Using network pharmacology and molecular docking to explore the underlying anti-inflammatory mechanism of Wuyao-Danshen to treat endometriosis

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Background: This study sought to explore the anti-inflammatory mechanism of Wuyao (radix linderae)–Danshen (salviae miltiorrhiza) in endometriosis (EMS) based on network pharmacology and molecular docking.

Methods: The active constituents of Wuyao-Danshen were collected and identified using the Traditional Chinese Medicine Systems Pharmacology Database, and used to predict and identify the protein targets. The EMS targets and anti-inflammatory targets were obtained from Genecards, Online Mendelian Inheritance in Man, and Drugbank. The Search Tool for the Retrieval of Interacting Genes/Proteins database was used to analyze the protein interactions (PPIs) and core targets, and a target PPI network was constructed by importing the software of Cytoscape. The Metascape database was used to conduct enrichment analyses of the Gene Ontology (GO) functions and the Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathways for the key anti-inflammatory targets of EMS. Finally, Autodock Vina software was used to verify the results of the active ingredients and key anti-inflammatory targets.

Results: There were 8 active components in Wuyao, 65 in Danshen, and 591 corresponding protein targets in Danshen, and 375 in Wuyao, including luteolin, quercetin, vancomyl alcohol, and salvianol. One thousand and six hundred eighty-nine disease targets, 1,216 anti-inflammatory targets, and 144 key anti-inflammatory targets were identified, including the (signal transduction and transcriptional activator 3) *STAT3*, phosphatidyl inositol-3 kinase regulates subunit 1 (*PIK3R1*) and mitogen-activated protein kinase 1 (*MAPK1*) protein kinase B. Three hundred and fifty-three biological processes (BPs), 21 cellular components, and 25 molecular functions (MFs) were enriched with GO functions, and 108 KEGG pathways were enriched and analyzed, including the *MAPK* and *PI3K-Akt* signaling pathways. Molecular docking confirmed that luteolin, coumarin, and quercetin could bind to the key target proteins (i.e., *STAT3*, *PIK3R1*, and *MAPK1*).

Conclusions: Based on network pharmacology and molecular docking, Wuyao-Danshen was found to act on EMS through anti-inflammatory targets and related signaling pathways. Our findings provide a basis for further research.

Keywords: Network pharmacology; molecular docking; Wuyao-Danshen; endometriosis (EMS); anti-inflammatory

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Introduction

Endometriosis (EMS) is a common gynecological disorder characterized by the implantation of endometrial tissue outside the uterus, resulting in chronic inflammation, pain, and infertility (1). EMS is a refractory disease with extensive lesions, diverse forms, and it is extremely invasive and recurrent. It affects 10–15% of women of childbearing age in their lifetime (2). The incidence of EMS has increased in recent years, and seriously affects women's health and quality of life. EMS has a long history, but its exact pathogenesis is not yet known. The long-term abnormal inflammatory environment in the human body is an important factor in the development and progression of the disease, and the most typical histological features are local inflammation and peritoneal fibrosis (3). Western medicine treatment methods focus on estrogen suppression, including gonadal hormone analogues and progesterone (4), or surgical treatment if necessary. However, estrogen suppression has many side effects, and the condition repeats after drug withdrawal, and removing the lesion by surgery does not provide a cure, which is often unacceptable to patients. The traditional Chinese medicine (TCM) treatment has no inhibitory effect on the pituitary gland and ovary, does not interfere with the normal menstrual cycle, has an obvious curative effect (i.e., relieves pain, controls the development of lesions, and regulates menstruation), and does not affect pregnancy.

The main treatment for EMS in TCM is to activate blood and remove blood stasis. Professor Wang Ziyu, a nationally renowned veteran TCM practitioner, also believes that blood stasis is the main pathology of EMS, and the Qi stagnation and cold clotting of blood stasis is common. Wu Dan pill is a famous formula of Professor Wang to treat EMS by invigorating blood, benefiting kidney, dispersing cold and relieving pain. It consists of more than 10 herbal flavors, including Wu Yao, Dan Shen, Tao Ren, Red Peony, Curcuma longa, Leech, Yuan Hu, Cinnamon, and Chuan Jie. Wuyao-Danshen is combination as the monarch medicine, which plays a key role.

Network pharmacology (5) is a new type of biological system network analysis that is based on systems biology theory. It seeks to understand the nature of diseases from the holistic perspective of network balance, and systematically explores the efficacy and mechanisms of TCM with multi-component, multi-target, and multi-mechanism effects. A network pharmacology research method was used in this study to explore the effect of the inflammatory reaction of the wudan pill gentleman medicine (i.e., radix linderae-

salvia miltiorrhiza), and the potential anti-inflammatory mechanism of EMS. Molecular docking technology, key efficacy components, and docking target validation were used to further elucidate the clinical curative effect of the medicine and the underlying mechanism.

Methods

Materials

Table 1 sets out the databases, analysis software, and analysis platform information used in this experiment.

Study methods

Screening of drug active ingredients and corresponding targets

The Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) database was searched using the following terms: "Radix linderae" and "salvia miltiorrhiza", with an OB (oral availability) ≥30%, and DL (similarity of patent medicine) ≥0.18 to determine the active compound composition information for "Radix linderae–Salvia miltiorrhiza". Additionally, PubChem was searched for details of the compound composition of the TCM not included in the TCMSP database. Next, swisStar prediction was used to predict potential target proteins of the drug compounds. Then, after logging into the Uniprot database, the biological species was limited to "human", and the data type was set to be reviewed according to the full name of the candidate target, and the corresponding gene name and Uniprot KB (UniProt Knowledgebase) of the target were obtained.

Collection of EMS and anti-inflammatory targets

The GeneCards database was searched using the following keywords: "Endometriosis" and "anti-inflammatory". Correlation scores >20 were set to retrieve information on the related genes. The Online Mendelian Inheritance in Man (OMIM), DRUGBANK, Therapeutic Target Database (TTD), and DISGENET databases were then searched using the same keywords. The targets of the 5 databases were uploaded to the UniProt database to identify the corresponding gene information and correct the targets. Next, the intersection of the EMS target with the anti-inflammatory related targets and compound targets was obtained to identify the common anti-inflammatory targets in the treatment of EMS with Wuyao-Danshen. The study was conducted in accordance with the Declaration of

Table 1 Database and software information used in the experiment

Name	Website
TCMSP	https://tcmspw.com/tcmsp.php
PubChem	https://www.ncbi.nlm.nih.gov/pccompound
Swiss Target Prediction	http://www.swisstargetprediction.ch/
Uniprot	https://www.un iprot.org/
GeneCards	https://www.genecards.org/
OMIM	https://omim.org/
DRUGBANK	https://go.drugbank.com/
TTD	http://db.idrblab.net/ttd/
DISGENET	https://www.genecards.org/
STRING	www.string-db.org
Cytoscape	–
Venny	http://www.bioinformatics.com.cn
Metascape	https://metascape.org
Autodock vina	–
Autodock tool	–
Discovery	–
Python	–
Pymol	–
PDB	http://www.pdb.org/

Helsinki (as revised in 2013).

Analysis of common anti-inflammatory targets

The obtained common anti-inflammatory targets were imported into the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database in the form of Genes YMBOL to analyze the interaction between proteins and obtain the key anti-inflammatory targets for the treatment of EMS with Wuyao-Danshen. Dig name url TCMSP dug core targets and protein interaction (PPI) network diagram. The species was selected as "human", and the minimum interaction threshold was set as "high confidence (≥0.9)". To improve the confidence of the PPI, a combined score >0.7 was selected, and nodes (Node 1, and Node 2), and the combined score information data were saved. Next, Cytoscape version 3.7.1 software was imported and a topology analysis was conducted using the NetworkAnalyzer plug-in. The core targets were screened according to the median centrality, compact density, and node connection degree.

Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis

Metascape (6) contains details of biological pathways and protein complexes, applies a powerful analytical pipeline to produce easily interpretable results, provides an annotated gene list, and rich and interactive resources, and supports the integrated analysis of multiple omics data sets. GO enrichment analysis and a KEGG pathway enrichment analysis were conducted to examine the potential role of "radix linderae-salvia miltiorrhiza" in the treatment of EMS target genes, and determine the corresponding molecular functions (MFs), biological processes (BPs), cellular compositions (CCs) and KEGG pathway-related data. To improve the confidence of the related pathways, the top 10 genes from a large proportion of genes enriched in the pathways were then screened, and data visualization was performed using the online platform of WeChat biogenesis to determine their main biological functions (BFs) and related signaling pathways.

Molecular docking

The core target protein structure was downloaded from the Protein Data Bank (PDB) database, and the water molecule was removed using PyMol1.8 software, and the original ligand was separated. After saving, it was imported into AutodockTools1.5.6 software for hydrogenation, total charge calculation, and atomic type setting, and saved in "PDBQT" format. The MOL2 structure of the core component (ligand) screened above was downloaded from the TCMSP database, and the rotable key was set by Autodock Tools and saved as a file in "PDBQT" format. Finally, molecular docking was performed by Autodock-Vina1.1.2 software. PyMol software visualizes the docking results and creates docking interaction model diagrams.

Results

Collection of drug active ingredients and corresponding targets

Through TCMSP and PubChem, "radix linderae-salvia miltiorrhiza" compounds were retrieved, and 8 radix linderae and 65 salvia miltiorrhiza effective compounds were identified. The Wiss Target Prediction database predicted and identified 591 salvia miltiorrhiza and 375 aconite protein

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Table 2 Main components of radix linderae-salvia miltiorrhiza

TCMS number	Compound name	OB%	DL
MOL000006	Luteolin	36.16	0.25
MOL007050	2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	62.78	0.40
MOL007077	Sclareol	43.67	0.21
MOL007081	Danshenol B	57.95	0.56
MOL007082	Danshenol A	56.97	0.52
MOL007155	(6S)-6-(hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho(8,7-g)benzofuran-10,11-dione	65.26	0.45
MOL000098	Quercetin	46.43	0.28
MOL000358	Beta-sitosterol	36.91	0.75
MOL000495	6,7-dimethoxy-2-(2-phenylethyl)chromone	31.93	0.30
MOL000496	DMPEC	32.38	0.39
MOL000907	Norboldine	40.92	0.46
MOL000917	Boldine	31.18	0.51
MOL007036	Isopropyl-1,1-dimethyl-2,3-dihydrophenanthren-4-one	33.77	0.29
MOL007085	Salvilenone	30.38	0.38
MOL007041	2-isopropyl-8-methylphenanthrene-3,4-dione	40.86	0.23

OB, oral availability; DL, similarity of patent medicine.

Figure 1 Drug-compound-target network diagram.

targets corresponding to the active ingredients (see *Table 2*). The drug active components and their corresponding targets were imported into Cytoscape 3.7.1 to construct a drug-component-target network diagram. The network comprised 271 nodes, including 122 active ingredient nodes, 81 target nodes, and 1,244 edges. As *Figure 1* shows, "radix linderae-salvia miltiorrhiza" mainly acts through multiple components corresponding to multiple targets.

Collection of disease-related targets

DISGENET, DRUGBANK, GeneCards, OMIM, and

TTD were used to search and obtain EMS target genes. Next, the 1,689 target genes were converted into UNIPROt by UNIPROt. A total of 1,216 anti-inflammatory targets were identified using the same method. A total of 559 targets were identified from the intersection of EMS with anti-inflammatory targets, and 144 targets were identified from the intersection of EMS with effective compound targets (see *Figure 2*).

PPI analysis of key targets

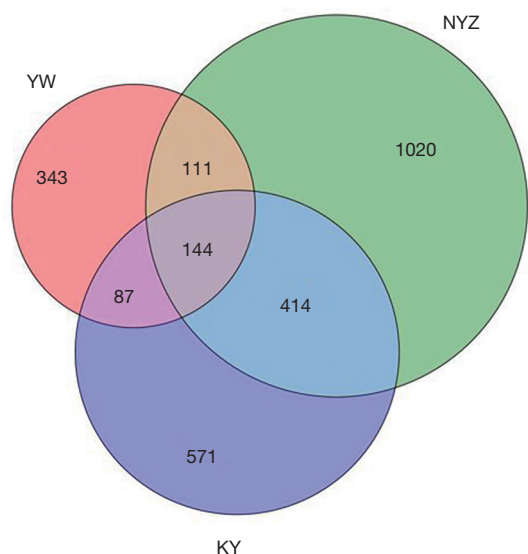Homo sapiens were selected to construct the PPI network

Figure 2 Venn diagram of the intersection of drug—EMS—anti-inflammatory. EMS, endometriosis; NYZ, Endometriosis; YW, drug (wuyao-danshen); KY, anti-inflammatory.
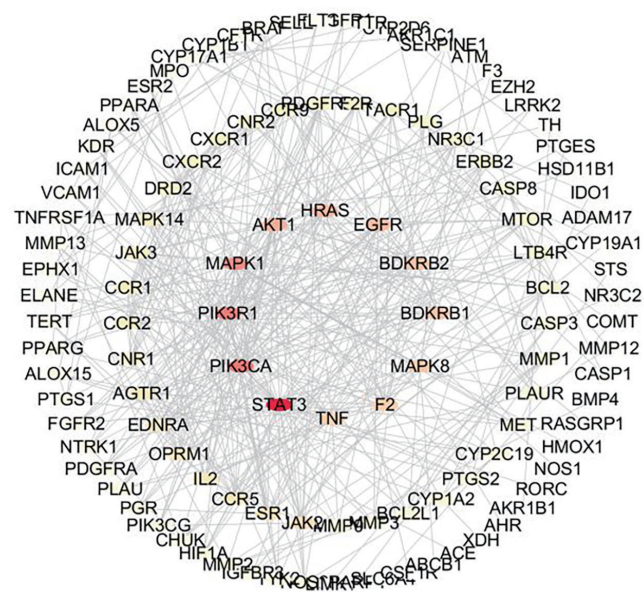

Figure 3 Interaction network diagram of key anti-inflammatory target proteins.

map, which was visualized using Cytoscape software. The nodes in the network map represented the protein molecules of the anti-inflammatory targets, and the edges represented the relationship between each target. The color in the figure represents the value of the degree of the anti-

inflammatory target; the intensity of the color (from light to dark) indicates the degree value of the anti-inflammatory target (from smaller to larger). The higher the de-GRE values of signal transduction and transcriptional activator 3 (*STAT3*), alpha catalytic subunit of phosphoinositol-3-kinase (*PIK3CA*), *PIK3R1*, *MAPK1*, *AKT1*, (GTPase Hras) *HRAS*, epidermal growth factor receptor (*EGFR*) and Bradykinin receptor B1 (*BDKRB1*), the more likely they are to be the key anti-inflammatory targets of endopathy (see *Figure 3*).

GO and KEGG pathway enrichment analyses

A GO enrichment analysis was conducted to describe the gene functions, and a KEGG pathway enrichment analysis was conducted to identify the significantly enriched signal pathways and explore the BPs and signal pathways of EMS treatment by radix linderae–salvia miltiorrhiza. The Metascape platform was used for the GO and KEGG pathway analyses (P<0.05), and a pathway annotation analysis was combined with the database. There were 399 GO items (P<0.05), including 353 BP items, 21 CC items, and 25 MF items. The top 10 items of each component were imported into the bioinformation platform for visualization (see *Figure 4*). The BP entries occurred before the response to lipopolysaccharide (the reaction), the response to the molecule of bacterial origin (the bacteria source molecular reaction), and the positive regulation of the response to external stimulus (external stimulating reaction positive adjustment). At present, the 2 CC strips were the Caveola and Plasman embrace raft. MF article 2 was currently the transmembrane receptor protein tyrosine kinase activity transmembrane receptor protein kinase activity (transmembrane receptor protein kinase activity). The color intensity represents the size of P; the darker the color, the smaller the P. The bubble size represents the number of enrichment targets in this pathway; the larger the number of points, the greater the number. There were 108 KEGG signaling pathways. The mitogen-activated protein kinase (*MAPK*) signaling Pathway and Phosphoinositide 3-kinase-Protein kinase B (*PI3K-AKT*) signaling pathway were the most significant (see *Figure 5*).

Molecular docking

To further validate the effect of the wudan pill gentleman medicine (i.e., radix linderae-salvia miltiorrhiza) in the treatment of EMS candidate compounds, the compounds 7 8 and participation key pathways targets before docking,
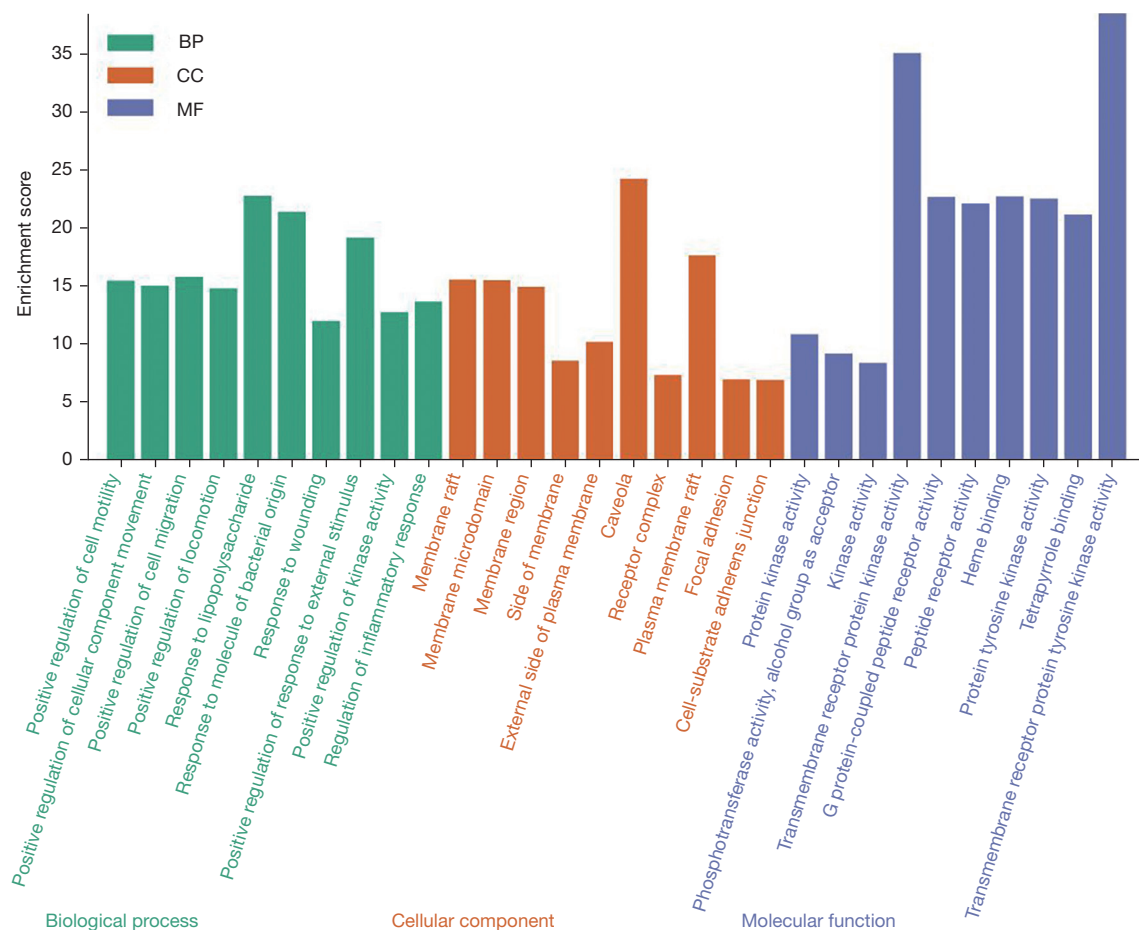
Figure 4 Histogram of GO enrichment at key targets. GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function.

found the lowest binding energy, –5.0 kJ mol, 1 or less, results demonstrate the validity of efficacy of molecular docking with the protein, the lower the binding energy, show that molecular and protein binding ability is poor (see *Table 3*). The docking results of luteolin, sclareol, quercetin, and the potential target proteins of *STAT3* (PDB:6NUQ), *PIK3R1* (PDB:6PYR), and *MAPK1* (PDB:6G8X) were visualized using PyMol software (see *Figure 6*). The docking analysis successfully predicted that luteolin, vanillyl alcohol, and quercetin bind well to the activity sites of the 3 target proteins. These results further prove that these 3 proteins can be used as therapeutic targets of luteolin, vanillyl alcohol, and quercetin in the inflammatory response of EMS.

Discussion

EMS is an immune inflammatory disease, which is mainly treated by surgery and drug therapy, but it produces adverse reactions, and EMS is prone to recurrence. Research on long-term chronic disease management needs to be conducted. TCM has played a positive role in safe guarding the fertility of EMs patients by taking the advantages of holistic concept and evidence-based treatment in the treatment of EMs, but the exact mechanism of action is unknown and needs to be explored by applying modern research methods in order to take advantage of natural herbal medicines. Scholars at home and abroad have turned their attention to the development and use of TCM to identify safe and effective drugs. Ziyu's empirical prescription of wudan pills has been clinically proven to improve the symptoms of EMS patients. Many studies have shown that aconite (7,8) has anti-inflammatory and analgesic effects, and salvia miltiorrhiza (9,10) has anti-inflammatory and blood-activating effects. which participate
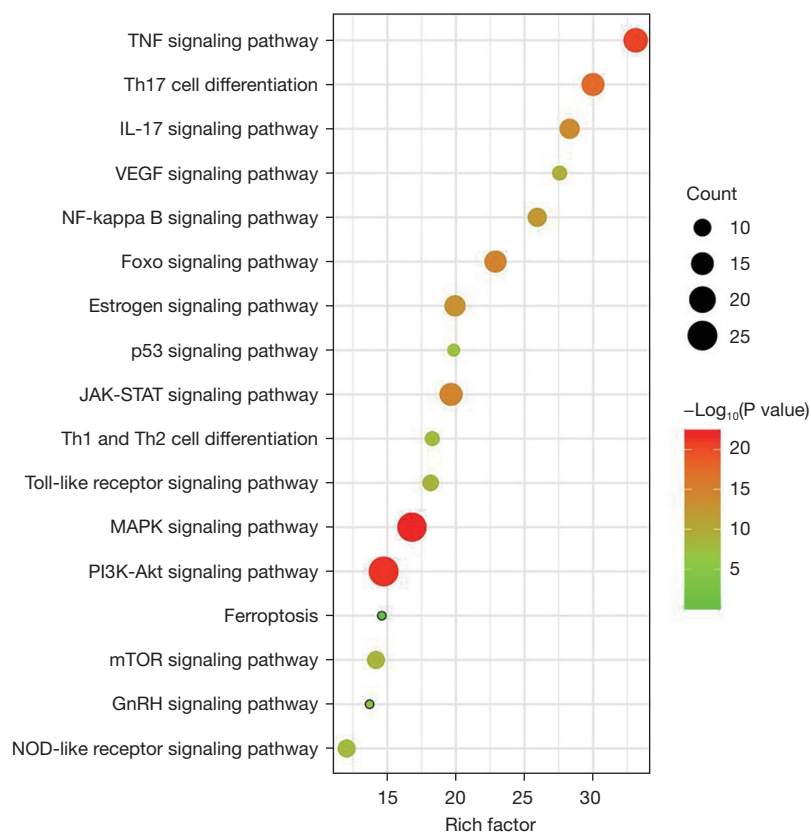
Figure 5 KEGG enrichment pathway analysis of key targets. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 3 Binding capacity of core compounds to core proteins (Kcal·mol^{-1})

MOL ID	Compound	STAT3	PIK3CA	PIK3R1	MAPK1	AKT1	HRAS	EGFR
MOL000006	Luteolin	−8.4	−7.2	−8.0	−7.4	−6.5	−6.6	−8.0
MOL007050	2-(4-hydroxy...	8.1	−8.0	−7.2	−7.5	−6.6	−6.1	−6.3
MOL007077	Sclareol	−5.5	−5.9	−6.9	−6.5	−6.5	−6.2	−7.4
MOL007081	Danshenol B	−7.2	−5.8	−6.4	−6.8	−6.3	−7.2	−7.7
MOL007082	Danshenol A	−6.5	−7.5	−7.3	−7.5	−7.1	−5.9	−8.1
MOL007155	(6S)-6-(hydrox...	−6.2	−6.5	−7.0	−7.0	−6.4	−5.7	−6.6
MOL000098	Quercetin	−8.7	−5.9	−8.1	−7.5	−6.6	−3.7	−8.0
MOL000358	Beta-sitosterol	−7.1	−6.6	−7.0	−5.9	−6.4	−7.5	−6.3

2-(4-hydroxy..., 2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde; (6S)-6-(hydrox..., (6S)-6-(hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho(8,7-g)benzofuran-10,11-dione.

in the inflammatory response of EMS, and inhibit the growth of ectopic endometrium. Thus, this study explored and analyzed the anti-inflammatory mechanism of radix linderae-salvia miltiorrhiza in the treatment of EMS from an anti-inflammatory perspective.

In this study, effective active compounds, such as luteolin of salvia miltiorrhiza, vanillyl alcohol, salviol, and quercetin of aconitum, were identified in the TCMSP database. Luteolin (11) has anti-inflammatory and antioxidant effects, this study (12) has shown that luteolin affects the
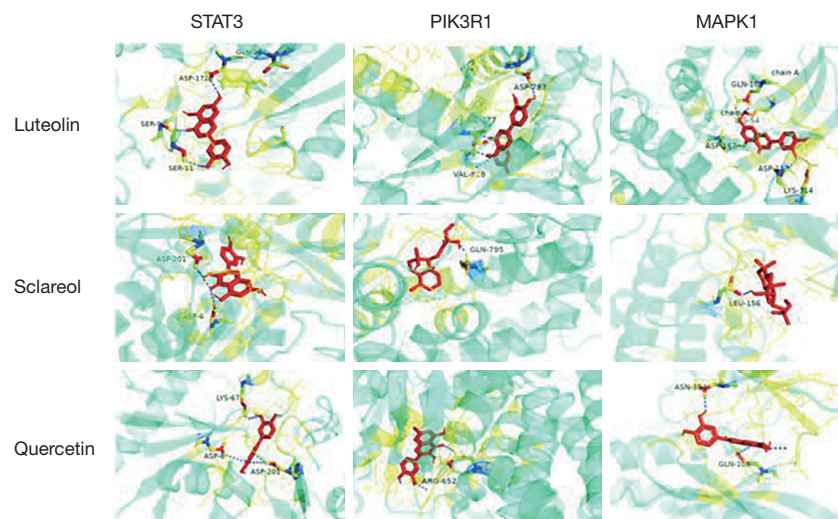
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Figure 6 Molecular docking diagram of some key targets and active ingredients.

expression of inflammatory mediators. Quercetin is a natural flavonoid, and the study (13) has shown that it has antibacterial, anticancer, antioxidant, and anti-inflammatory effects. Tian *et al.* (14) found that quercetin inhibits End1/E6E7 proliferation and promotes the apoptosis of EMS cells, and this inhibition is effective in the treatment of EMS. Some experiments have also shown that quercetin inhibits inflammation. Notably, Tang *et al.* (15) showed that quercetin inhibited the expression of NOD-like receptors family pyrin domain containing 3 (*NLRP3*) inflammasome-related proteins in (Henrietta Lacks) *HeLa* cells in a dose-dependent manner, thus inhibiting inflammation.

In this study, drug, disease and anti-inflammatory related targets were identified through the database number, and 144 anti-inflammatory targets for EMS treatment by radix linderae–salvia miltiorrhiza were identified by mapping their intersections with each other. The PPI results for the anti-inflammatory targets indicated that *STAT3*, *PIK3CA*, *PIK3R1*, *MAPK1*, *AKT1*, *HRAS*, *EGFR*, and *BDKRB1* may be the key anti-inflammatory targets of black herb and salviae miltiorrhiza in EMS treatment.

STAT3 (16), a member of the *STAT* protein family, plays an important role in various physiological processes, such as cell growth, differentiation, immune function, and hematopoiesis, and its activation may promote the infiltration and metastasis of active endometrium by regulating a variety of cytokines and proteins. Research (17) has shown that *STAT3* is over-activated in the ectopic endometrium of EMS patients, which may promote cell growth and resist apoptosis, angiogenesis, and invasion by

regulating the expression of matrix metalloproteinase-2 (*MMP-2*), matrix metalloproteinase-9 (*MMP-9*), and vascular endothelial growth factor (*VEGF*). Another study (18) has found correlations between EMS combined with infertility and signal transduction and *STAT3* gene polymorphism. Most studies of EMS (19) have noted that due to the continuous stimulation of external cytokines, activated *STAT3* regulates the transcription of relevant downstream target genes, promoting the abnormal proliferation, invasion, and metastasis of ectopic endometrial cells, and participating in the formation of an inflammatory microenvironment, epithelial-mesenchymal transformation, extracellular matrix degradation, and other processes. When *STAT3* in the cytoplasm is activated by Janus kinase 2 (*JAK2*), it can enter the nucleus via the direct and indirect regulation of related genes and promote angiogenesis and induce cell proliferation, differentiation, or apoptosis. Some scholars (20) have found that the inhibition of the *JAK2/STAT3* signaling pathway has a positive effect on EMS treatment.

PIK3CA and *PIK3R1* are common oncogenes, and it has been reported (21) that PIK3R1 has high frequency mutations in endometrial cancer, and the PI3K family plays a key role in the transduction of intracellular signals and the pathogenesis of inflammation, obesity, tumors, and immune diseases (22). The P110 catalytic subunit encoded by the *PIK3CA* gene and the P85 regulatory subunit encoded by the PIK3R1 gene mainly control important cellular activities, such as protein synthesis, cell growth and proliferation, angiogenesis, cell cycle, and cell survival.

When PI3 kinase is phosphorylated, its level is regulated by phosphatase and tensin homolog (*PTEN*) phosphatase activity. The signal transmission activates *AKT*, which regulates the downstream effector activation of mechanistic target of rapamycin (*mTOR*). *MTOR* is a serine-threonine kinase that plays an important role in cell growth, proliferation, and regulation. In recent years, the study of EMS and endometrial cancer through the *PI3K/Akt/mTOR* pathway (23,24) has become a popular area of research. The *PI3K/Akt/mTOR* pathway plays an important role in inflammatory diseases (25). Currently, very few studies have been conducted on the inflammatory response of EMS.

MAPK1 (26) is mainly involved in the regulation of cell proliferation, differentiation, growth, and apoptosis. The activation of the *MAPK* signaling pathway affects the activity of a variety of transcription factors, and thus regulates the expression of tumor necrosis factors (*TNF*), interleukin-1 (*IL-1*), interleukin-6 (*IL-6*), and other inflammatory cells. Wang *et al.* (27) confirmed that the Shaofu Zhuyu Decoction downregulates the expression of tumor necrosis factor alpha (*TNF-α*), *IL-6*, *IL-8*, nuclear factor kappa-light-chain-enhancer of activated B cells (*NF-κB*), *MAPK*, Extracellular Signal Regulated Kinase (*ERK*), and *VEGF*, etc., through the *MAPK/ERK* signaling pathway, which is mediated by estrogen, inhibits inflammatory factors and angiogenesis in ectopic tissues, and slows the occurrence and development of EMS. In the enrichment of KEGG pathways, *MAPK1* is directly or indirectly involved in the TNF signaling pathways, kuohaonucleotide-binding oligomerization domain protein (*NOD*) receptors signaling pathways, and *MAPK* signaling pathways, and forms a complex interaction relations of "wu medicine danshen active ingredient-anti-inflammatory target-signal path-endometriosis" network.

Conclusions

In this study, through the method of network pharmacology, it was found that the main active ingredients of radix linderae–salvia miltiorrhiza in the treatment of EMS inflammation may include luteolin, vanillyl alcohol, quercetin, and salviol B. These active ingredients inhibit the inflammatory response of EMS by acting on *STAT3*, *PIK3R1*, and *MAPK1*, and regulating the *MAPK*, *PI3-Akt* signaling pathway, *TNF*, *NF-KB*, and other signaling pathways. Additionally, molecular docking was also used to verify that the active drug ingredients have a strong binding force with the key targets. We further confirmed

the anti-inflammatory mechanism of a multi-target, multi-path, and coordinated treatment of radix linderae–salvia miltiorrhiza in the treatment of EMS, and provided a basis for subsequent experimental verification.

Footnote

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