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Mechanism research of *Salvia miltiorrhiza* on treating myocardial ischemia reperfusion injury according to network pharmacology combined with molecular docking technique

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Abstract

Myocardial ischemia reperfusion injury (MIRI) is a kind of complicated disease with an increasing incidence all over the world. Danshen was shown to exert therapeutic effect on MIRI. However, its chemical and pharmacological profiles remain to be elucidated. Network pharmacology was applied to characterize the mechanisms of Danshen on MIRI.

The active compounds were screened from the online database according to their oral bioavailability and drug-likeness. The potential proteins of Danshen were collected from the TCMSP database, whereas the potential genes of MIRI were obtained from Gene Card database. The function of gene and pathways involved were researched by GO and KEGG enrichment analysis. The compounds-targets and protein–protein interaction networks were constructed by Cytoscape software. The affinity between active components and potential targets was detected by molecular docking simulation.

A total of 202 compounds in Danshen were obtained, and 65 were further selected as active components for which conforming to criteria. Combined the network analysis and molecular docking simulation, the results firstly demonstrated that the effect of Danshen on MIRI may be realized through the targeting of vascular endothelial growth factor A, interleukin-6, and AKT1 by its active components tanshinone IIA, cryptotanshinone, and luteolin. The main regulatory pathways involved may include PI3K/ Akt signaling pathway, HIF-1 signaling pathway, and interleukin-17 signaling pathway. The present study firstly researched the mechanism of Danshen on MIRI based on network pharmacology.

The results revealed the multicomponents and multi-targets effects of Danshen in the treatment of MIRI. Importantly, the study provides objective basis for further experimental research.

Abbreviations: CAHD = coronary atherosclerotic heart disease, CHD = coronary heart disease, CPT = cryptotanshinone, DS = Danshen, GO = Gene Ontology, MIRI = myocardial ischemia reperfusion injury, PPI = protein–protein interaction, TSA = tanshinone IIA.

Keywords: Danshen, molecular docking, molecular mechanisms, myocardial ischemia reperfusion injury, network pharmacology

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

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1. Introduction

Cardiovascular diseases have an accumulating incidence worldwide caused mainly by the change of people's lifestyle. High fat diet and less exercise often increase the burden of heart, which leads to the occurrence of cardiovascular disease.[1–3] Notably, cardiovascular diseases, especially the coronary heart disease (CHD), have a high mortality rate in urban population.[4,5] It is generally realized that the pathogenesis of CHD is closely related to coronary atherosclerosis. In pathological condition, the blocked coronary blood flow will lead to myocardial ischemia, resulting in myocardial tissue damage or dysfunction, further inducing acute myocardial infarction and endangering life. Prompt restoration of blood flow to the ischemic myocardium can limit the infarct size, reduce mortality, and avoid further myocardial injury. Actually, thrombolytic therapy and coronary intervention are indeed the most effective treatment for reducing the size of a myocardial infarct and improving the clinical outcome. Unfortunately, reconstructing blood flow to the heart can also cause cardiovascular damage, which is called myocardial ischemia/reperfusion injury (MIRI).[6–8] Coping with adverse outcomes caused by MIRI, there is no effective treatment at present, and reducing the abnormal apoptosis of myocardial cells caused by ischemia/reperfusion and preventing from myocardial infarction are the main purposes of treatment.

Salvia miltiorrhiza (Danshen in Chinese) is the dried root and rhizome of the labiaceae plant *Salvia miltiorrhiza Bge*. Studies have shown that Danshen (DS) is the most widely used Chinese herbal medicine for the treatment of ischemic heart disease and ischemic stroke.[9,10] DS is a kind of multi-targets herbal medicine, which can be used alone or combined with other herbal medicine for the treatment of cardiovascular and cerebrovascular diseases.[11–13] Especially, DS and its compound preparation have been reported that take crucial part in the improvement of symptoms of MIRI in vivo and invitro experiments.[14,15] Indeed, >200 compounds have been identified from DS, demonstrating its multi-target pharmacological action; however, it is pretty difficult to clarify these complex pharmacological mechanisms by the conventional research strategies.

For the view of chemistry, traditional Chinese herbal medicine are complicate systems with multiple targets as well as interactions among their substances.[16] Different from western medicine of "one target, one drug," herbal medicines always exert multi-targets. This trait is a double-edged sword. Studies have shown that multi-target drugs can avoid the shortcomings of single-target drugs, such as single targeting and easy drug resistance.[17,18] However, due to the complex characteristics of multi-target drugs, it is difficult to clarify the mechanism of action. Network pharmacology combines the systems biology with pharmacokinetic and pharmacodynamics to research medicines, targets and their pharmacological activities.[19] Systematically, network pharmacology is on the basis of the interactions of disease-gene-targets-drug networks to observe the effect of herbs on diseases. Therefore, network pharmacology is realized as a promising method to cast light upon traditional Chinese herbs or formulas.[20]

In the present study, I used computational tools and resources to investigate the pharmacological network of DS on MIRI to predict the active compounds and potential protein targets. Molecular docking approach was applied to detect the interactions between activated compounds and protein targets. The detailed technical strategy of the present study was shown in Figure 1.

2. Methods

2.1. Screening of active compounds and targets

Based on the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmspw.com/tcmsp.php),[21] the chemical components of DS were searched, and the compounds with oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 were collected. Oral bioavailability (OB), which is the relative amount of a drug entering the bloodstream in prototype form after oral administration, is a key indicator of drug bioactivity and can be used as a screening value of ≥30%. DL is based on the similarity principle that generally similar molecules should have similar biological activity and DL ≥0.18 is the selection criteria for compounds.[22,23] At the same time, their action targets were screened in TCMSP. And then, the targets were transformed into the official gene symbols using R software according to the Uniprot (https://www.uniprot.org/), one of the most informative and resourceful protein databases.

2.2. Collection of overlapping genes of "MIRI-DS"

MIRI-related genes of homo-sapiens were collected from online database: Gene Cards is a searchable, integrative database that provides comprehensive information on all annotated and predicted human genes (https://www.genecards.org). The overlapping genes between MIRI-related genes and targets of active compounds were obtained using R software. A venn diagram was drew by R software to show the association between MIRI and active compounds related genes.

2.3. Creating a protein–protein interaction network and screening of hub genes

Importing the overlapping genes of "MIRI-DS" into the STRING online database (www.string-db.org/), a well-known database for protein–protein interaction (PPI) analysis,[24] to get the interactions between proteins. The results were then visualizing

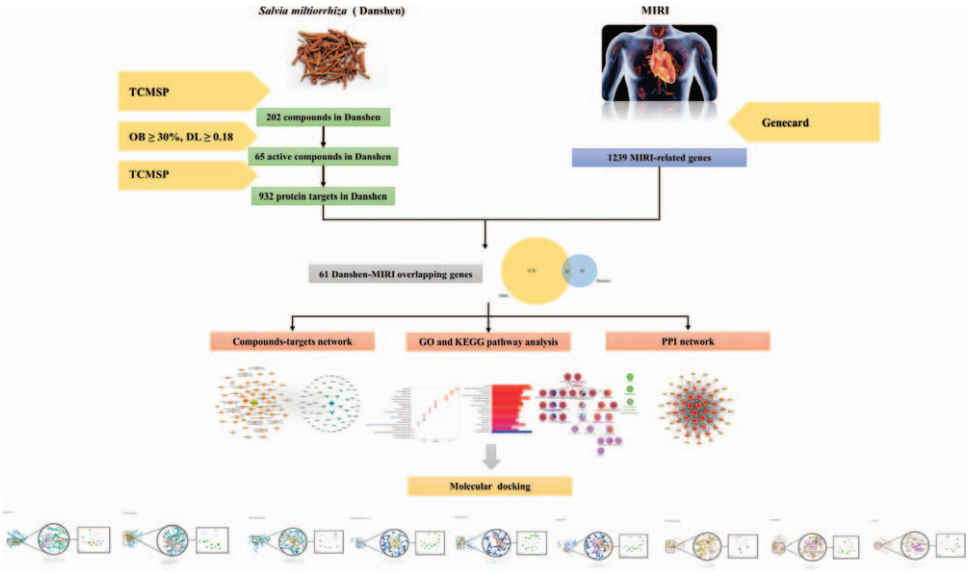

Figure 1. The detailed technical strategy of the present study.

Table 1

Information of active compounds in Danshen with OB ≥30% and DL ≥0.18.

Mol ID	Mol Name	MW	OB (%)	DL (%)
MOL001601	1,2,5,6-Tetrahydrotanshinone	280.34	38.75	0.36
MOL001659	Poriferasterol	412.77	43.83	0.76
MOL001771	Poriferast-5-en-3beta-ol	414.79	36.91	0.75
MOL001942	Isoimperatorin	270.3	45.46	0.23
MOL002222	Sugiol	300.48	36.11	0.28
MOL002651	Dehydrotanshinone II A	292.35	43.76	0.4
MOL002776	Baicalin	446.39	40.12	0.75
MOL000569	Digallate	322.24	61.85	0.26
MOL000006	Luteolin	286.25	36.16	0.25
MOL006824	α-amyrin	426.8	39.51	0.76
MOL007036	5,6-Dihydroxy-7-isopropyl-1,1-dimethyl-2,3-dihydrophenanthren-4-one	298.41	33.77	0.29
MOL007041	2-Isopropyl-8-methylphenanthrene-3,4-dione	264.34	40.86	0.23
MOL007045	3α-Hydroxytanshinone IIa	310.37	44.93	0.44
MOL007048	(E)-3-[2-(3,4-Dihydroxyphenyl)-7-hydroxy-benzofuran-4-yl]acrylic acid	312.29	48.24	0.31
MOL007049	4-Methylenemiltirone	266.36	34.35	0.23
MOL007050	2-(4-Hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	356.4	62.78	0.4
MOL007051	6-o-Syringyl-8-o-acetyl shanzhiside methyl ester	628.64	46.69	0.71
MOL007058	Formyltanshinone	290.28	73.44	0.42
MOL007059	3-Beta-hydroxymethyllenetanshiquinone	294.32	32.16	0.41
MOL007061	Methylenetanshinquinone	278.32	37.07	0.36
MOL007063	Przewalskin a	398.49	37.11	0.65
MOL007064	Przewalskin b	330.46	110.32	0.44
MOL007068	Przewaquinone B	292.3	62.24	0.41
MOL007069	Przewaquinone c	296.34	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	312.34	41.31	0.45
MOL007071	Przewaquinone f	312.34	40.31	0.46
MOL007077	Sclareol	308.56	43.67	0.21
MOL007079	Tanshinaldehyde	308.35	52.47	0.45
MOL007081	Danshenol B	354.48	57.95	0.56
MOL007082	Danshenol A	336.41	56.97	0.52
MOL007085	Salvilenone	292.4	30.38	0.38
MOL007088	Cryptotanshinone	296.39	52.34	0.4
MOL007093	Dan-shexinkum d	336.41	38.88	0.55
MOL007094	Danshenspiroketallactone	282.36	50.43	0.31
MOL007098	Deoxyneocryptotanshinone	298.41	49.4	0.29
MOL007100	Dihydrotanshinlactone	266.31	38.68	0.32
MOL007101	Dihydrotanshinone I	278.32	45.04	0.36
MOL007105	Epidanshenspiroketallactone	284.38	68.27	0.31
MOL007107	C09092	286.5	36.07	0.25
MOL007108	Isocryptotanshi-none	296.39	54.98	0.39
MOL007111	Isotanshinone II	294.37	49.92	0.4
MOL007115	Manool	304.57	45.04	0.2
MOL007118	Microstegiol	298.46	39.61	0.28
MOL007119	Miltionone I	312.39	49.68	0.32
MOL007120	Miltionone II	312.39	71.03	0.44
MOL007121	Miltipolone	300.43	36.56	0.37
MOL007122	Miltirone	282.41	38.76	0.25
MOL007123	Miltirone II	272.32	44.95	0.24
MOL007124	Neocryptotanshinone ii	270.35	39.46	0.23
MOL007125	Neocryptotanshinone	314.41	52.49	0.32
MOL007127	1-Methyl-8,9-dihydro-7H-naphtho[5,6-g]benzofuran-6,10,11-trione	280.29	34.72	0.37
MOL007130	Prolithospermic acid	314.31	64.37	0.31
MOL007132	(2R)-3-(3,4-Dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl)acryloyl]oxy-propionic acid	360.34	109.38	0.35
MOL007140	(Z)-3-[2-[(E)-2-(3,4-Dihydroxyphenyl)vinyl]-3,4-dihydroxy-phenyl]acrylic acid	314.31	88.54	0.26
MOL007141	Salvianolic acid g	340.3	45.56	0.61
MOL007142	Salvianolic acid j	538.49	43.38	0.72
MOL007143	Salvilenone I	270.4	32.43	0.23
MOL007145	Salviolone	268.38	31.72	0.24
MOL007149	NSC 122421	300.48	34.49	0.28
MOL007150	(6S)-6-Hydroxy-1-methyl-6-methylol-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-quinone	312.34	75.39	0.46
MOL007151	Tanshindiol B	312.34	42.67	0.45
MOL007152	Przewaquinone E	312.34	42.85	0.45
MOL007154	Tanshinone iia	294.37	49.89	0.4
MOL007155	(6S)-6-(Hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-dione	310.37	65.26	0.45
MOL007156	Tanshinone VI	296.34	45.64	0.3

DL = drug-likeness, MW = molecular weight, OB = oral bioavailability.

by Cytoscape 3.6.1. Cytohubba was applied to analyze the degree of interactions between proteins and obtained the hub genes based on the degree values.

2.4. Gene ontology and kyoto encyclopedia of genes and genomes analysis

In order to further analyze the biological function process and signal transduction involved in the DS on MIRI, R software was used to annotate the gene ontology (GO) biological function of the "drug-disease" intersection genes. Moreover, ClueGo plug-in in Cytoscape software was used to enrich the kyoto encyclopedia of genes and genomes (KEGG) pathway of the overlapping genes, and the enrichment results were screened with $P < .01$, kappa score = 0.4 as the standard for clustering and visualization analysis.

2.5. Interaction network construction and analysis

To investigate the in-depth molecular mechanism of DS on MIRI, the DS—compound – MIRI—targets (DCMT) network was constructed by Cytoscape software (Version. 3.6.1). In this network, the components and proteins were represented as nodes, whereas the interactions between nodes were represented as edges.

2.6. Molecular docking

To study the association between active compounds and hub genes in the network, molecular docking approach was applied to analyzed the strength and mode of interactions between active compounds screened form networks and hub genes from PPI. The protein crystal structures were obtained from Protein Data Bank database (PDB, http:// www. rcsb.org/). The protein structures were screened based on the criteria as follows: the protein structure must be from *Homo sapiens*; the structure is obtained by X-crystal diffraction; the resolution is <3 Å; priority is given to protein structures that have been previously reported in molecular docking study. The process is simply described as follows: The structures of active compounds were obtained from TCMSP database. Autodock Tools and autodock vina (version: 1.1.2)[25] were applied for molecular docking. Pymol and Discovery Studio were used to analyze the results of docking and presented as final figures.

3. Results

3.1. Identification of bioactive compounds in Danshen

Screening through TCMSP database, a total of 202 components of Danshen were obtained. As shown in Table 1, there were 65 active ingredients that met the criteria of OB ≥30% and DL ≥0.18. The molecular IDs of these 65 active compounds were input into TCMSP to obtain their potential protein targets. A total of 932 protein targets were obtained and after removing duplicate; 108 targets were screened to the further study.

3.2. Acquisition of MIRI related targets and "disease-drug" overlapping targets

Through the GeneCard database, a total of 1239 human related genes were retrieved with the key word "Myocardial ischemia reperfusion injury." The targets of DS were intersected with MIRI related genes, and a total of 61 "disease-drug" intersection genes were obtained (Fig. 2), which were the potential targets of DS acting on MIRI. The detailed contents of the overlapping genes are shown in Table 2.

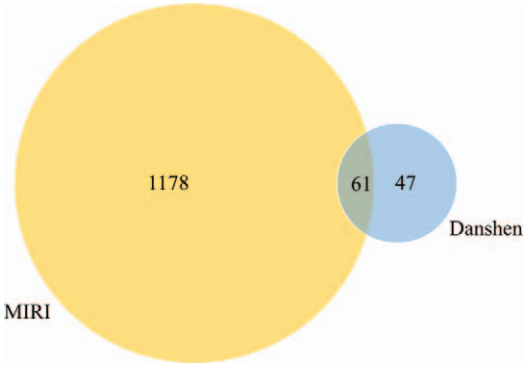

Figure 2. The venn diagram of "Danshen-MIRI" overlapping genes.

3.3. Construction and analysis of protein interaction (PPI) network

The PPI network of 61 overlapping genes was constructed through the String database. Analysis showed that there were a total of 61 nodes and 624 edges in this network (Supplementary figure 1, http://links.lww.com/MD/G514). The PPI network was imported into Cytoscape 3.6.1 software and Cytohubba plug-in was used to analyze the key nodes of the above 61 genes. As shown in Figure 3, AKT1, IL-6, vascular endothelial growth factor A (VEGFA), MAPK1, STAT3, CASP3, PTGS2, FOS, MYC, JUN, MAPK14, and BCL2L1 interacted with the other proteins in the highest frequency, and the interaction between targets was stronger and the degree value was larger, which indicated to play an important role in the PPI network. The results revealed that these targets might be potential targets of active components of DS in the treatment of MIRI, which were worthy of further discussion.

3.4. Construction and analysis of DS-compounds-MIRI-targets network

The obtained active compounds-MIRI-targets and the interactions between them were analyzed by R software, and the results were visualized by Cytoscape 3.6.1 to get the network of "DS - compounds - MIRI - targets" (DCMT) as shown in Figure 4. The network consisted of 118 nodes, including 1 traditional Chinese medicine—Danshen, 55 active compounds, 61 targets and 1 disease—MIRI. The results indicated the multitarget action of active components in DS on MIRI. Through the analysis of the network, the top 3 active components with the highest correlation with the treatment of MIRI were: luteolin, tanshinone IIA, cryptotanshinone.

3.5. GO and KEGG Pathway Enrichment Analysis

In order to further clarify the biological characteristics of the potential targets of Danshen on MIRI, GO, and KEGG pathway enrichment analysis were performed by R/Bio-conductor platform. All of 142 pathways shown in supplementary Table 1,

Table 2

Targets of Danshen action on MIRI.

No.	Target Symbol	Target Name	ID	Degree
1	AKT1	RAC-alpha serine/threonine-protein kinase	207	48
2	IL-6	Interleukin-6	3569	43
3	VEGFA	Vascular endothelial growth factor A	7422	42
4	MAPK1	Mitogen-activated protein kinase 14	1432	40
5	STAT3	Signal transducer and activator of transcription 3	6774	38
6	CASP3	Caspase-3	836	38
7	PTGS2	Prostaglandin G/H synthase 1	5742	38
8	FOS	Proto-oncogene c-Fos	2353	37
9	MYC	Myc proto-oncogene protein	4609	37
10	JUN	Transcription factor AP-1	3725	35
11	MAPK14	Mitogen-activated protein kinase 14	1432	34
12	BCL2L1	Bcl-2-like protein 1	598	33
13	CCND1	G1/S-specific cyclin-D1	595	33
14	IL-10	Interleukin-10	3586	32
15	MMP9	Matrix metalloproteinase-9	4318	32
16	RELA	Transcription factor p65	5970	31
17	PPARG	Peroxisome proliferator activated receptor gamma	5468	31
18	ICAM1	Intercellular adhesion molecule 1	3383	29
19	IL-4	Interleukin-4	3565	28
20	MMP2	72 kDa type IV collagenase	4313	28
21	ESR1	Estrogen receptor	2099	28
22	IL-2	Interleukin-2	3558	27
23	HMOX1	Heme oxygenase 1	3162	27
24	CASP9	Caspase-9	842	27
25	NR3C1	Glucocorticoid receptor	2908	26
26	IFNG	Interferon gamma	3458	25
27	APP	Amyloid beta A4 protein	351	25
28	NFKBIA	NF-kappa-B inhibitor alpha	4792	25
29	CDKN1A	Cyclin-dependent kinase inhibitor 1	1026	25
30	MDM2	E3 ubiquitin-protein ligase Mdm2	4193	24
31	NOS2	Nitric oxide synthase, inducible	4843	22
32	MMP1	Interstitial collagenase	4312	21
33	CD40LG	CD40 ligand	959	20
34	GSK3B	Glycogen synthase kinase-3 beta	2932	19
35	OPRM1	Mu-type opioid receptor	4988	15
36	CASP7	Caspase-7	840	14
37	ADRB2	Beta-2 adrenergic receptor	154	14
38	BIRC5	Baculoviral IAP repeat-containing protein 5	332	12
39	ACHE	Acetylcholinesterase	43	12
40	OPRD1	Delta-type opioid receptor	4985	11
41	BCL2	Apoptosis regulator Bcl-2	596	10
42	PTGS1	Prostaglandin G/H synthase 1	5742	10
43	GSTP1	Glutathione S-transferase P	2950	9
44	SLC6A4	Sodium-dependent serotonin transporter	6532	9
45	HTR3A	5-hydroxytryptamine receptor 3A	3359	9
46	ITGB3	Integrin beta-3	3690	8
47	EDNRA	Endothelin-1 receptor	1909	7
48	PTGES	Prostaglandin E synthase	9536	7
49	AKR1B1	Aldose reductase	231	7
50	TYR	Tyrosinase	7299	6
51	SLC6A3	Sodium-dependent dopamine transporter	6531	6
52	INSR	Insulin receptor	3643	5
53	PCNA	Proliferating cell nuclear antigen	5111	5
54	CHRM2	Muscarinic acetylcholine receptor M2	1129	5
55	FASN	Fatty acid synthase	2194	4
56	ECE1	Endothelin-converting enzyme 1	1889	4
57	SLC6A2	Sodium-dependent noradrenaline transporter	6530	3
58	NR3C2	Nuclear Receptor Subfamily 3 Group C Member 2	4306	3
59	KCNH2	Potassium voltage-gated channel subfamily H member 2	3757	2
60	CHRM3	Muscarinic acetylcholine receptor M3	1131	2
61	SCN5A	Sodium channel protein type 5 subunit alpha	6331	1

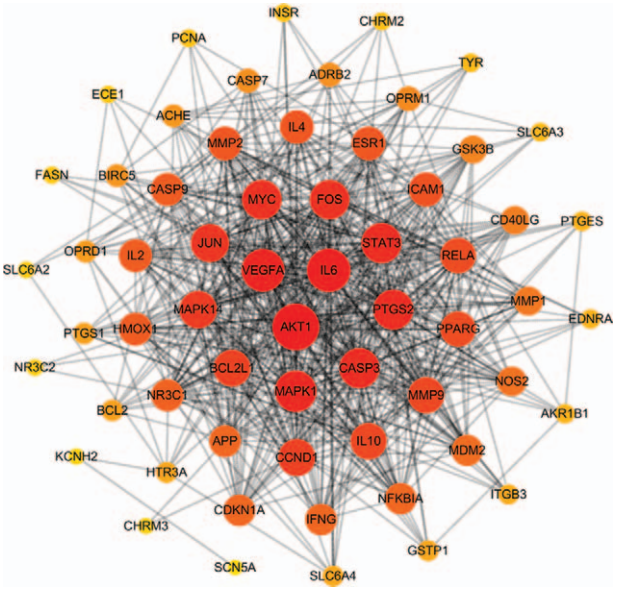

Figure 3. Protein–protein interaction (PPI) network.

http://links.lww.com/MD/G515 were enriched. The top 20 (Count ≥2 and $P < .05$) significantly enriched terms in functions of these targets were shown in Figure 5A and B, which indicated that DS may regulate the development of MIRI via amide binding, cytokine receptor binding and peptide binding to exert its therapeutic effect on MIRI. To explore the underlying involved pathways of DS on MIRI, KEGG analysis was conducted. As results, the top 20 significantly enriched pathways of DS on MIRI were shown in Figure 6A and B. Among these pathways enriched, PI3K-Akt signaling pathway, interleukin (IL)-17 signaling pathway, AGE-RAGE signaling pathway in diabetic complications, T cell receptor signaling pathway, tumor necrosis factor signaling pathway, and HIF-1 signaling pathway were mostly associated with MIRI, which have been reported in number of studies. Furthermore, a cluster analysis by using ClueGo indicated that pathways enriched from KEGG were mainly classified in IL-17 signaling pathway, apoptosis, and fluid shear stress atherosclerosis (Fig. 7).

3.6. Molecular docking between active compounds and potential targets

Because 61 potential targets were obtained, the top 3 targets (AKT1, IL6, and VEGFA), which had higher degree, were selected for molecular docking with top 3 active components screened from "DS-MIRI" network. The ranking of the affinity of the 3 molecule components was achieved, as shown in Table 3. The results revealed that TSA and CPT have the higher affinity to VEGFA. The details of docking were performed in Figure 8A–I. I observe that the molecule could bind with the protein by H-bong or π-π accumulation, respectively. For instance, the analysis of TSA indicated that TSA bound to the active pocket of VEGFA and interacted with the H-bond of the amino group on CYS-54, ASP-56, and ASN-55. Besides, it has Pi-Alkyl with amino group of ILE-39 and PHE-29 (Fig. 8I).

4. Discussion

MIRI belongs to the category of coronary atherosclerotic heart disease (CAHD). When the blood supply from the coronary artery is contradictory to the blood demand of the myocardium, the coronary blood flow is insufficient to supply the myocardial tissue to complete the normal metabolic function, which will lead to the occurrence of myocardial ischemia and hypoxia.[26] Sudden short-term ischemia and hypoxia can induce angina pectoris, whereas long-term severe ischemia and hypoxia can lead to critical cardiovascular diseases such as acute myocardial

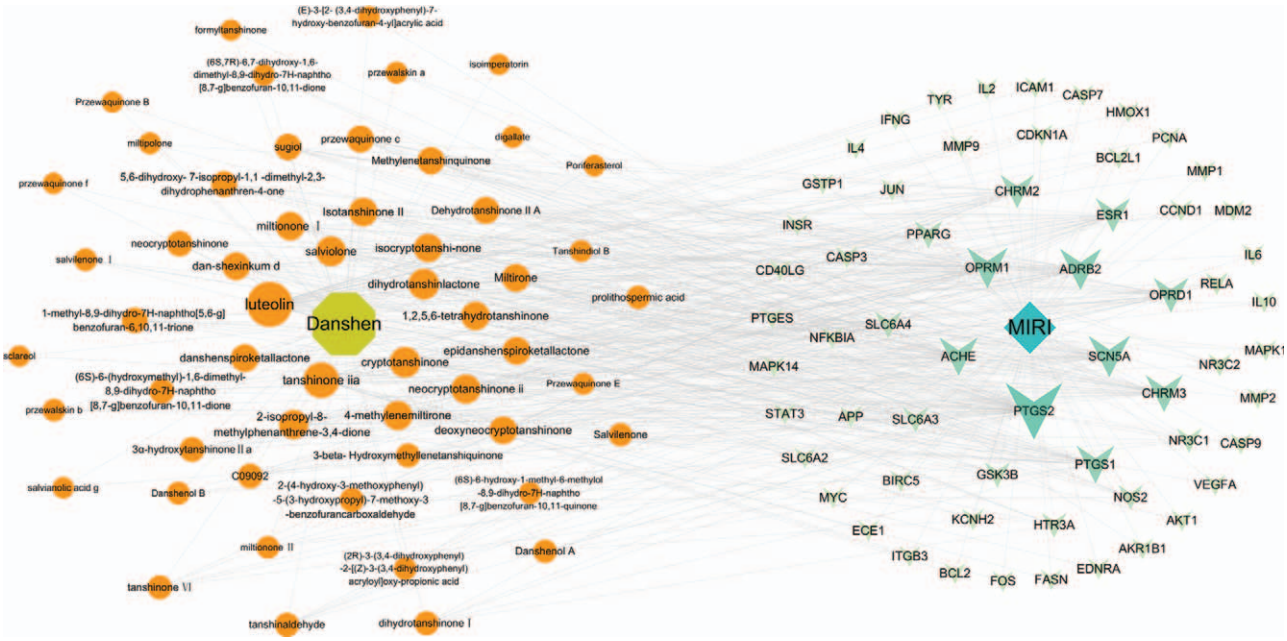

Figure 4. "DS-compounds-MIRI-targets" (DCMT) network.

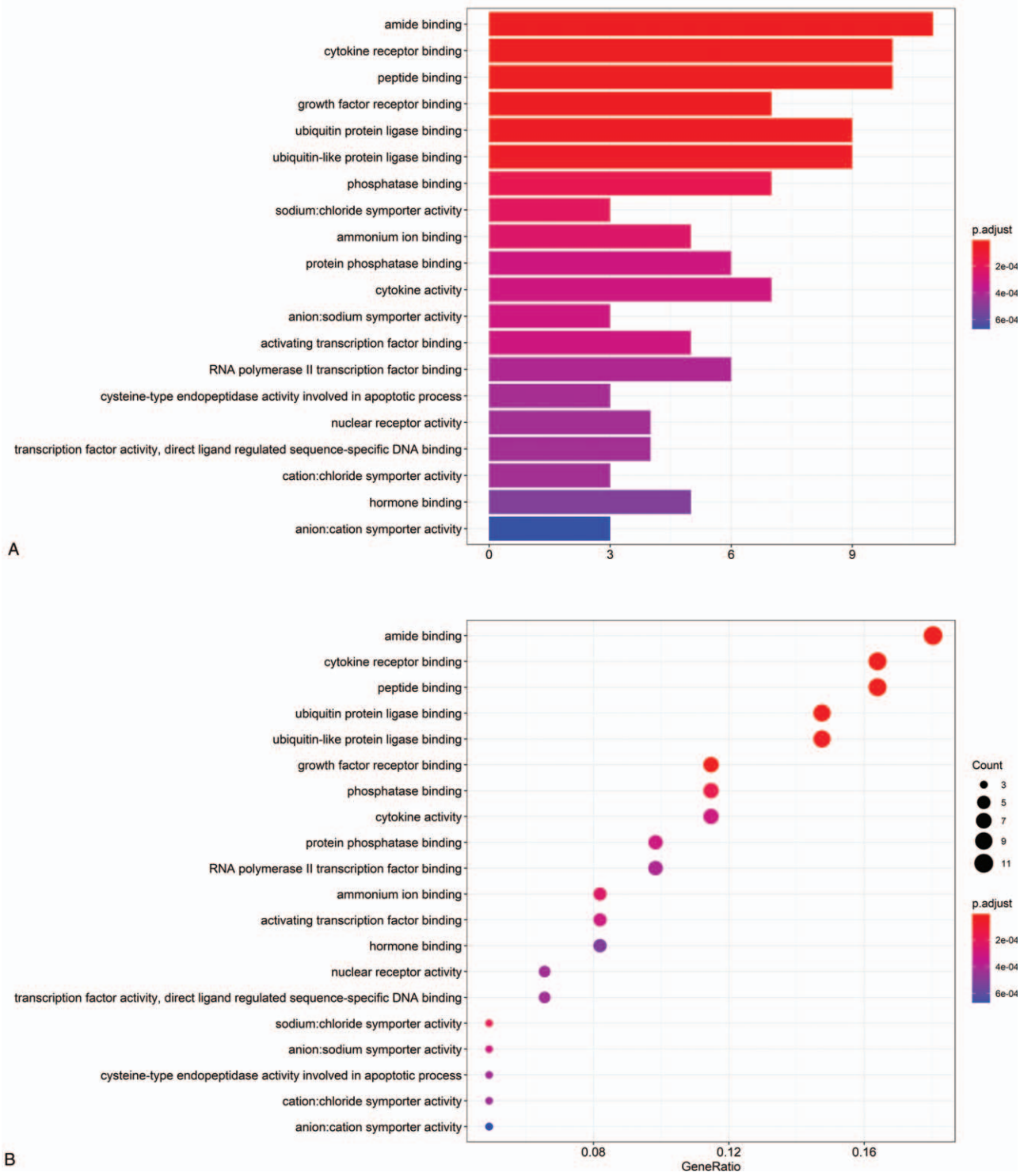

Figure 5. Gene Ontology (GO) enrichment analysis. (A) Top 20 enriched GO terms selected from 6 common targets. (*P*-adjust value <.01) (B) A bubble plot to describe *P*-adjust value range of to 20 enriched results.

infarction.[27] In recent years, the incidence of CAHD has been increasing, following with high mortality. Interventional therapy is one of the main methods for the treatment of CAHD, but MIRI is the main complication after thrombolysis and coronary interventional therapy in clinical patients with CHD.[7,28] The occurrence of MIRI usually results in poor prognosis and even endangers the life of the patients. Therefore, it is crucial to find safe and effective methods to prevent the occurrence of MIRI.

Traditional Chinese herbal medicine Danshen (DS) is the dried root of *Salvia miltiorrhiza* Bunge, a kind of labiaceae plants. It is a famous herbal medicine in China. Due to the diversity and effectiveness of its pharmacological activities, DS has become one

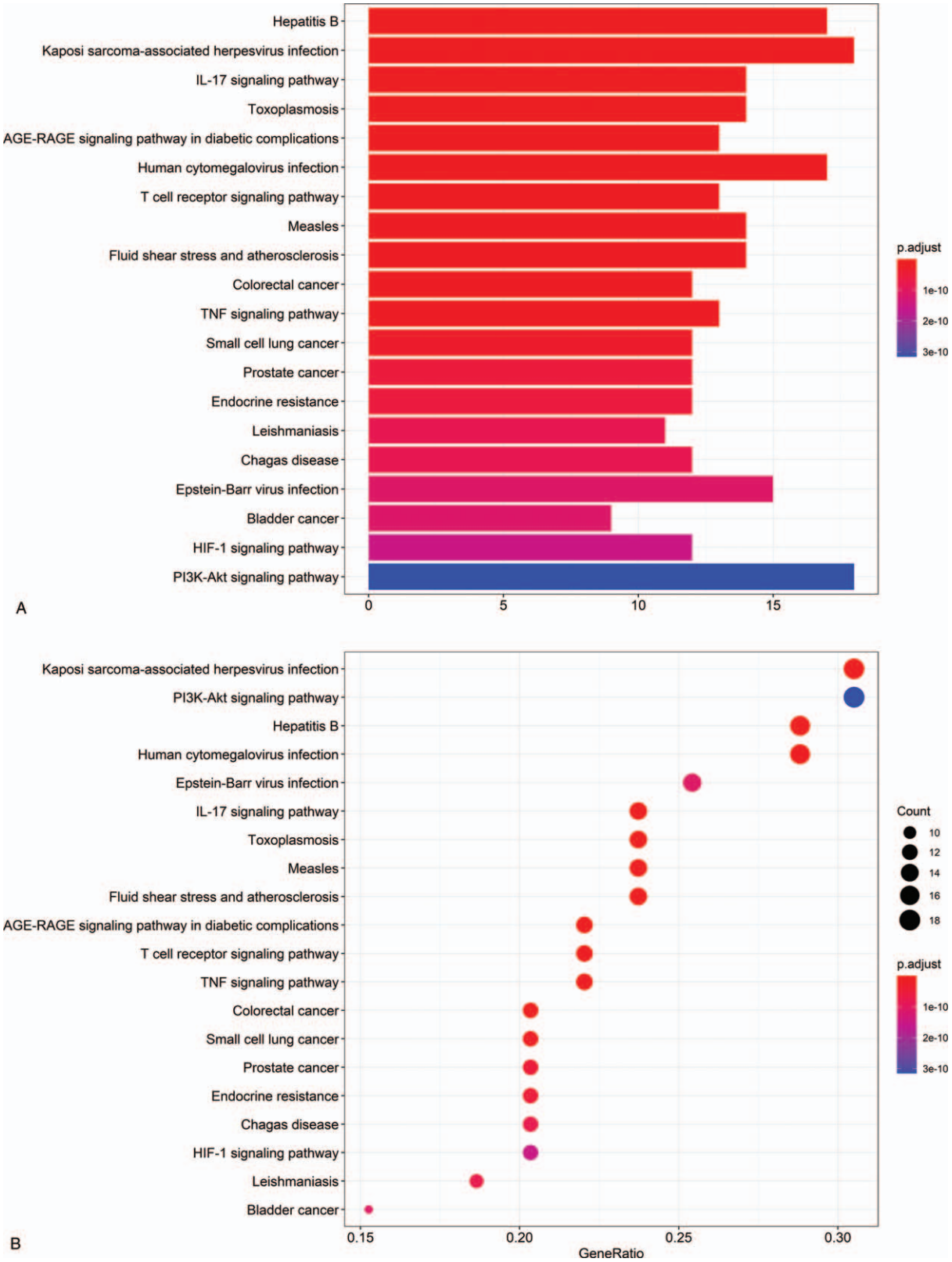

Figure 6. KEGG pathway enrichment analysis. (A) Top 20 pathways from KEGG (*P*-adjust value <.01). (B) A bubble plot to describe *p*-adjust value range of to 20 pathways.

Figure 7. Clustering analysis of signal pathways. The pathways were presented by circles. The main clusters are shown in bold labels.

of the visiting cards of Chinese medicines in contemporary China.[9,11] At present, DS is mainly used in the prevention and treatment of metabolic disorders such as cardiovascular and cerebrovascular diseases and hyperlipidemia, and plays important roles in the prevention and adjuvant treatment of MIRI.[29,30] With the deepening research of its pharmacology and molecular biology, researchers have found that the mechanism of DS's clinical therapeutic effect is varied. To clarify the multi-targets effects of herbal medicines, network pharmacology provides a new method for the study of explaining the pharmacological mechanisms of traditional Chinese herbal medicine.

Based on the network pharmacology, the present study systematically elaborated the pharmacological mechanism of DS in the treatment of MIRI. At first, through the analysis of the DCMT network, it was suggested that the main components of DS in the treatment of MIRI might be tanshinone IIA (TSA),

Table 3

Affinity of 3 active compounds of Danshen.

Affinity, kcal/mol	AKT1 (1UNQ)	IL6 (1ALU)	VEGFA (3QTK)
Luteolin	−6.3	−6.6	−8.1
Cryptotanshinone	−7.7	−6.6	−9.6
Tanshinone IIA	−7.3	−6.3	−9.8

cryptotanshinone (CPT) and luteolin. Tanshinone IIA, a kind of fat-soluble diterpenoids extracted from Danshen, has been widely applied in adjunctively treating cardiovascular diseases in China for a long time.[9,31] Accumulating evidence have shown the protective effects of TSA in ischemic myocardium. For instance, study of Yuan et al indicated that TSA protects the MIRI through PI3K/Akt-eNOS signaling pathway and blocking the expression of mitochondrial permeability transition pore in rats.[32] Moreover, Hu et al demonstrated that TSA could prevent myocardial tissue from ischemia reperfusion injury by inhibiting the expression of high mobility group box-B1 (HMGB1) and inducing the inflammatory reaction.[33] By whatever mechanism of action, TSA plays crucial role in the improvement of the outcomes of MIRI. As a natural quinone component, the medicinal value of CPT has received increasing attentions. In previous study, Jin et al detected the cardiovascular protective effects of CPT in vivo experiments. The results revealed that CPT reduced myocardial infarct size and improved cardiac function after ischemia and reperfusion injury. Importantly, their study suggested that CPT could protect myocardial injury from ischemia and reperfusion by inhibiting the NF-κB activation, cytokine production and adhesion molecules.[34] The disruption of calcium homeostasis is one of the important factors leading to myocardial injury. Calcium overload can occur in cardiomyocytes as a result of myocardial infarction or other stress, such as hypoxia and reoxygenation, leading to myocardial systolic dysfunction. Study of Liu et al have shown that CPT can suppress calcium overload in vitro cell experiments, and in vivo animal experiments have further verified that CPT plays a protective role in myocardium through antioxidant and anti-inflammatory

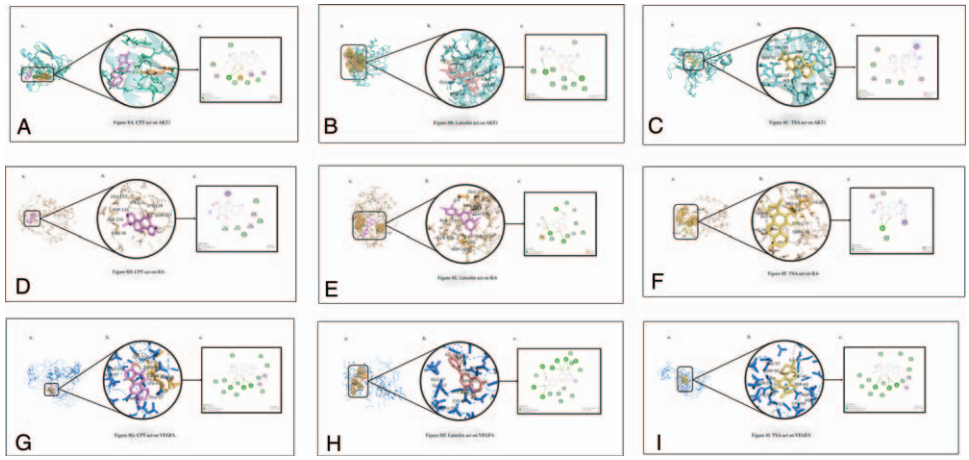

Figure 8. Molecular docking between active compounds and main potential targets. (A) CPT act on AKT1; (B) Luteolin act on AKT1; (C) TSA act on AKT1; (D) CPT act on IL6; (E) Luteolin act on IL6; (F) TSA act on IL6; (G) CPT act on VEGFA; (H) Luteolin act on VEGFA; (I) TSA act on VEGFA. In every figure of molecular docking: a. holistic view of docking model; b. enlarged view of the docking section; c. 2D diagram of docking results. CPT = cryptotanshinone, TSA = tanshinone IIA, VEGFA = vascular endothelial growth factor A.

activities.[35] Luteolin is a flavonoid with molecular formula of $C_{15}H_{10}O_6$ and relative molecular weight of 286.23. Luteolin is found in many medicinal plants, which has reported to play important roles in myocardial protection. Kim et al studied the protective effect of flavonoids on H92C cardiomyocytes in the simulation of MIRI, and the experimental results showed that luteolin could significantly increase the expression of anti-apoptotic protein Bcl-2 and inhibit the expression of pro-apoptotic protein Bax.[36] In addition, luteolin can reduce the frequency and duration of ventricular tachycardia and ventricular fibrillation during ischemia and reperfusion in rat model of MIRI, and significantly reduce the size of myocardial infarction.[37]

Protein interactions play important roles in important biological processes such as cell cycle control, metabolism and signaling pathways, and disease pathways. Therefore, the systematic mapping of protein interactions is essential for the interpretation of protein function and the understanding of complex cellular processes.[38,39] In the present study, after analysis of PPI network, the top 3 proteins calculated by degrees were obtained. Respectively, they are AKT1 (RAC-alpha serine/threonine-protein kinase), IL-6, and VEGFA. AKT1 can mediate PI3K-dependent cell adhesion, invasion, metastasis, and regulate cell apoptosis. AKT cascading activation is central to a variety of conditioning strategies and plays a related cardioprotective role in the reperfusion injury saving kinase pathway, which is blocked by inhibitors of upstream kinase PI3K.[40,41] Inflammatory is closely related to the pathogenesis of MIRI. IL-6 is one of the common inflammatory cytokines. Studies have shown that IL-6 can cause neutrophils to adhere to vascular endothelial cells, and thus induce neutrophils to exude and infiltrate, and make cardiomyocytes damaged. Suppressing the production of inflammatory cytokines such as IL-6 could protect against the MIRI.[42,43] If the role of paracrine mechanisms is taken as a starting point, the administration of growth factors to promote tissue revascularization represents an attractive option that has been explored in animal models of limb ischemia or myocardial infarction. Proangiogenic cytokines such as VEGF.[44] VEGFA is a member of the VEGF growth factor family. The VEGF family play an important role in the regulation of angiogenesis networks. VEGF directly affects vascular endothelial cells by promoting the proliferation and migration of vascular endothelial cells.[45] Ai et al indicated that upregulation of VEGFA could accelerate cardiac angiogenesis and improves cardia function.[46] Similarly, Guo et al also demonstrated that myocardial protective effect could be exerted via upregulating the VEGFA.[47]

The results of enrichment analysis of GO and KEGG pathways showed that the significant enrichment pathways were mainly concentrated in PI3K/ Akt signaling pathway, hepatitis B, cancer related pathway, tumor necrosis factor signaling pathway, IL-17 signaling pathway, fluid shear stress and atherosclerosis, virus infection and HIF-1 signaling pathway, among others. Among them, PI3K/Akt signaling pathway can regulate the activation of inflammatory cells, release of inflammatory mediators, and play a regulatory role in chronic inflammation.[48] HIF-1 is a hypoxia-inducible factor that activates genes encoding proteins involved in hypoxic homeostasis response and induces the expression of proteins controlling glucose metabolism, cell proliferation, and angiogenesis, playing a key role in ischemic and hypoxic myocardium.[49] Cluster analysis by using ClueGO showed that the pathways mainly classified in IL-17 signaling pathway, apoptosis, fluid shear stress, and atherosclerosis. IL-17 is an inflammation-related cytokine. Studies have shown that the activation of IL17 and its mediated signaling pathway leads to end-stage myocardial cell necrosis and loss of apoptosis.[50–52] Therefore, inhibiting the apoptosis of myocardial cells after ischemia reperfusion can effectively protect the myocardium from the injury. Endothelial cells transduce the frictional force from blood flow (fluid shear stress) into biochemical signals that regulate gene expression and cell behavior via specialized mechanisms and pathways. These pathways shape the vascular system during development and during postnatal and adult life to optimize flow to tissues.[53,54] This fluid shear stress is closely related to the occurrence and development of atherosclerosis.[55] A study in vivo revealed this association between alignment and atherosclerosis, which ECs fail to align in flow; under the condition of hypercholesterolemic, these syndecan4$^{-/-}$ mice show increased atherosclerosis, including lesions in regions of laminar flow that are normally protected.[56] Furthermore, the results of molecular docking suggested that the vina scores of TSA, CPT, and VEGFA were -9, 8 and -9.6 kcal/mol, respectively. It is generally believed that when the vina score is >-7 kcal/mol, there is a strong binding force between molecules. As active components contenting more in Danshen, the role of TSA and CPT in the treatment of MIRI deserves further study.

5. Conclusion

To be summarized, the present study suggests that luteolin, TSA, CPT might be the crucial active compounds in DS on MIRI. AKT1, IL6, VEGFA, MAPK1, STAT3, CASP3, PTGS2, FOS, MYC, JUN, MAPK14, and BCL2L1 might be the potential targets. Moreover, the effect of DS on MIRI might be achieved by regulating the IL-17 signaling pathway, PI3K/Akt signaling pathway or HIF-1 signaling pathway. Molecular docking indicated that the better affinity between TSA and potential target VEGFA, which was worthy to be further researched.

The present study based on network pharmacology and molecular docking simulation could provide a systematic analysis for the research of DS on MIRI. But nevertheless, it is noted that there were limitations in the present study. First, focusing on proven target genes may preclude potential targets that have not been experimentally validated. Secondly, the verification analyses which lack of in the present study will be completed in the further study to elucidate the molecular mechanism in the treatment of Danshen on MIRI.

Author contributions

Zhiyan Jiang contributed to the study conception and design. Zhiyan Jiang collected the data and performed the data analysis. Zhiyan Jiang contributed to the interpretation of the data and the completion of figures and tables. Zhiyan Jiang contributed to the drafting of the article and final approval of the submitted version.
Conceptualization: Zhiyan Jiang.
Data curation: Zhiyan Jiang.
Formal analysis: Zhiyan Jiang.

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