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Exploring the mechanism of action of DHI on myeloproliferative neoplasms based on network pharmacology

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Abstract

Objective: The aim of this study is to explore the active ingredients and mechanism of action of danhong injection (DHI) in treating myeloproliferative neoplasms using network pharmacology. **Methods:** The TCMSP platform and relevant literature were used to search for the active ingredients and targets of Radix Salviae and Carthami Flos in DHI. Disease targets related to myeloproliferative neoplasms were obtained from the GEO database, GeneCards, and DisGeNET database. The queried component targets were normalized using the UniProt database. Potential targets were identified by constructing protein-protein interactions networks using STRING 11.5 and visualized and analyzed using Cytoscape 3.9.1. GO and KEGG analysis were performed using the Metascape platform, and visualization was done using the built-in plug-in CluoGO or SangerBox platforms with Cytoscape 3.9.1. **Results:** The active ingredients of DHI for treating myeloproliferative neoplasms mainly consist of flavonoids and o-benzoquinones, including quercetin, luteolin, kaempferol, stigmasterol, tanshinone iia, cryptotanshinone, beta-carotene, 2-isopropyl-8-methylphenanthrene-3,4-dione, and neocryptotanshinone ii. The potential targets are JUN, TP53, STAT3, AKT1, MAPK1, RELA, TNF, MAPK14, IL6, and FOS. The relevant signaling pathways involved are mainly TNFα signaling pathway, PI3K-Akt signaling pathway, apoptosis, IL-17 signaling pathway, cellular senescence, MAPK signaling pathway, p53 signaling pathway, JAK-STAT signaling pathway, and NF-kappa B signaling. **Conclusions:** DHI acts mainly through flavonoids and o-benzoquinones to treat myeloproliferative neoplasms in a multi-targeted and multi-pathway manner.

Keywords: danhong injection; myeloproliferative neoplasms; network pharmacology; effective material basis; molecular mechanism

Author contributions

Ming-Jie Liu conducted the study and wrote the original draft; Ming-Jie Liu, Pu Wang, Zhi-Da Shi and Bao-Bing Zhao designed the study; Ming-Jie Liu, Rui-Fen Dong, Pu Wang, Zhi-Da Shi, Yuan Li, Qian Zhou, Shu-Jing Zhang and Bao-Bing Zhao discussed and analyzed the data; Chun-Hua Lu, Tao Shen, Rui-Fen Dong, Pu Wang, Zhi-Da Shi and Bao-Bing Zhao reviewed and edited the paper. All authors have read and approved the final the manuscript.

Competing interests

The authors declare no conflicts of interest.

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Peer review information

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Abbreviations

DHI, danhong injection; MPNs, myeloproliferative neoplasms; PMF, Primary Myelofibrosis; TCM, Traditional Chinese medicine; OB, Oral Bioavailability; DL, Drug-Like; DEGs, differentially expressed genes; PPI, protein-protein interactions; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological processes; CC, cellular components; MF, molecular functions.

Citation

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Introduction

Myeloproliferative neoplasms (MPNs) are clonal disorders involving hematopoietic stem and progenitor cells that are associated with myeloproliferation, splenomegaly, and systemic symptoms. The main characteristic is the malignant proliferation of one or more groups of myeloid cells, which can progress to thrombosis and acute leukemia [1]. Classical MPNs include Polycythemia Vera, Essential Thrombocythemia, and Primary Myelofibrosis (PMF) based on clinical symptoms. The current treatment of MPNs typically involves targeted or chemotherapeutic drugs combined with antithrombotic drugs, with the main goal of relieving symptoms and preventing disease progression. However, this approach cannot achieve a cure and has side effects, and long-term application can lead to tolerance [2]. Traditional Chinese medicine (TCM) believes that MPNs are caused by "blood stasis", "accumulation", "blood paralysis", and "obstruction", and classifies Polycythemia Vera, Essential Thrombocythemia, and PMF as "bone marrow blood accumulation disease", "bone marrow blood solid disease", and "bone marrow blood crux disease", respectively. TCM treatment for MPNs is mainly based on classical prescriptions that promote blood circulation and remove blood stasis, clear liver and remove blood stasis, and supplement deficiency and remove blood stasis [3–5].

Danhong injection (DHI) is a novel Chinese medicine injection made from the extraction of two Chinese herbs, Radix Salviae and Carthami Flos, in a 3:1 ratio. DHI is known for its ability to promote blood circulation and remove blood stasis, clear veins and relax collagalories, making it suitable for treating blood stasis caused by blood blockage. DHI is widely used in the clinical treatment of various cardiovascular and cerebrovascular diseases, including angina, acute coronary syndrome, acute myocardial infarction, stroke, transient ischemic attack, and hypertension. Radix Salviae, as an herbal medicine herb, has been reported to possess multiple effects such as scavenging antioxidants, antiplatelet aggregation, inhibiting inflammatory responses, and promoting vascular regeneration. Carthami Flos exhibits the ability to dilate blood vessels, improve microcirculation, enhance immune function, and also has anti-inflammatory, anti-tumor, and anti-aging effects. Radix Salviae is cold in nature as descending, while Carthami Flos is warm in nature as ascending. Both herbs complement each other and work together to activate blood circulation, remove blood stasis, and relieve pain. According to the theory of "treating different diseases with the same treatment" in Chinese medicine, activating blood circulation, dredging the meridians, and resolving blood stasis are also the basic treatment methods for MPNs. In this study, we applied network pharmacology analytical methods to screen potential targets of action and pathway analysis, providing a rational basis for validating this formula for the treatment of MPNs.

Methods

Collection and analysis of active constituents and targets of DHI

The active ingredients of DHI were screened using the TCMSP (http://tcmspw.com/tcmsp.php) and literature search with "Radix Salviae" and "Carthami Flos" as keywords [6]. The main active ingredients were selected based on the criteria of Oral Bioavailability (OB) ≥ 30% and Drug-Like (DL) ≥ 0.18 [7]. The active ingredients were then screened for their targets of action in TCMSP. The targets of the active ingredients were normalized using the UniProt database (https://www.uniprot.org/).

Collection of targets related to MPNs

The targets related to MPNs were obtained from the DisGeNET database (http://www.disgenet.org/) and the Genecards database (https://www.genecards.org/) using the search terms "Myeloproliferative Neoplasm", "Polycythemia Vera", "Essential Thrombocythemia", and "Primary Myelofibrosis". For the Genecards database, a relevance score ≥ 0.01 was used as the screening condition, while for the DisGeNET database, a score value ≥ 9.11 was used. In addition, we also selected differentially expressed genes (DEGs) from the GSE54644 dataset to comprehensively collect disease targets in MPNs. The GSE54644 dataset comprises transcriptomic data obtained from peripheral blood cells of 93 MPN patients and 11 healthy volunteers. DEGs linked to MPNs were identified based on the criteria of (logFC) ≥ 1 and adj. $P < 0.5$.

Protein-Protein interactions (PPI) network and core target analysis

The PPI network with common targets for DH and MPNs was constructed using the STRING database (version 11.5, https://cn.string-db.org/). The species was set to "Homo sapiens", and the minimum required interaction score was set to "highest confidence (0.9)". Cytoscape 3.9.1 was used for visualization and analysis of the PPI networks. The NetworkAnalyzer function was used to analyze the PPI networks, adjusting the thickness and color of edges based on the combined score value, and adjusting the target size and color based on the degree value. The core targets of DHI acting on MPNs were filtered based on the degree value magnitude.

Analysis of pathway enrichment

The Metascape database (https://Metascape.org) was used to perform pathway enrichment analysis of drug-disease common targets and core targets [8]. The species was set to human, and a significance threshold of $P < 0.05$ was applied for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. The data were saved and the results were visualized and analyzed using the built-in plugin CluoGO from Cytoscape 3.9.1 and the online web platform (http://www.sangerbox.com/).

Construction of herb-component-target-pathway network

The main signaling pathways related to DHI intervention in disease progression were identified based on the KEGG-enriched signaling pathways involved in MPNs. These pathways were then mapped back to the active ingredients based on the target genes enriched in the pathways. The "herbal-component-target-pathway" interactions were visualized using the built-in tools in Cytoscape 3.9.1 software. The main active substances were identified based on the key core components in the network.

Results

Identification of components in DHI and target prediction

A total of 391 DHI components were collected from TCMSP and literature. Among them, Salvia had 202 main components and Safflower had 189 main components. After screening with OB ≥ 30% and DL ≥ 0.18, a total of 73 active ingredients with targets were obtained, 65 for Radix Salviae and 22 for Carthami Flos (Table 1). The targets of these 73 active compounds in DHI were collected from the TCMSP database. After integrating UniProt database entries and removing duplicates, a network of "DHI-component-target" was established, consisting of 249 DHI targets (Figure 1).

Screening targets related MPNs

We obtained gene expression data for 471 targets from the GeneCards database, 539 targets from the DisGeNET database, and 562 targets from the GEO database. The GSE54644 microarray dataset, which contained transcriptomic data from 93 MPN patients and 11 healthy human participants in PBMC, was also used for differential gene analysis using [logFC] ≥ 1 and adj. $P < 0.5$ as the threshold. A total of 562 DEGs were identified, with volcano plots showing 512 DEGs upregulated and 50 DEGs downregulated in MPNs. In total, 1327 disease targets were collected from the three databases (Figure 2 and Table 2).

Table 1 The active ingredients of DHI

Mol ID	ID	Molecule Name	OB (%)	DL	Drug
MOL000006	A1	luteolin	36.16	0.25	Radix Salviae, Carthami Flos
MOL001771	A2	poriferast-5-en-3beta-ol	36.91	0.75	Radix Salviae, Carthami Flos
MOL002776	A3	Baicalin	40.12	0.75	Radix Salviae, Carthami Flos
MOL007154	DS60	tanshinone iia	49.89	0.40	Radix Salviae
MOL007088	DS29	cryptotanshinone	52.34	0.40	Radix Salviae
MOL007041	DS9	2-isopropyl-8-methylphenanthrene-3,4-dione	40.86	0.23	Radix Salviae
MOL007124	DS46	neocryptotanshinone ii	39.46	0.23	Radix Salviae
MOL000569	DS1	digallate	61.85	0.26	Radix Salviae
MOL001601	DS2	1,2,5,6-tetrahydrotanshinone	38.75	0.36	Radix Salviae
MOL001659	DS3	Poriferasterol	43.83	0.76	Radix Salviae
MOL001942	DS4	isoimperatorin	45.46	0.23	Radix Salviae
MOL002222	DS5	sugiol	36.11	0.28	Radix Salviae
MOL002651	DS6	Dehydrotanshinone II A	43.76	0.40	Radix Salviae
MOL007036	DS8	5,6-dihydroxy-7-isopropyl-1,1-dimethyl-2,3-dihydrophenanthren-4-one	33.77	0.29	Radix Salviae
MOL007045	DS10	3α-hydroxytanshinoneIIa	44.93	0.44	Radix Salviae
MOL007048	DS11	(E)-3-[2-(3,4-dihydroxyphenyl)-7-hydroxy-benzofuran-4-yl]acrylic acid	48.24	0.31	Radix Salviae
MOL007049	DS12	4-methylenemiltirone	34.35	0.23	Radix Salviae
MOL007050	DS13	2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	62.78	0.40	Radix Salviae
MOL007058	DS15	formyltanshinone	73.44	0.42	Radix Salviae
MOL007059	DS16	3-beta-Hydroxymethyllenetanshiquinone	32.16	0.41	Radix Salviae
MOL007061	DS17	Methylenetanshinquinone	37.07	0.36	Radix Salviae
MOL007063	DS18	przewalskin a	37.11	0.65	Radix Salviae
MOL007064	DS19	przewalskin b	110.32	0.44	Radix Salviae
MOL007068	DS20	Przewaquinone B	62.24	0.41	Radix Salviae
MOL007069	DS21	przewaquinone c	55.74	0.40	Radix Salviae
MOL007070	DS22	(6S,7R)-6,7-dihydroxy-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-dione	41.31	0.45	Radix Salviae
MOL007071	DS23	przewaquinone f	40.31	0.46	Radix Salviae
MOL007077	DS24	sclareol	43.67	0.21	Radix Salviae
MOL007079	DS25	tanshinaldehyde	52.47	0.45	Radix Salviae
MOL007081	DS26	Danshenol B	57.95	0.56	Radix Salviae
MOL007082	DS27	Danshenol A	56.97	0.52	Radix Salviae
MOL007085	DS28	Salvilenone	30.38	0.38	Radix Salviae
MOL007093	DS30	dan-shexinkum d	38.88	0.55	Radix Salviae
MOL007094	DS31	danshenspiroketallactone	50.43	0.31	Radix Salviae
MOL007098	DS32	deoxyneocryptotanshinone	49.40	0.29	Radix Salviae
MOL007100	DS33	dihydrotanshinlactone	38.68	0.32	Radix Salviae
MOL007101	DS34	dihydrotanshinoneI	45.04	0.36	Radix Salviae
MOL007105	DS35	epidanshenspiroketallactone	68.27	0.31	Radix Salviae
MOL007107	DS36	C09092	36.07	0.25	Radix Salviae
MOL007108	DS37	isocryptotanshi-none	54.98	0.39	Radix Salviae
MOL007111	DS38	Isotanshinone II	49.92	0.40	Radix Salviae
MOL007115	DS39	manool	45.04	0.20	Radix Salviae
MOL007119	DS41	miltionone I	49.68	0.32	Radix Salviae
MOL007120	DS42	miltionone II	71.03	0.44	Radix Salviae
MOL007121	DS43	miltipolone	36.56	0.37	Radix Salviae
MOL007122	DS44	Miltirone	38.76	0.25	Radix Salviae
MOL007125	DS47	neocryptotanshinone	52.49	0.32	Radix Salviae
MOL007127	DS48	1-methyl-8,9-dihydro-7H-naphtho[5,6-g]benzofuran-6,10,11-trione	34.72	0.37	Radix Salviae
MOL007130	DS49	prolithospermic acid	64.37	0.31	Radix Salviae
MOL007132	DS50	(2R)-3-(3,4-dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl)acryloyl]oxy-propionic acid	109.38	0.35	Radix Salviae
MOL007141	DS52	salvianolic acid g	45.56	0.61	Radix Salviae
MOL007142	DS53	salvianolic acid j	43.38	0.72	Radix Salviae
MOL007143	DS54	salvilenone I	32.43	0.23	Radix Salviae
MOL007145	DS55	salviolone	31.72	0.24	Radix Salviae
MOL007150	DS57	(6S)-6-hydroxy-1-methyl-6-methylol-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-quinone	75.39	0.46	Radix Salviae
MOL007151	DS58	Tanshindiol B	42.67	0.45	Radix Salviae
MOL007152	DS59	Przewaquinone E	42.85	0.45	Radix Salviae
MOL007155	DS61	(6S)-6-(hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-dione	65.26	0.45	Radix Salviae
MOL007156	DS62	tanshinone VI	45.64	0.30	Radix Salviae
MOL000098	HH1	quercetin	46.43	0.28	Carthami Flos
MOL000422	HH3	kaempferol	41.88	0.24	Carthami Flos
MOL000449	HH4	Stigmasterol	43.83	0.76	Carthami Flos
MOL002773	HH19	beta-carotene	37.18	0.58	Carthami Flos
MOL000358	HH2	beta-sitosterol	36.91	0.75	Carthami Flos
MOL000953	HH5	CLR	37.87	0.68	Carthami Flos
MOL002694	HH7	4-[(E)-4-(3,5-dimethoxy-4-oxo-1-cyclohexa-2,5-dienylidene)but-2-enylidene]-2,6-dimethoxycyclohexa-2,5-dien-1-one	48.47	0.36	Carthami Flos
MOL002695	HH8	lignan	43.32	0.65	Carthami Flos
MOL002710	HH12	Pyrethrin II	48.36	0.35	Carthami Flos
MOL002712	HH13	6-Hydroxykaempferol	62.13	0.27	Carthami Flos
MOL002714	HH14	baicalein	33.52	0.21	Carthami Flos
MOL002717	HH15	qt_carthamone	51.03	0.20	Carthami Flos
MOL002721	HH17	quercetagetin	45.01	0.31	Carthami Flos
MOL002757	HH18	7,8-dimethyl-1H-pyrimido[5,6-g]quinoxaline-2,4-dione	45.75	0.19	Carthami Flos

DHI, danhong injection; OB, Oral Bioavailability; DL, Drug-Like.

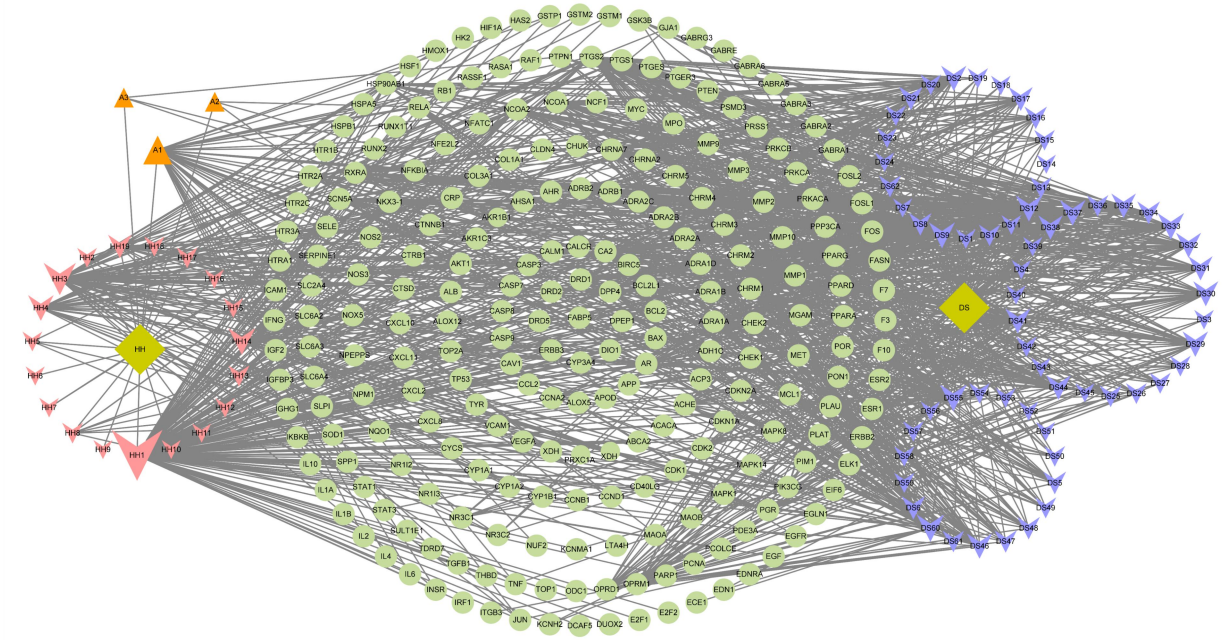

Figure1 Herb-Compound-Target network of DHI. DHI, danhong injection.

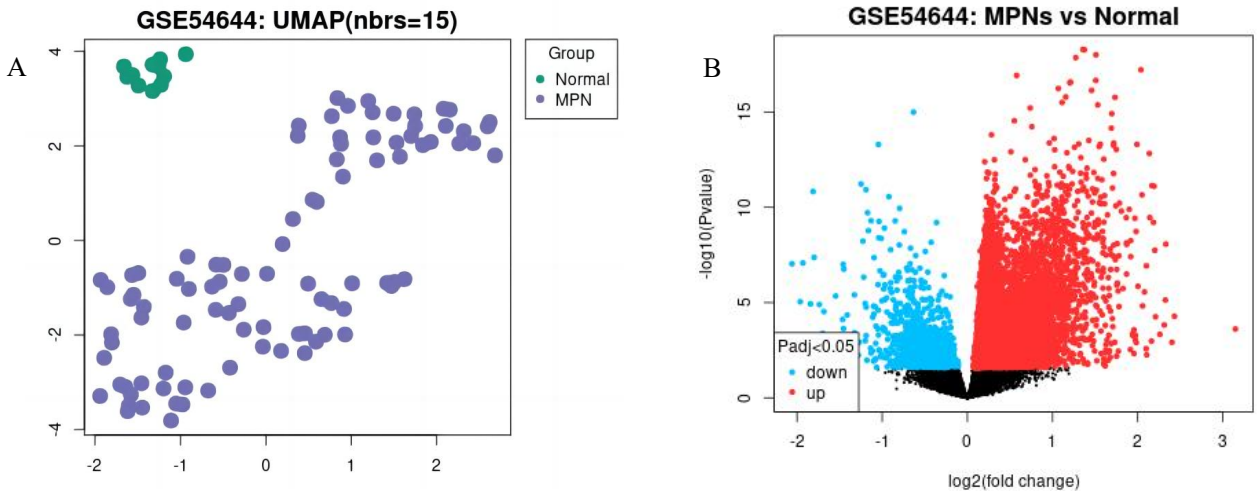

Figure 2 Screening for MPNs-related targets. (A) UMAP analysis of gene from the GSE54644 dataset; (B) Volcano plot map of DEGs. MPNs, myeloproliferative neoplasms; DEGs, differentially expressed genes.

Table 2 The union of MPNs targets from different databases

Data base	Targets
GSE54644 (93 vs 11samples) ([logFC] ≥ 1, adj. P < 0.5)	562
DisGeNET (Score ≥ 9.11)	539
Genecards (Relevance score ≥ 0.01)	471
Union	1327

MPNs, myeloproliferative neoplasms.

Identification of 99 common targets between DHI and MPNs

To investigate potential avenues for DHI treatment of MPNs, we intersected 241 DHI-related targets with 1372 MPNs-related targets, resulting in 99 shared targets (Figure 3A) that could serve as potential targets for DHI treatment of MPNs. We utilized Cytoscape 3.9.1 to construct a drug-compound-potential target-disease interaction network (Figure 3B).

Analysis of core targets of DHI on MPNs

To further refine the core targets, we created a PPI network comprising the 99 shared targets (Figure 4A). Additionally, we employed Cytoscape to visualize the correlation among these common targets (Figure 4B). The size and color of the nodes in the network graph correspond to the degree value, while the thickness and color of the edges correspond to the combined fraction value. A higher degree of nodes indicates that the target has more biological functions in the interacting network. Based on the findings, JUN, TP53, STAT3, AKT1, MAPK1, RELA, TNF, MAPK14, IL6, and FOS exhibited the highest degree of cross-linking with other targets in the PPI network (Table 3), suggesting that these targets may be potential core targets for DHI treatment of MPNs.

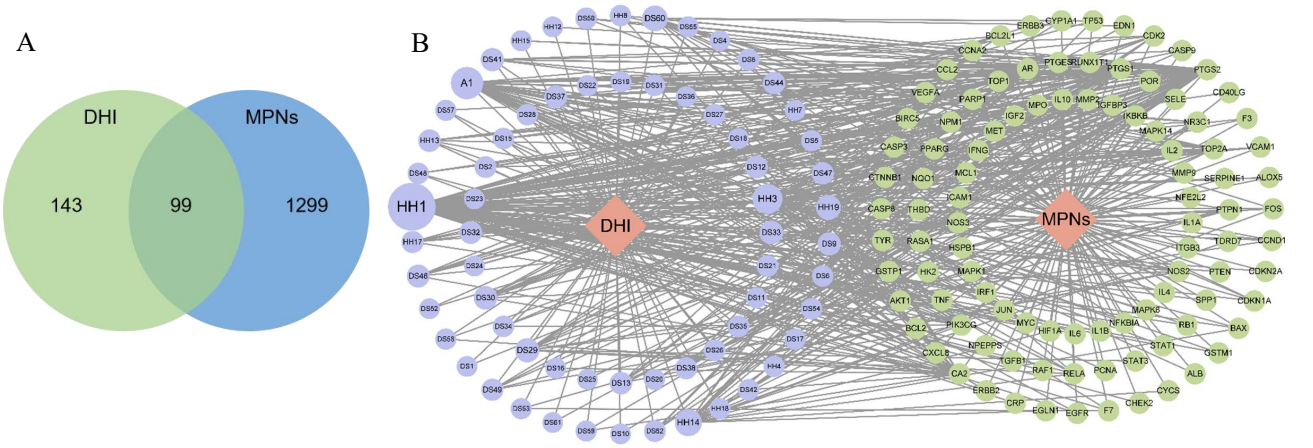

Figure 3 Venn diagram of common target of DHI-MPNs and interaction diagram of compound-target. (A) Venn diagram of the target of DHI and the target of MPNs; (B) The drug-compound-target-disease interaction network. DHI, danhong injection; MPNs, myeloproliferative neoplasms.

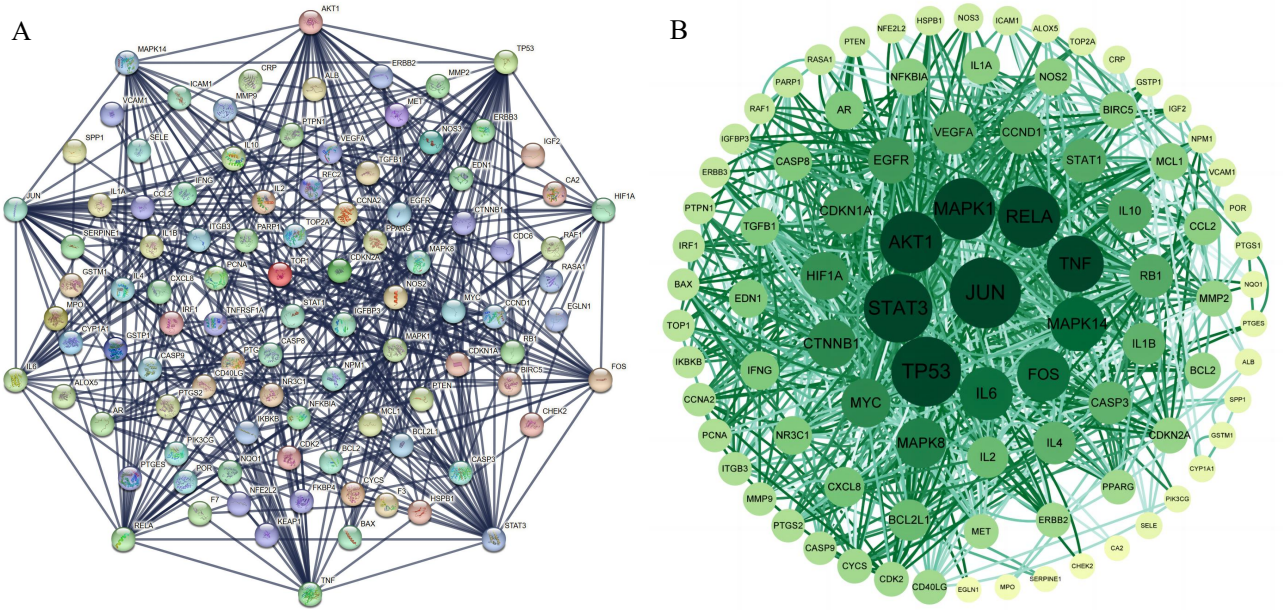

Figure 4 PPI networks with common targets for DHI-MPNs. (A) The PPI network of DHI in the treatment of MPNs; (B) Visual network diagram from A. PPI, protein-protein interactions; DHI, danhong injection; MPNs, myeloproliferative neoplasms.

Table 3 Top ten targets information of PPI network

Name	Degree	BC	CC
JUN	72	5.039850	0.584416
TP53	70	7.893127	0.555556
STAT3	68	5.472315	0.566038
RELA	58	2.128682	0.552147
MAPK1	58	3.260453	0.538922
AKT1	58	3.694440	0.545455
TNF	50	2.676746	0.500000
MAPK14	46	1.081399	0.523256
IL6	44	1.815680	0.494505
FOS	44	1.469773	0.511364

PPI, protein-protein interactions; BC, Betweenness Centrality; CC, Closeness Centrality.

Enrichment of pathway analysis of common targets

To investigate the mechanism of DHI on MPNs, we conducted GO enrichment analysis using the Meatascape database, which includes biological processes (BP), cellular components (CC), and molecular functions (MF). A total of 289 GO terms were highly enriched, comprising 86 MFs, 59 CCs, and 144 BPs. The top 10 significantly enriched terms in MFs, CCs, and BPs are presented in Figure 5A. The findings revealed that the primary MFs were kinase binMding, growth factor activity, cyclin-dependent protein kinase regulator activity, protein domain-specific binding, protein homodimerization activity, protein phosphatase binding, ubiquitin protein ligase binding, and protein kinase binding. The primary CCs were transcription regulator complex, vesicle lumen, mitochondrial envelope, membrane raft, and endoplasmic reticulum lumen. The primary BPs were response to

lipopolysaccharide, regulation of protein kinase activity, and cell proliferation.

Moreover, KEGG pathway enrichment analysis identified multiple signaling pathways that have been reported to be involved in the pathogenesis of MPNs, such as JAK-STAT signaling and PI3K/Akt signaling pathways (Figure 5B). Other signaling pathways, including TNF signaling, cellular senescence, MAPK signaling, p53 signaling, and NF-kappa B signaling, were also significantly enriched.

GO BP visual analytics and MCODE network analysis

Furthermore, we utilized the built-in Cytoscape plug-in ClueGO to visualize the GO BP enrichment analysis of the 99 potential targets (Figure 6A). The results indicated that the primary gene clusters were divided into three major directions, including regulation of inflammatory response, regulation of programmed cell death, and regulation of cell proliferation and growth. Consistent with this, GO enrichment analysis of the PPI network was conducted to extract "biological significance" (Figure 6B), and the primary BP modes were found to be involved in the regulation of cell proliferation and apoptosis.

"Herb-Components-Targets-Pathways" network analysis

Using the above eight enriched KEGG signaling pathways that are closely associated with MPN disease progression, we identified the target genes enriched in the pathways and mapped them back to the active ingredients and corresponding medicinal plants. The "herbal-component-target-pathway" interaction network was constructed using Cytoscape, and the built-in plug-in was utilized for degree analysis, with nodes exhibiting a higher degree representing a pivotal role in the network (Figure 7). Based on the analysis of the results, the key active components of DHI for the treatment of MPN were identified as quercetin (HH1), luteolin (A1), kaempferol (HH3), stigmasterol (HH14), tanshinone iia (DS60), cryptotanshinone (DS29), beta-carotene (HH19), 2-isopropyl-8-methylphenanthrene-3,4-dione (DS9), and neocryptotanshinone ii (DS46).

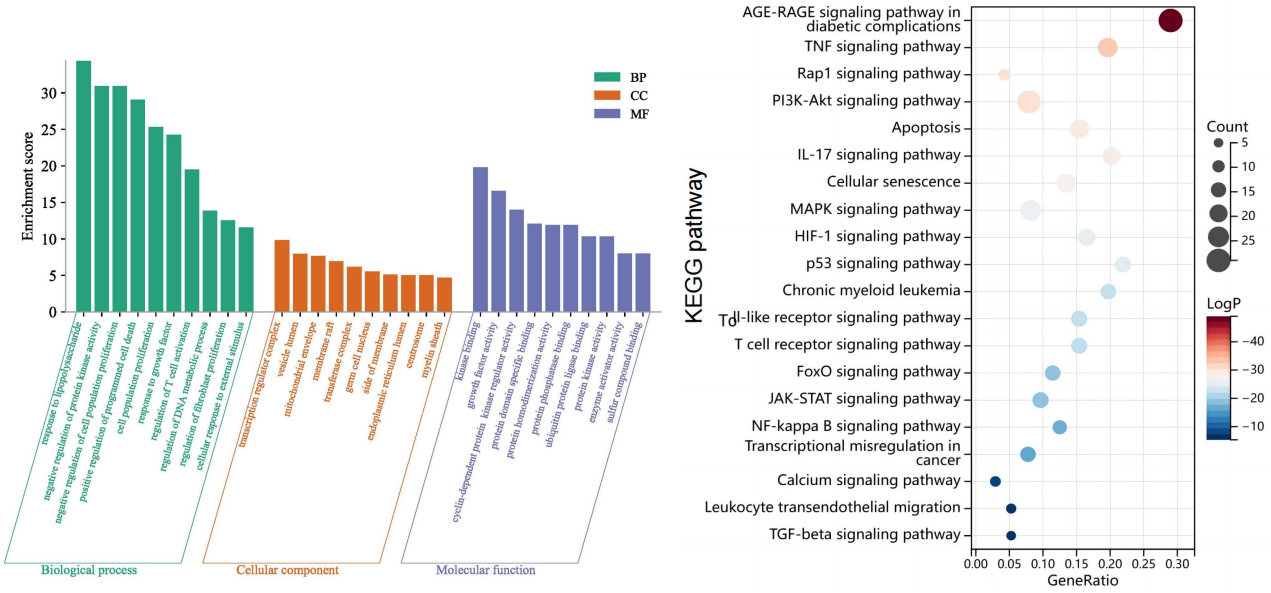

Figure 5 Enrichment analysis of common targets of DHI in treatment of MPNs. (A) GO enrichment analysis of the biological process, cellular components, and molecular function of DHI-MPNs; (B) KEGG bubble diagram of potential targets of DHI-MPNs. DHI, danhong injection; MPNs, myeloproliferative neoplasms; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

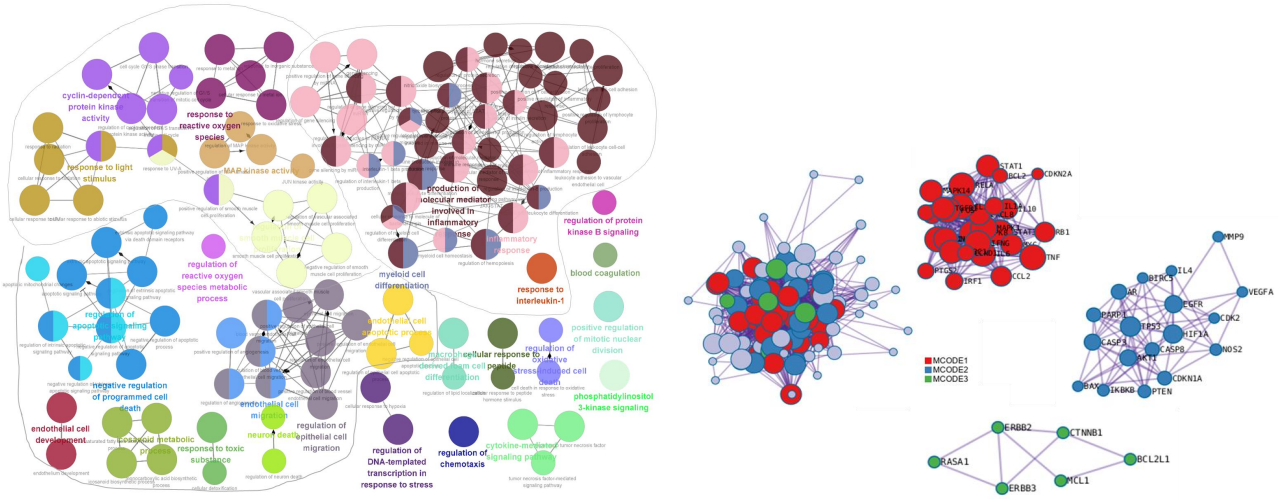

Figure 6 Visual analysis of the GO biological process and MCODE components identified in the gene lists. (A) Visual analysis of the GO biological process by using ClueGO within Cytoscape software; (B) MCODE analysis of GO biological process: MCODE1, negative regulation of cell population proliferation; MCODE2, regulation of apoptotic signaling pathway; MCODE3, extrinsic apoptotic signaling pathway in absence of ligand. GO, Gene Ontology.

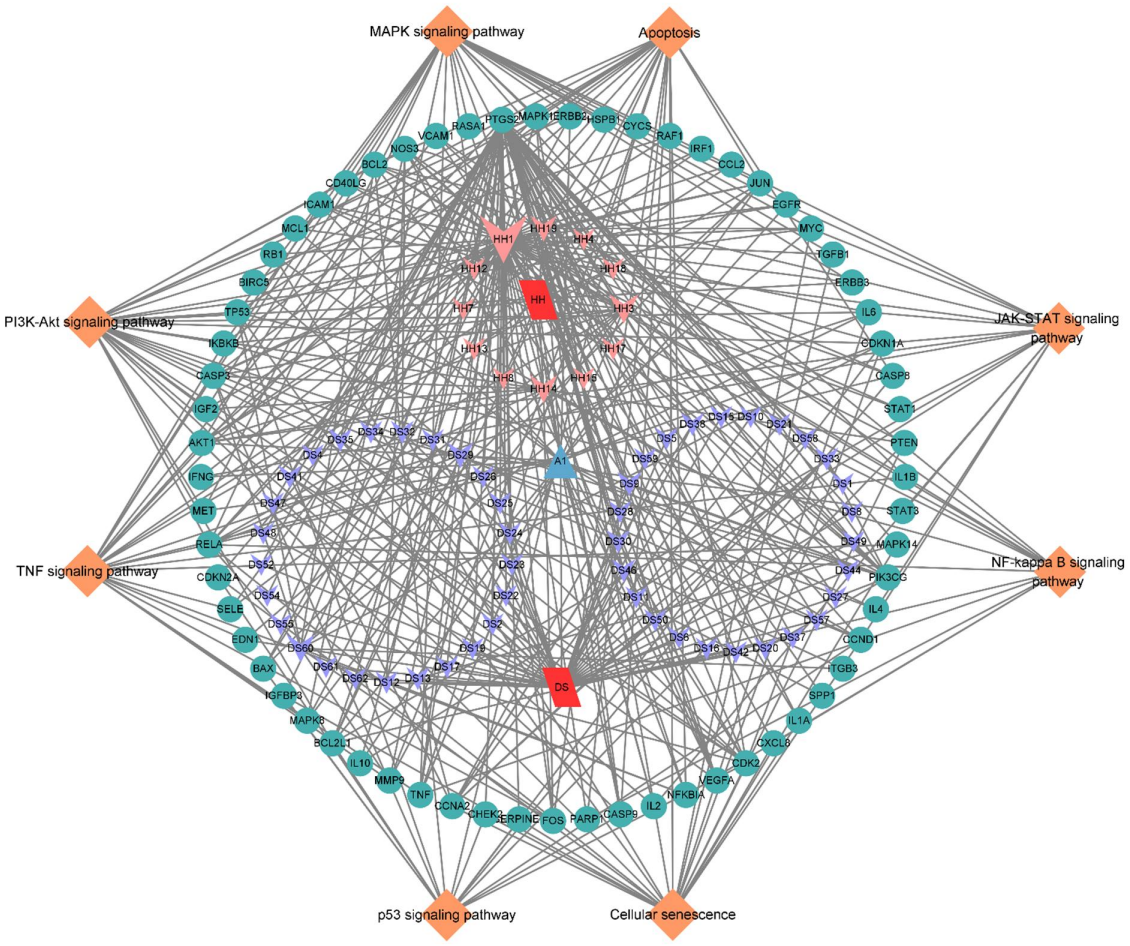

Figure 7 "Herb-compound-target-function" network of DHI for MPNs.

Discussion

In this study, the network pharmacology approach was used to integrate the main active ingredients in DHI with potential disease targets, resulting in the establishment of an effective herbal-component-target-pathway network relationship. The herbal-component-target-pathway network relationship identified Quercetin, luteolin, kaempferol, stigmasterol, tanshinone iia, cryptotanshinone, beta-carotene, 2-isopropyl-8-methylphenanthrene-3,4-dione, and neocryptotanshinone ii. Most of these components are flavonoids and o-benzoquinones, indicating that flavonoids and o-benzoquinones may be the main substance basis for the treatment of MPNs.

The PPI protein interaction network was used to identify the core proteins of MPNs that were interfered by Salvia injection. These proteins include JUN, TP53, STAT3, AKT1, MAPK1, RELA, TNF, MAPK14, IL6, and FOS. TP53 mutations are one of the most common genetic variants in all human mutations and are a poor prognostic marker for myeloid malignancies. They are found in chronic MPN cases and are believed to be a key factor in the conversion to myeloid leukemia [9–12]. STAT3 is a signal transduction and transcriptional activator that can be activated by JAK and enter the nucleus to play a role in transcriptional regulation. The constitutive activation of the JAK-STAT signaling pathway is the main manifestation of MPNs. The signal transduction pathway of STAT3 is related to the drive of malignant cell proliferation and inflammatory response [13, 14]. Additionally, AKT activation has been shown to be a characteristic of MPNs, and the use of selective AKT inhibitors has demonstrated therapeutic effects in MPN mice, suggesting that AKT is a potential therapeutic target for MPNs [15, 16].

The literature has increasingly reported on the role of inflammation in MPNs, with overproduction of inflammatory cytokines being a clinical feature of MPN patients [17–23]. The core proteins screened for inflammation-related factors include RELA, TNF, and IL6. IL6 is an important cytosolic inflammatory factor in vivo and the most commonly overexpressed inflammatory factor in myeloid malignancies, which promotes STAT3 phosphorylation [24, 25]. Activated IL6/STAT3 signaling can further mediate the production of TNFα, IL1β, and other inflammatory factors, which in turn continuously enhance the inflammatory response. TNF mediates the clonal advantage of mutant cells in MPNs, and inhibition of TNFR has shown therapeutic effects in MPN mice [20, 26]. The above data suggest a central role of inflammation-related factors in MPNs. Taken together, it suggests that DHI may have a role in the treatment of MPNs through multiple targets.

Enrichment analysis of the core targets of DHI intervention in MPNs has revealed that multiple signaling pathways are closely associated with the pathogenesis of MPNs. JAK/STAT signaling is the main mediator for the development of blood cells, which is always elevated in MPN patients. 90% of MPN patients carry mutations in JAK2, CALR, or MPL that lead to the phosphorylation of JAK2 independent of cytokine [27]. Activated JAK2 recruits and phosphorylates substrate molecules, including STAT, PI3K, MAPK, and AKT, ultimately leading to the constitutive activation of cellular signaling pathways [15, 28–30]. Additionally, MPN patients have elevated inflammatory cytokines, which reach the highest levels in patients with advanced PMF. Studies of patients and preclinical mouse models of MPN have linked inflammation and MPN progression to myelofibrosis [31]. TNF/NF-κB-related signaling pathways are also closely associated with the development of MPNs [32]. Blocking TNF/NF-kappaB signaling has shown positive effects in the treatment of MPNs and even reversed myelofibrosis, indicating an important role

of inflammation-related signaling pathways in the pathogenesis of MPNs [33, 34]. In summary, our study, combined with published reports, suggests that DHI exerts therapeutic effects on MPNs through multiple signaling pathways.

Conclusion

In summary, DHI, which is clinically used for promoting blood circulation and removing blood stasis, smoothing veins, and relaxing collagens, plays a positive role in the treatment of MPN. The underlying mechanism of DHI against MPN is related to its main components, flavonoids, and o-benzoquinones, which act on the JAK-STAT signaling, PI3K/AKT signaling, and MAPK signaling pathways. Additionally, inflammation-related signaling pathways and cellular senescence are also involved in the action of DHI on MPN.

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