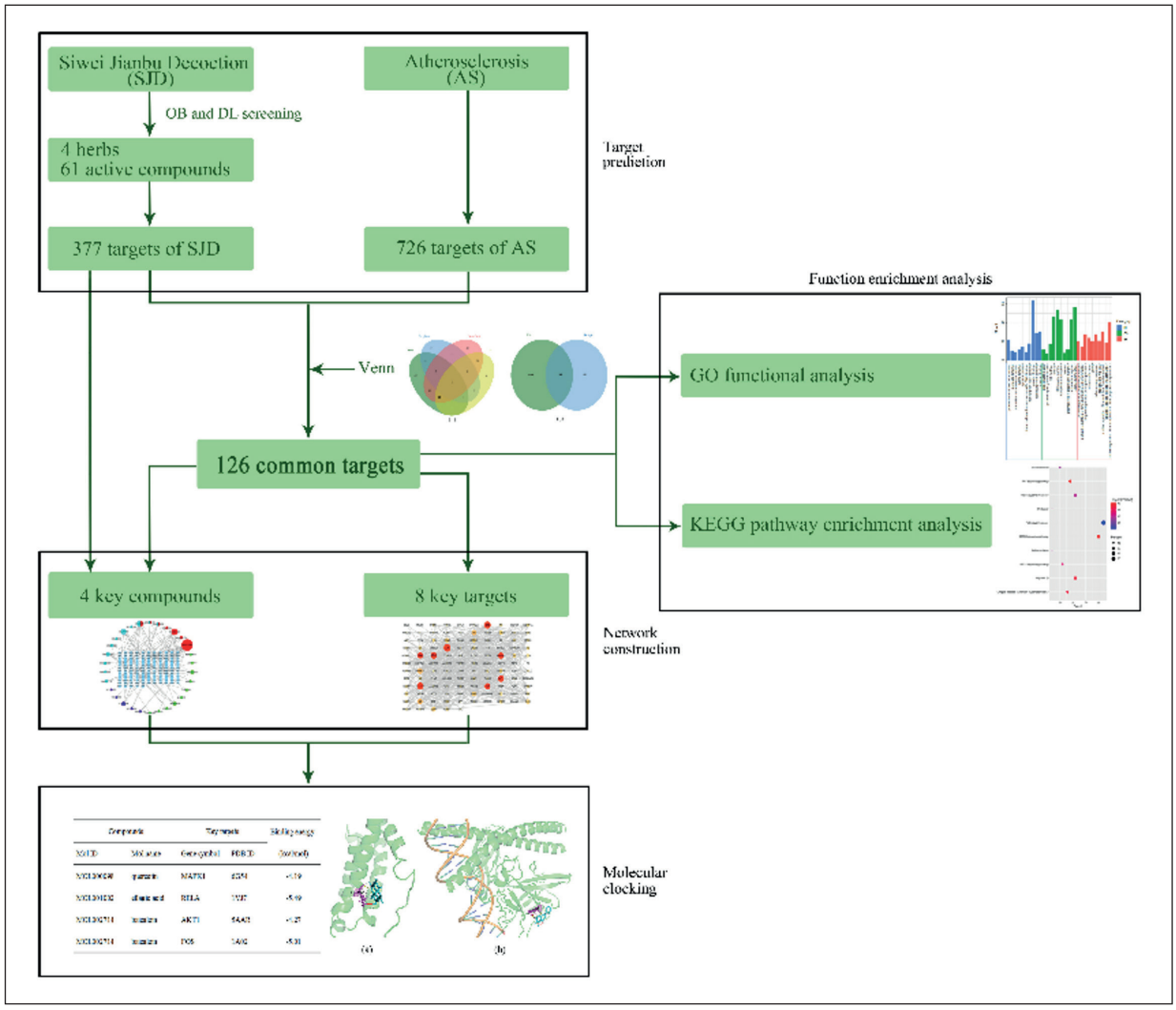

Figure 1. Flowchart of SJD in treating AS.

anism of SJD in the treatment of AS using network pharmacology and molecular docking technologies. The findings of our study are expected to provide valuable insights to guide further research and clinical applications of SJD. The study workflow is shown in Figure 1.

Materials and Methods

Screening for Active Compounds and Corresponding Targets in SJD

The Traditional Chinese Medicine System Pharmacology Database (TCMSP, https://old.tcmsp-e.com/index.php, version: 2.3) is a pharmacology database which provides information about active herb components and their corresponding targets[11]. In the present study, TCMSP database was searched to identify active compounds of SJD. Oral bioavailability (OB) is defined as the rate and percentage of pharmaceutical agents orally absorbed into the systemic circulation[12]. Drug-likeness (DL), a concept based on the physical and chemical properties of existing drugs, is often used to estimate whether compounds have reached the threshold to be considered a new drug[12]. Active compounds of SJD were screened using the following criteria: OB≥30% and DL≥0.18. Next, we screened the corresponding target genes of active compounds from the TCMSP database. The "Reviewed" and "Human organisms" terms were used as filters. Gene symbols of the targets were downloaded from UniProt (https://www.uniprot.org/)[13]. Active compounds of all herbs which could not be identified in the TCMSP database were obtained from relevant literature. The structural data of these

herbs were uploaded into the SwissTargetPrediction online tool (http://www.swisstargetprediction.ch/) to screen for their corresponding targets[14]. All compounds whose corresponding targets could not be found were excluded from our analysis.

Screening for AS Targets

To obtain AS targets, we searched Online Mendelian Inheritance in Man (OMIM) (https://omim.org/)[15], GeneCards (https://www.genecards.org/, version: 5.6)[16], Therapeutic Target Database (TTD) (http://db.idrblab.net/ttd/)[17], and DrugBank (https://go.drugbank.com/)[18] databases, using "atherosclerosis", "AS", and "ASO" as keywords. The disease targets retrieved from different databases were integrated, and duplicate records were removed to obtain candidate AS targets.

Construction of "Herb-Compound-Target" Network and Screening for Key Compounds

The Jvenn online tool (http://jvenn.toulouse.inra.fr/app/index.html)[19] was used to identify common target genes between SJD and AS. All active compounds associated with these common targets were also identified. The common targets and their corresponding active compounds were uploaded into the Cytoscape software version 3.7.2 to construct a "Herb-Compound-Target" network[20]. The relationships between common targets and their corresponding active compounds were then visualized. Nodes in the network represented compounds and targets, whereas edges indicated interactions between them[21]. Thereafter, we selected median of Betweenness centrality (BC), closeness centrality (CC) and Degree centrality (DC) as the criteria for identifying key compounds. Those with BC, CC and DC simultaneously greater than the median value were considered as key compounds.

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

All common target genes were uploaded onto the STRING online tool (https://string-db.org/, version 11.5), a data platform that hosts almost all known and predicted interactions between different proteins[22]. The species parameter was set as "*Homo sapiens*," while the confidence score was set to 0.9. Thereafter, a PPI network was constructed after hiding disconnected nodes, which was then visualized and analyzed by Cytoscape software version 3.7.2. Key targets in the PPI network were identified using the "Analyze network" tool of Cytoscape. In this process, BC, CC, and DC were used as parameters for calculating topological features in the network. Median of BC and CC, 4 folds of the median of DC were set as the threshold for filtering central nodes, and screening for key targets[23].

Enrichment Analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/summary.jsp, Version 6.8) is an online database that provides systematic, comprehensive bio-functional annotation information for large-scale genes or proteins for identification of most significantly enriched biological annotations[24]. To further explore the underlying mechanism of SJD in AS, we uploaded the identified common targets to DAVID for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. The top 10 terms for biological process (BP), molecular function (MF), cellular component (CC), and KEGG pathways were selected and visualized in R.

Molecular Docking

The binding capability of key compounds with their targets was evaluated via molecular docking. Briefly, 2-dimensional (2D) and 3-dimensional (3D) structures of the key compounds were acquired from ZINC (http://zinc.docking.org/)[25] and PDB (http://www.rcsb.org/)[26] databases, respectively. Next, we employed AutoDockTools software to convert the file of key compounds and related targets into "pdbqt" format and define the location of their "Grid Box". Finally, the AutoDockTools and PyMOL software were used to perform molecular docking between key compounds and their targets. A binding energy of less than 0 kcal/mol implies that a ligand can spontaneously bind to a protein receptor, whereas a binding energy <-5.0 kcal/mol implies good binding ability[27].

Results

Identification of Active Compounds in SJD

A total of 61 active compounds in 4 herbs of SJD (Chishao, Shihu, Niuxi, and Danshen) were acquired from the TCMSP database. Analysis of these herbs revealed that Chishao and Niuxi had three common active compounds, whereas Niuxi and Danshen had one common active compound. Details of the 61 active compounds are shown in Table I.

Table I. Characteristics of 61 active compounds identified from the TCMSP database.

Mol ID/CAS	Mol name	OB (%)	DL	Herb
MOL001002	ellagic acid	43.06	0.43	Chishao
MOL001918	paeoniflorgenone	87.59	0.37	Chishao
MOL001924	paeoniflorin	53.87	0.79	Chishao
MOL001925	paeoniflorin_qt	68.18	0.40	Chishao
MOL002714*	baicalein	33.52	0.21	Chishao
MOL000358*	beta-sitosterol	36.91	0.75	Chishao
MOL000449*	Stigmasterol	43.83	0.76	Chishao
MOL000359	sitosterol	36.91	0.75	Chishao
MOL000492	(+)-catechin	54.83	0.24	Chishao
MOL006992	(2R,3R)-4-methoxyl-distylin	59.98	0.30	Chishao
MOL006994	1-o-beta-d-glucopyranosyl-8-o-benzoylpaeonisuffrone_qt	36.01	0.3	Chishao
MOL007004	Albiflorin	30.25	0.77	Chishao
MOL007016	Paeoniflorigenone	65.33	0.37	Chishao
2115-91-5	Dendrobine	-	-	Shihu
156951-82-5	Chrysotoxine	-	-	Shihu
108853-09-4	Chrysotobibenzyl	-	-	Shihu
95041-90-0	Erianin	-	-	Shihu
108853-14-1	Moscatilin	-	-	Shihu
67884-30-4	Gigantol	-	-	Shihu
53076-61-2	Dehydroorchinol-5,7-dimethoxyphenanthren-2-ol	-	-	Shihu
135545-86-7	Ephemeranthol A	-	-	Shihu
82526-36-1	Denbinobin	-	-	Shihu
8031-72-9	Cypripedin	-	-	Shihu
MOL010161	Confusarin	-	-	Shihu
86630-46-8	Nudol	-	-	Shihu
97915-34-9	Dendroflorin	-	-	Shihu
MOL001543	Vicenin II	-	-	Shihu
886747-60-0	Phoyunnanin E	-	-	Shihu
MOL000431	Coumarin	-	-	Shihu
MOL010245	Flavone	-	-	Shihu
MOL000098	quercetin	46.43	0.28	Niuxi
MOL000173	wogonin	30.68	0.23	Niuxi
MOL000358*	beta-sitosterol	36.91	0.75	Niuxi
MOL000422	kaempferol	41.88	0.24	Niuxi
MOL000449*	Stigmasterol	43.83	0.76	Niuxi
MOL001006	poriferasta-7,22E-dien-3beta-ol	42.98	0.76	Niuxi
MOL001454	berberine	36.86	0.78	Niuxi
MOL002714*	baicalein	33.52	0.21	Niuxi
MOL002776**	Baicalin	40.12	0.75	Niuxi
MOL003847	Inophyllum E	38.81	0.85	Niuxi
MOL000006	luteolin	36.16	0.25	Danshen
MOL000569	digallate	61.85	0.26	Danshen
MOL001601	1,2,5,6-tetrahydrotanshinone	38.75	0.36	Danshen
MOL001659	Poriferasterol	43.83	0.76	Danshen
MOL002222	sugiol	36.11	0.28	Danshen
MOL002651	Dehydrotanshinone II A	43.76	0.40	Danshen
MOL002776**	Baicalin	40.12	0.75	Danshen
MOL007036	5,6-dihydroxy-7-isopropyl-1,1-dimethyl-2,3-dihydrophenanthren-4-one	33.77	0.29	Danshen
MOL007041	2-isopropyl-8-methylphenanthrene-3,4-dione	40.86	0.23	Danshen
MOL007049	4-methylenemiltirone	34.35	0.23	Danshen
MOL007050	2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	62.78	0.40	Danshen
MOL007063	przewalskin a	37.11	0.65	Danshen
MOL007082	Danshenol A	56.97	0.52	Danshen
MOL007088	cryptotanshinone	52.34	0.40	Danshen
MOL007093	dan-shexinkum d	38.88	0.55	Danshen
MOL007094	danshenspiroketallactone	50.43	0.31	Danshen
MOL007119	miltionone I	49.68	0.32	Danshen
MOL007122	Miltirone	38.76	0.25	Danshen
MOL007142	salvianolic acid j	43.38	0.72	Danshen
MOL007145	salviolone	31.72	0.24	Danshen
MOL007154	tanshinoneiia	49.89	0.40	Danshen

Common compounds between Chishao and Niuxi; **: Common compounds between Niuxi and Danshen. OB: Oral bioavailability. DL: Drug-likeness.

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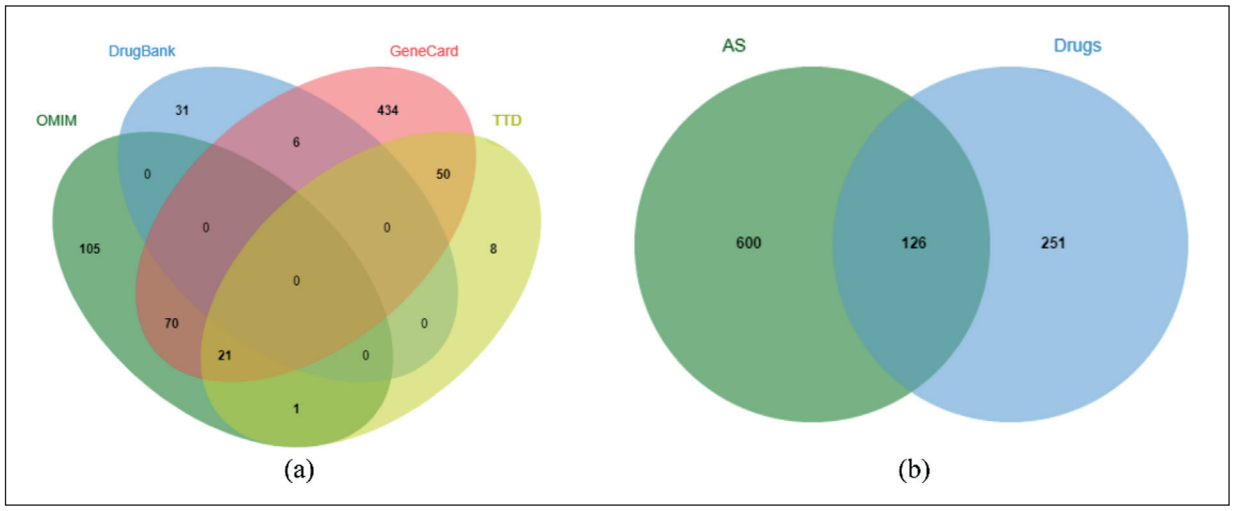

Figure 2. Venn diagrams showing the intersection of (**a**), Disease targets of AS. (**b**), Common targets between SJD and AS.

Target Prediction of Herbs and Diseases

For Chishao, Shihu, Niuxi, and Danshen, the number of identified targets of active compounds was 131, 115, 209, and 133, respectively. After removal of duplicates, 377 targets of active compounds in SJD were obtained. For the disease category, 80, 37, 581 and 197 AS-related targets were identified in TTD, DrugBank, GeneCards, and OMIM, respectively. A total of 726 therapeutic target of AS were identified after removal of duplicates (Figure 2a). Results of intersection analysis revealed that SJD and AS had 126 common targets (Figure 2b), with a corresponding 30 active compounds in SJD.

Construction of "Herb-Compound-Target" Network

A "herb-compound-target" network, including 156 nodes (30 active compounds and 126 common targets) and 126 edges, was constructed using Cytoscape 3.7.2 (Figure 3). Network analysis, using the "Analyze network" tool in Cytoscape, revealed four key compounds (out of the 30 active ones), namely MOL000098 (quercetin, degree=29), MOL000422 (kaempferol, degree=13), MOL002714 (baicalein, degree=13), and MOL001002 (ellagic acid, degree=12). These results indicated that SJD may prevent AS through these compounds. A summary of the relationships among herbs, active compounds and common targets is shown in Figure 3.

Construction of PPI Network

To further elucidate the underlying mechanisms of SJD in the treatment of AS, a PPI network was constructed which comprised the 126 common targets, which were then visualized and analyzed using the "Analyze network" tool in Cytoscape 3.7.2. The network contained 115 nodes and 564 edges (Figure 4). Analysis of topological features of the network, using the median of BC and CC, 4 folds of the median of DC (BC $\geq$ 0.0003, CC $\geq$ 0.2990, and DC $\geq$ 14), revealed several key targets, including TNF, SRC, RELA, AKT1, STAT3, JUN, MAPK1, and FOS (Figure 4).

GO Terms and Pathway Enrichment Analysis

The 126 common targets were subjected to GO functional and KEGG pathway enrichment analyses. Results showed that 769 GO terms were significantly enriched, among which 604, 116, and 49 were enriched in biological process (BP), molecular function (MF), and cellular component (CC), respectively. The top 10 terms of BP, MF and CC are illustrated in Figure 5a. BP terms were mainly involved in positive regulation of RNA polymerase-2 promoter, negative regulation of apoptotic process, inflammatory response, and positive regulation of proliferation of smooth muscle cells. On the other hand, CC terms were mainly enriched in plasma membrane, extracellular exosome, extracellular region, and extracellular space, whereas MF terms were mainly involved in protein binding, enzyme binding, and protein homodimerization activity. Moreover, KEGG pathway analysis revealed enrichment of 115 pathways. The top 10 enriched KEGG pathways are shown in Figure 5b. Potential SJD targets in treating AS were principally enriched in pathways regulating MAPK, cancer and TNF signaling.

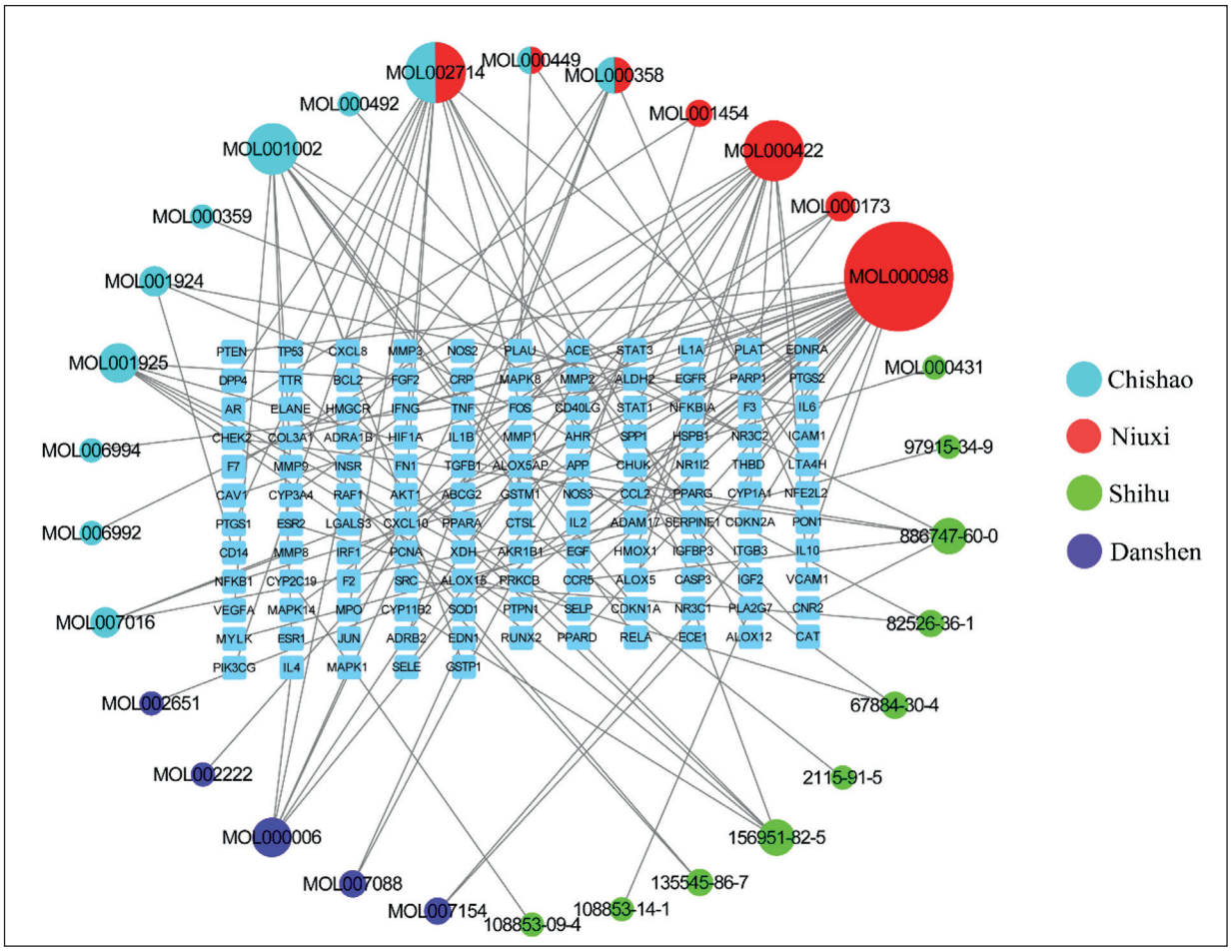

Figure 3. "Herb-compound-target" network. The circular nodes indicate active compounds, and the square nodes indicate the possible therapeutic targets in SJD. Different colors of the circular nodes indicate active compounds for different herbs.

Molecular Docking

To validate the results from network analysis, we performed molecular docking between the 4 key compounds (MOL000098, MOL000422, MOL001002 and MOL002714) and their corresponding key targets. Docking simulations revealed four pairs of docking results (Table II). In summary, the binding energy of two pairs (MOL002714 and FOS, MOL000098 and MAPK1) was less than -5.0 kcal/mol, indicating that they could bind tightly. Molecular docking results for the 2 key compounds (MOL002714 and MOL000098) and their corresponding key targets are shown in Figure 6. More information about molecular docking is shown in Table II. Collectively, molecular docking results indicated that a combination of these targets may play an essential role in the treatment of AS.

Discussion

AS is a common disease that negatively affects the health and quality of life in old patients. Although SJD has been shown to be a potential adjuvant or alternative medicine for the prevention of AS[28,29], its underlying mechanism of action is unclear. Previous studies have shown that network pharmacology, a new approach in drug discovery, can reveal the interactions among herbs, diseases, and targets[30,31]. Moreover, the characteristics of this tool are consistent with the theory of "multi-compounds, multi-targets, and multi-pathways" of TCMs.

Screening of TCMSP database identified 377 and 726 targets of SJD and AS, respectively, while results from the Venn diagram revealed 126 common targets between them. Moreover,

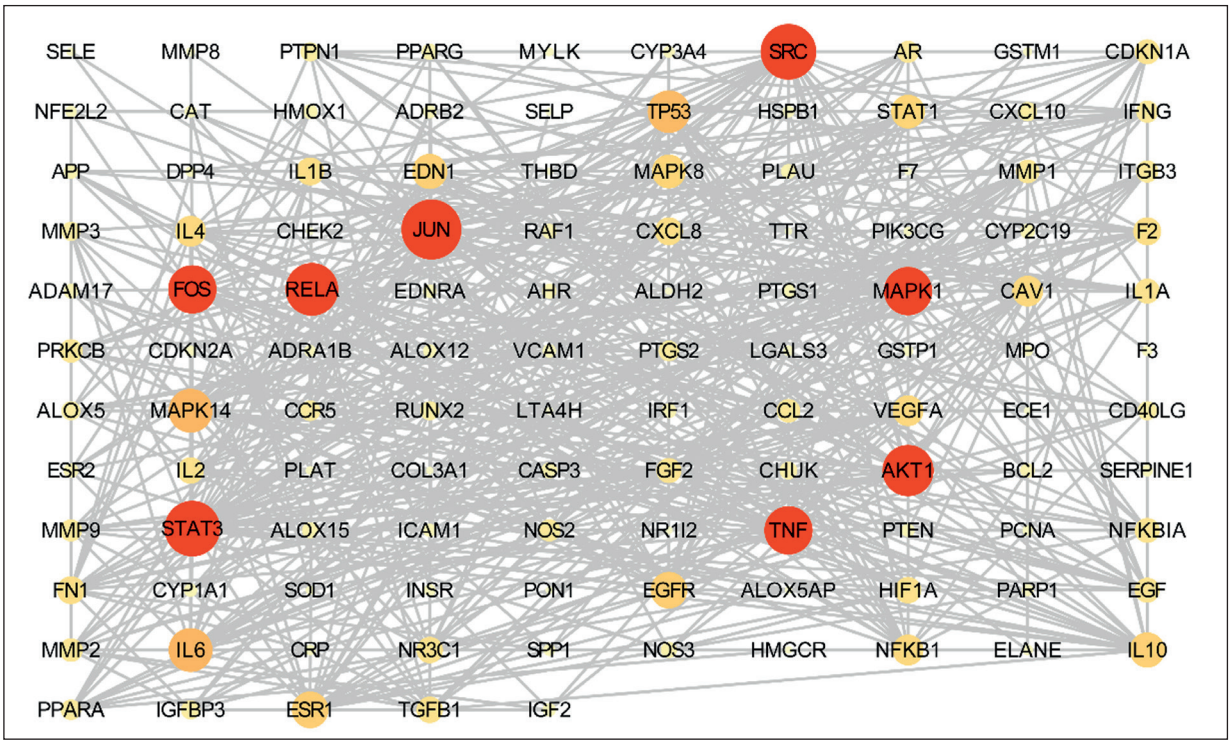

Figure 4. PPI network among common targets between SJD and AS. Red circular nodes indicate the key targets of PPI network.

analysis of the constructed network revealed four key compounds, namely quercetin, kaempferol, baicalein, and ellagic acid, and 8 key targets, namely TNF, SRC, RELA, AKT1, STAT3, JUN, MAPK1 and FOS. Furthermore, results of KEGG pathway enrichment analysis indicated that the MAPK signaling pathway may play an important role. Moreover, five of the eight key targets (TNF, RELA, JUN, MAPK1, and FOS) were involved in MAPK signaling pathway. Molecular docking analysis indicated that RELA, MAPK1, and FOS can bind to their corresponding key compounds (ellagic acid, quercetin, baicalein) strongly. Taken together, the results of present study suggested that SJD may exert anti-AS effect by inhibiting expression of relative genes regulating MAPK signaling pathway.

MAPK signaling pathway plays a key role in the regulation of gene expression and cellular activities. Previous studies have demonstrated that this pathway also promotes endothelial proliferation and neovascularization, accelerates tumor growth and enhances metastasis of tumor cells. Overall, this pathway may play important roles in many systems of human body[32]. Numerous studies have explored the relationships among key compounds (quercetin, ellagic acid, baicalein, and kaempferol) and MAPK signaling pathway in tumors. Results from some studies indicate an association between the activation of MAPK pathway and the occurrence as well as progression of AS[33-35]. Notably, Yin et al[36] reported that quercetin exerted anti-oxidative and anti-inflammatory effects by inhibiting MAPK signaling pathways, while Won et al[37] demonstrated that quercetin suppressed VSMC migration by downregulating phosphorylation of p38 MAPK and ERK1/2 pathways leading to the inhibition of neointimal hyperplasia during vascular remodeling. This may partly explain the anti-AS effect of SJD. Kowshik et al[38] demonstrated that ellagic acid may exert an anti-angiogenic effect in tumors by suppressing HDAC6 and HIF-1α responses *via* the MAPK signaling pathway. To date, however, the effect of ellagic acid on endothelium and intimal hyperplasia has not been elucidated, necessitating further exploration. Several studies have demonstrated that baicalein can inhibit anti-tumor effects by inhibiting proliferation, migration and invasion of tumor cells. This effect has been reported in patients with lung, breast, and cervical cancers[39-41]. However, the precise role of baicalein in the treatment of AS warrants further investigation.

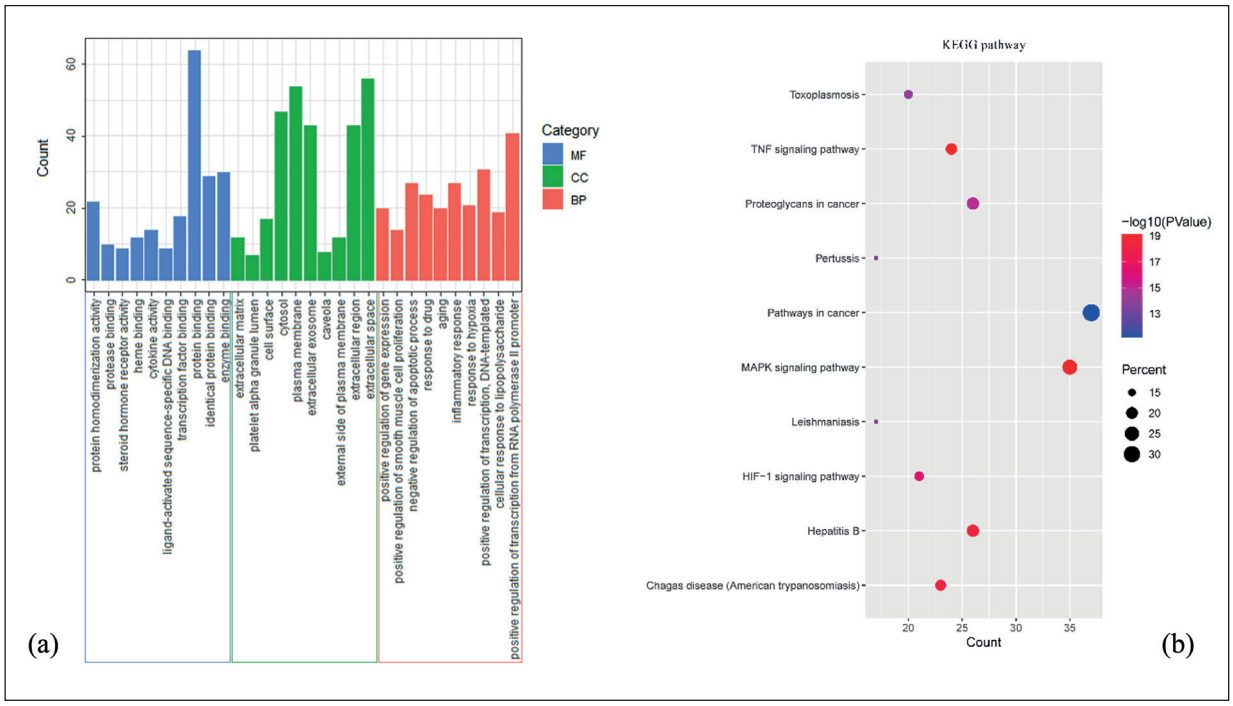

Figure 5. GO functional and KEGG pathway enrichment analyses. (**a**), Top 10 terms of BP, MF, and CC in GO functional enrichment analyses. (**b**), Top 10 terms of KEGG enrichment analyses.

Table II. Results of molecular docking of key compounds and their corresponding key targets in SJD.

Key compounds		Key targets		Binding energy (kcal/mol)
Mol ID	Mol name	Gene symbol	PDB ID	
MOL000098	Quercetin	MAPK1*	6G54	-4.19
MOL001002	Ellagic acid	RELA*	1VJ7	-5.49
MOL002714	Baicalein	AKT1	5AAR	-4.27
		FOS*	1A02	-5.01
MOL000422	kaempferol	–	–	–

*indicates that the molecule is involved in the MAPK signaling pathway.

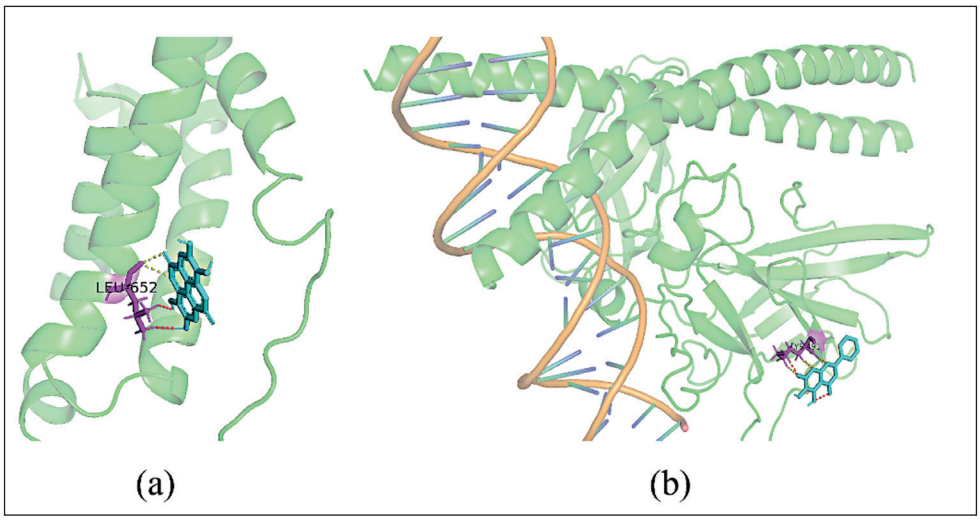

Figure 6. Results of molecular docking for interactions with binding energy of less than -5.0 kcal/mol. (**a**), ellagic acid (MOL001002) and RELA (**b**), baicalein (MOL002714) and FOS

Limitation

This study also had some limitations. The results obtained by our study were not experimentally validated. Therefore, further studies are warranted to explore the relationships among key compounds and their corresponding key targets.

Conclusions

In summary, results of pres ent study demonstrate that SJD prevents the progression of AS by inhibiting the expression of genes associated with the MAPK signaling pathway such as MAPK1, RELA, and FOS. Taken together, these findings lay the foundation for further experimental and clinical studies on the effect of SJD on AS.

Conflict of Interest

The authors have declared that no conflict of interest exists.

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Ethical statement

All data are available in public database.

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None.

Authors' Contributions

(I) Conception and design: Tao Luo, Linzhong Zhu; (II) Administrative support: Tao Luo, Linzhong Zhu; (III) Provision of study materials or patients: Jinming Yang, Yu Li; (IV) Collection and assembly of data: Yu Li, Wenhao Cui, Jukun Wang, Xin Chen; (V) Data analysis and interpretation: Yu Li, Wenhao Cui, Jukun Wang, Xin Chen; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Availability of Data and Materials

The datasets used or analyzed during the current study will be available from the corresponding author upon reasonable request. Meanwhile, the datasets generated and/or analysed during the current study are available in the following public repository:
Traditional Chinese Medicine System Pharmacology (TCMSP): https://old.tcmsp-e.com/index.php
UniProt: https://www.uniprot.org/
Online Mendelian Inheritance in Man (OMIM): https://omim.org/
GeneCards: https://www.genecards.org/
Terapeutic Target Database (TTD): http://db.idrblab.net/ttd/
DrugBank: https://go.drugbank.com/
ZINC: http://zinc.docking.org/
Worldwide Protein Data Bank (PDB): http://www.rcsb.org/

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