Exploring the Mechanisms Underlying the Therapeutic Effect of *Salvia Miltiorrhiza* Against Diabetic Nephropathy Using Network Pharmacology and Molecular Docking

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Research

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Abstract

Background

The mechanisms underlying the therapeutic effect of *Salvia Miltiorrhiza* (SM) against diabetic nephropathy (DN) using systematic network pharmacology and molecular docking methods were examined.

Methods

TCMSP database was used to screen the active ingredients of SM. Gene targets were obtained using Swiss Target Prediction and TCMSP databases. Related targets of DN were retrieved from the Genecards and DisGeNET databases. Next, a PPI network was constructed using the common targets of SM-DN in the STRING database. The Metascape platform was used for GO function analysis and Cytoscape plug-in ClueGO was used for KEGG pathway enrichment analysis. Molecular docking was performed using iGEMDOCK and AutoDock Vina software. Pymol and LigPlos were used for mapping the network.

Results

Sixty-six active ingredients and 189 targets were screened from SM. Among them, 64 targets overlapped with DN targets. The PPI network diagram revealed that *AKT1*, *VEGFA*, *IL6*, *TNF*, *MAPK1*, *TP53*, *EGFR*, *STAT3*, *MAPK14*, and *JUN* were the top 10 relevant targets. GO and KEGG analyses mainly focused on advanced glycation end products, oxidative stress, inflammatory response, and immune regulation. Molecular docking revealed that the potential target genes closely related to DN, including *TNF*, *NOS2*, and *AKT1*, were more stable in combination with salvianolic acid B, and their stability was better than that of tanshinone IIA.

Conclusion

This study reveals the active components and potential molecular mechanisms involved in the therapeutic effect of SM against DN and provides a reference for the wide application of SM in clinically managing DN.

Background

Diabetic nephropathy (DN) is a serious complication common in diabetic patients. As the incidence and mortality of patients with diabetes increases year by year, the prevalence of patients with DN rises sharply [1] and has become one of the leading causes of chronic renal failure [2]. According to reports, DN accounts for approximately 40% of end-stage kidney disease [3]. DN mainly manifests as glomerular hyperfiltration, renal hypertrophy, albuminuria, and even sepsis [4], with persistent proteinuria and gradual

decline in renal function being its main characteristics [5]. However, its specific molecular mechanism is complicated and unclear, leading to a lack of effective therapies.

In recent years, research on the effects of traditional Chinese medicine (TCM) in regulating blood sugar and lipid metabolism, reducing kidney damage, delaying kidney disease, and preventing glomerular sclerosis and fibrosis have gradually been discovered. *Salvia Miltiorrhiza* (SM) has a longstanding history as a commonly used TCM for promoting blood circulation in China. Its main functions are reducing blood viscosity, improving hemorheological characteristics, accelerating fibrin degradation [6], antioxidant activity [7], anti-infection [8], and improving glucose metabolism disorders [9]. It is often used for microvascular-related diseases such as DN and diabetic retinopathy.

TCM have a multi-target and multi-path intervention strategy that can exert an overall regulatory and synergistic effects, this has certain advantages for DN prevention and individualized treatment. However, the mechanism of SM against DN is unclear.

Network pharmacology is an effective method for studying and clarifying the mechanisms behind drug actions. These methods include chemoinformatics, bioinformatics, network biology, and pharmacology [10,11]. The research strategy of network pharmacology is in line with the understanding of disease integrity in TCM [12,13], and provides new ideas and methods for research on TCM [14]. In this study, we attempted to utilize network pharmacology technology to explore the main bioactive components of SM, and predict their effective targets and potential mechanisms involved in the treatment of DN at the molecular level. The flowchart is as follows (Fig 1).

Materials And Methods

Determination and Screening of Active Components of SM

SM components were retrieved from the Traditional Chinese Medicine System Pharmacology Database [TCMSP, <http://tcmospw.com/tcmosp.Php>]. We used pharmacokinetic information retrieval filters for absorption, distribution, metabolism, and excretion (ADME) screening based on oral bioavailability (OB) greater than or equal to 30% and Drug-likeness (DL) greater than or equal to 0.18. and active compounds selected without potential target information were excluded. Supplementing the unpredicted active compounds based on published literature reports.

Construction of an Active Component-Target Network

Obtain targets from the TCMSP and Swiss Target Prediction database (<http://www.Swisstargetprediction.ch>). Afterwards, the targets were standardized in the UniProt (<https://www.uniprot.org>) database with the property set to “reviewed” and “human” [15]. After removing duplicates, a database of SM compounds and their targets was constructed. Finally, a visual network was established using Cytoscape v.3.6.0 software.

Determination of Potential DN-Related Targets

DN-related targets were retrieved from the Human Gene Database (GeneCards, <https://www.genecards.org/>), as well as from the DisGeNET Database (<https://www.disgenet.org/home/>). The keyword used was “diabetic nephropathy”.

Determination of DN-related Targets of the Active Components

The screened targets of the active components and DN-related proteins were imported into the Venn Diagram (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) platform for analysis, and the common ones were identified as DN-related targets of the active components for further analysis.

Protein-Protein Interaction (PPI) Network of DN-related Targets of the Active Components

In order to study the interactions between the active components of SM and their target proteins, the drug-disease intersection target genes were searched using the interaction database platform STRING v.11.0 (<https://string-db.org/>), and a PPI network was constructed. STRING is a comprehensive multifunctional data platform [16,17] and aims to provide protein-protein interaction evaluation and integration [18]. In our database search, the species was limited to “Homo sapiens”, the confidence score cutoff was set at 0.4, and the rest of the settings were default.

Network Construction and Analysis

The targets of SM among DN-related proteins identified using STRING were further analyzed using the Cytoscape v.3.6.0 software to visualize and analyze the interaction network. Using the network analysis plug-in in the software to count the nodes in the network graph and analyze its role in the graph.

Gene Ontology (GO) Functional Analysis

The Metascape Platform (<http://metascape.Org/gp/index.html>) has a comprehensive annotation function that updates the gene annotation data every month [19]. The SM regulates DN abnormalities entered into the Metascape platform, set $P < 0.01$, analyzes their main biological processes, and performs enrichment analysis. It saves the data results and uses biological online tools to visualize the data.

Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

KEGG pathway enrichment analysis was conducted using the Cytoscape plug-in ClueGO. The candidate DN-related genes targeted by SM were entered into the ClueGO plug-in, with P set to < 0.01 and kappa score set to ≥ 0.53 .

Molecular Docking

Using KEGG pathway enrichment analysis, we identified the potential DN-related genes targeted by SM components. These targets were further confirmed by molecular docking with the experimentally verified

active components. These components were Tanshinone IIA, which can improve kidney hypertrophy and 24-hour urine protein excretion [20], and salvianolic acid B, which can inhibit the proliferation of mesangial cells and the production of extracellular matrix induced by high glucose in a dose-dependent manner in SM [21]. The crystal structure of the core genes was obtained from the RCSB Protein Data Bank (PDB, <https://www.rcsb.org/>) [22]. The compound structure was saved as a docking ligand in MOL2 format. The iGEMDOCK software was used for molecular docking. The software automatically defaults parameters when setting the standard docking.

From the molecular docking results of the iGEMDOCK software, we selected the top 5 receptor proteins with the lowest energy value and the ligand that bound to these receptor proteins most stably and ran AutoDock Vina for docking. Pymol and LigPlos softwares were used for visualization and construction of the network, respectively.

Results

Screening for Active Components of SM

Using the TCMSP database to obtain 202 known active compounds in SM, we screened these through the conditions of $OB \geq 30\%$, $DL \geq 0.18$, and obtained 65 active ingredients that met the conditions. According to published literature reports, we also selected the known active compound Salvianolic acid B, which was screened out by ADME. Finally, 66 active components were selected. (Table 1).

Determination of Targets of SM Active Components and Active Component-Target Network Construction

Candidate targets of active components in SM were searched from the databases of TCMSP and Swiss Target Prediction. Then, after uniprot standardization, deduplication occurred. As a result, 189 targets were identified after removal of duplicate data (Table S1). Afterwards, we used cytoscape 3.6.0 software to build a "herbs-ingredients-targets" interaction Network, as shown in Figure 2.

Determination of DN-Related Targets

The DN-related disease targets obtained from the GeneCards and DisGeNET databases were 1189 and 3084, respectively.

Drug-Disease Intersection Targets

Venn analysis was performed on 189 targets of SM components, 1189 and 3084 DN target genes, and 64 drug-disease intersection gene targets were obtained for further analysis, as shown in Figure 3 and Table 2. These targets information were provided in Supplementary Table S2.

PPI Network Analysis

The drug-disease intersection gene targets (64) were analyzed using the STRING database PPI network, as shown in Figure 4A. From the analysis results, a total of 64 nodes and 704 edges were acquired, and the average node degree was 21.3, with a PPI enrichment p-value of $<1.0e^{-16}$ (Fig 4 A). Then, the Import STRING analysis results were analyzed by Cytoscape software. The network analysis plugin was used to count the nodes in the network graph and analyze its role in the graph according to degree. The greater the degree of freedom, the more biological functions the node has in the network. The network was constructed as shown in Figure 4B. Among them, the top ten targets AKT serine/threonine kinase 1 (AKT1), vascular endothelial growth factor A (VEGFA), Interleukin 6 (IL6), Tumor necrosis factor (TNF), Mitogen-activated protein kinase 1 (MAPK1), Tumor protein p53 (TP53), Epidermal growth factor receptor (EGFR), signal transducer and activator of transcription 3 (STAT3), Mitogen-activated protein kinase 14 (MAPK14), and Transcription factor AP-1 (JUN) explain their significance in the network (Fig 4 B).

GO Functional Analysis

The Metascape data platform was used to enrich and analyze the 64 relevant DN-related targets of SM, and the results were visualized using biological online tools.

A total of 1557 biological processes (BP) were enriched, and the first 20 significantly enriched BP terms were selected for analysis. The results showed that the biological processes involved in SM mainly included cytokine-mediated signaling pathway, apoptotic signaling pathway, positive regulation of cell migration, reactive oxygen species metabolic process, regulation of inflammatory response, regulation of cell-cell adhesion, response to oxygen levels, cellular response to growth factor stimulus, and regulation of protein serine/threonine kinase activity (Fig5 A).

A total of 90 molecular functions (MF) GO terms were enriched, and the first 19 significantly enriched MF terms were selected for analysis based on $P < 0.01$. The results showed that the intersection genes were mainly enriched in protein kinase binding, transcription factor binding, cytokine receptor binding, integrin binding, cysteine-type endopeptidase activity involved in apoptotic process, kinase regulator activity, heme binding, protein domain specific binding, endopeptidase activity, nitric-oxide synthase regulator activity, and many other molecular functions related to the above genes (Fig5 B).

A total of 38 cell components (CC) GO terms were enriched, and the first 13 significantly enriched CC terms were selected for analysis according to $P < 0.01$. The results showed that the intersection genes were mainly enriched in membrane rafts, RNA polymerase II transcription factor complex, external side of plasma membrane, extracellular matrix, adherens junction, and glutamatergic synapse (Fig5 C). Details of the node attribute information of GO analysis results are provided in the supplementary materials (Table S3).

KEGG Pathway Enrichment Analysis

In order to further reveal the potential mechanism underlying the therapeutic effect of SM against DN, we performed KEGG pathway enrichment analysis on 64 intersection gene targets using the Cytoscape plug-

in ClueGO. The screen was based on a $p < 0.01$ and kappa score ≥ 0.53 , in order to visualize the results of KEGG enrichment (Fig 6 A), and we used a pie chart to describe the percentage of genes involved in the different biological functions and signal pathways among the total number of genes that are intersected (Fig 6 B). The results showed that a total of 38 terms were enriched, including the AGE-RAGE signaling pathway in diabetic complications, TNF signaling pathway, JAK-STAT signaling pathway, FoxO signaling pathway, and HIF-1 signaling pathway. In addition, we also found some other pathways, such as Fluid shear stress and atherosclerosis, Platelet activation, Relaxin signaling pathway and so on. These results revealed that SM alleviated DN by improving the organism's immunity, anti-inflammation, relieving advanced glycation end products, antioxidant stress response, and other harmful alien organism-related pathways. Details of node attribute information of the KEGG analysis results are provided in the supplementary materials (Table S4).

Molecular Docking Study

According to the results of KEGG pathway enrichment analysis, we selected the AGE-RAGE signaling pathway in diabetic complications which had the largest percentage of genes involved in different biological functions and signaling pathways among the total number of intersection genes. According to the drug-target correspondence, the target protein in this pathway is molecularly docked with the corresponding drug components. The 16 target proteins enriched by the AGE-RAGE signaling pathway in diabetic complications are AKT1, BCL2, CASP3, EDN1, ICAM1, IL6, JUN, MAPK1, MAPK14, MMP2, NOS3, NOS2, RELA, STAT3, TNF, and VEGFA. We selected the experimentally verified tanshinone IIA and salvianolic acid B from among the SM active molecules, and molecularly docked with 16 target proteins. The matching energy of small molecules and large molecules determines the degree of binding. If the matching energy is relatively low, it indicates that the conformation of small molecules and large molecules is stable. The 16 potential targets of DN had better binding stability to salvianolic acid B than the tanshinone IIA (Table 3).

Using AutoDock Vina software, the five target proteins (AKT1, NOS2, TNF, JUN, and RELA) with the lowest energy value in the molecular docking by iGEMDOCK software were molecularly docked with Salvianolic acid B. Figure 7 shows the best docking combination for the target protein and the active component salvianolic acid B, including TNF, NOS2, and AKT1. These have the best combination with salvianolic acid B, and the binding energy is -9.3 kcal/mol, -6.6 kcal/mol, and -6.4 kcal/mol respectively. This shows that salvianolic acid B has good binding ability with the targets.

Discussion

TCM mechanisms of action are complicated with multiple components and targets. When the pathogenesis of a disease is not clear yet, it becomes more difficult to analyze the mechanism of action of Chinese medicine. Network pharmacology is a method that combines system network analysis and pharmacology. It can systematically study the effective components, targets, and pathways of drugs at

the molecular level, so as to understand the interaction between components, targets, and pathways. Therefore, network pharmacology research methods provide new ideas and methods for TCM research.

In this study, the results of the TCM-component-target network analysis showed that luteolin, tanshinone IIA, salvianolic acid B, dihydrotanshinlactone, and other active ingredients can act on multiple targets in the network. This finding suggests that these components may be important for the therapeutic effect of SM against DN and warrant further exploration. Luteolin has the most potential targets, followed by Tanshinone IIA. According to reports, luteolin can not only increase insulin-mediated glucose uptake and enhance insulin sensitivity [23], but also inhibit high glucose-induced vascular endothelial growth factor (VEGF) [24], reducing reactive oxygen species (ROS) generation, and reducing lipid accumulation [25]. This shows that luteolin can improve insulin resistance and regulate glucose and lipid metabolism. Tanshinone IIA reduces vascular intimal hyperplasia, improves tissue blood perfusion, improves kidney microcirculation, removes intracellular oxygen free radicals, improves blood lipids, promotes anticoagulation and various other functions [26], such as anti-inflammatory and antioxidant effects [27,28], thereby reducing kidney damage. Salvianolic acid B is a water-soluble compound with the highest activity and highest salvianolic acid content [29]. A number of basic studies have shown that salvianolic acid B has potential therapeutic effects on renal microcirculation. Salvianolic acid B has anti-oxidation [30], anti-inflammatory [31], neuroprotection [32], and anti-fibrosis [33] effects. Current research reports show that salvianolic acid B can be delivered to the kidney to reduce the progression of renal fibrosis [34], thereby protecting renal function and delaying DN progression.

The PPI network of 64 targets of SM acting on DN showed that *AKT1*, *VEGFA*, *IL6*, *TNF*, *MAPK1*, *TP53*, *EGFR*, *STAT3*, *MAPK14*, and *JUN* are the top 10 targets in the PPI network according to degree value. These genes are regarded as core genes and may play an important role in the therapeutic effect of SM against DN. These genes are involved in oxidative stress, inflammation, vascular permeability, and immune regulation. For example, the activation of *AKT1* has the effect of promoting cell proliferation and inhibiting cell apoptosis. It is an important gene in the immune inflammatory mechanism of DN [35]. It is closely related to mesangial matrix proliferation, basement membrane thickening, podocyte damage, and renal tubular epithelial cell transdifferentiation [36]; *IL6* and *TNF* have immunomodulatory and pro-inflammatory effects [37]; *VEGFA* is related to the vascular permeability of patients with DN [38]; the activation of *EGFR* upregulates the production of ROS and endoplasmic reticulum stress, and the onset of DN plays an important role in this mechanism [39]. Therefore, it can be inferred that luteolin, tanshinone IIA, and salvianolic acid B, the main components of salvianolic acid B, can reduce oxidative stress and inhibit the expression of inflammatory mediators such as IL-10, IL-6, TNF, etc., thus delaying DN progression.

To predict the mechanism underlying the therapeutic effect of SM against DN, we performed GO enrichment analysis of 64 potential targets. As shown in Figure 5A, the first 20 terms of BP are mainly related to cytokines, apoptosis, reactive oxygen species, and inflammation regulation. For example, cytokine-mediated signaling pathway, apoptosis signaling pathway, reactive oxygen species metabolic

process, regulation of inflammatory response, etc. Relevant studies have shown that the occurrence and development of DN is related to cell dysfunction and damage [40,41], chronic inflammatory infiltration [42], cell apoptosis, and oxidative stress [43]. This indicates that the main target genes are important for multiple BP. MF enrichment analysis mainly included cytokine receptor binding, integrin binding, endopeptidase activity, transcription factor binding, protein kinase binding, and heme binding (Figure 5B). The target genes involved mainly include *VEGFA*, *PTGS2*, *DDP4*, *TNF*, and *NOS2*, which mainly focused on oxidative stress, inflammatory response, and immune regulation. Among them, DDP4 inhibitors is of great significance for reducing the blood sugar levels in diabetic patients and delaying the occurrence and development of DN [44,45]. In addition, as shown in Figure 5C, cellular components mainly include membrane raft, RNA polymerase II transcription factor complex, external side of plasma membrane, extracellular matrix, and adherens junction. These enriched functions also involve top targets, such as TNF and JUN. At the same time, this also illustrates the complexity of the pathological mechanism of DN.

To further explore the potential mechanism of SM in treating DN, we conducted KEGG analysis on 64 potential targets of SM acting on DN. As shown in Figure 6, the pathways related to DN, including the AGE-RAGE signaling pathway in diabetic complications, TNF signaling pathway, JAK-STAT signaling pathway, and FoxO signaling pathway, mainly involve three aspects: (1) accumulation of advanced glycation end products: Normally, the glycation reaction proceeds very slowly. However, the response is obviously accelerated in the hyperglycemic state, and the aggregation of AGEs in tissues and their binding with RAGE, a specific receptor, produces cytotoxic effects and damages the kidneys, which may be the key factors contributing to DN. Most studies have shown that the AGE/RAGE signaling pathway can promote the expression of NF- κ B [46], upregulate TGF-beta 1, VEGF [47], and activate NADPH oxidases, etc., cause the expression and release of inflammatory factors and adhesion factors, increase vascular permeability, increase the expression of connective tissue growth factor, and enhance oxidative stress, thus increasing proteinuria, promoting renal fibrosis, leading to the occurrence and development of DN. In addition, other studies have shown that the interaction between AGEs and RAGE leads to vasoconstriction, procoagulant state [48], accelerates renal vascular aging and injury [49], and further promotes DN progression. (2) Immune inflammation regulation: TNF has immunomodulatory and pro-inflammatory effects [37]. TNF- α stimulates the aggregation and adhesion of inflammatory cells, increases the permeability of microvessels, and impairs glomeruli through an inflammatory response [50]. Some studies have confirmed that TNF- α levels are significantly increased in DN patients and positively correlated with the course of disease [51,52]. In addition, many studies have shown that activation of the JAK/STAT signaling pathway can cause immune inflammation in renal tissue [53,54], and mediates mesangial proliferation and renal tissue fibrosis associated with DN [55]. (3) Oxidative stress: FoxO mainly regulates oxidative stress, apoptosis, and immune response through transcription and transmission of various growth factors and cytokine signals, among which FoxO1 plays an important role in the pathogenesis of kidney disease [56]. FoxO1 activation can inhibit podocyte epithelial-mesenchymal cell transformation induced by high glucose and improve proteinuria and renal damage in diabetic mice [57]. In addition, we found other pathways, such as proteoglycans in cancer, fluid shear

stress, and atherosclerosis. This indicates that SM has potential applications in tumors, arteriosclerosis, and other diseases. Based on the above multiple pathways, it is speculated that SM may delay the progression of DN and protect renal function by participating in advanced glycation end-products, oxidative stress, inflammatory response, immune regulation, and other processes.

To further explore the potential molecular mechanism of SM in the treatment of DN, we conducted molecular docking studies on 16 target genes closely related to DN screened by KEGG, using the corresponding experimentally validated key components Tanshinone IIA and Salvianolic acid B as ligands. The results showed that 16 potential target genes had good binding with salvianolic acid B, and their stability was better than the Tanshinone IIA. The binding energies of these docking results further helped to refine the targets for SM.

Conclusions

In conclusion, this study analyzed the mechanisms underlying the therapeutic effect of SM against DN using network pharmacology and analysis of a PPI network showing the interactions between SM active components and targeted DN-related proteins and determined the synergistic effects between the herbs in SM. Our findings revealed that SM exerted its pharmacological effects against DN through "multi components-multi targets-multi pathways" that were mainly involved in advanced glycation end-products, oxidative stress, inflammatory response, and immune regulation. Further, our findings offer a reference for further investigation of the mechanism underlying the therapeutic effect of SM against DN. This study also had some limitations. We only explored the effect of SM on DN at the level of network pharmacology. However, the current network information technology is not comprehensive, and the accuracy of database data and real-time updates needs to be improved. Therefore, the results obtained from this analysis need further verification with respect to the corresponding pharmacodynamics, and mechanistic experiments are needed to explain the complex multi-target, multi-pathway, and synergistic interactions involved in the therapeutic effects of TCM.

Abbreviations

TCM: Traditional Chinese medicine; TCMSP: Traditional Chinese Medicine Systems Pharmacology; OB: Oral bioavailability; DL: Drug likeness; PPI: Protein–protein interaction; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins database; GO: Gene Ontology; BP: Biological processes; MF: Molecular functions; CC: Cellular components; KEGG: Kyoto Encyclopedia of Genes and Genomes; PDB: Protein Data Bank; SM :Salvia Miltiorrhiza; DN: Diabetic Nephropathy.

Declarations

Acknowledgements

Not applicable.

Authors' Contributions

X-LT, L-HZ, and L-LZ conceived and designed the research methods. L-LZ, L-H, X-MW, Y-W, and J-HZ collected the data. L-LZ and L-H analysed the data. L-LZ, L-H, and X-MW wrote the original draft. X-LT and L-HZ reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Tables

Table 1. Basic information on the main active ingredients of SM

Mol ID	Molecule Name	OB%	DL
MOL001601	1,2,5,6-tetrahydrotanshinone	38.75	0.36
MOL001659	Poriferasterol	43.83	0.76
MOL001771	poriferast-5-en-3beta-ol	36.91	0.75
MOL001942	isoimperatorin	45.46	0.23
MOL002222	sugiol	36.11	0.28
MOL002651	Dehydrotanshinone II A	43.76	0.4
MOL002776	Baicalin	40.12	0.75
MOL000569	digallate	61.85	0.26
MOL000006	luteolin	36.16	0.25
MOL007036	5,6-dihydroxy-7-isopropyl-1,1-dimethyl-2,3-dihydrophenanthren-4-one	33.77	0.29
MOL007041	2-isopropyl-8-methylphenanthrene-3,4-dione	40.86	0.23
MOL007045	3 α -hydroxytanshinone	44.93	0.44
MOL007048	(E)-3-[2-(3,4-dihydroxyphenyl)-7-hydroxy-benzofuran-4-yl]acrylic acid	48.24	0.31
MOL007049	4-methylenemiltirone	34.35	0.23
MOL007050	2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	62.78	0.4
MOL007058	formyltanshinone	73.44	0.42
MOL007059	3-beta-Hydroxymethyllenetanshinquinone	32.16	0.41
MOL007061	Methylenetanshinquinone	37.07	0.36
MOL007063	przewalskin a	37.11	0.65
MOL007064	przewalskin b	110.32	0.44
MOL007068	Przewaquinone B	62.24	0.41
MOL007069	przewaquinone c	55.74	0.4
MOL007070	(6S,7R)-6,7-dihydroxy-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-dione	41.31	0.45
MOL007071	przewaquinone f	40.31	0.46
MOL007077	sclareol	43.67	0.21
MOL007079	tanshinaldehyde	52.47	0.45

MOL007081	Danshenol B	57.95	0.56
MOL007082	Danshenol A	56.97	0.52
MOL007085	Salvilenone	30.38	0.38
MOL007088	cryptotanshinone	52.34	0.4
MOL007093	dan-shexinkum d	38.88	0.55
MOL007094	danshenspiroketallactone	50.43	0.31
MOL007098	deoxyneocryptotanshinone	49.4	0.29
MOL007100	dihydrotanshinlactone	38.68	0.32
MOL007101	dihydrotanshinone	45.04	0.36
MOL007105	epidanshenspiroketallactone	68.27	0.31
MOL007107	C09092	36.07	0.25
MOL007108	isocryptotanshi-none	54.98	0.39
MOL007111	Isotanshinone II	49.92	0.4
MOL007115	manool	45.04	0.2
MOL007119	miltionone	49.68	0.32
MOL007120	miltionone	71.03	0.44
MOL007121	miltipolone	36.56	0.37
MOL007122	Miltirone	38.76	0.25
MOL007124	neocryptotanshinone ii	39.46	0.23
MOL007125	neocryptotanshinone	52.49	0.32
MOL007127	1-methyl-8,9-dihydro-7H-naphtho[5,6-g]benzofuran-6,10,11-trione	34.72	0.37
MOL007130	prolithospermic acid	64.37	0.31
MOL007132	(2R)-3-(3,4-dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl)acryloyl]oxy-propionic acid	109.38	0.35
MOL007141	salvianolic acid g	45.56	0.61
MOL007142	salvianolic acid j	43.38	0.72
MOL007143	salvilenone	32.43	0.23
MOL007145	salviolone	31.72	0.24
MOL007150	(6S)-6-hydroxy-1-methyl-6-methylol-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-quinone	75.39	0.46

MOL007151	Tanshindiol B	42.67	0.45
MOL007152	Przewaquinone E	42.85	0.45
MOL007154	tanshinone iia	49.89	0.4
MOL007155	(6S)-6-(hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-dione	65.26	0.45
MOL007156	tanshinone Ⅱ	45.64	0.3
MOL006824	α -amyrin	39.51	0.76
MOL007118	microstegiol	39.61	0.28
MOL007123	miltirone Ⅱ	44.95	0.24
MOL007149	NSC 122421	34.49	0.28
MOL007140	(Z)-3-[2-[(E)-2-(3,4-dihydroxyphenyl)vinyl]-3,4-dihydroxyphenyl]acrylic acid	88.54	0.26
MOL007051	6-o-syringyl-8-o-acetyl shanzhiside methyl ester	46.69	0.71
MOL007074	salvianolic acid b	3.01	0.41

Table 2. Potential targets of SM against DN

Serial number	Protein name	Gene name	UniProt ID
1	peroxisome proliferator activated receptor gamma	PPARG	P37231
2	vascular endothelial growth factor A	VEGFA	P15692
3	insulin receptor	INSR	P06213
4	interleukin 6	IL6	P05231
5	nitric oxide synthase 3	NOS3	P29474
6	tumor necrosis factor	TNF	P01375
7	solute carrier family 2 member 4	SLC2A4	P14672
8	AKT serine/threonine kinase 1	AKT1	P31749
9	signal transducer and activator of transcription 3	STAT3	P40763
10	dipeptidyl peptidase 4	DPP4	P27487
11	intercellular adhesion molecule 1	ICAM1	P05362
12	tumor protein p53	TP53	P04637
13	interleukin 10	IL10	P22301
14	endothelin 1	EDN1	P05305
15	CD40 ligand	CD40LG	P29965
16	matrix metalloproteinase 9	MMP9	P14780
17	interleukin 4	IL4	P05112
18	nitric oxide synthase 2	NOS2	P35228
19	interferon gamma	IFNG	P01579
20	interleukin 2	IL2	P60568
21	heme oxygenase 1	HMOX1	P09601
22	matrix metalloproteinase 2	MMP2	P08253
23	mitogen-activated protein kinase 1	MAPK1	P28482
24	prostaglandin-endoperoxide synthase 2	PTGS2	P35354
25	mitogen-activated protein kinase 14	MAPK14	Q16539
26	nuclear receptor subfamily 3 group C member 2	NR3C2	P08235
27	caspase 3	CASP3	P42574
28	Jun proto-oncogene, AP-1 transcription factor subunit	JUN	P05412

29	xanthine dehydrogenase	XDH	P47989
30	estrogen receptor 1	ESR1	P03372
31	matrix metalloproteinase 1	MMP1	P03956
32	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma	PIK3CG	P48736
33	endothelin receptor type A	EDNRA	P25101
34	epidermal growth factor receptor	EGFR	P00533
35	serine protease 1	PRSS1	P07477
36	RELA proto-oncogene, NF-kB subunit	RELA	Q04206
37	caspase 9	CASP9	P55211
38	prostaglandin-endoperoxide synthase 1	PTGS1	P23219
39	5-hydroxytryptamine receptor 2A	HTR2A	P28223
40	fatty acid synthase	FASN	P49327
41	cyclin dependent kinase inhibitor 1A	CDKN1A	P38936
42	coagulation factor X	F10	P00742
43	glutathione S-transferase pi 1	GSTP1	P09211
44	BCL2 apoptosis regulator	BCL2	P10415
45	integrin subunit beta 3	ITGB3	P05106
46	protein tyrosine phosphatase non-receptor type 2	PTPN2	P17706
47	MDM2 proto-oncogene	MDM2	Q00987
48	integrin subunit alpha 2b	ITGA2B	P08514
49	matrix metalloproteinase 12	MMP12	P39900
50	nuclear receptor subfamily 1 group I member 2	NR1I2	O75469
51	caspase 7	CASP7	P55210
52	Lymphocyte differentiation antigen CD38	CD38	P28907
53	Glyoxalase I	GLO1	Q04760
54	Arachidonate 12-lipoxygenase	ALOX12	P18054
55	Aldose reductase (by homology)	AKR1B1	P15121
56	Protein-tyrosine phosphatase 1C	PTPN6	P29350
57	LXR-alpha	NR1H3	Q13133

58	Protein-tyrosine phosphatase 2C	PTPN11	Q06124
59	Arginase-1(by homology)	ARG1	P05089
60	Poly [ADP-ribose] polymerase-1	PARP1	P09874
61	Adenosine A1 receptor (by homology)	ADORA1	P30542
62	NADPH oxidase 4	NOX4	Q9NPH5
63	Tyrosine-protein kinase SYK	SYK	P43405
64	Cytochrome P450 19A1	CYP19A1	P11511

Table 3. Docking of Tanshinone IIA and Salvianolic Acid B with target protein molecules (kcal·mol⁻¹)

Target name	PDBID	Tanshinone IIA	Salvianolic acid B	Canagliflozin
MMP2	1EAK	-88.21	-127.25	-109.23
EDN1	1EDP	-62	-110.92	-92.83
RELA	1NFI	-81.98	-151.32	-92.46
NOS3	1NIW	-92.71	-131.05	-113.3
JUN	1S9K	-87.31	-147.78	-99.95
AKT1	1UNQ	-79.22	-148.03	-102.74
BCL2	1YSW	-78.34	-129.71	-113.85
TNF	2E7A	-90.62	-146.4	-113.05
MAPK14	2NPQ	-84.34	-139.24	-102.14
BCL2	2O2F	-82.16	-133.24	-99.02
BCL2	2O21	-89.69	-136.58	-105.02
BCL2	2O22	-97.25	-135.9	-104.91
NOS2	3E7G	-89.58	-162.49	-109.28
CASP3	3KJF	-81.44	-130.26	-91.52
VEGFA	3V2A	-76.85	-121.29	-91.19
IL6	4CNI	-89.55	-125.52	-98.18
MAPK1	4IZ5	-78.5	-144.95	-97.71
STAT3	4ZIA	-84.1	-136.45	-103.68
ICAM1	5MZA	-80.39	-125.56	-98.36

Figures

Figure 1

Flowchart based on network pharmacology and molecular docking.

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Flowchart based on network pharmacology and molecular docking.

Figure 2

Network construction of “ingredients-targets”. The light cyan prism nodes represent the targets and the light purple round nodes represent the ingredients in SM.

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Network construction of “ingredients-targets”. The light cyan prism nodes represent the targets and the light purple round nodes represent the ingredients in SM.

Figure 3

SM-DN common target genes.

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SM-DN common target genes.

[illegible]

PPI network analysis. A: PPI networks of targets by STRING 11.0. Nodes represent proteins. Edges represent protein-protein associations. B: Potential target genes are arranged counterclockwise according to the degree value from large to small.

[illegible]

PPI network analysis. A: PPI networks of targets by STRING 11.0. Nodes represent proteins. Edges represent protein-protein associations. B: Potential target genes are arranged counterclockwise according to the degree value from large to small.

Figure 5

GO enrichment analysis. These include (A) biological process (BP) terms, (B) molecular function (MF) terms (C) cellular component (CC) terms. A, B: The color of the node is displayed in a gradient from red to green according to the descending order of the P value. The size of the nodes is arranged in ascending order of the number of genes. C: Sort by the importance of $-\log_{10}(P)$ of each lane.

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A

B

Figure 6

KEGG pathway analysis of potential targets of SM among DN-related proteins using the ClueGO plug-in. The KEGG term is expressed as a node, and the size of the node indicates importance. Only the most significant terms in the group have been labeled.

A

B

Figure 6

KEGG pathway analysis of potential targets of SM among DN-related proteins using the ClueGO plug-in. The KEGG term is expressed as a node, and the size of the node indicates importance. Only the most significant terms in the group have been labeled.

A

B

C

Figure 7

Molecular docking. Molecular models of the binding of salvianolic acid B with (A) TNF, (B) NOS2, and (C) AKT1 shown as 3D diagrams and 2D diagrams.

Figure 7

Molecular docking. Molecular models of the binding of salvianolic acid B with (A) TNF, (B) NOS2, and (C) AKT1 shown as 3D diagrams and 2D diagrams.

Figure 8

The network structure of "SM-component-target-pathway-DN". "Oval" represents SM and DN, "diamond" represents components, "hexagon" represents target, and "rectangle" represents pathway.

Figure 8

The network structure of "SM-component-target-pathway-DN". "Oval" represents SM and DN, "diamond" represents components, "hexagon" represents target, and "rectangle" represents pathway.

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