Published By Guangdong Pharmaceutical University, Guangzhou

Journal of Holistic Integrative Pharmacy

2021,2(1): 56-68

EXPERIMENTAL RESEARCH

A network pharmacology study on the main active constituents and key pharmacological pathways of Shaoyao Gancao decoction on Osteoarthritis

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[Abstract] **Objective** This study identifies the active constituents and molecular mechanism of Shaoyao Gancao decoction (SGD) in the treatment of osteoarthritis (OA) and provides new ideas and targets for the prevention and treatment of OA with traditional Chinese medicine (TCM). **Methods** The chemical constituent profile of SGD was identified by UHPLC-Q-TOF assay. Based on the candidate active compounds, combining LC-MS with the TCM database, putative targets of SGD drug-like chemical constituents were predicted using TCMSP. The targets of disease were obtained from the Gene database. Subsequently, the compound-target network was constructed and the core potential targets were screened out by a Cytoscape plug-in. **Results** Thirty-two bioactive compounds of 87 drug-like chemical constituents of SGD were identified by UHPLC-Q-TOF: 279 genes were found to be the putative targets of these compounds. In a protein-protein interaction (PPI) network, according to the comprehensive analysis of the topological parameters, 62 key targets were found. Subsequently, 23 core genes were further screened out by the cytohubba plug-in of Cytoscape resulting from a synthesis of 11 algorithms. Functional enrichment analysis suggested that SGD exerted its pharmacological effects in OA by modulating multiple pathways. Eighteen active components, 23 key targets and eight key regulatory pathways were identified, among which, CCND1, MYC, MAPK8, INS, EGF, serpine1, and AGE-RAGE signaling pathway are new targets (non-OA genes) and new mechanisms. **Conclusion** This study systematically and comprehensively studied bioactive compounds and core targets and pathways of SGD on OA by LC-MS-MS and network pharmacology. These results suggest that SGD is likely to treat OA by regulating metabolic pathways, which provides important information and direction for further research.

[Key words] Shaoyao Gancao decoction (SGD); Osteoarthritis (OA); Network Pharmacology; Pharmacological pathways

1 Introduction

Osteoarthritis (OA) is a complex chronic disease, characterized by degeneration and loss of cartilage, secondary synovial inflammation, alteration to articular bone, osteophyte formation, and changes to subchondral bone. OA is a leading cause of disability, and has a societal cost in older adults[1-2]. The pathogenesis and etiology of OA remains unclear. Strong evidence indicates a variety of risk factors including heredity, race, age,

[Research funding] This work was supported by National Natural Science Foundation of China (No.81703845); Key project for the thirteenth five-year plan (No.ZZ10022) and the Fundamental Research Funds for the Central public welfare research institutes (ZZ13-YQ-036).

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These authors have no conflict of interest to declare.

female sex, obesity, and previous injury[3]. Senility is one of the most evident risk factors for OA[4]. Oral NSAIDs[5-6] have been the main therapeutic recommendation, in terms of clinically relevant improvement of both pain and function. However, serious gastrointestinal or renal adverse events are an important consideration in their use. Considering the individual and societal burden of OA, it is extremely urgent to identify new drugs and better approaches towards management.

In traditional Chinese medicine (TCM), OA is categorized as "arthromyodynia" (Bi Zheng) or "bone arthralgia". Kidney deficiency and liver stagnancy are the main pathogenesis for bone arthralgia[7]; therefore, treatment should focus on the hepato-renal system, and improving strength and energy to treat the underlying cause. Meanwhile, therapy for expelling wind, dissipating cold to overcome dampness, and promoting blood circulation for analgesia is important for relieving the secondary symptoms.

Shaoyao Gancao decoction (SGD) contains two Chinese herbal medicines: shaoyao (SY) derived from *Radix Paeoniae Alba*, and gancao (GC) derived from *Glycyrrhizae Radix et Rhizoma*, in a 1:1 ratio. SGD has been used for thousands of years, and is recorded in "Typhoid and Miscellaneous Diseases", a clinical TCM book written by Zhang Zhong Jing around 200 B.C[8]. Pain is the dominant symptom, and is a major driver of clinical decision making and health service use, and is best framed within a biopsychosocial model[9-10]. Pharmacological studies have shown that the two compounds in the SGD formulation have a synergistic effect in reducing inflammation, pain, and swelling, and improving joint function in patients with OA [11-12]. However, the underlying pharmacological mechanisms of SGD and its components in the treatment of OA complicated with pain remain unclear, and the pharmacodynamic properties of its components and key targets remain to be identified.

Network pharmacology is emerging as a promising strategy[13-15], closely related to the application of multiple omics- and systems biology-based technologies. It is a valuable tool for achieving a holistic view of comprehensive and systematic insights into the mechanisms of multi-ingredient medicine.This study uses a comprehensive, network pharmacology-based approach to discover the key active constituents and pharmacological mechanisms of SGD action on OA complicated with pain.

2 Materials and methods

2.1 Database construction of candidate compounds

From TCM systems pharmacology database and analysis platform (https://tcmspw.com/tcmsp.php), 365 compounds were collected from SY and GC including 280 compounds in GC and 85 in SY. Oral bioavailability (OB) and drug-likeness were primarily used to screen potent pharmaceutical compounds from SGD.The higher the OB of the compound, the more likely it is to be developed for clinical application. DL is the sum of the pharmacokinetic properties and safety, which comes from the interactions of physicochemical properties and structural factors, including solubility, permeability, and stability. According to TCMSP recommendations, molecules with OB $\geqslant$ 30% and DL $\geqslant$ 0.18 were considered to be better pharmacologically and were screened out as candidate compounds for further analysis. Of the 83 active constituents collected from TCMSP, 16 were for *Radix Paeoniae Alba* and 67 were for *Glycyrrhizae Radix et Rhizoma*. Furthermore, predicted ingredients with OB $\geqslant$ 30% and DL $\geqslant$ 0.18% of *Paeoniae Radix Alba* and *Glycyrrhizae Radix et Rhizoma* were collected from the Hit database[16]. Some molecules with OB < 30% and DL < 0.18% were selected because reported pharmacological data demonstrated they play an important role in the therapeutic effect of drugs.

2.2 Instrument and UHPLC-Q-TOF conditions

2.2.1 Sample preparation

Radix Paeoniae Alba (5.0 g) and glycyrrhiza (5.0 g) were ground into a powder and dissolved in 100 mL water. The sample solution was heated to reflux for 1 h after weighing. The lost weight was made up with water after hot reflux extraction. Solutions were filtered through a 0.22 μm millipore membrane and 5 μL of the filtrate was directly injected into the UPLC system.

2.2.2 UPLC system

A Nexera UHPLC LC-30A (Shimadzu) UPLC system was used to analyze all the samples. The flow rate was 300 μL/min and the injection volumes were 1 μL. Chromatographic separation was carried out using a reverse-phase column ACQUITY UPLC C_{18} Column (1.7 μm, 2.1 mm×100 mm) at 40 ℃. For C_{18} separation, mobile phase A was water and mobile phase B was acetonitrile; both A and B contained 0.1% formic acid and 5 mmol/L ammonium acetate. The gradient conditions for reversed phase C18 separation is shown in Table 1.

2.2.3 Mass spectrometry and data processing

The LC-ESI-MSn experiments were performed using TripleTOF5600+, AB SCIEX™. The positive and negative spray voltages were 3.7 kV and 3.5 kV, respectively. The heated capillary temperature was 320 ℃, the sheath gas pressure was 206.8 kPa, the auxiliary gas setting was 68.9 kPa, and the heated vaporizer temperature was 300 ℃. Both the sheath gas and the auxiliary gas were nitrogen. The collision gas was also nitrogen, at a pressure of 1.5 mTorr. The parameters of the full mass scan were as follows: a resolution of 70 000, an auto gain control target under 1×10^6, a maximum isolation time of 50 ms, and an m/z range 50–1 500. The accuracy error threshold was fixed at 10 ppm.

2.3 Network construction and analysis

2.3.1 Known therapeutic targets for the treatment of OA

Known therapeutic targets for the treatment of OA were collected from NCBI and OMIM databases (www.omim.org; last updated 2019-07-22). OA was used as a key search term in the Gene database (last updated 2019-07-22) and 589 results for OA with variations of medical significance were reported via Variation Viewer.

2.3.2 Drug target prediction for SGD

Based on the candidate active compounds, combining LC-MS with the TCM database (OB ⩾ 0% and DL ⩾ 0.18), putative targets of candidate compounds in SY and GC were identified. The therapeutic targets of candidate compounds from SGD, obtained from the TCMSP database, were considered as putative targets of SGD.

2.3.3 Constructing the Network of Compound-Target

The composition of SGD is very complicated. The number of targets and signaling pathways they are involved in is very large. To screen out OA and pain-related targets and signaling pathways, putative SGD targets, known therapeutic targets of OA, and putative SGD networks were constructed.

2.3.4 Conducting PPI Network

Protein-protein interaction (PPI) data were conducted to better analyze and understand the mechanisms of SGD on OA, based on the study of PPIs using STRING software. The importance of key proteins is analyzed according to the degree of the node exported from the STRING database. The differing identification types of the proteins were target to the corresponding compound.

2.3.5 Gene ontology (GO) functional enrichment analysis and pathway enrichment analysis

Table 1 Characterization of 32 chemical constituents of Shaoyao Gancao decoction (SGD) by UHPLC-ESI-Q-TOF

Compound	Molecular formula	RT /min	Adduct	*m/z*	OB /%	DL
11alpha,12alpha-epoxy-3-*β*-23-dihydroxy-30-norolean-20-en-28,12-*β*-olide	$C_{29}H_{42}O_5$	4.96	$[M+H]^+$	470.250 9	48.14	0.43
Glabranin	$C_{20}H_{20}O_4$	4.98	$[M+H]^+$	324.119	52.90	0.31
Glycyrol	$C_{21}H_{18}O_6$	4.98	$[M+H]^+$	366.106 8	90.78	0.67
Nicotiflorin	$C_{27}H_{30}O_{15}$	5.02	$[M+2H]^{2+}$	594.110 9	3.64	0.73
8-(6-hydroxy-2-benzofuranyl)-2,2-dimethyl-5-chromenol-Kanzonol U	$C_{19}H_{16}O_4$	5.03	$[M+H]^+$	308.097 6	58.44	0.38
Glyasperin F	$C_{20}H_{18}O_6$	5.06	$[M+2H]^{2+}$	354.095	75.84	0.54
Semilicoisoflavone B	$C_{20}H_{16}O_6$	5.11	$[M+H]^+$	352.056 5	48.78	0.55
1,3-dihydroxy-9-methoxy-6-benzofurano[3,2-c] chromenone	$C_{16}H_{10}O_5$	5.22	$[M+H]^+$	282.040 3	48.14	0.43
Liquiritin	$C_{21}H_{22}O_9$	5.24	$[M+H]^+$	418.086 5	65.69	0.74
Glypallichalcone	$C_{17}H_{16}O_4$	5.38	$[M+H]^+$	284.086 5	61.60	0.19
Jaranol	$C_{17}H_{14}O_6$	5.64	$[M+H]^+$	314.051 8	50.83	0.29
Euchrenone	$C_{26}H_{30}O_4$	6.48	$[M+H]^+$	406.155 9	30.29	0.57
Glyasperin B	$C_{21}H_{22}O_6$	6.66	$[M+H]^+$	370.140 9	65.22	0.44
Gancaonin H	$C_{25}H_{24}O_6$	7.12	$[M+H]^+$	420.120 7	50.10	0.78
Paeoniflorin	$C_{23}H_{28}O_{11}$	13.96	$[M+H]^+$	480.170 7	53.87	0.79
Medicarpin	$C_{16}H_{14}O_4$	14.48	$[M+H]^+$	270.075	49.22	0.34
Sigmoidin-B	$C_{20}H_{20}O_6$	16.09	$[M+H]^+$	356.134 5	34.88	0.41
18-*β*-glycyrrhetinic acid	$C_{30}H_{46}O_4$	17.65	$[M+H]^+$	470.318 9	64.77	0.38
Mairin	$C_{30}H_{48}O_3$	18.45	$[M+H]^+$	456.335 4	55.38	0.78
Glycyrrhizic acid	$C_{42}H_{62}O_{16}$	22.58	$[M+H]^+$	822.360 8	19.62	0.11
(2S)-2-[4-hydroxy-3-(3-methylbut-2-enyl)phenyl]-8,8-dimethyl-2,3-dihydropyrano[2,3-f]chromen-4-one	$C_{25}H_{26}O_4$	5.06	$[M-H]^-$	390.142 6	31.79	0.72
Malic acid	$C_4H_6O_5$	5.24	$[M-H]^-$	133.019 9	59.62	0.02
Xambioona	$C_{25}H_{24}O_4$	5.64	$[M-H]^-$	388.136	54.85	0.87
Gallic acid (3,4,5-trihydroxybenzoic acid)	$C_7H_6O_5$	5.87	$[M-H]^-$	170.025 4	31.69	0.04
Hydroxyferulic acid	$C_{10}H_{10}O_5$	6.50	$[M-H]^-$	209.056 6	59.99	0.07
Paeonol	$C_9H_{10}O_3$	6.50	$[M-H]^-$	166.067 5	28.79	0.04
(+)-catechin	$C_{15}H_{14}O_6$	7.29	$[M-H]^-$	290.087 8	54.83	0.24
Acetic acid	$C_2H_4O_2$	7.71	$[M-H]^-$	59.989 59	47.87	0.001 7
Formononetin	$C_{16}H_{12}O_4$	16.07	$[M-H]^-$	268.080 6	69.67	0.21
Isoliquiritigenin	$C_{15}H_{12}O_4$	17.45	$[M-H]^-$	255.078 2	85.32	0.15
Licochalcone G	$C_{21}H_{22}O_5$	26.46	$[M-H]^-$	354.123	49.25	0.32
Gancaonin A	$C_{21}H_{20}O_5$	28.30	$[M-H]^-$	352.108 2	51.08	0.40

To further clarify the biological roles of the screened major targets, DAVID[17] (database for annotation, visualization and integrated discovery) was used to perform the gene ontology (GO) biological process enrichment analysis and kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis (KEGG, http://www.genome.jp/kegg/; last updated 2019-07-20). $P < 0.05$ was set as the significance threshold. The nodes with centrality measures that were more than the median centralities of all nodes represent putative major targets. The compound-target network, pathway-gene network, and constituent-pathway-target network were intersected and analyzed using the

cytohubba plug-in of Cytoscape[18] by calculating topological parameters, including degree centrality (DC), closeness centrality (CC), and betweenness centrality (BC)[19]. Among them, CC was considered the most important element to screen the putative targets for topological importance.

3 Results

3.1 Characterization and identification of chemical constituents containing SGD

The total ion chromatograms (TIC) of the SGD sample corresponding to the positive and negative signals are shown in Fig.1.

Four standard available constituents were identified as gallic acid, malic acid, hydroxyferulic acid, and isoliquiritigenin. For the standard unavailable constituents, a series of continuous procedures was used to increase the credibility for structure identification by calculating the molecular formulas using high-resolution and high-accuracy mass spectra, searching in KNApSAcK_database and TCMSP, and determining structure characterization and confirmation using MSn mass spectrometry. Overall, 32 constituents, including paeoniflorin, mairin, gallic acid, isoliquiritigenin, glycyrol, kaempferol, and formononetin were identified or

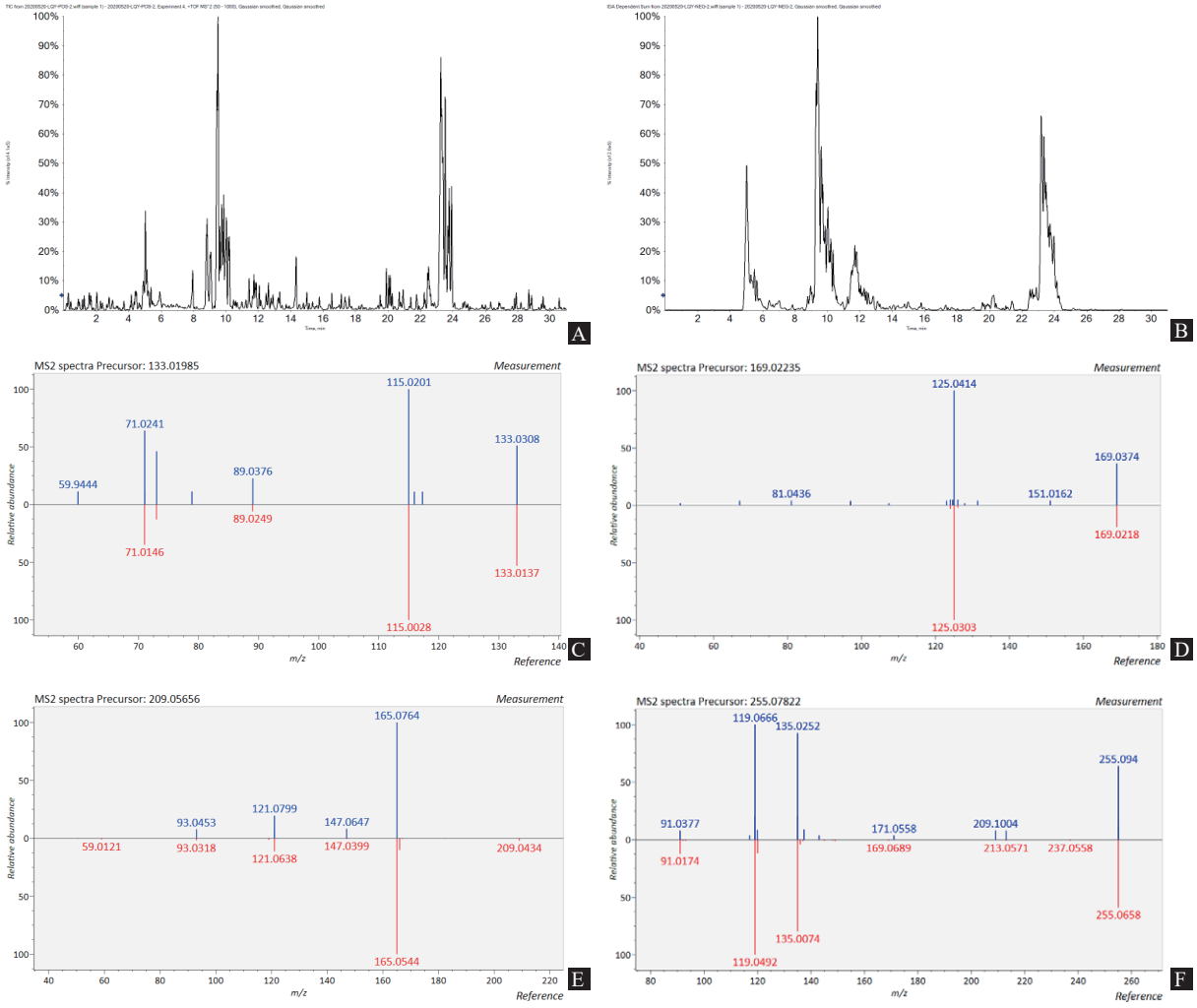

Fig. 1 Total ion chromatogram (TIC) of Shaoyao Gancao decoction (SGD) and secondary mass spectrum matching diagram of reference material and determined material

A. positive ion mode; B. negative ion mode using UHPLC-Q-TOF; C. malic acid; D. gallic acid; E. hydroxyferulic acid; F. isoliquiritigenin.

tentatively characterized with their retention times and MSn data. The detailed information of these constituents is summarized in Table 1.

3.2 Prediction of the oral absorption and bioavailability of chemical constituents containing SGD using the TCMSP database

The TCMSP database can provide information on the absorption, distribution, metabolism, and excretion (ADME) properties of a drug, as well as potential biological effects at a systematic level, for example, OB, DL, Caco-2 permeability (Caco-2), and blood-brain barrier (BBB). OB and DL are the foremost features. In addition to the 32 water-soluble compounds detected by mass spectrometry, there were a further 55 chemical ingredients screened out with the criteria of OB $\geqslant$ 30% and DL $\geqslant$ 0.18%. Further analysis conclusively showed paeoniflorin (DL=0.787, OB=68.18) and Lactiflorin (DL=0.79, OB=58.87) from *Paeoniae Radix Alba*, Mairin (DL=0.776 1, OB=55.37) from the same component of *Paeoniae Radix Alba* and *Glycyrrhizae Radix et Rhizoma*, and Kanzonol F (DL=0.893 6, OB=32.47) and Gancaonin H (DL=0.784 2, OB=50.10) had satisfactory pharmacokinetic profiles, which suggested these molecules as DL constituents.

3.3 Putative targets for SGD

A total of 279 putative targets, corresponding to the active ingredients in SGD, were predicted using PubChem/TCMSP/HIT. Among these, there were 105 known therapeutic targets for OA and 174 for non-OA genes, including estrogen receptor beta (ESR2), mitogen-activated protein kinase 3 (MAPK3), interleukin-10 (IL-10), matrix metalloproteinase-2 (MMP2), Myc proto-oncogene protein (MYC), and cellular tumor antigen p53 (TP53). The obtained compounds and targets were used to construct the pharmacology network.

3.4 Pharmacological mechanisms of SGD acting on OA

To illustrate the underlying associations between drugs and disease, the molecular network-based analysis was performed by building an interaction network of disease-related genes and drug target genes. PPI networks of acquired drug targets were constructed by the STRING database (https://string-db.org/). In the PPI network, according to the comprehensive analysis of the topological parameters (CC > median, BC > median, degree greater than twice the average) there are 62 targets meeting the three conditions at the same time. These are likely to be the key targets of SGD for the treatment of OA. Subsequently, 23 core genes (FOS, CCND1, IL1β, ESR1, TP53, SERPINE1, MMP2, CXCL8, MYC, MAPK14, IL6, MAPK3, IL10, JUN, CASP3, MMP9, AKT1, MAPK8, insulin, VEGFA, EGF, CCL2, MAPK1) were further screened out by the cytohubba plug-in of Cytoscape resulting from synthesis of 11 algorithms.

3.5 GO enrichment analysis

GO enrichment analysis results consist of biological processes (BP), cell components (CC), and molecular functions (MF). The top 20 biological processes of 62 key targets, by FDR, is shown in Fig. 2, including regulation of immune system process, positive regulation of cellular metabolic process, cytokine-mediated signaling pathway, response to cytokine, and regulation of apoptotic process.

3.6 KEGG pathways enrichment analysis

To further study the biological function of 62 key targets, enrichment analysis of KEGG pathway was carried out for key targets (Fig. 3).

The top 20 signaling pathways of key targets included pathways AGE-RAGE signaling pathway in diabetic complications, MAPK signaling pathway, p13k-AKT signaling pathway, TNF signaling pathway, apoptosis, cytokine-cytokine receptor

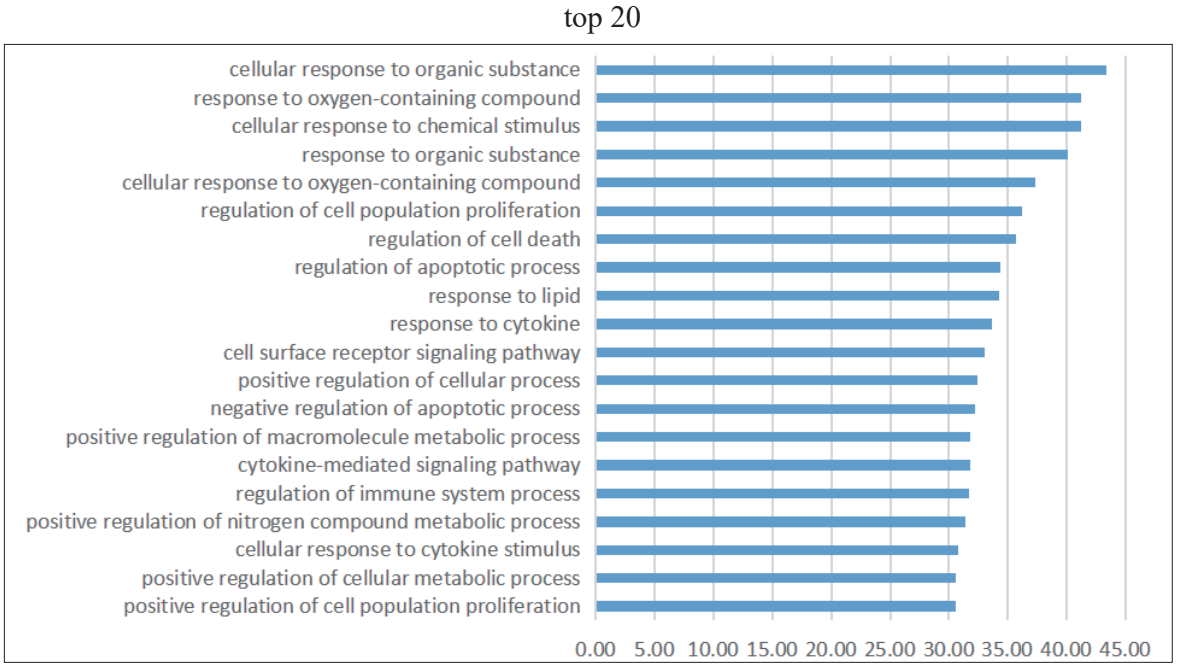

Fig. 2 The functional analysis for identified compounds related targets

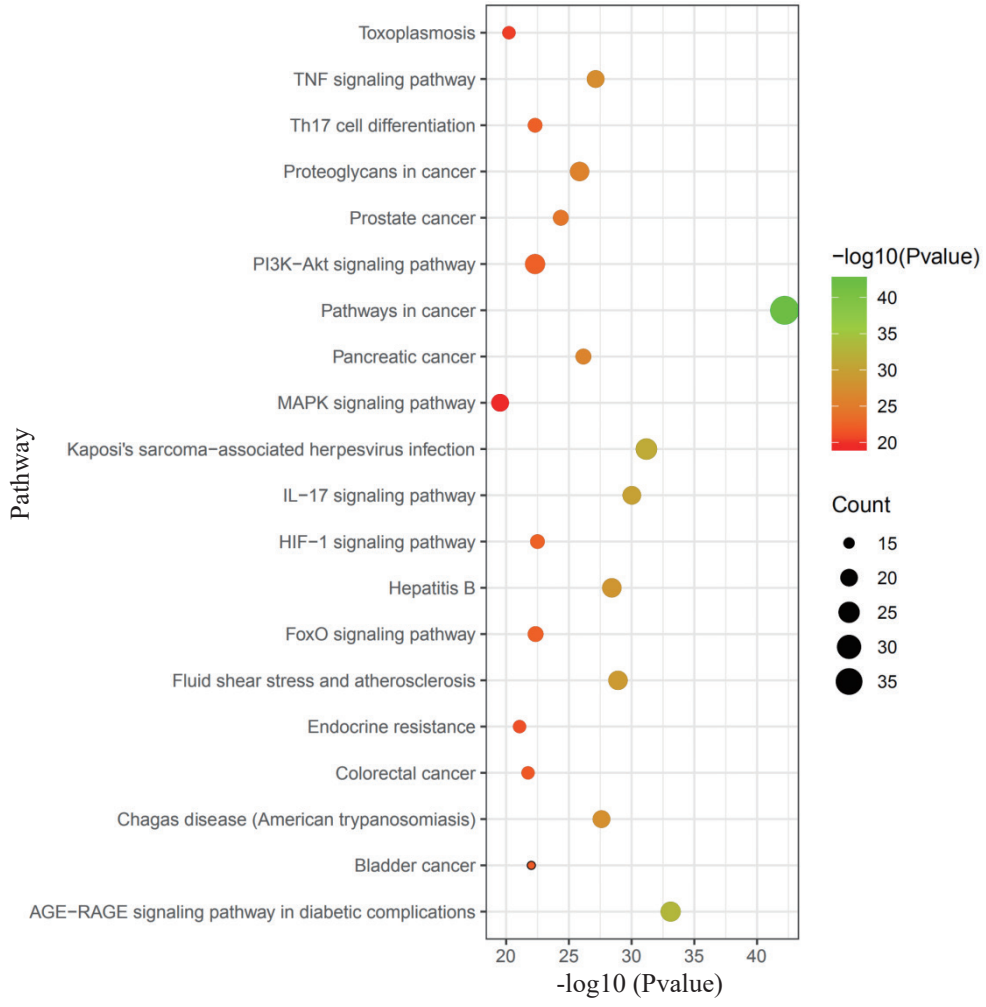

Fig. 3 The functional analysis for identified compounds related targets. The docking targets relate to KEGG pathways distribution

interaction, VEGF signaling pathway, and NF-kappa B signaling pathway. The AGE-RAGE signaling pathway in diabetic complications is of interest because this pathway has rarely been studied in osteoarthritis before, but plays a key role in the treatment of OA by SGD. Twenty-one hub genes of the 62 key genes participate in the regulation of this pathway. The AGE-RAGE signaling pathway in diabetic complications interacts with classic OA pathways (TLR pathway, MAPK pathway, p13k-akt pathway) and can also regulate OA by regulating downstream apoptosis and the NF kappa B signaling pathway, etc.

3.7 Construction of compound-target network

The network relationship of 23 key targets and active compounds of SGD are shown in Fig. 4. In this network, the larger the node is, the more compounds the target involves. Known OA genes are shown in red, and new potential targets are shown in yellow. Related chemical components of SGD corresponding to MAPK14 among OA genes are the most, while ESR, as a potential target of SGD on OA, also corresponds to a lot of active ingredients. In addition, INS (insulin), EGF, VEGFR, and MMPs are of interest.

3.8 Identification of key active constituents of SGD acting on OA

In this study, a drug-like chemical constituent with more key targets and important pathways was regarded as a key active constituent of SGD acting on OA. Eight out of 18 key active constituents were identified by LC-MS: quercetin, kaempferol, naringenin, formononetin, isorhamnetin, 3,4,5-trihydroxybenzoic acid(Gallic acid), licochalcone, medicarpin, 7-methoxy-2-methyl isoflavone, Glyzaglabrin, rutin, glypallichalcone, acetic acid, licoagroisoflavone, 18 beta-glycyrrhetinic acid, glycyrol, (+)-catechin, and Gancaonin A.

According to the KEGG results, the major putative targets of the key active constituents were

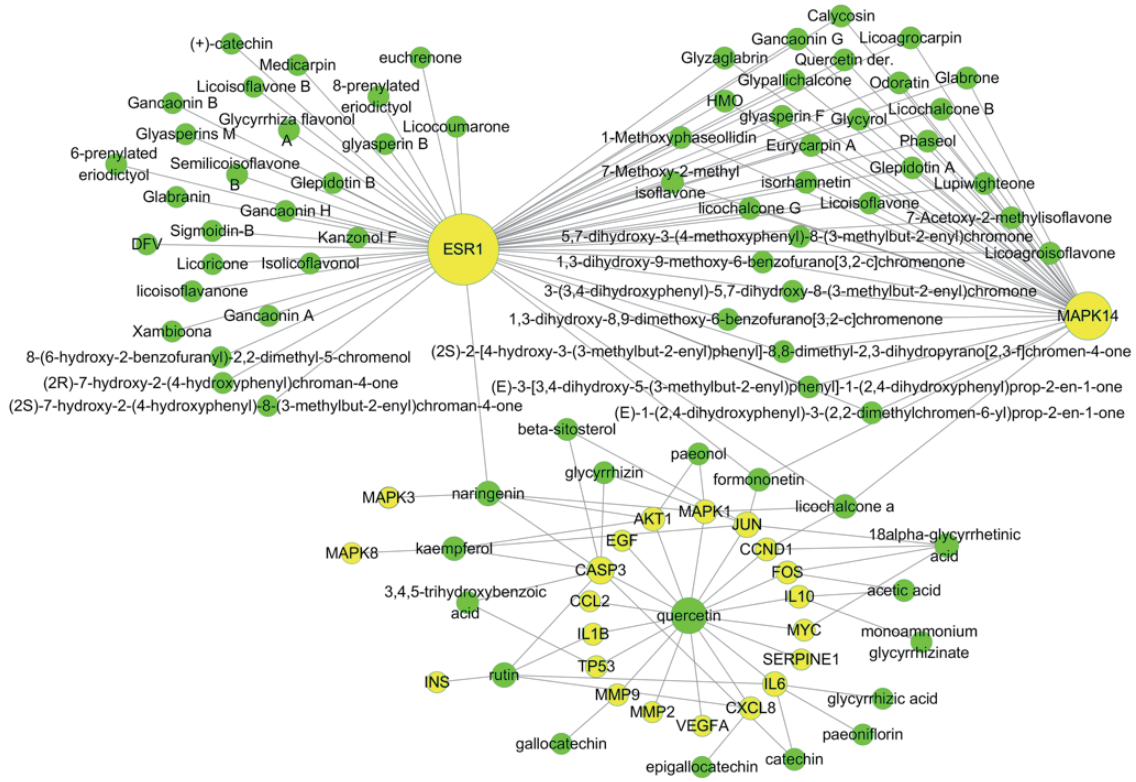

Fig. 4 Twenty-three key targets-compound network

mainly involved into the following eight critical pathways: AGE-RAGE signaling pathway in diabetic complications, MAPK signaling pathway, p13k-AKT signaling pathway, TNF signaling pathway, apoptosis, cytokine-cytokine receptor interaction, VEGF signaling pathway, and NF-kappa B signaling pathway.

4 Discussion

In the current study, 87 SGD drug-like chemical constituents were identified, with 32 water-soluble compounds detected by mass spectrometry and 279 genes predicted as putative targets of these molecules. According to the topological feature values of the 62 key putative targets, 18 key active constituents were found because the corresponding putative targets had topological importance in the SGD putative target-known OA therapeutic target network. These were defined as the candidate targets. Functionally, these candidate targets were significantly involved in several OA-related pathways, including regulation of immune system processes and inflammation, regulation of cell population proliferation/cell death/apoptotic processes, metabolic processes and affecting patient pain[20-22].

As the main clinical patterns of Chinese medicine, formula is a complex chemical system, which may be significantly varied because of different origins, sources, harvest time, pretreatments, and medicinal material manufacturing[23]. In recent years, UPLC-MS-MS techniques have been accepted as a powerful tool for the evaluation and quality control of multi-component TCMs and their finished products[24-25]. In this paper, LC-ESI-MSn experiments were performed to characterize and identify the chemical profiles of SGD. The oral absorption and bioavailability are two important pharmacokinetic parameters for the constituents of TCM, and growing evidence suggests that chemical constituents with Papp and OB (F) values higher than 7×10^6 cm/s and 30%, respectively, may have excellent DL. Eight out of 18 key chemical constituents of SGD with good absorption and DL were identified in this study[26-27].

To investigate the pharmacological mechanisms of SGD acting on OA, the putative targets of SGD were predicted based on the structures and functions of the chemical constituents. Following the construction of a hub interaction network and the calculation of the four topological features: "degree", "betweenness", "closeness", and "coreness", this study focussed on 62 key targets of SGD. After that, 23 core targets were further screened out by the cytohubba plugin of Cytoscape. These core targets were classified into four categories. IL1β, IL-6, IL-10, and CCL2 participated in or regulated inflammatory reaction (for example the NF–κB signaling pathway and TNF signaling pathway), leading to articular cartilage damage and cartilage matrix degradation[28-31]. Csaki et al.[32] have shown that resveratrol and curcumin have synergistic protective effects on human articular cartilage by inhibiting the IL-1β induced NF-κB signal pathway, which could activate the mediated inflammatory response and apoptosis. The second classification is the MAPK signaling pathway, which is associated with proliferation and differentiation of OA cartilage and apoptosis of chondrocytes, such as MAPK1. MAPK3, MAPK8, FOS, JUN, and CASP3[33-34].

In recent years, along with in-depth studies on the pathogenesis of OA, the Notch signaling pathway[35] as a potential molecular regulator of catabolism and anabolism of cartilage ECM, has been shown to play an important role in the development of OA. Overexpression of notch increases MMP13 and VEGF in chondrocytes, resulting in OA. The network pharmacology research identified MMP2 and MMP9 as critical targets. MMP9[36] plays a crucial part in the dynamic balance of degradation and remodeling of extracellular matrix, and the expression of MMP13[37] can lead to the degradation of type II collagen and promote cartilage damage. Knockout of serpine1 can enhance the activity of MMPs[38]. These studies indicate that MMPs, VEGF, and serpine1are key targets involved in the regulation

of extracellular matrix degradation and bone metabolism and affect OA.

One of the important innovations of this study is the significance of the AGE-RAGE signaling pathway for SGD treatment of OA. With China becoming the country with highest rates of diabetes mellitus, it is very important to clarify the relationship between diabetes mellitus and OA, for the health of the elderly in China. This pathway may be a significant breakthrough to pay further attention to. This pathway has high significance in the KEGG analysis (TOP3). Although this pathway is new to the study of the pathological mechanism of OA, it has rarely been reported before, there are not only 21 key targets involved in the regulation of this pathway, but also this pathway and the classic OA pathway[39] (TLR pathway, MAPK pathway, p13k-akt pathway) are interlaced and affect each other. The AGE pathway can also affect OA by regulating the downstream apoptosis, and NF kappa B signaling pathways (Fig. 5).

Two proto oncogenes (CCND1 and MYC) related to cell cycle were also identified from the 23 key targets, which suggest SGD may treat OA by cell proliferation. Studies[40] have shown that the incidence rate of OAs in postmenopausal women is significantly higher. SGD may also treat OA by targeting ESR.INS (insulin); as one of the 23 key targets, it is also an interesting target of SGD for OA. IGF is the ligand of INS, which may aggravate OA by affecting diabetes-related metabolic pathways[41]. However, the key targets of INS with AGE-RAGE signaling pathway in diabetic complications, CCND1, MYC, and serpine1 are new targets and new mechanism for SGD on OA.

SGD, as a classic famous prescription, is applied for leg and foot spasms, abdominal pain caused by blood deficiency, body fluid consuming, and the unmoistened muscles and veins. It is highly acclaimed and has been used by medical experts throughout the ages, with increasing application scope. This formula is used to treat spastic diseases,

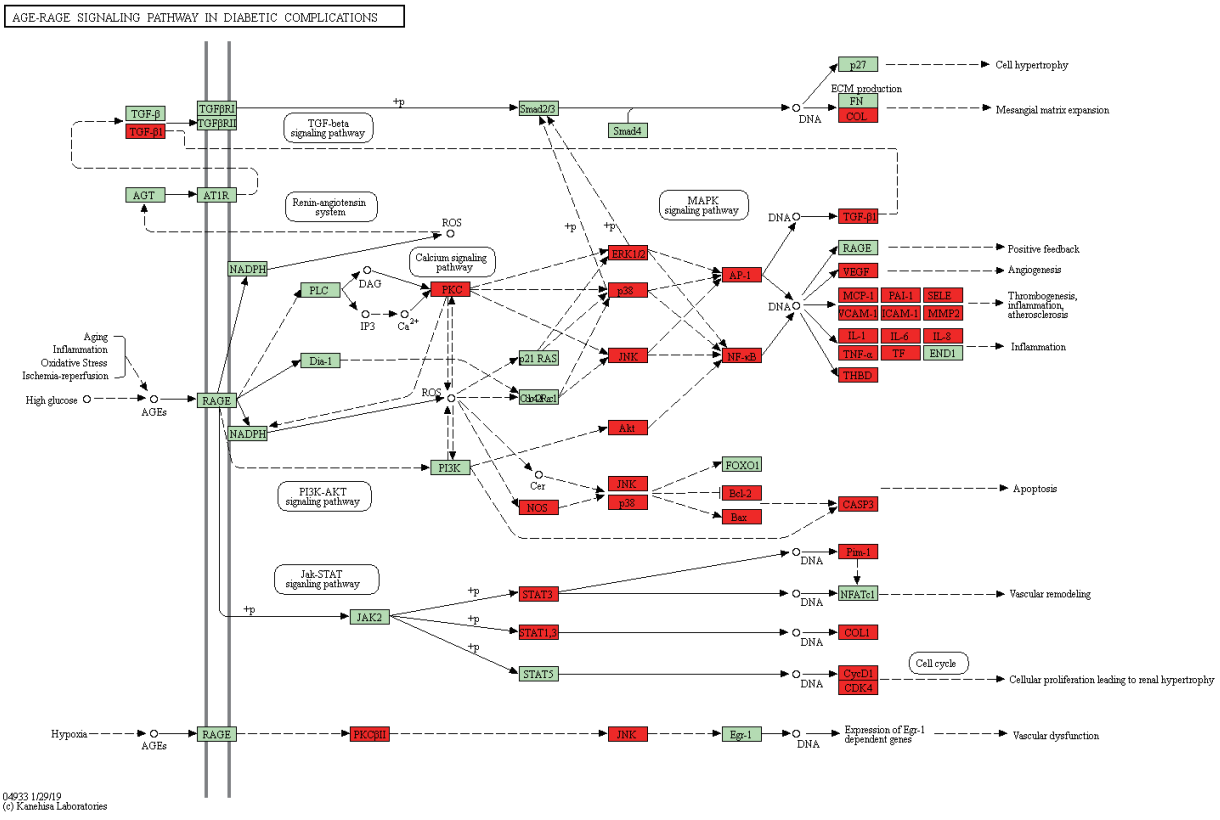

Fig. 5 AGE-RAGE signaling pathway in diabetic complications

painful diseases, and inflammatory diseases, among others. Pain is the dominant symptom of OA and is a major driver of clinical decision making and health service use for OA. Supplementary Table S4 shows that the genes for pain and OA are highly coincident, such as MMP9, MMP2, TLR4, NF-*k*B, and PTGS2. Therefore, SGD may not only relieve pain, but also play a role in the treatment of OA by these overlapping targets. This study has identified that SGD treats OA by regulating INS, MAPK, MMPs, ESR, EGF, and other core target involved in the AGE-RAGE pathway, MAPK pathway, metabolic pathway, and immune inflammation related pathway. Quercetin, kaempferol, naringenin, formononetin, isorhamnetin, 3,4,5-trihydroxybenzoic acid (Gallic acid), and other 12 components may be the main active components of SGD in the treatment of OA.

5 Conclusion

In conclusion, this study identified a list of key active constituents of SGD acting on OA, using an integrative pharmacology-based approach, which combined active chemical constituent identification, drug target prediction and network analysis. This method may offer an efficient way to understand the pharmacological mechanisms of traditional Chinese medicine prescriptions.

The present study demonstrates, for the first time, that INS with AGE-RAGE signaling pathway in diabetic complications, CCND1, MYC, and serpine1 are new targets and new mechanisms for SGD on OA, besides the classic targets and pathways related to immune, inflammation, MAPK pathways, and MMPs. This research is the first to propose that INS with AGE-RAGE signaling pathway, CCND1, MYC, and serpine1may represent potential therapeutic targets for the future development of clinical drugs for the treatment of OA. This research systematically identifies the active compounds and molecular mechanism of SGD in the treatment of OA, providing new ideas and targets for the prevention and treatment of this disease with TCM.

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