Regular article

Exploring the potential mechanism of drug pair Salviae Miltiorrhizae Radix et Rhizoma and Notoginseng Radix et Rhizoma in the treatment of thrombosis after polycythemia vera by network pharmacology

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Abstract

Thrombosis is a leading cause of mortality and morbidity in patients suffering from polycythemia vera (PV). Drug pair Salviae Miltiorrhizae Radix et Rhizoma (Danshen, DS) and Notoginseng Radix et Rhizoma (Sanqi, SQ) is common traditional Chinese medicine (TCM) used in clinical practice to promote blood circulation and eliminate blood stasis. In this study, network pharmacology and molecular docking were used to analyze the potentially active ingredients and underlying mechanisms of drug pair DS-SQ against thrombosis after PV. These results show that 54 targets are related to both disease and the drug pair. Nineteen core targets, including IL-6 and AKT1, were screened. Luteolin and tanshinone IIa from DS as well as quercetin from SQ might be the major substances in the treatment of thrombosis after PV. KEGG enrichment analysis demonstrated that the lipid and atherosclerosis signaling pathway might play a significant role. These results provide valuable insights and a reference for the use of drug pair DS-SQ in management of thrombosis after PV and lay a foundation for further exploration of pharmacological effects.

Keywords: thrombosis; polycythemia vera; Salviae Miltiorrhizae Radix et Rhizoma; Notoginseng Radix et Rhizoma; network pharmacology; mechanism

1 Introduction

Polycythemia vera (PV) is a subtype of chronic Philadelphia-negative (Ph-) myeloproliferative neoplasm (MPN) characterized by JAK2 V617F driver mutation [1]. PV cause the body to produce too many red blood cells (RBC), white blood cells (WBC) and platelets (PLT), thus increasing the risk of thrombosis [2]. Direct oral anticoagulants (DOACs) are the key to the PV clinic management. Many antithrombotic therapies, including aspirin intake are often used to reduce thrombosis risk, but they also result in several clinical disadvantages, including gastrointestinal adverse effects, drug resistance and hemorrhage [3]. Therefore, it is urgent and necessary to explore the optimal strategies of

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anti-thrombosis and treatment duration [4].

The natural products Salviae Miltiorrhizae Radix et Rhizoma (Danshen, DS) and Notoginseng Radix et Rhizoma (Sanqi, SQ) are widely used to treat thrombosis, with the characteristics of safety and few side effects, and have been included in the Chinese Pharmacopoeia as Compound Danshen Tablet. According to the compatibility theory of traditional Chinese medicine (TCM), drugs have enhanced or synergistic effects in specific combinations and their toxicity is dramatically reduced. For example, the compatibility of DS and Panax SQ can significantly improve the efficacy of promoting blood circulation and removing blood stasis and the intestinal absorption level of the main components [5]. The drug pair DS-SQ is involved in antiinflammatory, antiplatelet aggregation and adhesion, inhibition of cell apoptosis, regulation of lipid metabolism and energy metabolism, as well as improving endothelial cell function, etc. [6,7]. It has a synergistic effect in protecting endothelial cells from oxidative stress damage induced by hydrogen peroxide (H_2O_2). Sheng et al. confirmed that Fufang Xueshuantong Capsule with DS and SQ as the main components, significantly improved coagulation, fibrinolysis, and platelet aggregation in rats with disseminated intravascular coagulation (DIC) induced by lipopolysaccharides (LPS), correcting abnormalities in the coagulation system [8]. Kasimu et al. observed that Salviae Miltiorrhizae Radix et Rhizoma extract inhibited thrombosis formation [9]. Compound Danshen dripping pills (CDDP) composed of DS, SQ and Borneol can prolong bleeding time, reduce fibrinogen levels, and decrease the incidence of thromboembolic complications, and prolong PT and APTT. Similarly, compound Xueshuantong, including DS and SQ, is able to reduce blood viscosity, eliminate blood stasis, and improve blood circulation. It also alters proteins expression relevant to blood coaqulation, PT and APTT, and decreases fibrinogen levels [10]. However, the core active ingredients and disease targets of DS-SQ drug pair and its potential molecular mechanisms in the treatment of thrombosis are still unclear and need further investigations.

Network pharmacology has been used to explore the relationship between action ingredients of DS-SQ drug pair and the role and mechanism of the bioactive ingredients in disease treatment [8]. In this study, we conducted a network pharmacological analysis and molecular docking to evaluate the therapeutic action and potential molecular mechanism of DS-SQ drug pair on thrombosis secondary to PV. The insights gained from this study will help us better understand the actions and mechanisms of the DS-SQ drug pair on the management of thrombosis after PV, and provide scientific basis for further pharmacological research. The workflow diagram is shown in Fig. 1.

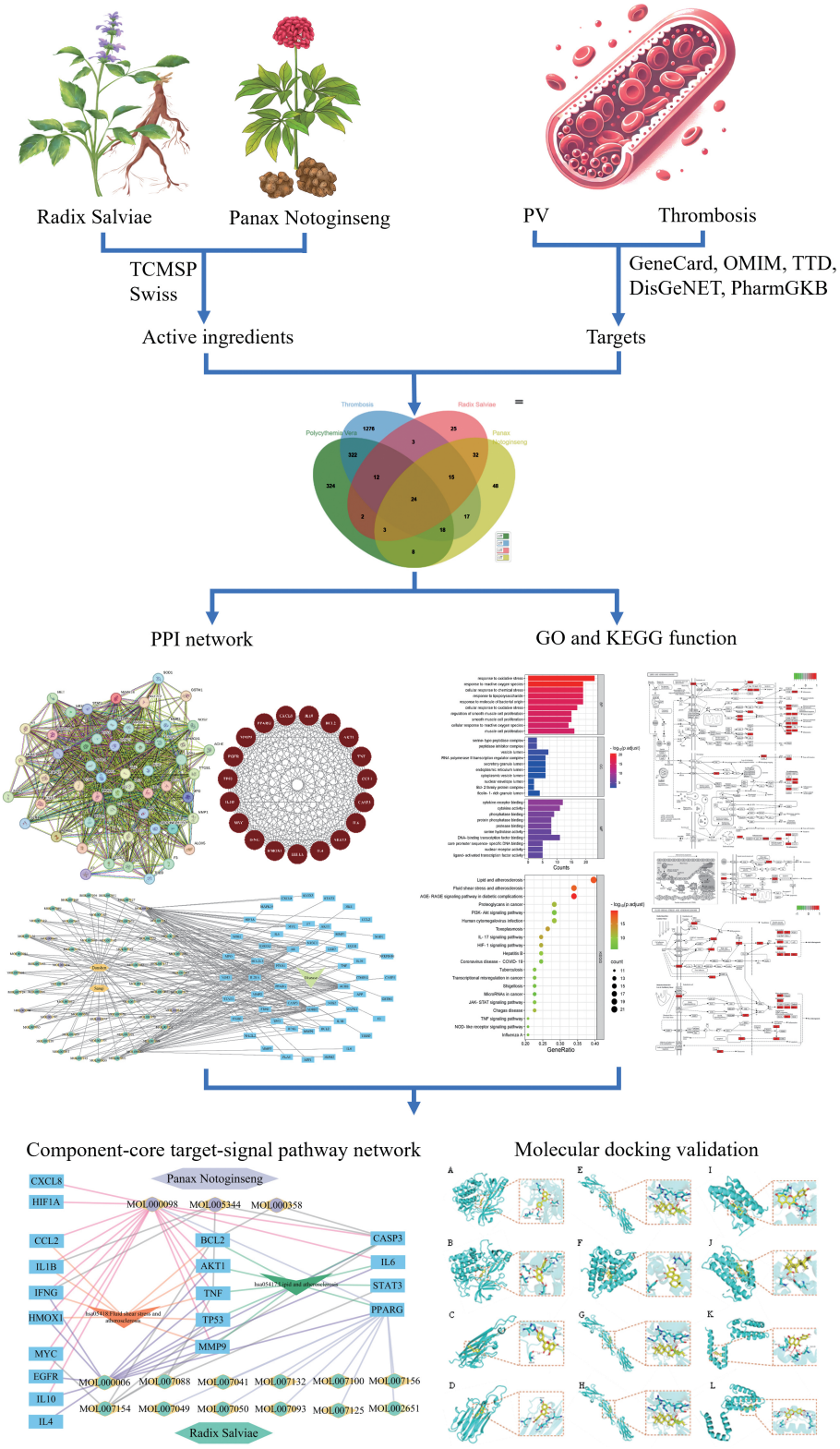


Fig. 1 Research Framework Diagram

2 Methods

2.1 Acquisition of drug targets

Using "Radix Salviae" and "Panax Notoginseng" as keywords, the chemical ingredients of these traditional Chinese medicines were obtained from TCMSP database. Based on oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18, the active components of Salviae Miltiorrhizae Radix et Rhizoma (DS) and Notoginseng Radix et Rhizoma (SQ) were screened. TCMSP pharmacology database (https://old.tcmsp-e.com/tcmsp.php) and SwissTargetPrediction database [11] (http://www.swisstargetprediction.ch/) were used to search for the targets of DS-SQ drug pair.

2.2 Acquisition of disease targets

GeneCards [12] (https://www.genecards.org/), OMIM databases [13] (https://www.omim.org), TTD [14] (https://db.idrblab.net/ttd/), DisGeNET [15] (https://www.disgenet.org/), and PharmGKB [16] (https://www.pharmgkb.org/) were used to retrieve targets related to thrombosis and PV.

2.3 Screening of common targets between drugs and diseases and construction of the network

The common targets for drug pair DS-SQ and thrombosis and PV were considered as potential targets of this drug pair against thrombosis after PV. The venn diagram was generated using the Jvenn website (http://jvenn.toulouse.inra.fr/app/example.html) to identify the overlapping targets of DS and SQ against thrombosis after PV. The active components and their corresponding targets of DS and SQ, which may have potential effects on PV-related thrombosis, were identified and used to construct a "drug-active component-target-disease" network using Cytoscape software.

2.4 Construction of protein-protein interaction network and screening of core targets

The intersection targets between drugs and diseases obtained from Venny were uploaded to the STRING online database to construct protein-protein interaction (PPI) network. The species was set to "Homo sapiens" with the highest confidence (confidence score > 0.9) and non-interacting genes were hidden to construct the PPI network. The PPI network was then imported into Cytoscape software, and topological analysis was performed using the CytoNCA plugin based on network centrality to construct the component-target-disease network. The degree value was screened to identify the core targets of the drug pair DS-SQ in the treatment of thrombosis after PV.

2.5 GO functional and KEGG pathway enrichment analysis

The intersection targets were input to the DAVID platform [17] (DAVID, https://david.ncifcrf.gov/) for GO enrichment analysis and KEGG pathway enrichment analysis. GO enrichment analysis covers biological processes, cellular components, and molecular functions. KEGG pathway enrichment analysis was applied to clarify the mechanisms of DS and SQ against PV-related thrombosis. The top 20 GO terms and KEGG pathways with P-value < 0.05 were selected and visualized using bar and bubble charts in R Studio. Topological property analysis was conducted to explore the multi-target and multi-pathway mechanisms of the drug pair in the treatment of thrombosis secondary to PV.

2.6 Construction of key active ingredient -core target-crucial pathway network

To further explore the multi-targets mechanisms of key active ingredients of the drug pair against

thrombosis of PV, the key active ingredients screened by core targets identified in section "1.4s" and the first two pathways related to thrombosis obtained in section "1.5s" were imported into Cytoscape software to construct an "Active Ingredient-Core Target-Crucial Pathway" network map.

2.7 Molecular docking

The main compounds of Luteolin, Quercetin and their key protein targets were analyzed by molecular docking using the AutoDockTools 1.5.7 software. The 3D structures of Luteolin and Quercetin were obtained from TCMSP database. The 3D structures of key protein targets were obtained from RCSB Protein Data Bank (RCSB PDB) database 54 (https://www.rcsb.org/). The figures of the active binding site were generated by PyMOL 2.2.0 software (https://pymol.org/2/).

3 Results

3.1 Acquisition of drug targets

A total of 65 bioactive ingredients were identified in DS (Supplementary Table 1), while 8 bioactive ingredients were identified in SQ (Supplementary Table 1). 165 gene targets were identified from DS and 116 from SQ using TCMSP pharmacology database. There were 74 gene targets related to both DS and SQ (Fig. 2A, Supplementary Table 2).

3.2 Acquisition of disease targets

Disease targets related to polycythemia vera (PV) were retrieved from GeneCards, OMIM, TTD, DisGeNET, and PharmGKB databases, resulting in 554, 1, 10, 17, and 291 targets, respectively. For thrombosis, 1552, 4, 42, 63, and 230 targets were obtained from these databases, respectively. After removing the duplicates, there were 376 overlapping gene targets between polycythemia vera and thrombosis (Fig. 2B, Supplementary Table 3).

3.3 Screening of common targets between drug pair and diseases

A total of 74 overlapping gene targets were considered as potential targets for the treatment of thrombosis secondary to PV by drug pair DS-SQ (Fig. 2C, Supplementary Table 4). Ingredient-target network was then constructed using Cytoscape shown in Fig. 3, which shows that these 54 gene targets might play important roles in the treatment of thrombosis after PV by drug pair DS-SQ. The bioactive ingredients in drug pair may act synergistically on different signaling pathways, thus achieving the effect of anti-thrombosis.

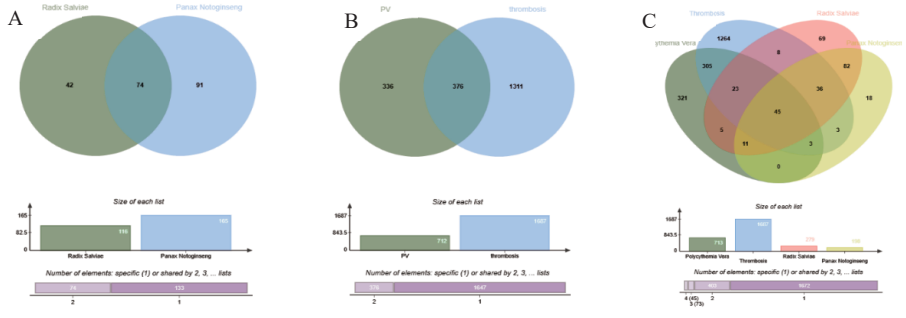


Fig. 2 Prediction of the targets of the drug pair DS-SQ against thrombosis after PV by network pharmacology. (A) Venn diagram showing the common targets between the drug pair DS-SQ; (B) Venn diagram showing the common targets of disease between PV and Thrombosis; (C) Venn diagram showing the common targets between the drug pair and the disease

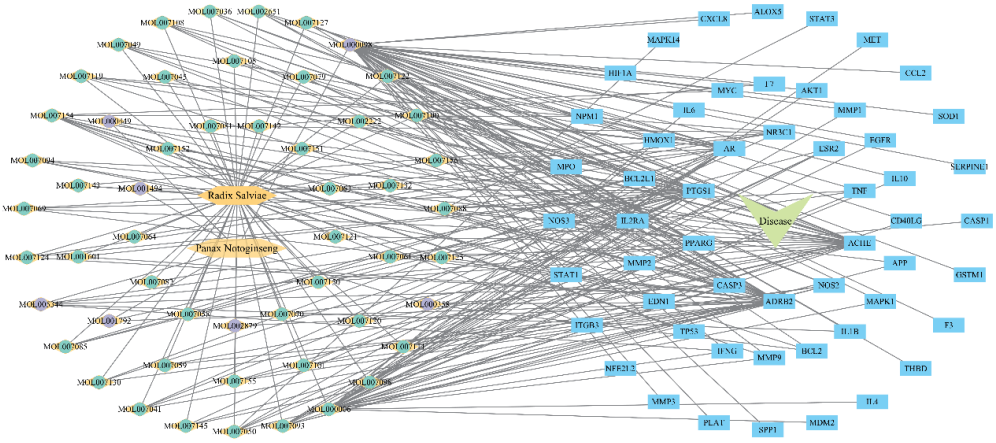


Fig. 3 The drug pair-ingredients-genes-disease network

3.4 Construction of PPI network for potential drug-disease targets

The overlapping gene targets between the drug pair and thrombosis secondary to PV were used to construct and analyze the protein-protein interaction (PPI) network via STRING database (Fig. 4). This network consists of 54 protein nodes and 220 edges, with an average node degree of 31.4 and a clustering coefficient of 0.804. Topological analysis was conducted using the CytoNCA plugin in Cytoscape software, and gene targets with all indicators greater than the median were selected for further analysis. Through twice screening and analysis, the top nineteen core targets in the PPI network based on degree value ≥ 20 and centrality > 0.02 are shown in Fig. 4 and Table 1, including TNF, IL-6, AKT1, MMP9, IL1*β*, IL-10, IFNG, BCL2, CASP3, CXCL8 (IL-8), TP53, EGFR, STAT3, PPARG, HIF1A, IL-4, CCL-2, HMOX1, and MYC. Then, the key active ingredients connected with the core targets were selected (Supplementary Table 5), including 12 ingredients derived from DS and the others from SQ. These key active ingredients indicate the diversity and effectiveness of compounds in the drug pair for the treatment of thrombosis after PV.

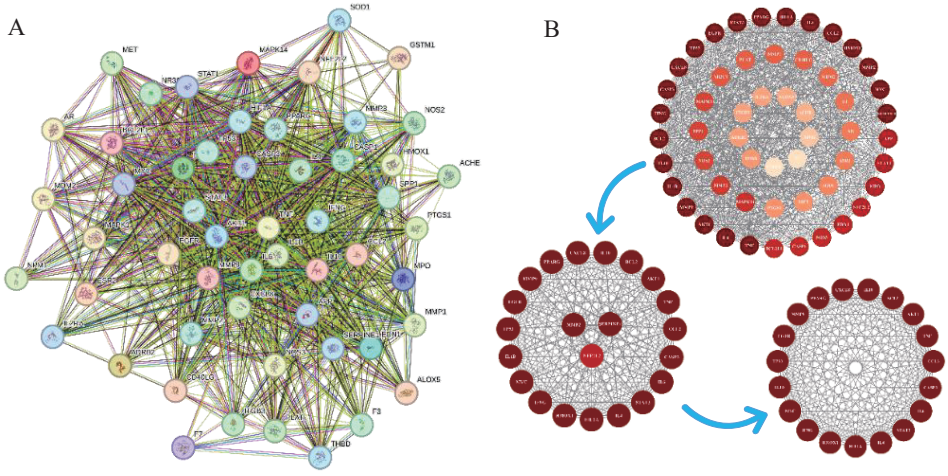
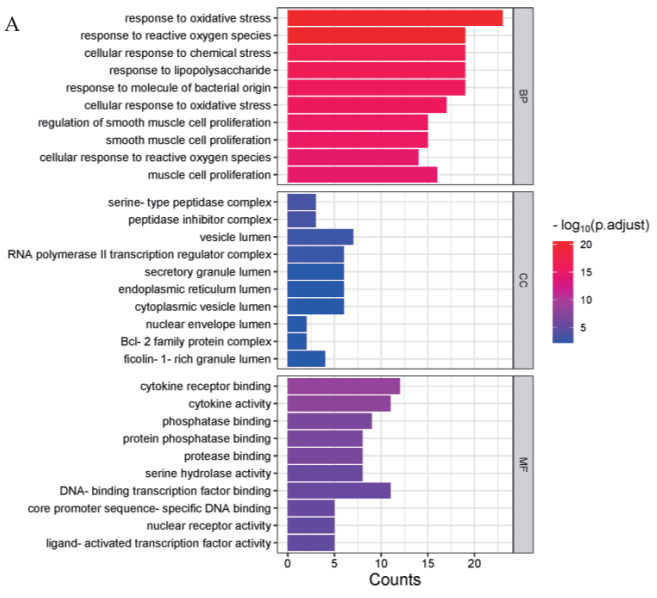


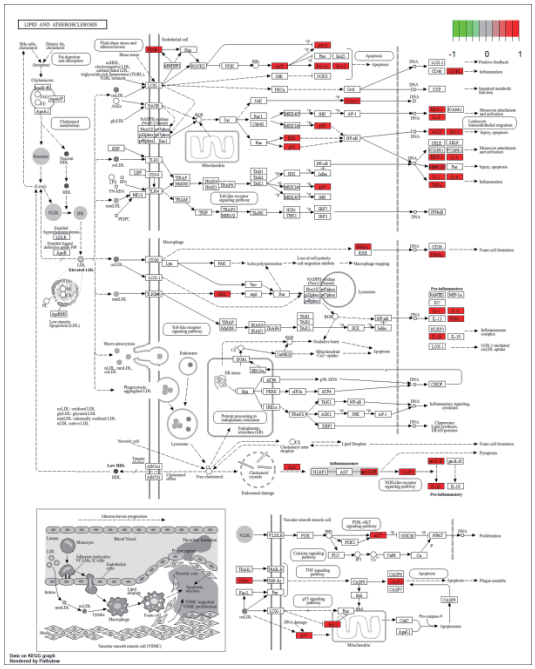
Fig. 4 Network of core ingredients in the drug pair and core targets of thrombosis after PV; (A) PPI network of potential drug pair ingredients-disease targets; (B) Topological analysis of the potential core targets of drug pair-disease target network

3.5 GO and KEGG enrichment analysis

GO and KEGG enrichment analyses were carried out to elucidate the underlying mechanism of the drug pair against thrombosis after PV. GO enrichment analysis revealed that the core targets were primarily enriched in biological processes (BP) such as oxidative stress, reactive oxygen species production, and cellular response to chemical stress. The involved cellular components (CP) include vesicle lumen, RNA polymerase II transcription regulator complex, peptidase inhibitor complex, and serine-type peptidase complex. Molecular functions (MF) include cytokine receptor binding, cytokine activity, phosphatase binding, etc. (Fig. 5A).

KEGG enrichment results showed that a total of 146 pathways were involved in the treatment of thrombosis secondary to PV by drug pair DS-SQ (Fig. 5B). Among the top 20 pathways ranked by *P*-value, lipid and atherosclerosis, fluid shear stress and atherosclerosis, AGE-RAGE in diabetic complications, proteoglycans in cancer, PI3K-Akt, IL-17, HIF-1, JAK-STAT, TNF and other signaling pathways were closely regulated by the drug pair in the treatment of thrombosis after PV. As to the pathways related to thrombosis, lipid and atherosclerosis, fluid shear stress and atherosclerosis are in the most prominent position as crucial pathways, and their relation to gene targets are shown in Fig. 5C, 5D and Supplementary Table 6. The network suggests that the drug pair treats thrombosis after PV through multiple pathways and targets.





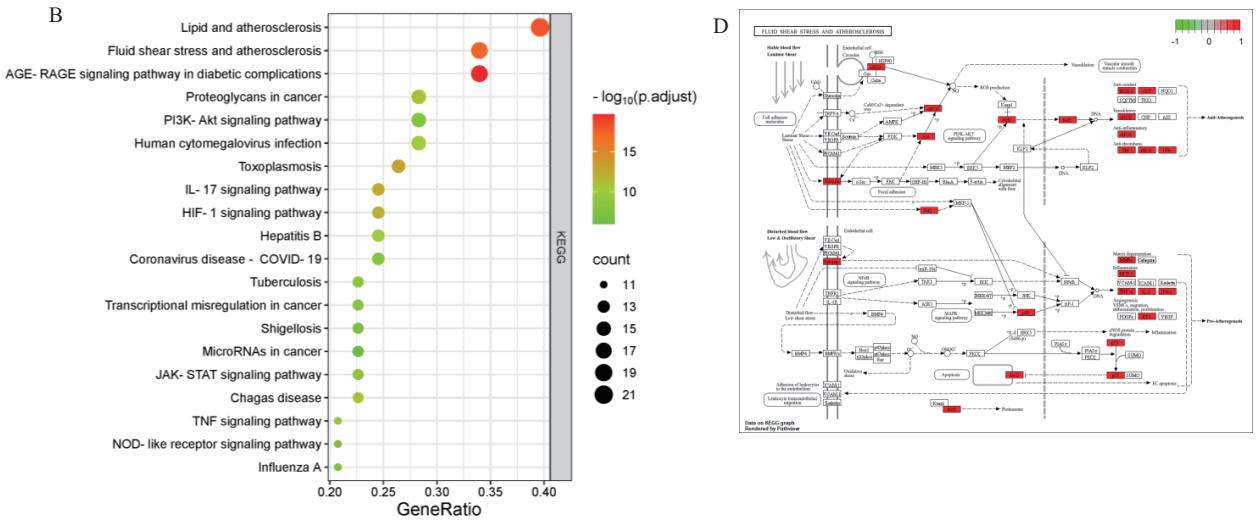


Fig. 5 GO and KEGG enrichment analysis of the core targets of the drug pair against thrombosis after PV. (A) GO enrichment analysis. GO annotations based on the three-dimension terms of biological process (BP), molecular function (MF), and cellular component (CC). The orange bars indicate the significance of the *P* values, represented as –log (p.adjust); (B) KEGG pathway enrichment analysis for the mechanisms of the drug pair against thrombosis after PV. A larger richness factor reflects greater enrichment. The size of the bubble represents the number of genes enriched in the different pathways. The color of the bubble indicates the range of *P* value; (C and D) the gene targets marked in red nodes represent those involved in the lipid and atherosclerosis pathway as well as the fluid shear stress and atherosclerosis pathway, respectively

3.6 Construction of key active ingredient -core gene target-crucial pathway network

In order to further understand the molecular mechanisms behind the pathways closely related to the treatment of thrombosis after PV by the drug pair, we constructed a "key active ingredient-core target-crucial pathway" network by Cytoscape software (Fig. 6). This network include 15 candidate active ingredients (Supplementary Table 6), 19 core targets (Table 1) and 2 crucial pathways (Fig. 5). A total of 14 core gene targets were shaped by three candidate active ingredients including quercetin (MOL000098) from SQ, luteolin (MOL000006) and tanshinone IIa (MOL007154) from DS (Supplementary Table 7). Notably, PPARG, one of the core gene target, is related to 10 candidate active ingredients from DS, but only to one from SQ (Fig. 6 and Supplementary Table 7). These results imply that quercetin, luteolin, and tanshinone IIa might be the major substances in the prevention and treatment of thrombosis after PV by the drug pair, involving two crucial pathways. Above all, drug pair DS-SQ demonstrated the capacity to concurrently influence multiple targets and multiple pathways, while certain targets and pathways were susceptible to modulation by multiple ingredients concurrently.

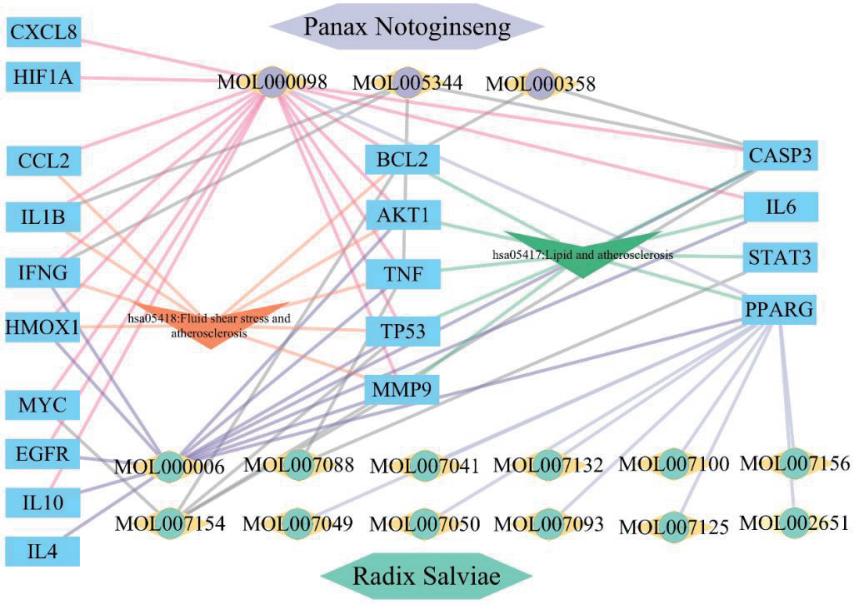


Fig. 6 Key active ingredients-core targets-crucial signal pathway network

Table 1 The core gene targets information and topological properties

Gene name	Protein name	BC	CC	DC
TNF	Tumor Necrosis Factor	115.03	0.98	52
IL6	Interleukin 6	88.05	0.96	51
AKT1	AKT Serine/Threonine Kinase 1	69.18	0.95	50
MMP9	Matrix Metallopeptidase 9	69.72	0.95	50
IL1B	Interleukin 1 Beta	60.48	0.93	49
IL10	Interleukin 10	60.10	0.88	46
IFNG	Interferon Gamma	41.55	0.87	45
BCL2	BCL2 Apoptosis Regulator	42.86	0.87	45
CASP3	Caspase 3	41.55	0.87	45
CXCL8	C-X-C Motif Chemokine Ligand 8	41.48	0.87	45
TP53	Tumor Protein P53	49.33	0.85	44
EGFR	Epidermal Growth Factor Receptor	42.23	0.84	43
STAT3	Signal Transducer And Activator Of Transcription 3	34.35	0.84	43
PPARG	Peroxisome Proliferator Activated Receptor Gamma	29.98	0.83	42
HIF1A	Hypoxia Inducible Factor 1 Subunit Alpha	25.00	0.83	42
IL4	Interleukin 4	26.09	0.82	41
CCL2	C-C Motif Chemokine Ligand 2	26.35	0.82	41
HMOX1	Heme Oxygenase 1	24.34	0.77	37
MYC	MYC Proto-Oncogene, BHLH Transcription Factor	18.25	0.76	36

3.7 Construction of molecular docking

Binding energy < -5.0 kcal/mol indicates good binding activity, and binding energy < -7.0 kcal/mol indicates strong binding activity. The results of molecular docking showed that the binding energies between the 6 key target proteins and Luteolin, Quercetin were all less than -5.0 kcal/mol (Table 2, Fig. 7) Especially, the binding energy of Luteolin and Quercetin and AKT1 was -9.8 kcal/mol, and -10.0 kcal/mol. Particularly, five hydrogen bonds were formed between ASP-292, GLN-79, THR-211, VAL-271 and SER-205 residues of the target protein from Luteolin.

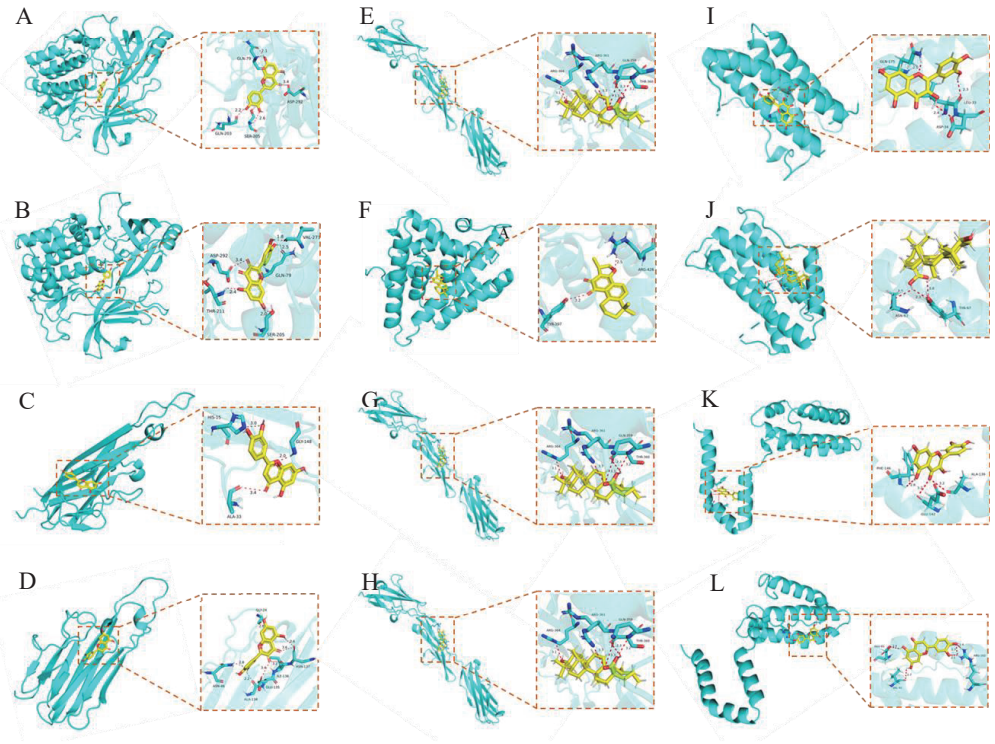


Fig. 7 Docking diagram of Luteolin and Quercetin and the core targets. (A) Quercetin-AKT1; (B) Luteolin-AKT1; (C) Quercetin-STAT1; (D) Luteolin-TNF; (E) Quercetin-TNF; (F) Cryptotanshinone-PPARG; (G) Luteolin-ICAM-1; (H) Quercetin-ICAM-1; (I) Luteolin-IL-6; (J) Quercetin-IL-6; (K) Luteolin-IL-10; (L) Quercetin-IL-10

Table 2 The minimum binding energy in molecular docking between Luteolin, Quercetin and the core targets

Ingredients	Target protein name	Binding energy (kcal/mol)
Quercetin	AKT1	-10.0
Luteolin	AKT1	-9.8
Quercetin	STAT1	-7.5
Luteolin	TNF	-7.0
Quercetin	TNF	-10.0

(to be continued)

Continued Table 2

Ingredients	Target protein name	Binding energy (kcal/mol)
Cryptotanshinone	PPARG	-7.1
Luteolin	ICAM-1	-7.4
Quercetin	ICAM-1	-6.7
Luteolin	IL-6	-7.3
Quercetin	IL-6	-6.7
Luteolin	IL-10	-6.9
Quercetin	IL-10	-6.7

4 Discussion

PV is a chronic and progressive myeloproliferative neoplasm characterized by an increased risk of thrombosis [18,19]. Antithrombotic therapy is one of the necessary treatment for PV [18,19]. However, the anticoagulation therapy with western medicine is accompanied by severe adverse effects. Thus, the optimal strategy of anti-thrombosis and treatment duration are needed. Drug pair DS-SQ of the natural products has been used to prevent and treat thrombosis in China for more than 2000 years [18,19]. However, the scientific basis as well as potential molecular mechanisms of drug pair DS-SQ in the treatment of thrombosis after PV are yet to be elucidated. In this study, network pharmacology and molecular docking validation were used to confirm the molecular mechanism of drug pair DS-SQ in the treatment of thrombosis after PV.

4.1 Major substances of drug pair DS-SQ in the treatment of thrombosis after PV

In the present study, a total of 54 potential gene targets common to the drug pair and thrombosis after PV were acquired. Correlation analysis between the drug pair and disease unveiled fifteen potential key active ingredients and 19 crucial gene targets for the treatment of thrombosis after PV. There are reports of the top three active ingredients, quercetin, luteolin and tanshinone IIa, against thrombosis [20-22]. For example, Tang et al. reported that luteolin from Salvia miltiorrhiza could attenuate zebrafish caudal vein thrombosis in a dose-dependent manner in a chemical-induced zebrafish thrombosis model [20]. Wang revealed that Tanshinone IIA (TS IIA), as a diterpene quinone isolated from dried roots of DS, had anti-platelet activation effect induced by platelet-derived microvesicles (PMVs) and down-regulated CD36 and MKK4/JNK2 signaling pathway [20]. Saviano et al. reported that Tanshinone IIA (TIIA) was able to interact significantly with the key proteins, COX-2, 5-lipoxygenase (5-LO), platelet-activating factor receptor (PAFR), and mPGES-1 using a combined in silico, *in vitro*, *in vivo*, and *ex vivo* assays, which were involved not only in the onset of inflammation but also in platelet activity [23]. Quercetin (QC) is one of effective ingredients in SQ and exhibits anticoagulant activity by reducing thromboxane B2 (TXB2) and endothelin-1 (ET-1) while increasing nitric oxide synthase (eNOS) and 6-keto prostaglandin F1α (6-keto-PGF1 α) in the acute circulatory stasis paradigm in mice [22]. Quercetin (QC) could decrease the platelet activation and the pro-aggregate effect of calcium ionophoret through the blocked of GPIIb/IIIa receptors [24]. All of these studies support the material basis for drug pair DS-SQ against thrombosis after PV.

4.2 The core targets of drug pair DS-SQ in the treatment of thrombosis after PV

PPI and topological network analysis demonstrated that nineteen potential core gene targets including TNF, IL-6, IL1β, AKT1, BCL2 and CASP3, were related to the treatment of thrombosis after PV by a substantial number of ingredients from DS and SQ. Most of them are mainly related to inflammation response and lipid metabolism and cell apoptosis, which are involved in thrombosis events [25-27].

The inflammatory response related to thrombosis is involved in all stages of PV development [28-30]. Cytokines act as information modulators in inter cellular communication regulating inflammatory responses after binding to their respective receptors. Among the core gene targets, TNF, IL-6, IL1β, CXCL8 (IL-8), CCL2, IFNG, STAT3 and HIF1A possessed pro-inflammatory effects. The core gene targets, AKT1, BCL2, CASP3, TP53 and MYC, were involved in cell survival and apoptosis [31]. The core gene targets, PPARG, EGFR, and MMP9 were associated with lipid metabolism and inflammation [32]. Especially, PPARG was related to ten active ingredients from DS, which were involved in regulating lipid and glucose metabolism, as well as inflammatory processes [33,34]. It implied DS in the drug pair might play a major role in treatment of metabolism inflammation after PV.

Zhou et al. reported that synergistic interactions of DS and SQ combination in LPS-induced RAW264.7 macrophages cell model, which inhibited the expression of pro-inflammatory mediators including TNF, NO (induced by iNOS), and MCP-1 [35]. The effects of Danqi Pill (DQP) containing DS and SQ on coronary artery disease (CAD) rat models *in vivo* and *in vitro* HUVEC exerts protective effects through fatty acids oxidation axis PPARα-CD36-CPT1A. The Fufang Xueshuantong (FXST) formula containing DS and SQ significantly decreases the levels of interleukin 1β (IL1β), matrix metalloproteinase 2 (MMP2), intercellular adhesion molecule 1 (ICAM1) and endothelial cell apoptosis [36]. These studies show that these gene-regulated bio-processes collectively lead to the endothelial dysfunction, and activate blood cells that produce thrombosis in patients with PV [37]. These studies also show that drug pair DS-SQ has multi-target effects on the regulation of vascular endothelial cells and blood cells such as neutrophil, monocyte, platelets and red blood cells involved in thrombosis. These results also indicate that cytokines act as the main players in the thrombosis after PV.

4.3 The molecular mechanism of drug pair DS-SQ in the treatment of thrombosis after PV

KEGG pathway enrichment analysis of 54 common gene targets demonstrated that they were related to lipid and atherosclerosis as well as fluid shear stress and atherosclerosis signaling pathways, which may be the crucial molecular mechanisms of drug pair DS-SQ in the treatment thrombosis after PV. The two signaling pathways play important roles in endothelial dysfunction, and lipid metabolism disorder have been identified as high risk factors of atherosclerosis and thrombosis [38]. Based on the key active ingredients and core gene targets and crucial pathways network, 5 core gene targets including TNF, BCL2, AKT1, TP53 and MMP9 were regulated by both pathways.

Tumor necrosis factor (TNF), a significant pro-inflammatory cytokines family closely related to thrombosis, can exacerbate inflammatory injury and trigger cell apoptosis signaling. TNF can stimulate endothelial cells to express adhesion molecules and inflammation factors, help LDL-C intake in vascular endothelial cells, lead to vascular endothelial dysfunction, which is a critical step in

thrombus development [35]. A clinical study showed that TNF-α levels in the serum increased compared to controls [39]. BCL2 and TP53 are involved in apoptotic pathways that influence both endothelial cell death and leukocyte activation, contributing to thrombosis. TP53 is also a tumor suppressor [40]. MMP9 promotes cell matrix remodeling and atherosclerosis plaque instability, increasing the risk of thrombosis by degrading the extracellular matrix [41,42]. AKT1 activation can initiate a downstream cascade reaction of the PI3K/Akt signaling pathway, and it further phosphorylates a series of downstream genes such as Caspase-3 and mTOR, thereby promoting cell survival and inhibiting mitochondrial damage-mediated apoptosis as well as involving the pathogenesis of endothelial dysfunction and atherosclerosis [43]. On the other hand, PI3K/Akt/NF-κB pathway can trigger the production of inflammatory cytokines. Yan et al, reported that Zhongfeng Capsules (ZFCs) containing Salviae Miltiorrhizae Radix et Rhizoma and Notoginseng Radix et Rhizoma have a definite therapeutic effect on cerebral ischemia-reperfusion injury of rat via mediating PI3K/Akt/NF-κB pathway by regulating the inflammatory factors expression including IL-1β, IL-6, and TNF-α [43]. Liu et al. observed that LPS stimulation of RAW264.7 cells resulted in a significant increase in ROS, NO, TNF-α, IL-6 and IL-1β, while the luteolin from Salviae Miltiorrhizae Radix et Rhizoma significantly reduced their expression via regulation of NF-κB signaling pathways [43]. These results suggest that the inflammatory cytokines involved in the thrombosis and the striking anti-inflammatory effect of DS and SQ are regulated through AKT pathway.

4.4 The limitation of drug pair DS-SQ in the treatment of thrombosis after PV

Above all, our study revealed that key active ingredients, core gene targets and crucial pathways of drug pair DS-SQ in the treatment of thrombosis after PV. However, there are still some limitations to this study. To begin with, the complex development of diseases and pharmacodynamics processes of active ingredients as well as unidentified and unrecorded ingredients and gene targets are dynamic, and this study is largely based on the existing databases. Some ingredients or gene targets may be omitted. Further scientific research regarding the treatment of drug pair DS-SQ against thrombosis after PV still needs experimental validation *in vitro* and *in vivo*. In addition, according to GO analysis and KEGG results, the mechanisms of drug pair DS-SQ in the treatment of thrombosis after PV may involve other biological processes and signaling pathways, but this needs to be verified in further research. We believe that further development of computational techniques combined with experimental validation will help to explore the underlying molecular mechanisms of the drug pair against thrombosis after PV.

4.5 Conclusion

In conclusion, the present study predicted the possible active ingredients and targets of compound-target-pathway-disease, and analyzed its possible mechanisms. Fifteen key active ingredients and 19 core gene targets exert anti-thrombotic effects mainly through lipid and atherosclerosis pathway regulated by drug pair DS-SQ, which are related to anti-inflammation, inhabiting cell apoptosis and lipid metabolism disorder. These results provide scientific basis and research direction for experimental studies and clinical application of the drug pair.

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A statement that all authors have seen and approved the manuscript

All the authors were involved in the manuscript writing process and approved it for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online.

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