

Network Pharmacology and Molecular Docking Analysis of Shanhaidan granules in treatment of coronary heart disease angina pectoris

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Research

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Abstract

Objective

To study the pharmacological mechanism of Shanhaidan granules for treatment of coronary heart disease angina pectoris(CHD-AP).

Methods

The active ingredients and their target genes of Shanhaidan granules were collected from TCMSP and TCMID. CHD-AP-related target genes were collected from GeneCards, OMIM and DurgBank database. The target PPI network was constructed by STRING. PPI network was used for topological analysis and core target screening. The hub target proteins and top core ingredients of Shanhaidan Granule on CHD-AP was analysed for the binding validation experiment by Molecular Docking.

Results

198 active components and 320 potential targets were obtained from Shanhaidan granule, 1208 related targets and 158 joints of CHD-AP were screened. PPI network analysis showed that AKT1, IL-6, VEGF, TNF, MAPK, Caspase-3, MMP, and EGFR were the core and key teagets in treatment of CHD-AP with Shanhaidan granule. KEGG-GO analysis indicated that Shanhaidan granules treated CHD-AP by regulating the TNF signaling pathway, IL-17 signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway and apoptosis-related signalling pathway. The results of the molecular docking showed that the core compounds and target proteins had strong binding activity.

Conclusion

The results predicted and verified mechanism of Shanhaidan granule gainst CHD-AP from a holistic perspective, which provided theoretical support for rational clinical application of CHD-AP.

1. Introduction

Coronary heart disease angina pectoris (CHD-AP) is a common clinical symptom in patients with coronary heart disease which characterized by insufficient blood supply to coronary arteries, sudden myocardial ischemia, and hypoxia, with episodic chest pain or chest discomfort [1]. According to the 2017 global research reports, coronary heart disease (CHD) is the main cause of disability and death globally, and the CHD-related morbidity and mortality remain increasing[2]. In the clinical treatment, western medicine for CHD-AP like β -receptor blocker, calcium channel blocker, angiotensin-converting enzyme inhibitor, angiotensin II receptor antagonist all have a good effect, but the demand for CHD-AP symptom control is still unsatisfied[3]. Traditional Chinese medicine (TCM) possesses significant

advantages in the treatment of CHD-AP, and the core treatment for CHD-AP is “syndrome differentiation” serves in TCM clinical practice[4]. CHA-AP belongs to “chest pain”, “heartache” (“Xiongbi”) majorly caused by “blood stasis”, “phlegm retention,” and “the deficiency in both Yang and Qi”[5]. The method of activating blood circulation and resolving stasis is commonly used for the treatment of CHD-AP in the TCM theory[6].

Many researches showed that Chinese patent medicine has the advantages of good effect and few adverse reactions in the treatment of CHD-AP [5]. Shanhaidan granule has the effect of activating blood circulation and resolving stasis, which used for “Xiongbi” and syndrome of heart vessel blocking. Shanhaidan granule containing 16 Chinese medicines including *Salvia miltiorrhiza* Bge., *Panax notoginseng*, *Carthamus tinctorius*, the blood of goats, *Panax ginseng* C. A. Meyer, *Astragalus propinquus* Schischkin, *Ganoderma lucidum*, *Fallopia multiflora*, *Ligusticum chuanxiong* Hort., *Cyperus rotundus* L., *Citrus medica sarcodactylis*, *Typha angustifolia* L., *Sargassum*, *Ophiopogon japonicus*, *Cassiae Semen*, *Radix Puerariae*. These components are effective in treating CHD-AP[7]. *Salvia miltiorrhiza* Bge., *Panax notoginseng*, *Carthamus tinctorius*, and the blood of goats have effect on promoting blood circulation by removing blood stasis and obstruction in collaterals, as well as relieving pain. *Panax ginseng* C. A. Meyer, *Astragalus propinquus* Schischkin, *Ganoderma lucidum*, and *Fallopia multiflora* have the effect of replenishing and nourishing qi, blood, and yin; *Ligusticum chuanxiong* Hort., *Cyperus rotundus* L., *Citrus medica sarcodactylis*, *Typha angustifolia* L., *Sargassum*, *Ophiopogon japonicus*, *Cassiae Semen*, and *Radix Puerariae* have the effect of nourishing blood and yin, promoting Qi, restoring pulse, and clearing heat [8].

Network pharmacology is a network perspective sharing much with the basic disciplines of TCM, which analyzed the relationship of biological systems, drugs, and diseases by systemic manner [9]. It could be applied to explore the pharmacological action, mechanism, and development of TCM [10]. This paper aimed to explore the potential mechanism of Shanhaidan granules on CHD-AP based on Network Pharmacology. The study design and workflow were presented in Fig. 1.

2. Materials And Methods

2.1 Ingredients collection of Shanhaidan Granule

The ingredients of all Chinese medicine in Shanhaidan Granule (Zhejiang Strong Pharmaceutical Co., Ltd., Z20153023) herbal medicines were collected from traditional Chinese medicine systems pharmacology (TCMSP) and traditional Chinese medicine information database (TCMID). According to the integrative ADME model, the bioavailability (OB) and drug-likeness (DL) permeability were applied to prescreen the available compounds. The active ingredients were adopted as the criteria of $OB \geq 30\%$ and $DL \geq 0.18$.

2.2 Predicted targets of Shanhaidan Granule

The potential targets of active compounds were obtained from TCMSP and STITCH databases. All the target gene names were standardized by UniProt knowledgebase and only those of Homo sapiens were reserved for further analysis.

2.3 Construction of Chinese medicine-target Network

The Chinese medicine-target network and ingredient-target network by Cytoscape (3.6.0), which explored the relationship among the drugs of Shanhaidan Granule, active ingredients, and corresponding targets.

2.3 Collection of related genes associated with CHD-AP

Genes related to CHD-AP were obtained from the databases of GeneCard Database, OMIM Database and DurgBank by searching with the terms “Coronary heart disease angina pectoris” and “Homo sapiens”.

The CHD-AP-target network was constructed by Cytoscape (3.6.0).

2.5 Protein-protein interaction (PPI) network and clustering analysis

The gene targets of Shanhaidan granule and CHD-AP were analyzed using the STRING database and “Homo sapiens” was limited. Two PPI network were obtained and merged by Cytoscape (3.6.0), which got the targets of Shanhaidan granule against CHD-AP. The network of Shanhaidan granule against CHD-AP was analyzed by Cytoscape (3.6.0). The 20 hub genes with plugcluster Cytohubba by maximal clique centrality (MCC) method were screened.

2.6 Core targets enrichment analysis

GO annotation and KEGG pathway enrichment analysis of Shanhaidan Granule against CHD-AP were carried out through the Matescape online database, which got the potential targets, signal pathways, related biological processes, cell components and molecular functions of the effective components in treatment of CHD-AP. The results of GO enrichment analysis and the bubble diagram of KEGG enrichment pathways were drawn by Bioinformatics online tools.

2.7 Molecular Docking Verification

The 3D structures of hub target proteins were downloaded from the database of RCSB Protein Data Bank. The 3D structures of top 10 ingredients of Shanhaidan Granule were downloaded from the database of TCMSP. AutoDock software was used to modify the structure including removing H₂O, adding hydrogen atoms, and calculating charge and atom type. Finally, AutoDock vina software was used for molecular docking.

3. Results

3.1 Composite ingredients of Shanhaidan Granule

A total of 293 ingredients in Shanhaidan Granule were retrieved from in TCMSP and TCMID, including 64 ingredients in *Salvia miltiorrhiza* Bge., 8 ingredients in *Panax notoginseng*, 22 ingredients in *Carthamus tinctorius*, 37 ingredients in *Panax ginseng* C. A. Meyer, 20 ingredients in *Astragalus propinquus* Schischkin, 61 ingredients in *Ganoderma lucidum*, 16 ingredients in *Fallopia multiflora*, 8 ingredients in *Ligusticum chuanxiong* Hort., 18 ingredients in *Cyperus rotundus* L., 6 ingredients in *Citrus medica sarcodactylis*, 8 ingredients in *Typha angustifolia* L., 4 ingredients in *Sargassum*, 3 ingredients in *Ophiopogon japonicus*, 14 ingredients in *Cassiae Semen*, 4 ingredients in *Radix Puerariae*. A total of 198 ingredients were identified after removing duplication.

3.2 Putative targets for Shanhaidan Granule

In this study, putative target genes of active ingredients in Shanhaidan Granule obtained from TCMSP database. The target genes of 198 ingredients were collected by TCMSP database and Uniport database. 1474 genes were identified, in which 1153 genes of the 15 Chinese medicine overlapped, and a total of 320 genes were identified after removing duplication.

3.3 Shanhaidan Granule ingredient-target network analysis

The Chinese medicine-target network contained 336 nodes and 1659 edges(Fig. 2A). The ingredient-target network of Shanhaidan granule contained 519 nodes and 2185 edges(Fig. 2B). A median of 198 candidate compounds was 5 degrees which indicating that most compounds of Shanhaidan granule were affected by multiple target genes. The top 10 effective ingredient according were Quercetin, Kaempferol, Luteolin, Isorhamnetin, 7-O-methylisomucronulatol, Formononetin, Tanshinone iia, Beta-sitosterol, Arachidonic acid and Baicalein. Hence, they might be the crucial effective compounds of Shanhaidan granule according the network(Table 1).

Table 1
Top 10 ingredients of Shanhaidan Granule

Name	Degree	OB (%)	DL
Quercetin	169	46.43	0.28
Kaempferol	68	41.88	0.24
Luteolin	64	36.16	0.25
Isorhamnetin	44	49.6	0.31
7-O-methylisomucronulatol	41	74.69	0.3
Formononetin	40	69.67	0.21
Tanshinone iia	39	49.89	0.4
Beta-sitosterol	38	36.91	0.75
Arachidonic acid	37	45.57	0.2
Baicalein	35	33.52	0.21

3.4 Known therapeutic targets acting on CHD-AP

The therapeutic targets for CHD-AP were collected from the databases of GeneCard Database, OMIM Database and DurgBank. After eliminating the redundancy, a total of 1208 known therapeutic targets in the treatment of CHD-AP were collected in this study. The CHD-AP-target network (Fig. 3) was constructed by Cytoscape (3.6.0).

3.5 PPI network

158 genes were eventually identified for Shanhaidan Granule against CHD-AP by PPI network analysis. The PPI network of Shanhaidan Granule, CHD-AP, and Shanhaidan Granule against CHD-AP related target genes was separately constructed in this study. The network of Shanhaidan Granule putative target genes contained 311 nodes and 5946 edges (Fig. 4A), the network of CHD-AP related target genes contained 1121 nodes and 43958 edges (Fig. 4B), and the network of Shanhaidan Granule against CHD-AP contained 158 nodes and 3511 edges (Fig. 4C). The top 20 targets of core network were showed in Table 2 and Fig. 5.

Table 2
Top 20 targets of core network

Gene	Protein	Degree	Betweenness Centrality
AKT1	RAC-alpha serine/threonine-protein kinase	122	0.05829036
IL-6	Interleukin-6	117	0.04206843
VEGFA	Vascular endothelial growth factor A	112	0.02932718
TNF	Tumor necrosis factor	108	0.02447763
TP53	Cellular tumor antigen p53	102	0.01818035
EGF	Pro-epidermal growth factor	97	0.03316086
MAPK1	Mitogen-activated protein kinase 1	96	0.02025876
CASP3	Caspase-3	96	0.01052758
CXCL8	Interleukin-8	95	0.01953467
PTGS2	Prostaglandin G/H synthase 2	94	0.0128017
MMP9	Matrix metalloproteinase-9	94	0.00962495
JUN	Transcription factor AP-1	93	0.00835769
MAPK8	Mitogen-activated protein kinase 8	93	0.00944918
EGFR	Epidermal growth factor receptor	92	0.01314838
SRC	Proto-oncogene tyrosine-protein kinase Src	90	0.01396553
STAT3	Signal transducer and activator of transcription 3	90	0.00756421
CCL2	C-C motif chemokine 2	88	0.0145556
MYC	Myc proto-oncogene protein	86	0.00705256
MMP2	72 kDa type IV collagenase	83	0.00737449
IL1B	Interleukin-1 beta	83	0.00826749

3.6 Core targets enrichment analysis

GO and KEGG pathway of the 158 candidate genes were analyzed by Matescape online database. GO of the 158 candidate genes was analyzed based on Biological process (BP), Cellular component (CC), Molecular function (MF). 2695 GO terms were significantly enriched, 2465 in BP, 89 in CC, 141 in MF ($P < 0.05$). Top 20 terms were shown in Fig. 6.

These GO terms data showed that Biological process, Cellular component and Molecular function in Shanhaidan Granule against CHD-AP. Biological process of Shanhaidan Granule against CHD-AP

contained response to oxidative stress, positive regulation of cell motility, apoptotic signalling pathway, response to lipopolysaccharide, cellular response to oxidative stress, reactive oxygen species metabolic process, blood vessel development, response to reactive oxygen species, blood circulation and blood vessel morphogenesis, et al. Cellular component of Shanhaidan granule against CHD-AP contained membrane raft, membrane microdomain, perinuclear region of cytoplasm, plasma membrane raft, cytoplasmic vesicle lumen, plasma membrane protein complex, platelet alpha granule, endoplasmic reticulum lumen, lytic vacuole and lysosome, et al. Molecular function of Shanhaidan granule against CHD-AP were contained kinase binding, protein kinase binding, protein domain specific binding, cytokine receptor binding, transcription factor binding, ubiquitin-like protein ligase binding, cytokine activity, ubiquitin protein ligase binding, receptor regulator activity and receptor ligand activity, et al.

3.7 Gene-pathway network analysis

335 KEGG pathways were significantly enriched ($P < 0.05$). Top 20 pathways were shown in Fig. 7, in which color represented P value and the size of spot represented count of genes. These results showed that the core pathway of Shanhaidan granule for CHD-AP included Fluid shear stress and atherosclerosis, AGE-RAGE signalling pathway, TNF signalling pathway, c-type lectin receptor signalling pathway, IL-17 signalling pathway, PI3K-Akt signalling pathway, endocrine resistance, apoptosis and MAPK signalling pathway, et al. Construction of the top 20 target-pathway network was used to analyze the key target genes of Shanhaidan granule against CHD-AP (Fig. 8). The network showed that the core target genes had larger degree such as AKT1, IL-6, VEGF, TNF, MAPK, Caspase-3, MMP, and EGFR. They might be the key target genes using Shanhaidan granule in the process of treating CHD-AP. All of the above analysis could reveal a new strategy for drug development on CHD-AP.

3.8 Molecular Docking

The hub target proteins and top core ingredients of Shanhaidan Granule on CHD-AP was analysed for the binding validation experiment by Molecular Docking. Generally, the lower the binding free energy, the more stable the binding between the ligand and protein receptor. In recently studies, the ingredient-target interaction with binding energy less than -5 kcal/mol would deem the effective docking [11, 12]. The result of binding energy showed in Fig. 9. According to the results, these targets have a good docking effect with the bioactive ingredients, which indirectly certified the results of network pharmacology.

4. Discussion

In this study, 158 targets of Shanhaidan granule on CHD-AP and 355 pathways were identified by network pharmacology. The results showed that Shanhaidan granule act a multi-target and multi-pathway on CHD-AP. The most ingredients of Shanhaidan granule were affected by multiple target genes, such as Quercetin, Kaempferol and Luteolin acted on 169, 68 and 64 target genes, respectively. Research shows that quercetin, kaempferol and luteolin are flavonoid antioxidants with 2-or 3-phenylchroman structures, which may reduce coronary disease and cancer [13]. Quercetin exhibits the capacity for cardioprotective as inhibition of LDL oxidation, endothelium-independent vasodilator effects, reduction of adhesion

molecules and other inflammatory markers, the protective effect on nitric oxide and endothelial function under conditions of oxidative stress[14]. Kaempferol possesses a wide range of therapeutic properties such as antioxidant, anticancer, and anti-inflammatory, which could act as a therapeutic agent in the treatment of cardiovascular diseases[15]. Luteolin possesses antioxidant activity, which may control of both NAFLD and cardiovascular risk[16]. These study shows that these active ingredients are significantly associated with CHD-AP, suggesting Shanhaidan granules in treatment of CHD-AP through these.

According to the results of the PPI network, GO and KEGG analysis, AKT1, IL-6, VEGF, TNF, MAPK, Caspase-3, MMP, EGFR and other targets have important implications in the treatment of CHD-AP with Shanhaidan granules. Shanhaidan granule could improve the CHD-AP by acting on inflammation, oxidative stress, apoptosis, vascular development and generation, circulation system and other processes, which regulating by the TNF signaling pathway, IL-17 signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway and apoptosis signalling pathway etc..

The basic pathology of CHD involves the dysfunction of both vascular and myocardial processes which was induced and exacerbated by oxidative stress, inflammation, and apoptosis [17]. IL-6 and TNF- α were important risk factors of coronary heart disease, which connects with the severity of coronary heart disease[18]. In coronary atherosclerosis, IL-6 could stimulate the expression of TNF- α , IL-1 β and other inflammatory factors which enhanced the inflammatory response and promoted the development of coronary heart disease[19]. In addition, IL-6 could accelerate the development of atherosclerosis by activating JAK-STAT signalling pathway and enhancing the proliferation of vascular smooth muscle cells [19]. In coronary heart disease, TNF- α might increase the expression of monocyte/macrophage tissue factor which could enhance thrombus activity. In atherosclerotic plaques, TNF- α cause plaque instability by enhancing apoptosis and degradation of matrix metalloproteinases (MMP), which aggravated the development of atherosclerosis and angina pectoris[20]. In patients with CHD and heart failure, TNF- α activated the transcription nuclear factor-kappa B which further induce inflammatory and oxidative stress responses[21]. Caspase-3 acted as the convergence of apoptosis-related signal pathways, and activation of Caspase-3 thereby amplify the apoptotic signalling pathway irreversibly [17]. Several studies showed that TNF- α , IL-6 and caspase-3 were closely related to the occurrence of angina pectoris, which can induce inflammatory factors, apoptosis pathway, oxidative stress, and aggravate the myocardial injury[19, 20, 22]. In the treatment of CHD-AP, Shanhaidan granule might improve the level of inflammation, apoptosis, and oxidative stress by affecting the expression of IL-6, TNF- α , and caspase-3.

VEGF played a key role in the development of angina pectoris, such as promoting inflammation and thrombosis, improving vascular permeability, and promoting angiogenesis. VEGFA could activate downstream signalling pathways, such as MAPK, Akt and eNOS to stimulate angiogenesis[23], and it can improve ischemic myocardium by affecting VEGF signalling pathway[24]. VEGF participated in myocardial cell proliferation, cardiac regeneration and prevent myocardial infarction through activating eNOS activity and promoting the synthesis of NO[25]. Rotllan, N et al. found that VEGF inhibited apoptosis through PI3K-Akt signalling, and promoted endothelial cell proliferation and angiogenesis through mTORc2 and FoxO1 [26].

The concentration of MMP in patients with CHD increased significantly [27]. The increase of MMP activity causes progressive cardiac expansion through degraded collagen, which eventually leads to ventricular remodeling, myocardial infarction, and heart failure [28]. MMP-9 could increase the thickness and inflammation of atherosclerotic plaques which caused plaque instability and rupture [29].

MAPK signalling pathway is the common pathway of various extracellular signal transmission including ERK, p38 MAPK and JNK signalling pathways, which could regulate proliferation, apoptosis, inflammation, immune response and other processes[30]. Previous study showed that p38 MAPK was involved in the ischemia, reperfusion, inflammation and apoptosis of myocardial cells, and the activation of p38 MAPK was implicated in the regulation of myocardial cell apoptosis[31, 32]. In the pathogenesis of coronary heart disease, MAPK was involved in myocardial hypertrophy, proliferation and apoptosis induced by various stimuli, and the drug targeting MAPK may be useful candidates for the treatment of myocardial hypertrophy[33].

PI3K/Akt signalling pathway played an important role in regulating cardiomyocyte survival and function [34]. Activation of PI3/Akt signalling pathway regulated glucose and lipid metabolism, and protected the heart which affected the occurrence and development of coronary heart disease[35]. In addition, Activation of PI3/Akt could reduce the activities of inflammatory factors such as TNF- α and IL-6, which reduced the inflammatory reaction in cardiovascular diseases[36]. Akt also increased VEGF secretion and mediated eNOS phosphorylation, vasorelaxation and angiogenesis[37].

It has been clinically confirmed that Shanhaidan granule had the effect of promoting blood circulation, removing blood stasis, and replenishing qi and nourishing blood, and could effectively treat CHD [38, 39]. A study showed that Shanhaidan granule could relieve the symptoms of coronary heart disease and improve left ventricular diastolic function, which had a good effect on angina pectoris of coronary heart disease [7]. These study showed that Shanhaidan granules had the good effect in treatment of CHD.

Collectively, these results revealed that Shanhaidan granules may decrease inflammation, oxidative stress, and apoptosis, improve vascular endothelial function, and protect cardiovascular. The network and docking analysis showed that Shanhaidan granules may regulate TNF signaling pathway, IL-17 signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway and apoptosis signalling pathway by acting on AKT1, IL-6, VEGF, TNF, MAPK, Caspase-3, MMP, and EGFR to treat CHD-AP. The results of molecular docking verified that the present network pharmacology method was reasonable, however, the prediction of targets and mechanism require further clinical validation.

5. Conclusions

This study revealed the potential pharmacological mechanism of Shanhaidan granule in the treatment of CHD-AP at a system level. The results showed that the anti-angina effect of Shanhaidan granule was played through regulating multi-targets by multi-components, which provided theoretical support for rational clinical application of Shanhaidan granule against CHD-AP. Moreover, we hope that our study will be useful for Anti-angina drug discovery.

Abbreviations

CHD-AP

Coronary heart disease angina pectoris

TCM

Traditional Chinese medicine

OB

Bioavailability

DL

Drug-likeness

TCMSP

Traditional chinese medicine systems pharmacology

TCMID

Traditional chinese medicine information database

AKT1

RAC-alpha serine/threonine-protein kinase1

IL-6

Interleukin-6

IL-17

Interleukin-17

VEGF

Vascular endothelial growth factor

TNF

Tumor necrosis factor

MAPK

Mitogen-activated protein kinase

MMP

Matrix metalloproteinase

EGFR

Epidermal growth factor receptor

Declarations

Authors' Contributions

Wanpeng Lu and Mengkai Zheng conceived and designed the experiments; Wenyang Wei, Yunfeng Yang, Wanpeng Lu and Mengkai Zheng performed the research; both Wenyang Wei and Wanpeng Lu wrote the manuscript with input from all authors. Mengkai Zheng and Jing Wang revised the language. All authors reviewed and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Figures

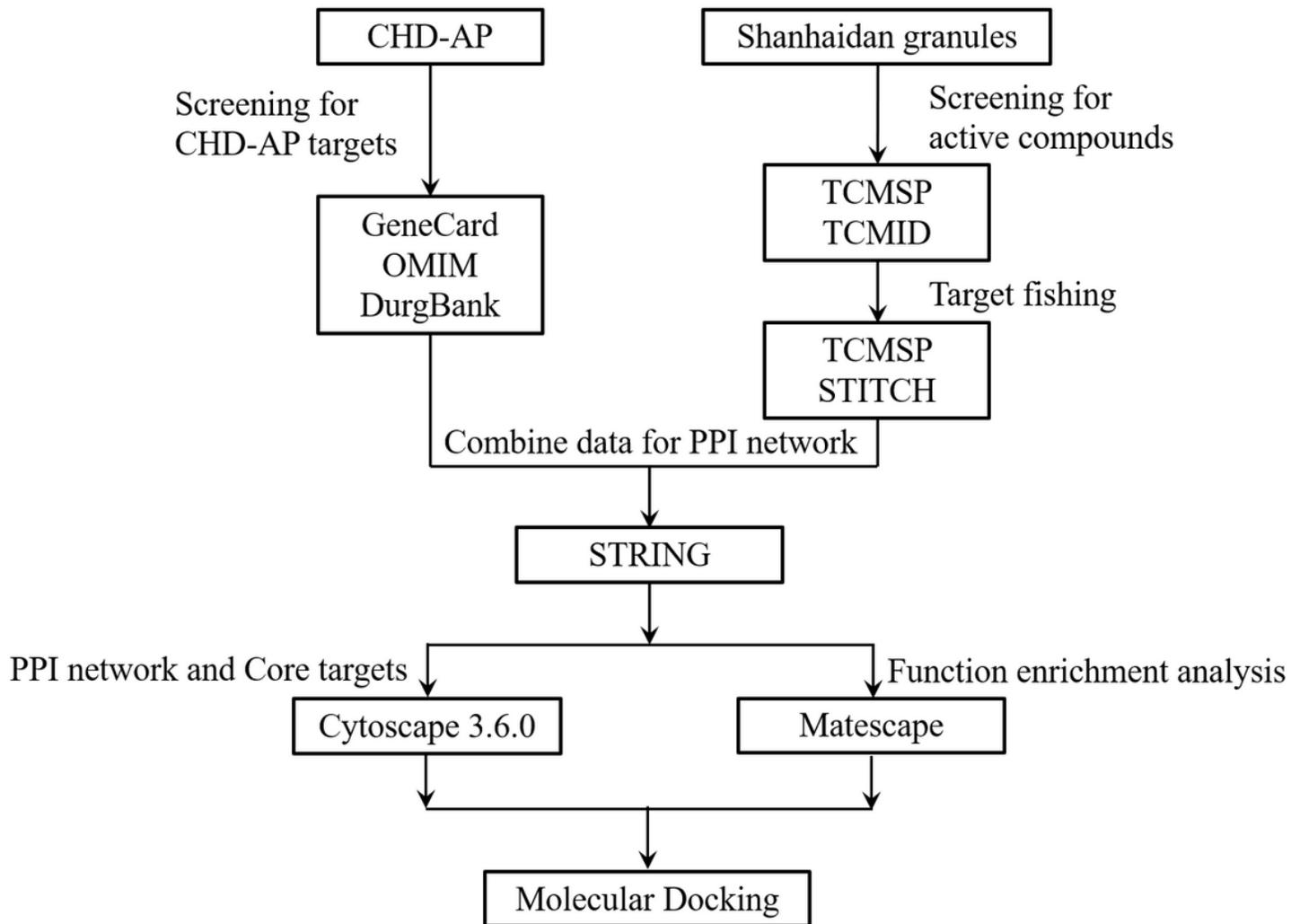


Figure 1

Detailed design and workflow of the present study

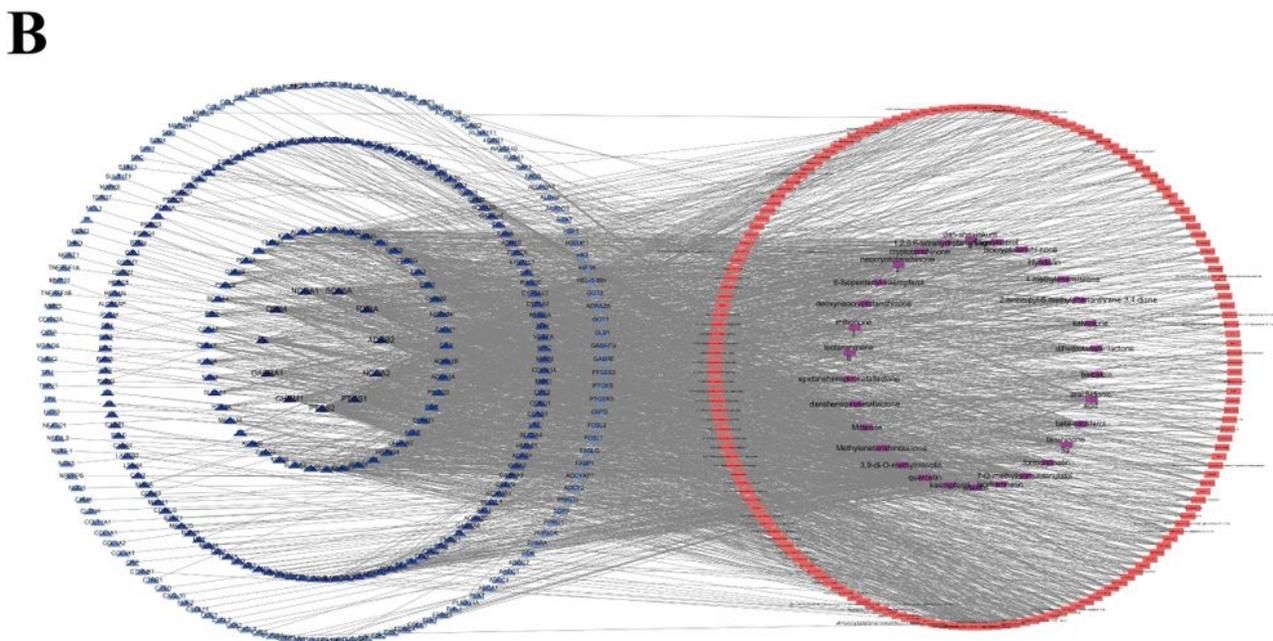
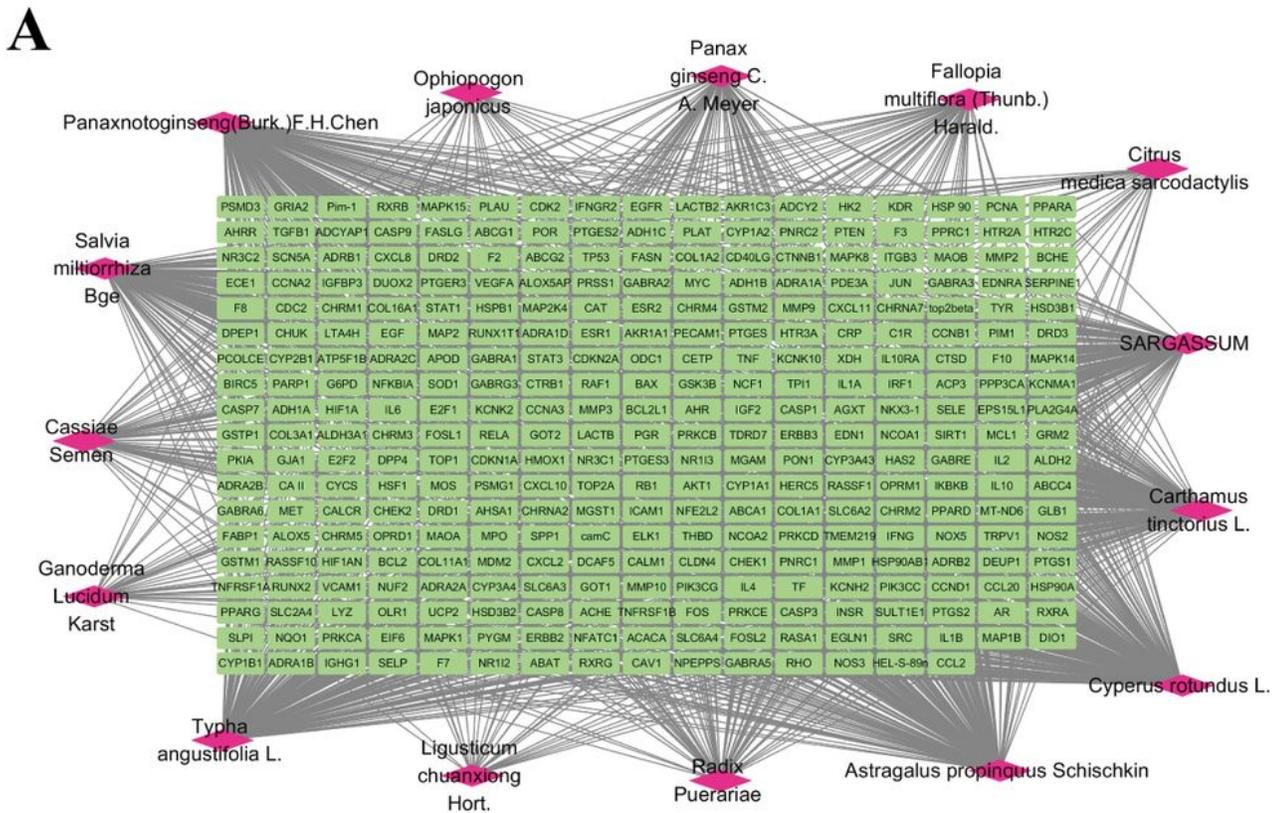


Figure 2

The network of Shanhaiidan granules. A. Chinese medicine-target network of Shanhaiidan granules. The blue diamond represent the Chinese medicine of Shanhaiidan granules, the green rectangle represent the targets. B. The ingredient-target network of Shanhaiidan granule. The blue triangle represent the ingredients of Shanhaiidan granules, the purple and orange rectangle represent the targets.

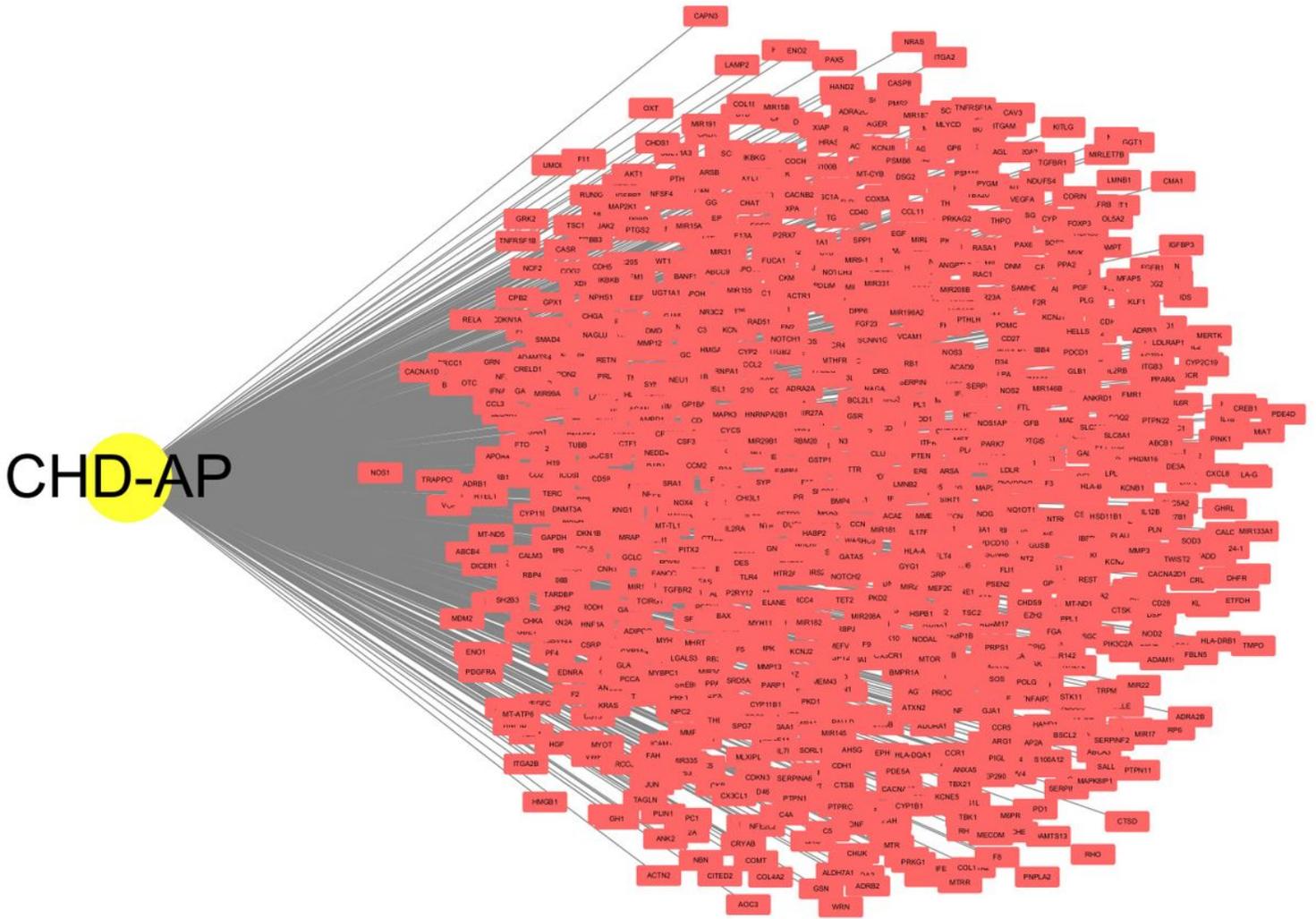


Figure 3

CHD-AP target network

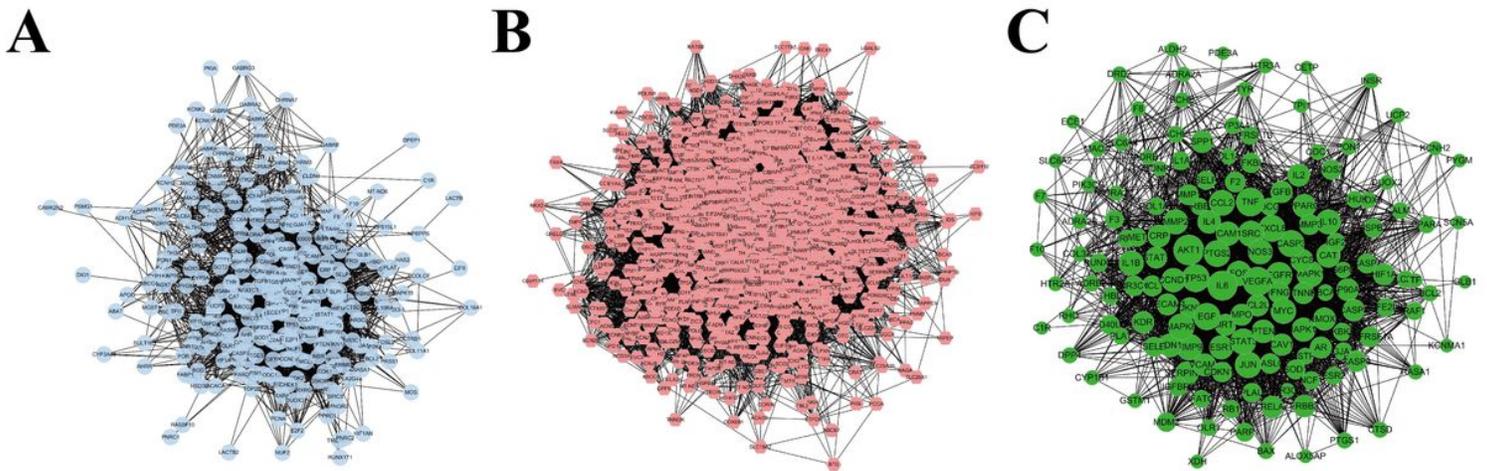


Figure 4

PPI network. A. PPI network of Shanhaidan granules, B. PPI network of CHD-AP, C. Core target intersection PPI network.

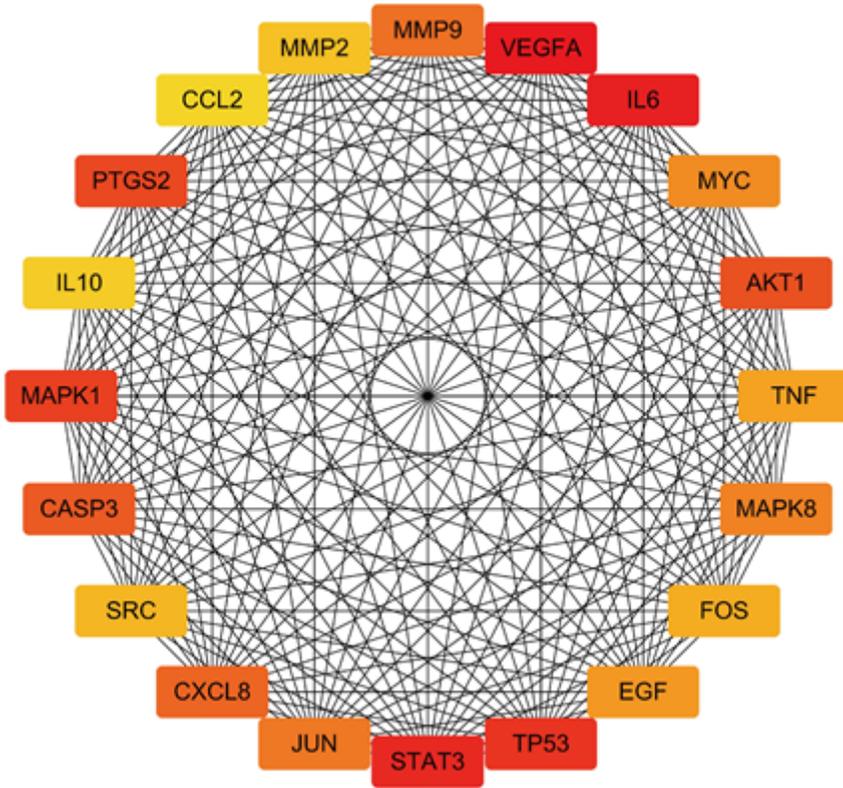


Figure 5

The top 20 hub genes of PPI network.

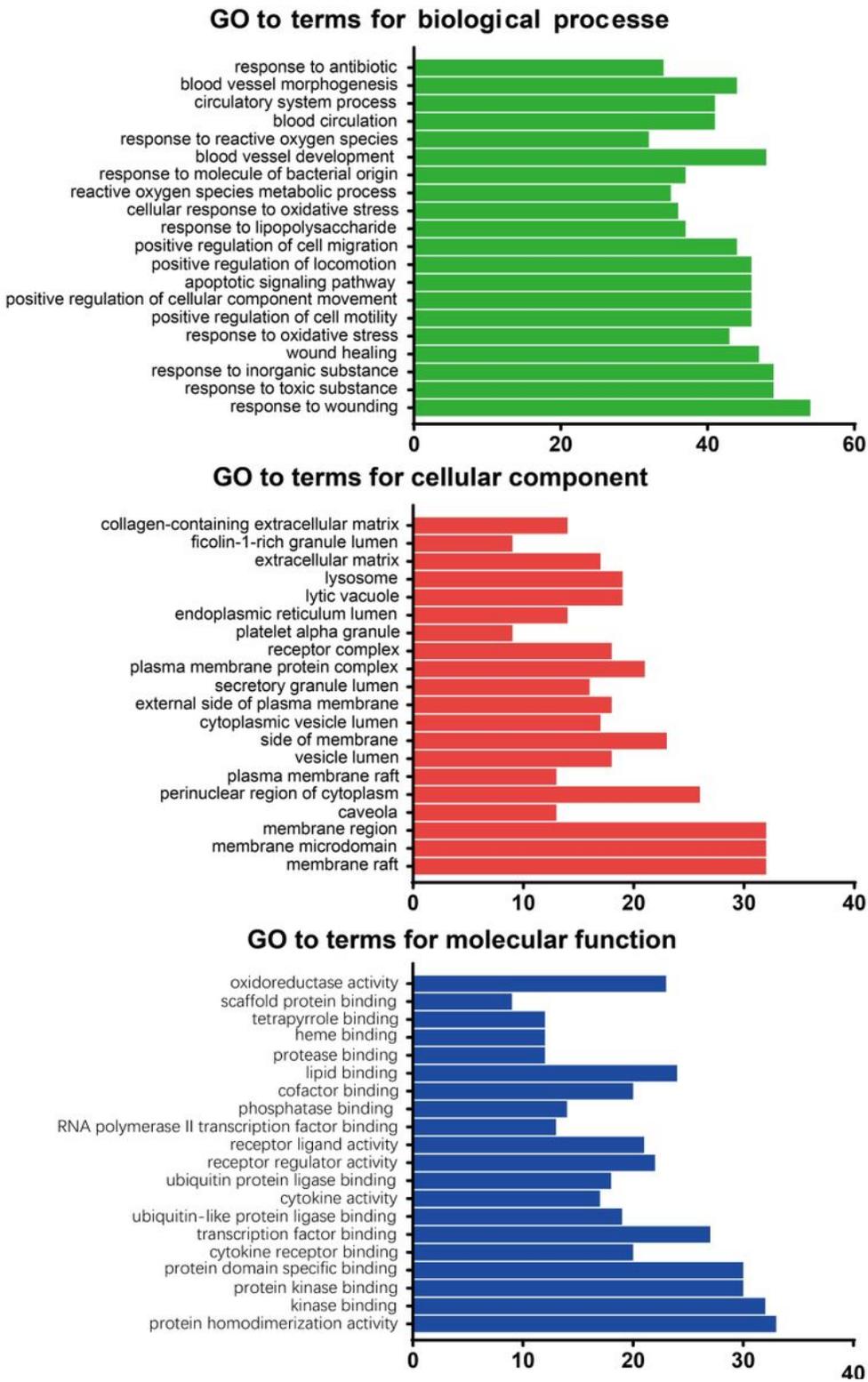


Figure 6

GO enrichment analysis of Shanhaidan granules in treating CHD-AP targets

Top 20 of pathway enrichment

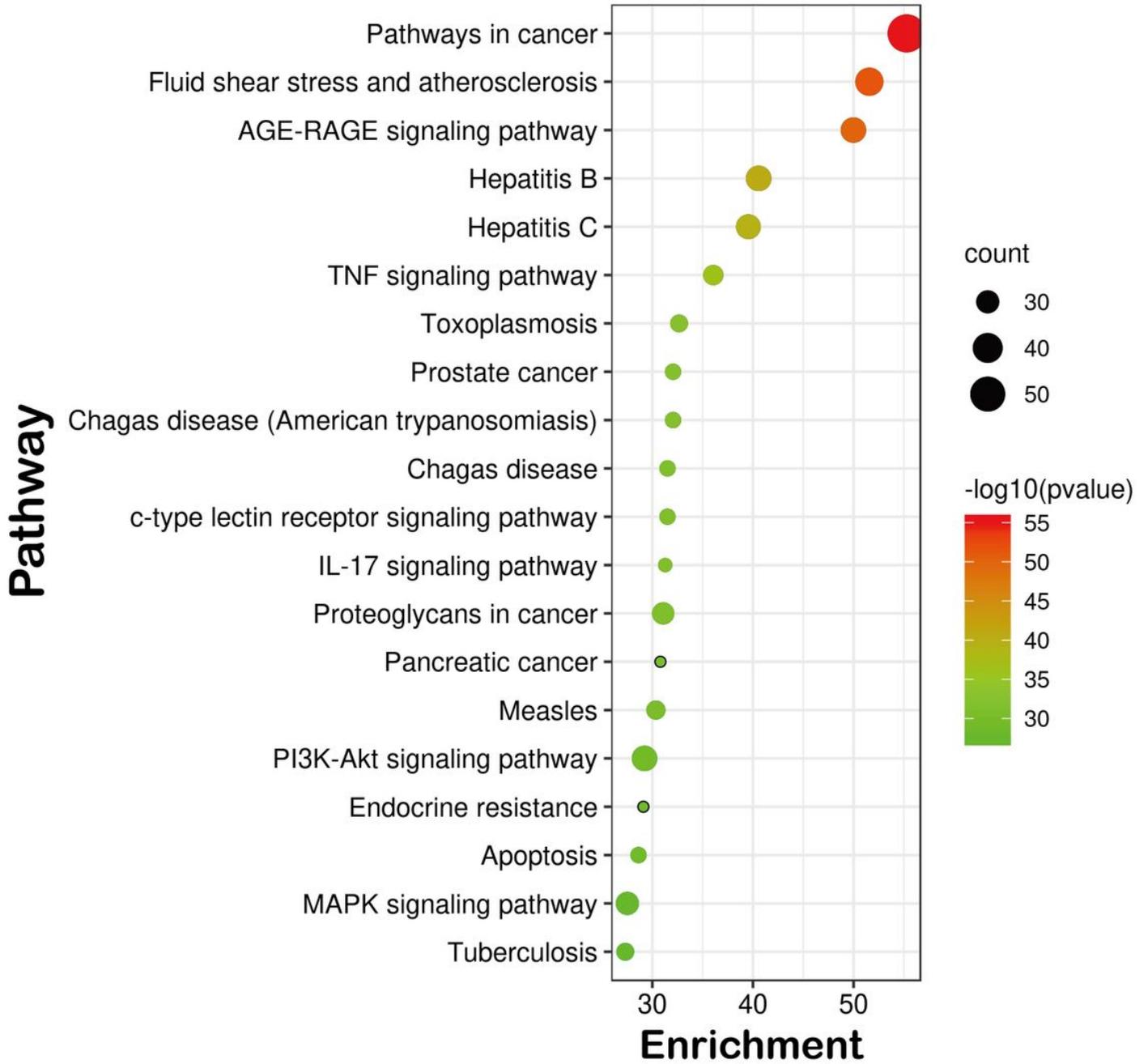


Figure 7

KEGG pathway in treatment of CHD-APtarget by Shanhaidan granules

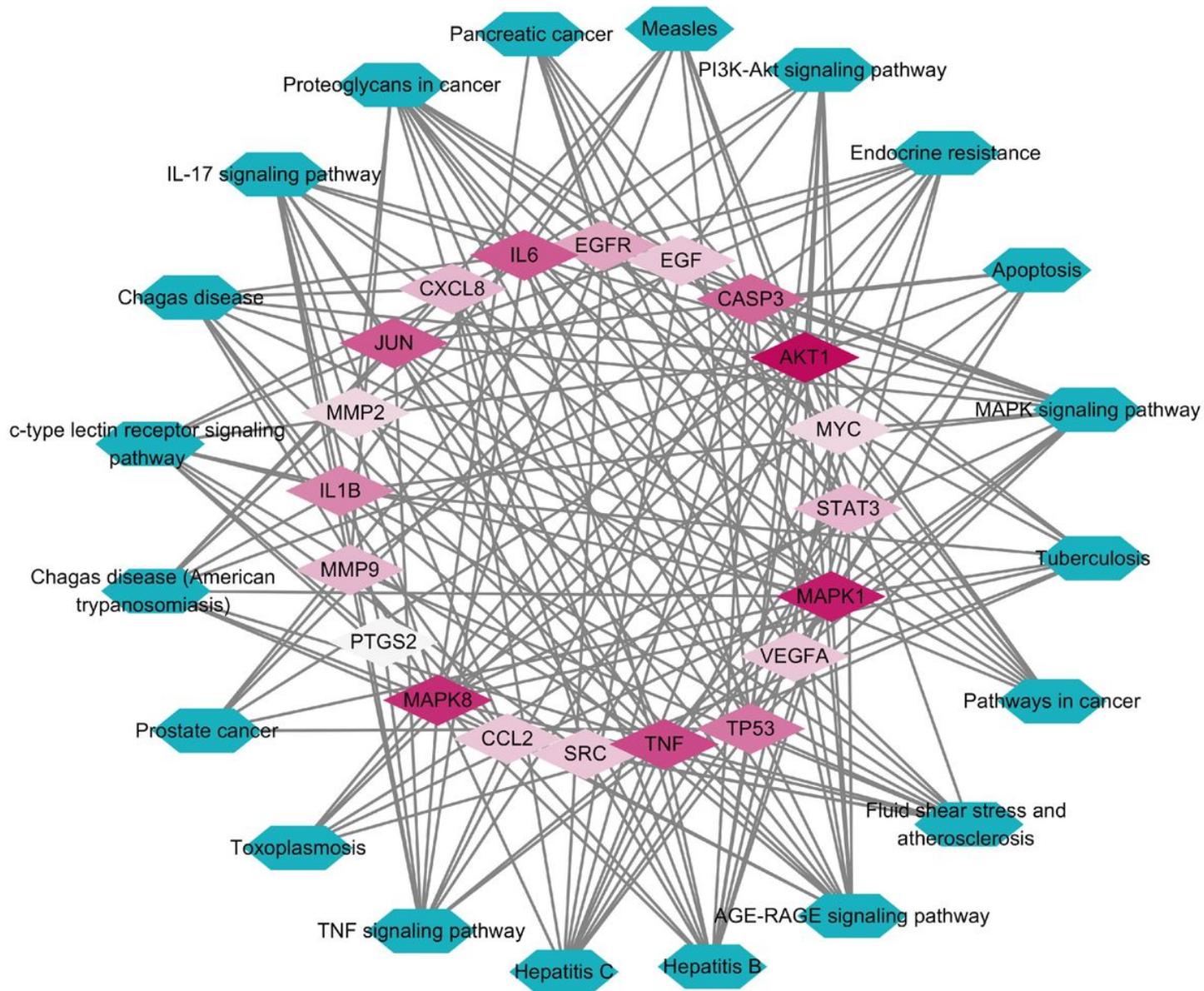


Figure 8

Top 20 targets-pathway network. The blue hexagon represent the signal pathways, the pink diamond represent the the targets. The color of nodes represents the value of degree centrality

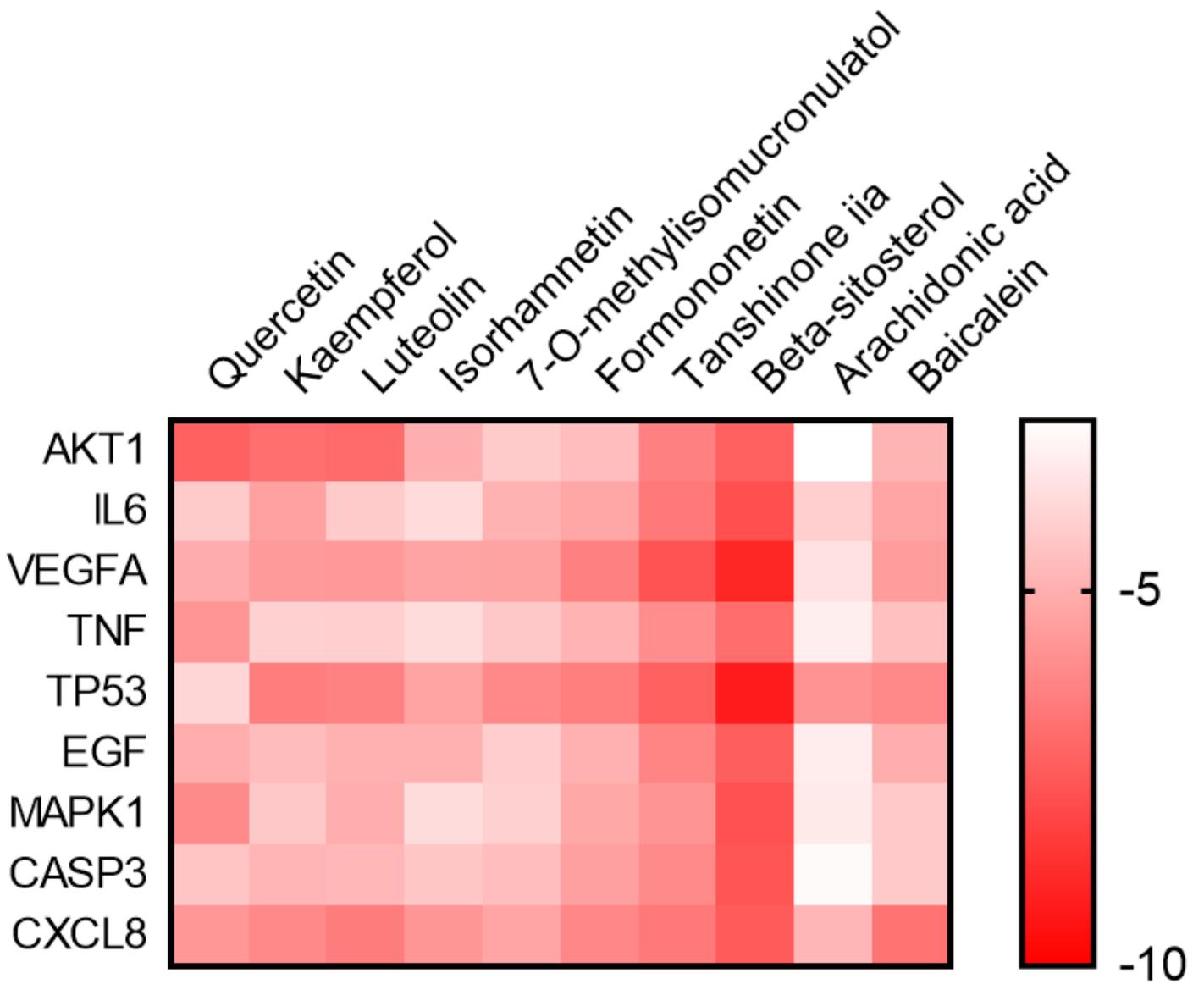


Figure 9

The heat map of molecular docking.