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Drug Combination Therapy 2023;5(2):8. https://doi.org/10.53388/DCT20230008

Exploring the potential molecular mechanism of JinlingziSan in the treatment of endometriosis based on network pharmacology system

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Author contributions

Ke-Feng Sun and Li-Jun Zhou conceived this study, carried out this study, and drafted the manuscript. Yuan Wang and Wen Li designed the study, collected and analyzed the data. Li-Jun Zhou directed the R software drawing and reviewed the article critically. Ke-Feng Sun helped accomplish the conception and design of the study. Ke-Feng Sun and Li-Jun Zhou were responsible for this manuscript and reviewed the article critically. All authors read and approved the final manuscript.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

This work has been supported by grants from the Heilongjiang Provincial Traditional Chinese Medicine Research Project: Mechanism study of Zhuyu Ning in treating endometriosis based on the Wnt/β-catenin signaling pathway (ZHY2022-130); and the National Traditional Chinese Medicine Clinical Research Base Business Construction Scientific Research Special Project (JDZX2015057) of the State Administration of Traditional Chinese Medicine: Clinical observation of the therapeutic effect of acupuncture on dysmenorrhea in endometriosis based on the VEGF/VEGFR pathway.

Peer review information

Drug Combination Therapy thanks Yu-Xi Huang and other anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations

EMs, endometriosis; TCMSP, Traditional Chinese Medicine Systematic Pharmacology Database and Analysis Platform database; PPI, protein-protein interactions; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, gene ontology.

Citation

Zhou LJ, Li W, Wang Y, Sun KF. Exploring the potential molecular mechanism of JinlingziSan in the treatment of endometriosis based on network pharmacology system. *Drug Comb Ther*. 2023;5(2):8. doi: 10.53388/DCT20230008.

Executive editor: Shan-Shan He.

Received: 10 February 2023; **Accepted:** 17 April 2023; **Available online:** 5 May 2023.

Abstract

Background: Chinese herbal medicines have been proven to be effective in clinical treatment and disease prevention. Jinlingzi San is a herbal medicine used for the treatment of endometriosis. However, a comprehensive network pharmacology approach has not been used to understand the active ingredients and therapeutic mechanisms behind Jinlingzi San in the treatment of endometriosis. **Methods:** A network pharmacology approach and a molecular docking approach were used to predict the active ingredients and potential targets of Jinlingzi San in the treatment of endometriosis. **Results:** The integrated network pharmacology approach successfully identified 60 active ingredients and 540 key targets of action in Jinlingzi San dispersion. Among them, STAT3, TP53, MAPK3, RELA, MAPK1, JUN and other key genes were mainly regulated through pathways in cancer, lipid and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, pancreatic cancer, prostate cancer, PI3K-Akt signaling pathway, Th17 cell differentiation, and other signaling pathways in the cell body, receptor complexes, cytoplasm, nucleoplasm, and plasma membrane. **Conclusion:** Based on a systems network pharmacology approach, our work successfully predicts the active components and potential targets of Jinlingzi San for the treatment of endometriosis and helps to illustrate the mechanism of action at an integrative level. This demonstrates that Jinlingzi San for the treatment of endometriosis is regulated through a multi-component, multi-target, multi-system co-regulation. This study identifies key genes and pathways associated with endometriosis and pathogenesis from new insights, and also provides a viable approach to the chemical basis and pharmacological studies of Jinlingzi San.

Keywords: JinlingziSan; network pharmacology; endometriosis; mechanism of action

Introduction

Endometriosis (EMs) refers to the presence of active endometrial tissue (glands and mesenchyme) outside the endometrium and is an estrogen-dependent disease, with a clinical incidence of approximately 2%–10% [1, 2]. The main manifestations are chronic pelvic pain, menstrual irregularities, dysmenorrhea, and painful intercourse [3]. The pathogenesis of EMs is not clear, but modern studies suggest that the development of EMs is related to genetic susceptibility, immune factors with inflammatory cytokines, estrogen dependence, and progesterone resistance [4]. EMs not only has physical effects but also psychological effects on the patient. It has been found that EMs can lead to depression, anxiety, and thus affect social relationships. In addition, EMs has a negative impact on sexuality and imposes a high financial burden on patients [5]. Chinese medicine has its own advantages and specificities in personalized treatment and early intervention of EMs.

Jinlingzi San was created by Liu Wansu, a famous physician in the Jin-Yuan period, and this formula consists of two herbs, chuanlianzi and yanhuosuo. It has the effect of regulating qi and relieving pain. It is said in *Suwen Sickness QiYi Bao Sheng Ji Collection* (Wan-Su Liu, 1110–1200 C.E.), "Heat syncope and heart pain, which may occur or stop, and which does not heal for a long time." Clinical studies have shown that Jinlingzi San can effectively relieve pain and has significant efficacy in treating infertility caused by EMs [6, 7].

Network pharmacology is regarded as a promising approach for discovering herbal medicines from a system perspective [8]. It is a predictive tool used to explore the chemical composition of Jinlingzi San and its relationship with EMs. Our study aims to investigate the relationship between Jinlingzi San dispersal and EMs. It is the first study to identify potential bioactive compounds in Jinlingzi San and to elucidate their mechanisms in the treatment of EMs.

Materials and methods

Construction of the database of Jinlingzi San

The chemical composition of two drugs, Jinlingzi and Yanhuosuo, was collected from the Traditional Chinese Medicine Systematic Pharmacology Database and Analysis Platform database (TCMSP) (https://old.tcmsp-e.com/index.php) [9]. The bioactive components that contribute to the therapeutic effect were selected based on absorption, distribution, metabolism, and excretion, while those compounds with poor pharmacological properties and poor drug-forming ability were removed. We screened the ingredients while drug composition oral bioavailability ≥ 30% and drug-like ≥ 0.18 to obtain compounds with higher oral absorption, utilization, and biological properties and built a database, which was supplemented by literature search for their major components. The names of the relevant genes symbol in the database of Jinlingzi were corrected for their names by UniProt KB (http://www.uniprot.org/) [10].

Target of action of Jinlingzi San

The activity of active compounds in drugs is dependent on their targets. Using the active compounds obtained above, we obtained a collection of their relevant targets directly from the TCMSP database. Complementary active ingredients were predicted using the Swiss Target Prediction database (https://www.swisstargetprediction.ch/).

Acquisition of EMs targets

Four databases, Gene Cards (https://www.genecards.org/), OMIM (https://omim.org/), DrugBank (https://go.drugbank.com/), and Therapeutic Target Database (http://db.idrblab.net/ttd/), were searched using the keyword "Endometriosis" to identify target genes related to EMs and establish the database of EMs targets [11–14].

Protein-protein interactions (PPI) of key targets

Bioinformatics & Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn/) was utilized to identify the intersection of the active ingredient action targets and disease target genes of Jinlingzi san drug, in order to obtain the key targets of goldbellsan for treating EMs. The key targets were then imported into the String (https://www.string-db.org/) database, with the species selected as "Homo sapiens", and a high confidence score of ≥ 0.900 chosen as the cutoff value for correlation, to obtain the protein interaction network. Subsequently, the data obtained by Cytoscape 3.8.2 software was used for visualization and analysis, to map the key target protein interaction network [15, 16].

Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

We used the DAVID database (https://david.ncifcrf.gov/) for gene ontology (GO) and KEGG enrichment analysis of key targets [17]. GO enrichment analysis encompasses three main aspects: biological process, molecular function and cellular composition. KEGG enrichment allows to obtain the gene KEGG enrichment allows to obtain the potential biological functions of genes and the biological pathways involved, and $P < 0.05$ was selected to screen the enrichment pathways for key targets. The results were later visualised using R Language 4.0 [18].

Molecular docking

Autodock software was utilized to perform molecular docking of the top 5 ranked targets in the PPI results with the major drug compounds of Jinlingzi san [19]. The molecular structures of the targets were obtained and downloaded from the PDB database (https://www.rcsb.org/), while the small molecule Mol2 structures were obtained from the TCMSP database and the PDB database [20]. Protein and small molecule structures were prepared in the Autodock tool for ligands prior to the docking process. The crystal structure of the target protein was pretreated, including removal of water molecules, Protonate 3D hydrogenation, protein structure correction, energy optimization, and retention of the target active region. The results were visualized after docking using PyMol software [21].

Results

Collection of chemical components and screening of active ingredients in Jinlingzi San

Jinlingzi San is composed of two herbs, chuanlianzi and yanhuosuo. Through a TCMSP database search, 33 chemical components of chuanlianzi and 77 chemical components of yanhuosuo were collected, resulting in a total of 110 chemical components. By limiting the values of oral bioavailability and drug-like with literature supplementation, we finally obtained 10 potential active ingredients of chuanlianzi and 60 potential active ingredients of yanhuosuo. These were integrated and de-weighted to finally obtain 70 potential active ingredients (Table 1), resulting in 2471 active ingredient action targets. According to Uniprot, the names of the active ingredient targets were combined and de-weighted, resulting in 540 targets. This suggests that the scatter compounds of Jinlingzi san may have synergistic effects on these targets, leading to pharmacological effects in EMs.

"Active ingredient-target" network construction analysis

The 60 active ingredients and 540 target genes were entered into Cytoscape 3.8.2 software for network construction and visualization (Figure 1). Among them, the top 10 Degrees include PTGS2, PTGS1, SCN5A, KCNH2, ADRA1B, ADRA1D, ADRB2, OPRM1, RXRA, and OPRD1. These indicate that the above ingredients and genes are important for the treatment of EMs with Jinlingzi san, and are crucial target genes for the treatment of EMs.

Collection of key targets for the treatment of EMs with Jinlingzi san

EMs-related target genes were collected from Gene Cards, OMIM, and Drugbank databases. From Gene Cards database, 1782 EMs-related targets were obtained, while 21 EMs-related targets were obtained

Table 1 Active ingredients of Jinlingzi San

Drug	MOLID	Active ingredient	OB	DL
Chuanlianzi	MOL001494	Mandenol	42.00	0.19
Chuanlianzi	MOL001495	Ethyl linolenate	46.10	0.20
Chuanlianzi	MOL002045	Stigmasterol	43.41	0.76
Chuanlianzi	MOL002047	Melianone	40.73	0.81
Chuanlianzi	MOL002048	Nimbolidin D	30.38	0.53
Chuanlianzi	MOL002053	Nimbolin A	32.11	0.34
Chuanlianzi	MOL002056	(E)-3-[(2S,3R)-2-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-3-methylol-2,3-dihydrobenzofuran-5-yl]acrolein	54.74	0.40
Chuanlianzi	MOL002058	40957-99-1	57.20	0.62
Chuanlianzi	MOL000098	Quercetin	46.43	0.28
Chuanlianzi	–	Kaempferol	–	–
Yanhusuo	MOL001454	Berberine	36.86	0.78
Yanhusuo	MOL001458	Coptisine	30.67	0.86
Yanhusuo	MOL001460	Cryptopin	78.74	0.72
Yanhusuo	MOL001461	Dihydrochelerythrine	32.73	0.81
Yanhusuo	MOL001463	Dihydrosanguinarine	59.31	0.86
Yanhusuo	MOL001474	Sanguinarine	37.81	0.86
Yanhusuo	MOL000217	(S)-Scoulerine	32.28	0.54
Yanhusuo	MOL002670	Cavidine	35.64	0.81
Yanhusuo	MOL002903	(R)-Canadine	55.37	0.77
Yanhusuo	MOL000359	Sitosterol	36.91	0.75
Yanhusuo	MOL004071	Hyndarin	73.94	0.64
Yanhusuo	MOL004190	(–)-alpha-N-methylcanadine	45.06	0.80
Yanhusuo	MOL004191	Capaurine	62.91	0.69
Yanhusuo	MOL004193	Clarkeanidine	86.65	0.54
Yanhusuo	MOL004195	CORYDALINE	65.84	0.68
Yanhusuo	MOL004196	Corydalmine	52.50	0.59
Yanhusuo	MOL004197	Corydine	37.16	0.55
Yanhusuo	MOL004198	18797-79-0	46.06	0.85
Yanhusuo	MOL004199	Corynoloxine	38.12	0.60
Yanhusuo	MOL004200	Methyl-[2-(3,4,6,7-tetramethoxy-1-phenanthryl) ethyl] amine	61.15	0.44
Yanhusuo	MOL004202	Dehydrocavidine	38.99	0.81
Yanhusuo	MOL004203	Dehydrocorybulbine	46.97	0.63
Yanhusuo	MOL004204	Dehydrocorydaline	41.98	0.68
Yanhusuo	MOL004205	Dehydrocorydalmine	43.90	0.59
Yanhusuo	MOL004208	Demethylcorydalmatine	38.99	0.54
Yanhusuo	MOL004209	13-methyldehydrocorydalmine	35.94	0.63
Yanhusuo	MOL004210	(1S,8'R)-6,7-dimethoxy-2-methylspiro[3,4-dihydroisoquinoline-1,7'-6,8-dihydrocyclopenta[g][1,3]benzodioxole]-8'-ol	43.95	0.72
Yanhusuo	MOL004763	Izoteolin	39.53	0.51
Yanhusuo	MOL004214	Isocorybulbine	40.18	0.66
Yanhusuo	MOL004215	Leonticine	45.79	0.26
Yanhusuo	MOL004216	13-methylpalmatrubine	40.97	0.63
Yanhusuo	MOL004220	N-Methyllaurotetanine	41.62	0.56
Yanhusuo	MOL004221	Norglaucing	30.35	0.56
Yanhusuo	MOL004224	Pontevedrine	30.28	0.71
Yanhusuo	MOL004225	Pseudocoptisine	38.97	0.86
Yanhusuo	MOL004226	24240-05-9	53.75	0.83
Yanhusuo	MOL004228	Saulatine	42.74	0.79
Yanhusuo	MOL004230	Stylopine	48.25	0.85
Yanhusuo	MOL004231	Tetrahydrocorysamine	34.17	0.86
Yanhusuo	MOL004232	Tetrahydroprotopapaverine	57.28	0.33
Yanhusuo	MOL004233	ST057701	31.87	0.56
Yanhusuo	MOL004234	2,3,9,10-tetramethoxy-13-methyl-5,6-dihydroisoquinolino[2,1-b]isoquinolin-8-one	76.77	0.73
Yanhusuo	MOL000449	Stigmasterol	43.83	0.76
Yanhusuo	MOL000785	Palmatine	64.60	0.65
Yanhusuo	MOL000787	Fumarine	59.26	0.83
Yanhusuo	MOL000790	Isocorypalmine	35.77	0.59
Yanhusuo	MOL000791	Bicuculline	69.67	0.88
Yanhusuo	MOL000793	C09367	47.54	0.69
Yanhusuo	MOL000098	Quercetin	46.43	0.28
Yanhusuo	–	DL-Tetrahydropalmatine	–	–
Yanhusuo	–	Tetrahydrocoptisine	–	–
Yanhusuo	–	Dehydroglaucine	–	–
Yanhusuo	–	Tetrahydrocolumbamine	–	–
Yanhusuo	–	Corybulbine	–	–
Yanhusuo	–	Tetrahydroberberine	–	–
Yanhusuo	–	Columbamine	–	–
Yanhusuo	–	Yuanhunine	–	–
Yanhusuo	–	Protopine	–	–
Yanhusuo	–	Alpha-Allocryptopine	–	–
Yanhusuo	–	Cryptopine	–	–

OB, oral bioavailability; DL, drug-like.

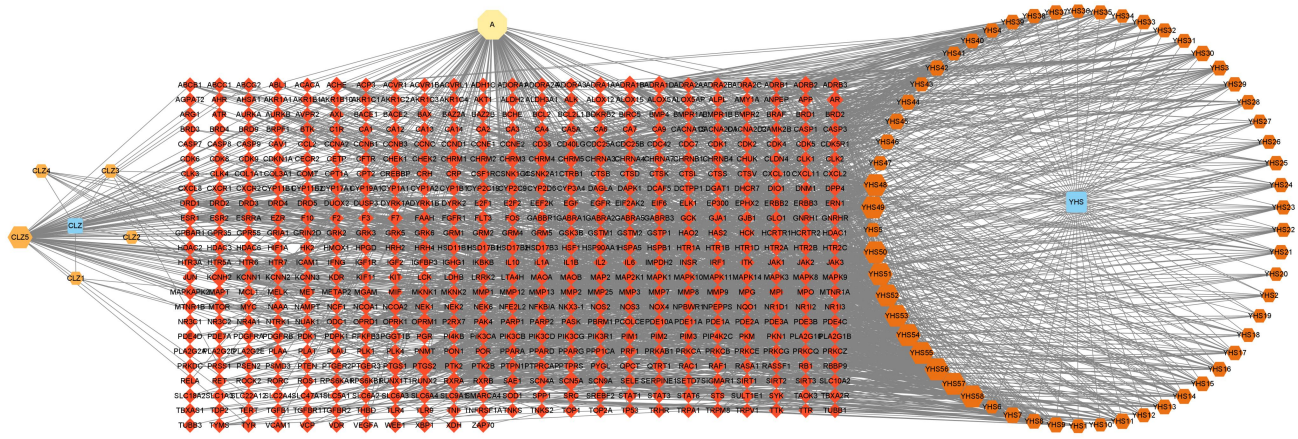

Figure 1 "Active ingredient-target" network diagram. Blue represents the drug, orange represents the chemical constituents of CLZ and YHS respectively, red diamond represents the drug target, octagon A (quercetin) is the common constituent of CLZ and YHS.

from OMIM database and 46 EMs-related targets were obtained from Drugbank database. In total, 1813 EMs-related targets were obtained, and only 1 duplicate target was retained, resulting in the final EMs-related target. The Venn diagram of the key target genes was obtained by taking the intersection of the active ingredient targets and the disease targets (Figure 2).

Protein interaction network analysis of key target genes

The 208 key target genes were imported into the String database to obtain protein-protein interactions (Figure 3). The results were then imported into Cytoscape 3.8.2 software, and the PPI network was plotted with the Degree value reflecting the size of the nodes, including MAPK1, AKT1, PIK3R1, and PIK3CA (Figure 4). Larger Degree values indicate that these targets play a crucial role in the treatment of EMs using Jinlingzi san.

GO analysis of key targets

The results of GO enrichment analysis revealed that the biological processes associated with key target genes mainly include negative regulation of apoptotic process, protein phosphorylation, positive regulation of gene expression, inflammatory response, positive regulation of cell proliferation, aging, and positive regulation of apoptotic process. In terms of molecular functions, enzyme binding, protein kinase activity, RNA polymerase II transcription factor activity, sequence-specific DNA binding, transcription factor binding, and heme binding play important roles. In cellular components, they mainly include cytosol, receptor complex, cytoplasm, nucleoplasm, and plasma membrane (Figure 5).

KEGG pathway analysis

Based on the KEGG pathway enrichment analysis (Figure 6), the key target pathways mainly focus on pathways in cancer, lipid and atherosclerosis, hepatitis B, AGE-RAGE signaling pathway in diabetic complications, pancreatic cancer, prostate cancer, PI3K-Akt signaling pathway, Th17 cell differentiation, and other signaling pathways. These pathways play crucial roles in human immunity, inflammation, and other related processes.

Molecular docking and results

The PPI results indicate that genes STAT3, SRC, TP53, JUN, and MAPK3 play a crucial role in the treatment of EMs using Jinlingzi san. The compounds with the top five Degree values were analyzed for their Jinlingzi san drug composition using Cyoscape 3.8.2, and molecular docking experiments were conducted using Autodock software to verify the binding activity of compounds and targets. The docking results were then imported into Pymol software to visualize the results (Figure 7). The binding energy of the docking results was less than − 5 KJ/mol, indicating a strong binding ability of the molecule to the protein (Figure 8).

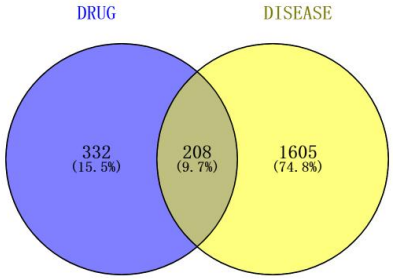

Figure 2 Venn diagram of Jinlingzi san targets and EMs targets. EMs, endometriosis.

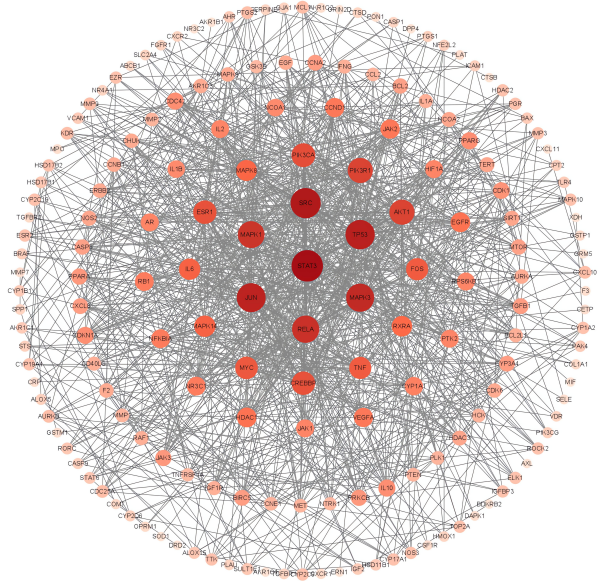

Figure 3 PPI network diagram of key targets and EMs targets Darker color and larger nodes represent larger Degree values. PPI, protein-protein interactions; EMs, endometriosis.

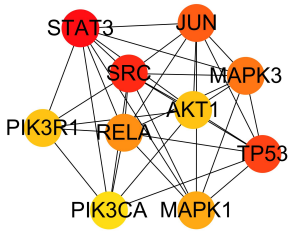

Figure 4 Core targets

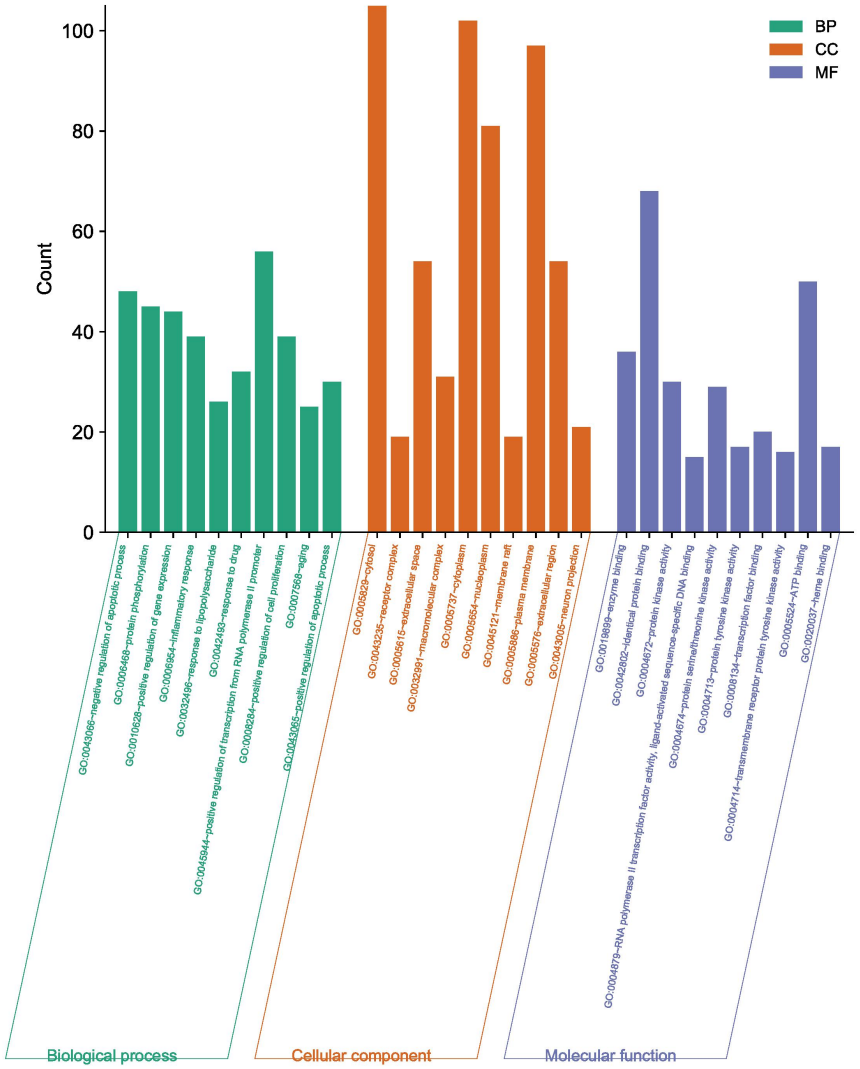

Figure 5 GO enrichment analysis. GO, gene ontology.

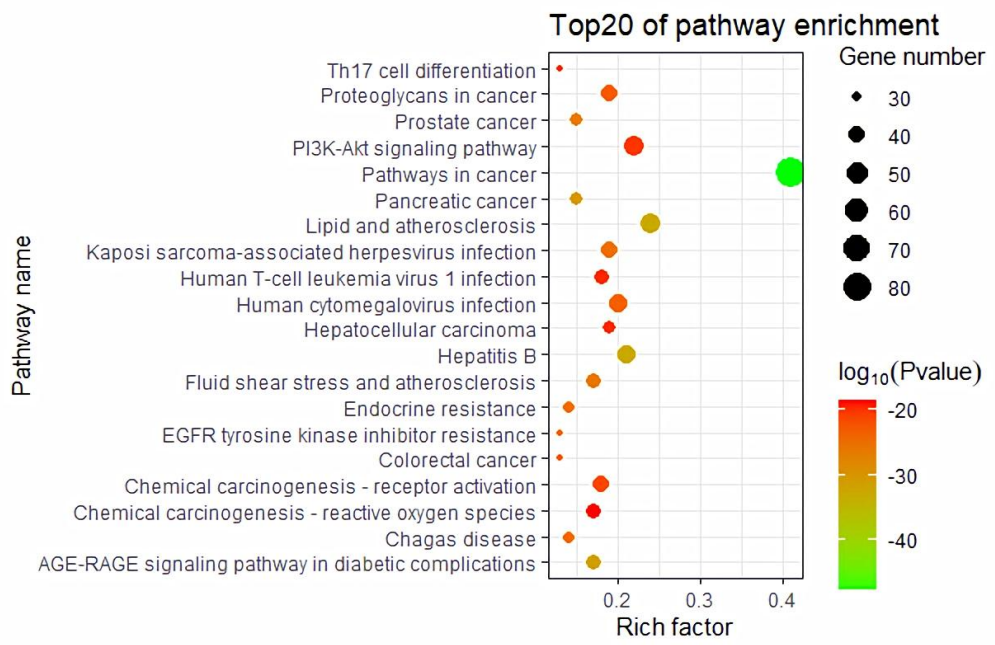

Figure 6 KEGG enrichment analysis. KEGG, Kyoto Encyclopedia of Genes and Genomes.

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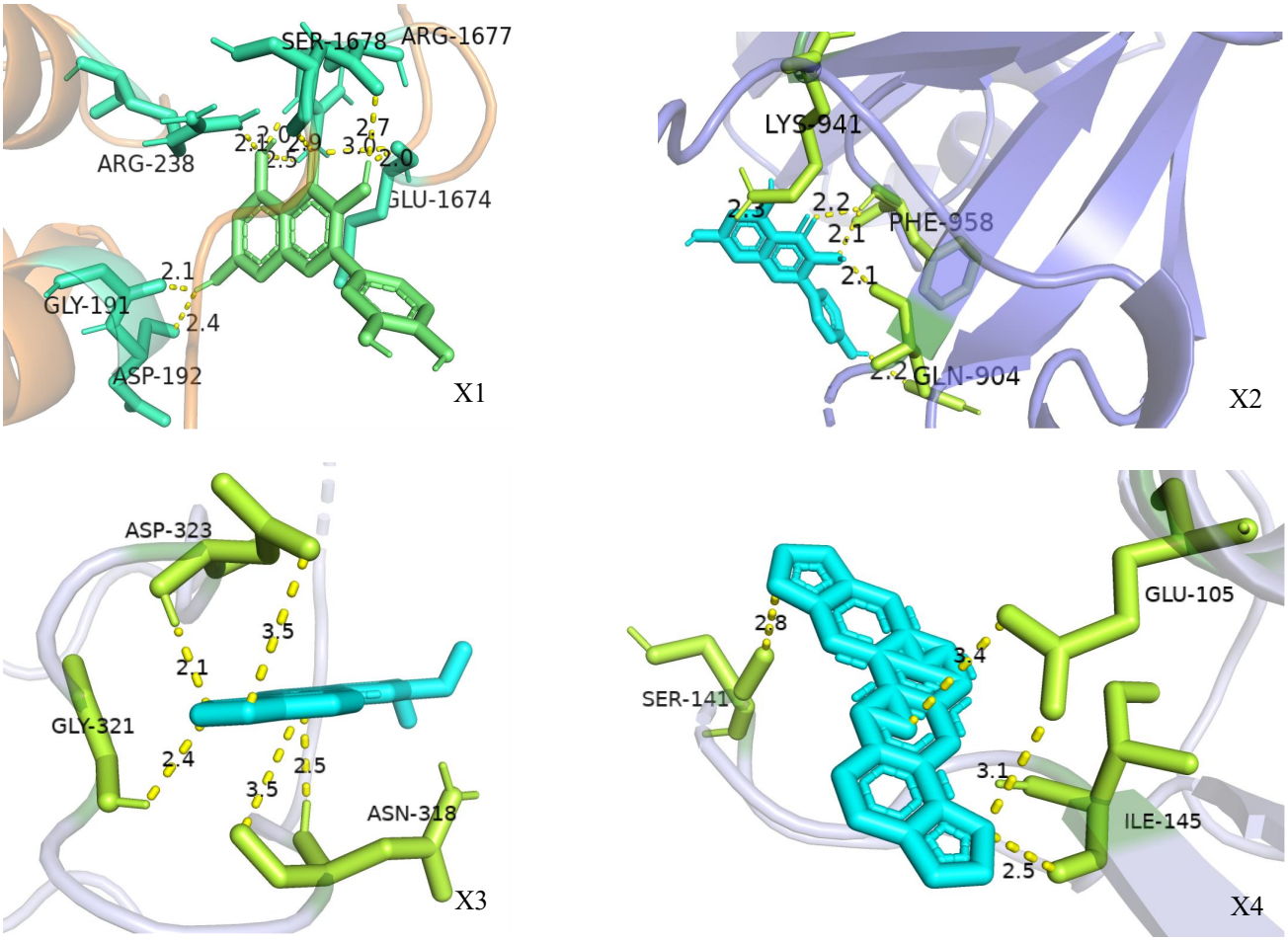

Figure 7 Molecular docking of the major compounds to their core targets. X1, Docking results of TP53 with quercetin; X2, Docking results of STAT3 with kaempferol; X3, Docking results of JUN with Alpha-Allocryptopine; X4, Docking results of SRC with Protopine.

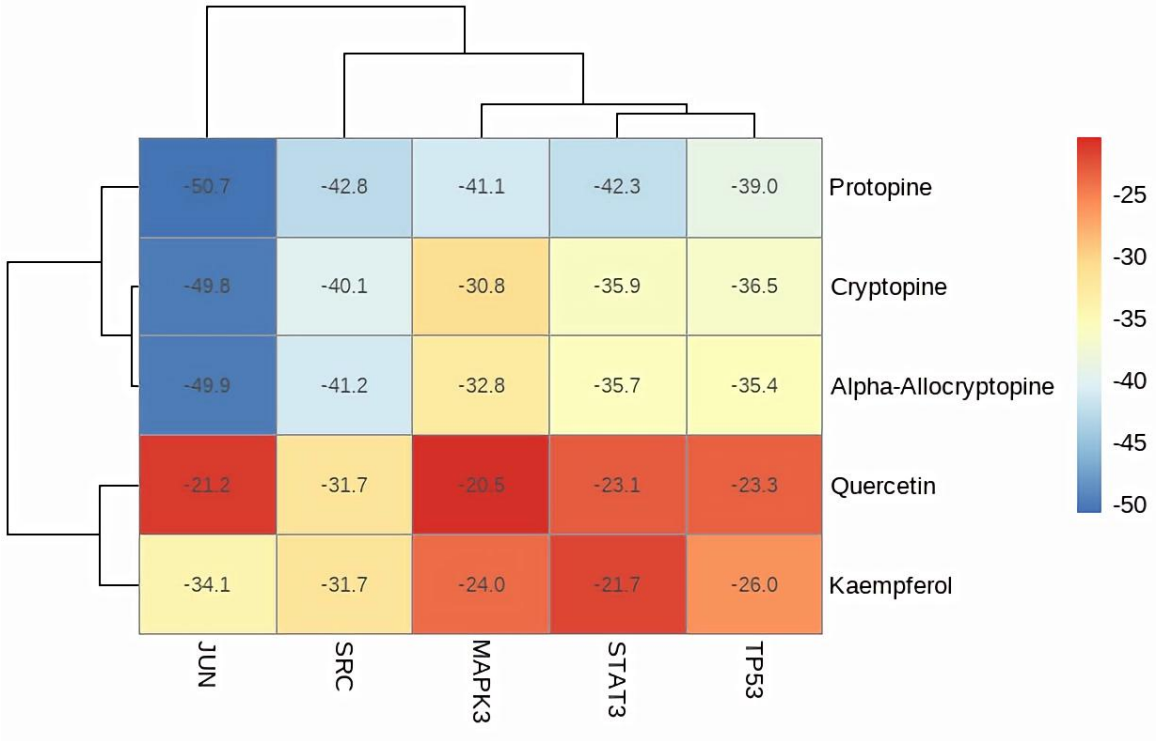

Figure 8 Molecular docking results

Discussion

Jinlingzi San possesses the characteristics of multi-component, multi-target, and multi-system regulation. Network pharmacology can analyze the multi-level and multi-faceted biological network relationship between "herbal compoun-herbal medicine-herbal active ingredient-drug target-disease", predict the effect of multi-target interaction in herbal medicine, and the pharmacological basis of herbal compound. It provides an essential reference for the discovery of pharmacological efficacy and mechanism of drugs. In this study, a systematic analysis was conducted to predict the potential active ingredients, targets, and related signaling pathways of Jinlingzi San in the treatment of EMs using network pharmacology. This analysis provides a theoretical basis for the mechanism of action of Jinlingzi San in the treatment of EMs.

In this study, we identified 60 main ingredients in Jinlingzi san, including quercetin, Cryptopine, Protopine, Alpha-Allocryptopine, Kaempferol, ethyl linolenic acid, maltol, and melphalanone. Among them, quercetin is a crucial ingredient with various pharmacological effects. Studies have shown that quercetin has antiproliferative and anti-inflammatory effects in a mouse model of EMs autotransplantation [22]. It inhibits the proliferation of VK2/E6E7 and End1/E6E7 cells, induces cell cycle arrest, and induces apoptosis through DNA fragmentation, loss of mitochondrial membrane potential, and reactive oxygen species production. Additionally, it downregulates ERK1/2, P38 MAPK, and AKT signaling molecules. Quercetin was also found to have an inhibitory effect on surgically induced EMs in rats [23]. It can inhibit the growth of ectopic endothelium in the rat model of EMs by decreasing serum FSH and LH levels, leading to a decrease in local estrogen content. Moreover, quercetin can reduce the expression of ERα, ERβ, and PR in hypothalamus, pituitary, and endometrium, thus inhibiting the binding of estrogen and progesterone to their receptors and exerting anti-estrogen and progesterone effects [24]. Kaempferol is another flavonoid that affects T-cell signaling and has anti-inflammatory, antioxidant, and anticancer effects. The combined application of quercetin and kaempferol enhances the antioxidant, anti-inflammatory, and anti-cancer effects [25]. In a study by Zhong Wenliang et al, kaempferol was found to be involved in the regulation of tumor cell signaling pathways, most notably the PI3K/Akt signaling pathway, which induces and maintains pain sensitivity and plays a crucial role in the formation and development of neuropathic pain [26]. Therefore, kaempferol can improve pain in EMs by modulating the PI3K/Akt signaling pathway [27]. Shao Jingbao et al found that the total alkaloids of yanhusuo were effective in inhibiting central and peripheral pain, and Cryptopine, Protopine, and Alpha-Allocryptopine were among the components of the alkaloids [28].

The drug's active ingredients and targets of Jinlingzi San were screened by constructing PPI networks to identify core proteins such as STAT3, TP53, SRC, and JUN, which were found to be involved in various bioregulatory processes, including substance metabolism, cell proliferation, differentiation, transformation, apoptosis, angiogenesis, and other aspects. Studies have shown that cytokine-stimulated STAT3 is continuously activated and overexpressed, leading to the activation of the JAK2/STAT3 pathway in the organism of EMs patients. This abnormal proliferation of ectopic endometrial cells and blocking of normal apoptosis can result in malignant behaviors such as adhesion, invasion, infiltration, and angiogenesis of endometrium in ectopic sites. The use of STAT3 inhibitors has been found to effectively inhibit various pathological processes, suggesting that they may be novel agents for the treatment of EMs [29]. TP53 is closely related to EMs and can inhibit cell proliferation, migration, and invasion while promoting apoptosis through targeted therapy [30]. Studies have shown that UCA1, which is highly expressed in in situ endothelial cells of patients with EMs, inhibits TP53 expression and suppresses autophagy for the purpose of treating EMs [31].

Pathway enrichment analysis revealed the molecular biological functions of these targets and the biological pathways in which they are located. It was found that their treatment of EMs may be mainly related to mechanisms such as inhibition of the inflammatory response, anti-angiogenesis, inhibition of cell proliferation and differentiation, anti-tumor, and inhibition of transcription factor binding. Yilmaz et al. showed that endometriotic mesenchymal cells from patients with EMs develop abnormalities in the expression of nuclear receptors, which in turn mimics the process of cancer metastasis [32]. Although EMs does not belong to the category of cancer, several cancer-related pathways such as prostate cancer, bladder cancer, and colorectal cancer are obtained in the KEGG. Studies have shown that downregulation of RGS2 and MYLK gene expression is involved in the malignant process of EMs-associated ovarian clear cell carcinoma [33]. Ectopic endothelial cells are particularly similar to tumor cells, with rapid implantation, proliferation, and involvement in angiogenesis. The PI3K-Akt signaling pathway is mainly associated with the suppression of inflammatory responses. It has been demonstrated that the PI3K-Akt pathway is altered in the endometrium of patients with EMs, is associated with ectopic tissue angiogenesis, and is involved in the development of EMs. Inhibition of the PI3K-Akt pathway has been found to reduce the volume of ectopic lesions as well as the inflammatory response [34, 35].

In conclusion, this study investigated the main active components and potential molecular mechanisms of action of Jinlingzi San for the treatment of EMs at the molecular level. The findings provide a theoretical basis for subsequent studies on the pharmacodynamic substance basis, quality control enhancement, and mechanism of action of this drug.

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