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Network pharmacology and molecular docking approach to explore the potential mechanisms of Xuefu Zhuyu Capsule in coronary heart disease

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Abstract

Xuefu Zhuyu Capsule (XFZYC) plays a pivotal role in treating coronary heart disease (CHD) because of its potent clinical effects and fewer side effects. However, the possible pharmacological effect on CHD is limited. Thus, this research systematically analyzes XFZYC and CHD through network pharmacology technology. We identified 139 active compounds and 127 overlapped target genes in 11 herbs of XFZYC by using network pharmacology, and these 127 genes regulated the major signaling pathways related to CHD. The analysis of protein–protein interaction networks demonstrated that 30 important gene targets, such as interleukin-6, signal transducer and activator of transcription 3, protein kinase B1, mitogen activated protein kinases 3, cellular tumor antigen p53, vascular endothelial growth factor A, transcription factor p65, and proto-oncogene c-Fos, participated in the regulation of 79 major signaling pathways related to CHD. On this basis, we found that protein kinase B1, cellular tumor antigen p53, and transcription factor p65, which played a role in multiple regulatory pathways of the network, were also regulated by more than 3 compounds and expressed in at least 4 herbs. Molecular docking showed that XFZYC had a good binding potential with the overlapped protein targets. Gene Ontology enrichment analysis revealed that the active targets involved a various of biological process, cellular component, and molecular function, which included the key ones such as positive regulation of transcription from RNA polymerase II promoter, plasma membrane, and protein binding. T cell receptor signaling pathway, programmed death-ligand 1 expression and programmed death cell receptor-1 checkpoint pathway in cancer, FOXO signaling pathway, Ras signaling pathway, interleukin-17 signaling pathway, and VEGF signaling pathway, which were selected from Kyoto Encyclopedia of Genes and Genomes, were closely related to XFZYC in the treatment of CHD. XFZYC has a potential pharmacological effect on CHD, which provides the value for further study of XFZYC's therapeutic effect on CHD.

Abbreviations: AKT1 = protein kinase B1, AS = atherosclerosis, BP = biological process, CC = cellular component, CHD = coronary heart disease, HIF-1 = hypoxia-inducible factor-1, IL-6 = interleukin-6, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function, MMP9 = matrix metalloproteinase-9, PD-1 = programmed death cell receptor-1, PD-L1 = programmed death-ligand 1, PPI = protein–protein interaction, RELA = transcription factor p65, STAT3 = signal transducer and activator of transcription 3, TCM = traditional Chinese medicine, TP53 = cellular tumor antigen p53, VEGFA = vascular endothelial growth factor A, XFZYC = Xuefu Zhuyu Capsule.

Keywords: coronary heart disease, integrating network pharmacology, molecular mechanism of pharmacological action, traditional Chinese medicine, Xuefu Zhuyu Capsule

HQ, B-BC, and MZ contributed equally to this work.

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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1. Introduction

As the most common type of organ lesions caused by atherosclerosis (AS), coronary heart disease (CHD) is a universal disease that seriously endangers human health, and it has become a major public health problem worldwide. An estimated 110 million people worldwide suffer from CHD, and the age-standardized average prevalence is 1663 per 100,000 (1.7 %). Central Asia has the highest CHD mortality rate (336 per 100,000), while high-income Asia held the lowest (45 per 100,000). Studies have shown that there are 290 million cases of cardiovascular diseases in China, including 11 million cases of CHD. Although CHD is clinically preventable, treatable and controllable, the progression of CHD is irreversible. It is necessary to carry out effective clinical treatment of CHD.

More and more attention has been paid to traditional Chinese medicine (TCM) for its remarkable clinical efficacy and slight side effects, which makes it a unique advantage in the regulation of CHD process. As a Chinese patent medicine listed in China, Xuefu Zhuyu Capsule (XFZYC) is developed on the basis of Xuefu Zhuyu decoction, which is established by Wang Qingren, a physician in the Qing Dynasty. This classical formula consists of TaoRen (TR, Persicae Semen), HongHua (HH, Carthami Flos), ChiShao (CS, Radix Paeoniae Rubra), ChuanXiong (CX, Chuanxiong Rhizoma), NiuXi (NX, Achyranthis Bidentatae Radix), DiHuang (DH, Rehmanniae Radix Praeparata), DangGui (DG, Angelicae Sinensis Radix), JieGeng (JG, Platycodon Grandiforus), ZhiQiao (ZQ, Aurantii Fructus), ChaiHu (CH, Radix Bupleuri), and GanCao (GC, licorice), which makes XFZYC an effective herb combination to have evident effect of promoting blood circulation, removing blood stasis, promoting qi circulation, and relieving pain. Based on the above, XFZYC is commonly applied to inhibit the development of CHD, such as AS, hypertension, stroke, and deep vein thrombosis. Some herbs in this prescription are often used in pairs in TCM formulae, which are called herb pairs. For example, the combined application of TaoRen and HongHua, which is named as TaoHong herb pair, has been used in TCM to promote blood circulation and dissipate blood stasis for many years in China.[1] The famous formulations containing TaoHong herb pair include Buyang Huanwu Decoction, Fuyuan Huoxue Decoction, Taoren Danggui Decoction, and so on. Similarly, ChiShao and ChuanXiong, named as ChiChuan herb pair, are often used together as the key herbs in promoting blood circulation. Hence, ChiChuan herb pair exert the functions of anti-AS, stabilizing plaque, improving myocardial ischemia, reducing restenosis rate after PCI, and protecting brain tissue and nerve through lipid-lowering, anti-inflammatory, bi-directional regulation of angiogenesis, and so on.[2–8]

With the development of TCM, Chinese medicine is playing an increasingly important role in the prevention and treatment of CHD. Clinical research shows that XFZYC has played an irreplaceable curative role in treating CHD. Modern research has concluded that XFZYC can treat CHD by regulating phospholipid, cardiac energy, polyunsaturated fatty acid, and amino acid metabolism.[9,10] The fact that cardiomyocytes can be protected from hypoxia/reoxygenation damage by inhibiting autophagy can be due to XFZYC.[11] XFZYC may also have a transient and bi-directional regulation effect on

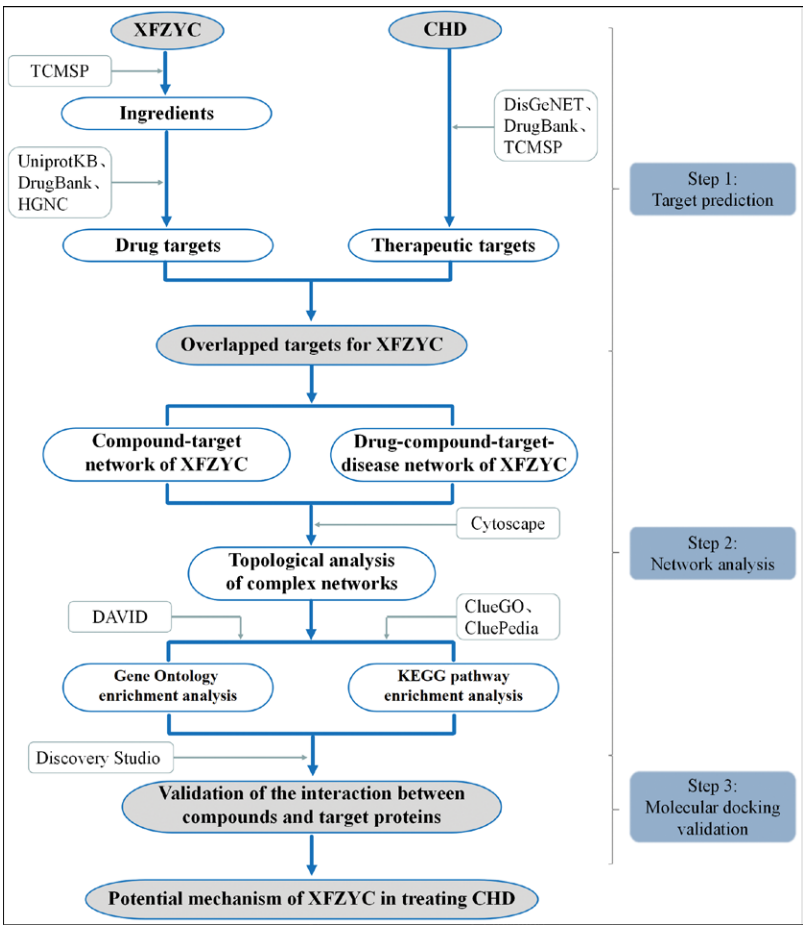

Figure 1. Flowchart of XFZYC on CHD by using network pharmacology. CHD = coronary heart disease, DAVID = Database for Annotation, Visualization and Integrated Discovery, GO = Gene Ontology, HGNC = HUGO Gene Nomenclature Committee, KEGG = Kyoto Encyclopedia of Genes and Genomes, TCMSP = Traditional Chinese Medicine Systems Pharmacology, UniprotKB = UniProt Knowledgebase, XFZYC = Xuefu Zhuyu Capsule.

vascular growth,[3,8] and can prevent myocardial cell apoptosis by increasing the mRNA and protein expression of silent information regulator1 and by inhibiting the mRNA and protein expression of tumor associated nuclear factor kappa-B, protein 53, fork box protein O1, fork box protein O3, and fork box protein O4.[12] Since TCM is mainly aimed at a range of clinical symptoms and different diseases may have the same pathological process, modern research has also revealed that XFZYC may still be used to intervene in the course of other diseases. For example, XFZYC has an effective clinical efficacy in the treatment of uterine fibroids, localized scleroderma, postcraniocerebral traumatic mental disorder, and so on.[13–15] Experimental studies have proven that XFZYC has neuroprotective effects through an anti-inflammatory pathway in a rat model of traumatic brain injury.[16] It can protect skeletal muscle atrophy of SD rat model under simulated microgravity by increasing the expression level of the proteins related to PI3K–AKT signaling pathway as well.[17] Besides, the protective effects of Xuefu Zhuyu decoction in a rat model of traumatic brain injury were revealed by using TMT-based proteomics analysis.[18] There is no doubt that TCM has solid effects in clinical practice and has attracted worldwide attention.[19]

Network pharmacology can be used with the support of various technologies such as omics, high-throughput screening, network visualization, and network analysis, and it can predict the molecular basis and mechanism of TCM in regulating diseases from multi-dimensional perspectives such as "drug–component–target," "disease–target," "drug–component–component target–disease target–disease" to elucidate the potential mechanism of the complex combination forms of the compounds and targets.[20] Network pharmacology has been widely used in the research field of TCM, which provides a more appropriate research path for exploring the multitarget effect of TCM.

Although XFZYC has significant clinical efficacy and its chemical composition has been confirmed, the pharmacological mechanism of XFZYC in the treatment of CHD still remains unclear. This study discussed the potential mechanism of XFZYC in the treatment of CHD at the molecular level by using network pharmacology, so as to provide reference for rational clinical application and theoretical basis for further experimental research of XFZYC. The flowchart for this network pharmacology study is shown in Figure 1.

2. Materials and methods

2.1. Constituents and targets of XFZYC

The data on the chemical ingredients of 11 herbs were screened from Traditional Chinese Medicine Systems Pharmacology[21] (TCMSP, https://www.tcmsp-e.com/#/home) Database and Analsis Platform. Considering the complexity and different absorbance of the Chinese herbal medicines, we selected oral bioavailability ≥ 30 %, druglikeness ≥ 0.18, halflife ≥ 4, and intestinal epithelial permeability ≥ -0.4 as the screening thresholds to pick out the potential active ingredients for further analysis, which is also the reference standard provided by TCMSP database. The targets of each component were then selected from UniProt Knowledgebase (UniprotKB, https://www.uniprot.org/), DrugBank[22] (https://www.drugbank.ca/), and HUGO Gene Nomenclature Committee (HGNC, https://www.genenames.org/) databases. The reduplicative targets were erased.

2.2. Significant genes collection for CHD

Significant genes associated with CHD were screened from 3 databases, that is DisGeNET[23] (https://www.disgenet.org/), TCMSP, and DrugBank databases. The repetitive genes collected from the 3 databases were removed.

2.3. Target protein interaction analysis

Venn analysis was carried out to find mapping relationship in the data set of the CHD targets and the targets of main chemical components of XFZYC. The overlapped genes were then obtained and STRING_v11.0 (https://string-db.org/) was used to construct the protein–protein interaction (PPI) network on the target proteins. The relationship between targets in the network were analyzed with the topology theory.

2.4. Network construction and analysis

The drug–compound–target network and the drug–compound–target–disease network were constructed by using Cytoscape_v3.7.1 software.[24] The network topological parameters were calculated to analyze the network,

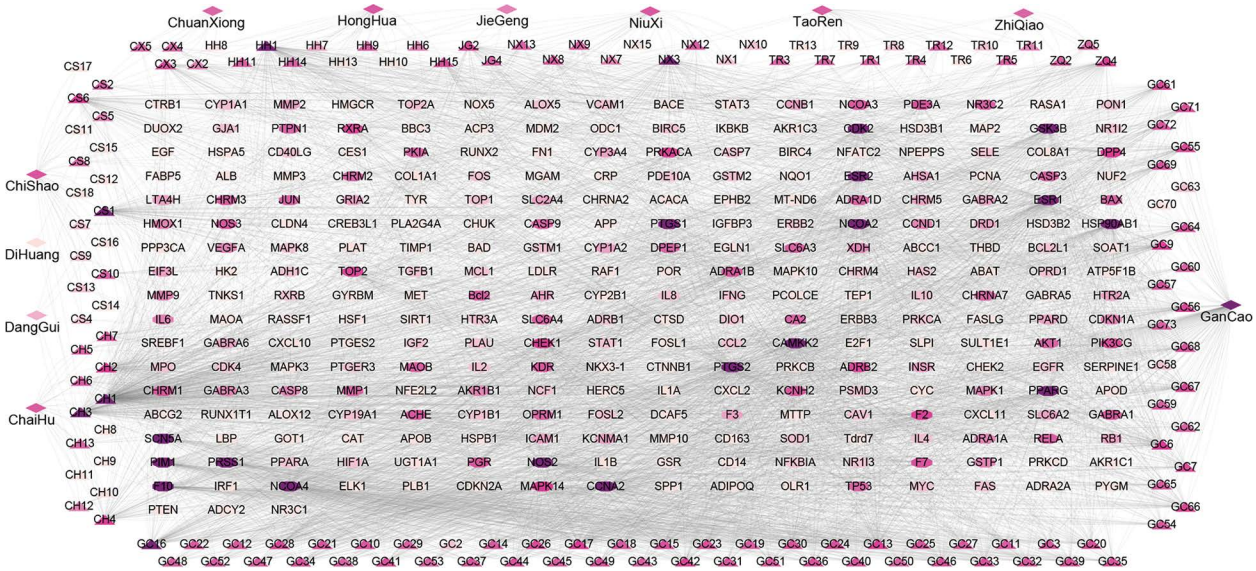

Figure 2. Drug–compound–target network. Octagon nodes represent targets, triangle nodes represent compounds, and diamond nodes represent drug. Edges represent interactions between ingredients, targets, and drug. Node color was regulated by degree centrality, and the nodes with purple tended to have a high degree.

Table 1

Information of the 19 key compounds in FXZYC.

Compound	Molecule ID	Molecule name	DC	BC	CC	Source of Chinese medicine
CH1	MOL000099	Quercetin	304	0.38040	0.49417	ChaiHu, GanCao, HongHua, NiuXi
CH2	MOL000354	Isorhamnetin	76	0.02524	0.38406	ChaiHu, GanCao
CH3	MOL000422	Kaempferol	126	0.08012	0.41006	ChaiHu, GanCao, HongHua, NiuXi
CH4	MOL000449	Stigmasterol	71	0.05193	0.39552	ChaiHu, ChiShao, DangGui, HongHua, NiuXi
CS1	MOL000358	Beta-sitosterol	86	0.06520	0.40076	ChiShao, DangGui, HongHua, NiuXi, TaoRen, ZhiQiao
CS6	MOL002714	Baicalein	78	0.06849	0.39259	ChiShao, HongHua, NiuXi
GC13	MOL002565	Medicarpin	70	0.01996	0.37390	GanCao
GC16	MOL003896	7-Methoxy-2-methylisoflavone	86	0.01896	0.38475	GanCao
GC17	MOL004328	Naringenin	76	0.09186	0.37522	GanCao
GC42	MOL004891	Shinpterocarpin	62	0.01195	0.37128	GanCao
GC6	MOL000392	Formononetin	78	0.03308	0.37655	GanCao
GC66	MOL005003	Licoagrocarpin	60	0.00713	0.37456	GanCao
GC9	MOL000497	Licochalconea	66	0.01708	0.37655	GanCao
HH1	MOL000006	Luteolin	116	0.08211	0.39700	HongHua, JieGeng
HH15	MOL002773	Beta-carotene	46	0.02385	0.36364	HongHua
JG2	MOL001689	Acacetin	54	0.01544	0.37128	JieGeng
NX3	MOL000173	Wogonin	92	0.04149	0.38475	NiuXi
TR5	MOL001328	2,3-DidehydroGA70	22	0.00789	0.36364	TaoRen
ZQ4	MOL005828	Nobiletin	70	0.03352	0.37655	ZhiQiao

CH1, CH2, CH3, CH4 are the compounds of ChaiHu. CS1 and CS6 are the compounds of ChiShao. GC13, GC16, GC17, GC42, GC6, GC66, and GC9 are the compounds of GanCao. HH1 and HH15 are the compounds of HongHua. JG2 is the compound of JieGeng. NX3 is the compound of NiuXi. TR5 is the compound of TaoRen. ZQ4 is the compound of ZhiQiao.

including degree centrality, betweenness centrality, and closeness centrality. The degree of a node is the total number of edges connected by the node. The greater the degree of a node means the higher degree centrality of the node, and thus the more critical the node is in the network. Betweenness centrality indicates the importance of the nodes in maintaining steady state of the network by measuring the ratio of a node to the other one in the shortest path. Closeness centrality represents the distance of the nodes to the center of the network. It is generally believed that the nodes with degree centrality value greater than the double median, closeness centrality, and betweenness centrality values greater than the median hold a vital role in the network.

2.5. Enrichment analysis

The CHD-related genes in XFZYC were input into the Database for Annotation, Visualization, and Integrated Discovery[25] (DAVID_v6.8, https://david.ncifcrf.gov/), which was applied to analyze the Gene Ontology (GO) enrichment, including biological process (BP), cellular component (CC), and molecular function (MF). Besides, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed by using ClueGO and CluePedia, the plug-ins of Cytoscape.[26,27] The advanced bubble chart is performed by using OmicShare,[28] which is a free online data analysis platform (http://www.omicshare.com/tools).

2.6. Molecular docking

The role of molecular docking is to place ligand molecules at the active site of receptor, and to evaluate the interaction between ligands and receptors in real time according to the principles of geometric complementarity and energy complementarity, so as to find the best binding mode. In order to verify the accuracy of the network analysis results in this study, we first downloaded the 3D structures of the top 6 target proteins from RCSB Protein Data Bank (http://www.rcsb.org/) database and the 3D structures of 19 core compounds from Chemspider (http://www.chemspider.com/), and PubChem NCBI (https://www.ncbi.nlm.nih.gov/pccompound) databases. After preprocessing the target proteins, Discovery

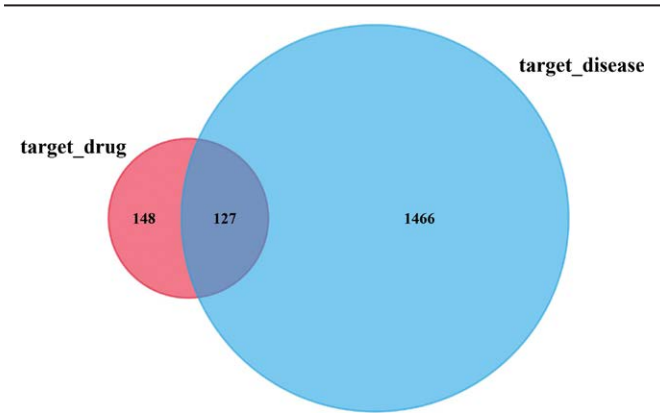

Figure 3. Intersection of drug targets and disease targets. The blue circle represents the CHD-related targets, and the red circle represents the XFZYC-related targets. CHD = coronary heart disease, XFZYC = Xuefu Zhuyu Capsule.

Studio software was used to define the active pockets according to the spatial position of the primitive ligand, and the interaction between the core compound and the key target was verified by CDOCKER. The parameter "pose cluster radius" of "top hits" was set as 0.5, and the rest parameters were the defaults.

The primitive ligand was extracted and then rejoined into the active pocket of the protein. The root-mean-square deviation between the primitive ligand molecule and the ligand after docking was taken into account because it could reflect the consistency and accuracy of the docking. It is generally believed that when root-mean-square deviation ≤ 2Å, the molecular docking model can reproduce the binding mode of the primitive ligand and protein well, and the docking results hold higher reliability.[29] The potential active ingredients were subsequently introduced into Discovery Studio for molecular docking, and the docking score (-CDOCKER ENERGY) was calculated. When -CDOCKER ENERGY value is negative and higher than the primitive ligand, it can be considered that the component has higher binding activity with the target protein, which is often used as a standard to evaluate the results of molecular docking.

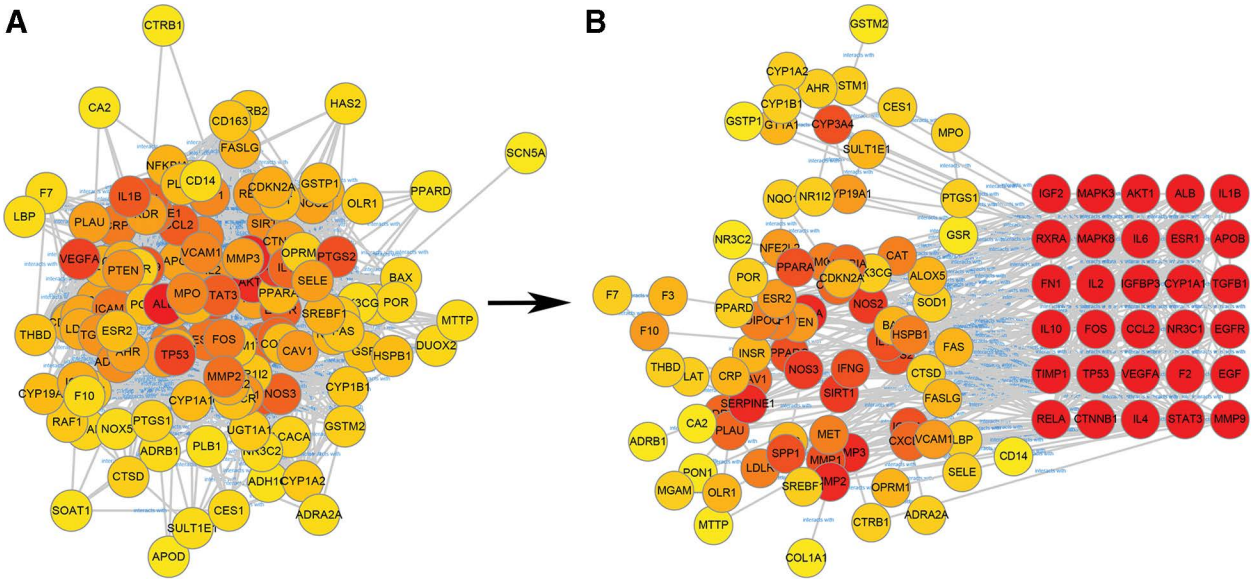

Figure 4. PPI network of XFZYC. (A) PPI network of the potential target proteins. (B) PPI network of the key target proteins. In the 2 figures, the nodes indicate the active proteins, and the edges represent the active protein–protein interactions. The colors of the nodes are illustrated from red to yellow in descending order of degree values. PPI = protein–protein interaction, XFZYC = Xuefu Zhuyu Capsule.

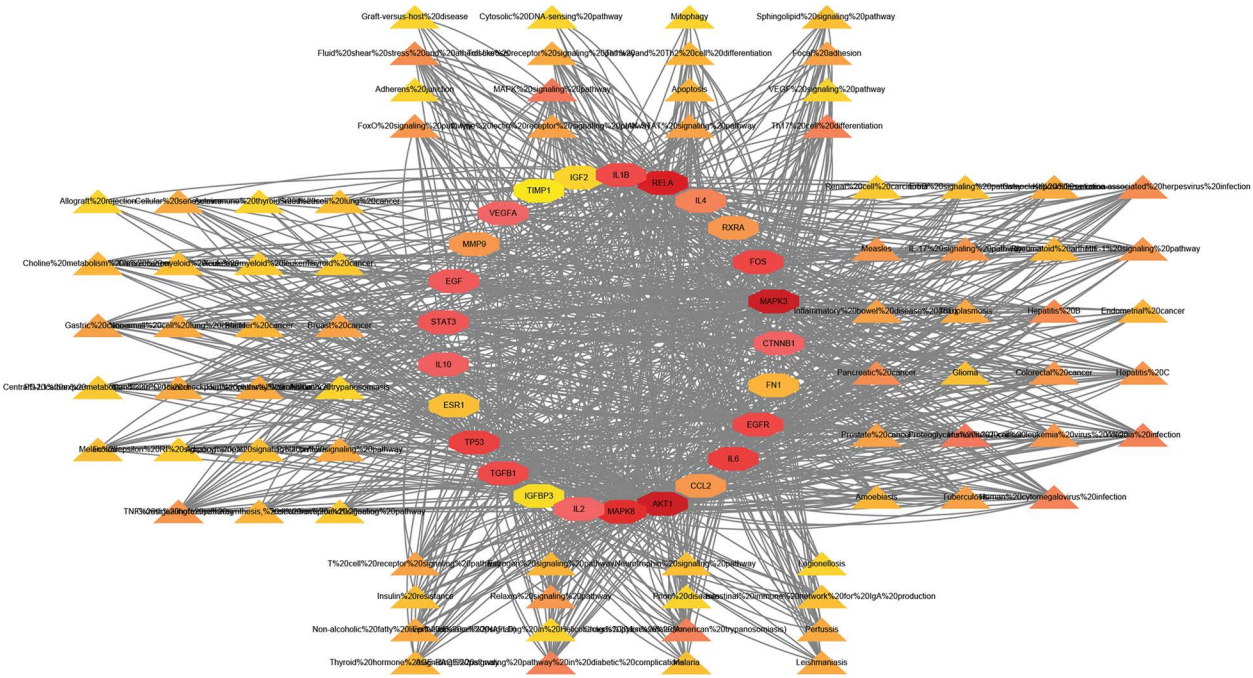

Figure 5. Network of the key target-pathways in Xuefu Zhuyu Capsule. Octagon nodes represent the key targets, triangle nodes represent the related signaling pathways. Edges represent the interactions between the key target and the signaling pathways. Node color was regulated by degree centrality, and the nodes with the deeper red tended to have a higher degree.

3. Results

3.1. Compounds in XFZYC

One thirty-nine ingredients from TCMSP with the criteria of oral bioavailability ≥ 30%, druglikeness ≥ 0.18, halflife ≥ 4, and intestinal epithelial permeability ≥ -0.4 were selected, including 5 ingredients in TaoRen, 15 ingredients in HongHua, 18 ingredients in ChiShao, 5 ingredients in ChuanXiong, 5 ingredients in ZhiQiao, 13 ingredients in ChaiHu, 4 ingredients in JieGeng, 2 ingredients in DangGui, 2 ingredients in DiHuang, 15 ingredients in NiuXi, and 73 ingredients in GanCao (Table S1, Supplemental Digital Content, http://links.lww.com/MD/O259).

3.2. Target compounds and target genes

A total of 3038 genes were collected from the DisGeNE, TCMSP, and DrugBank databases. Two hundred seventy-five significant genes were left after deleting the repetitions. The significant genes consisted of 46 genes in TaoRen, 221 genes in HongHua, 69 genes in ChiShao, 30 genes in ChuanXiong, 93 genes in ZhiQiao, 204 genes in ChaiHu, 74 genes in JieGeng, 53 genes in DangGui, 31 genes in DiHuang, 215 genes in NiuXi, and 238 genes in GanCao. As the interaction between targets plays crucial role in the interaction of TCM, we then drawed and analyzed drug–compound–target network of XFZYC (Fig. 2).

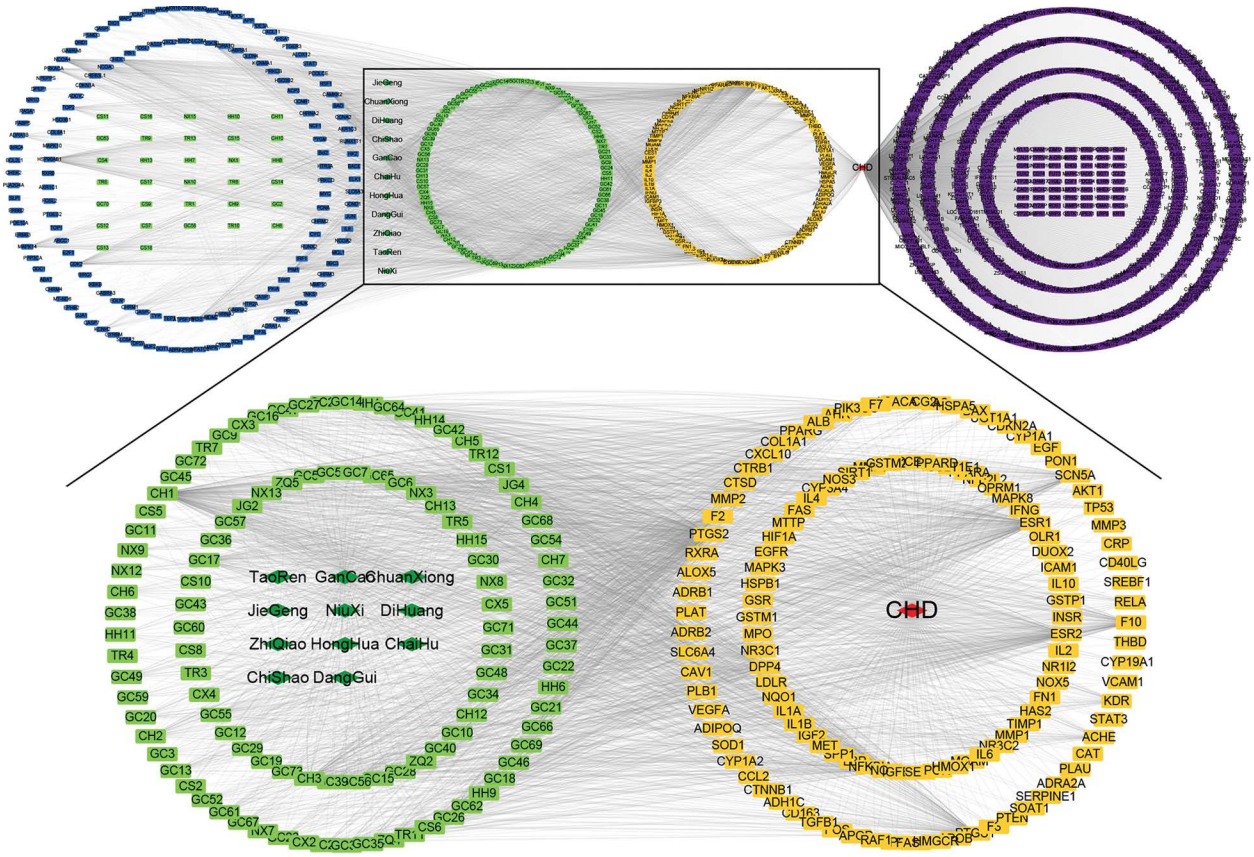

Figure 6. Drug–compound–target–disease network of Xuefu Zhuyu Capsule. Dark green nodes represent drugs, light green nodes represent compounds, dark blue nodes represent the compound-related targets, red nodes represent diseases, purple nodes represent the disease-related targets and yellow nodes represent the overlapped targets.

The above composite network contained 425 nodes and 4229 edges which showed the relationships between 11 Chinese herbal medicines, 139 compounds, and 275 compound-related targets. Taken GanCao as an example, there were 73 compounds and 238 corresponding targets in it when XFZYC regulated CHD, indicating that GanCao regulated a wide range of targets through complex components. Thus, the relationship between compounds and targets is not 1-to-one because 1 compound can correspond to multiple targets and vice versa. This result indicated that XFZYC might exert pharmacological effects on CHD through a complex network of compounds and targets.

The topology properties of the network were further analyzed by using the network analyze function in Cytoscape. The medians of the 3 topological indexes were calculated respectively (degree centrality = 5, betweenness centrality = 0.00607, closeness centrality = 0.33639), and the 19 key compounds were picked out with the standard of degree centrality > 10, betweenness centrality > 0.00607, and closeness centrality > 0.33639 (Table 1).

3.3. Analysis of targets in PPI network

One thousand five hundred seventy-six targets, 1 target, and 35 CHD-related targets were collected respectively by using DisGeNET, TCMSP, and Drug Bank databases. A total of 1593 targets were saved for further work after deleting repetitions, and 127 potential targets were identified as the active ones for XFZYC in the treatment of CHD (Fig. 3).

To illustrate the functional relationship between the CHD-related targets in XFZYC, STRING platform was arranged to construct the PPI network (Fig. 4A). The PPI network of the key targets with the highest confidence (≥0.900) was obtained from the above PPI network and the independent nodes were then excluded (Fig. 4B). The latter network induced by XFZYC was composed of 111 nodes and 388 edges. The results of further network topology analysis showed that the average degree centrality value of the nodes was 6.99099 and there were 46 nodes with degree centrality value higher than that. Similarly, the average betweenness centrality value of the nodes was 0.01912 and there were 32 nodes with betweenness centrality value higher than that. With degree centrality ≥ 10, 30 key nodes were selected as the key targets of XFZYC, and the value of the key targets was evaluated by degree centrality, betweenness centrality and closeness centrality (Table S2, Supplemental Digital Content, http://links.lww.com/MD/O259). The above results showed that 30 key nodes played a more critical role in the XFZYC target network, including interleukin-6 (IL-6), signal transducer and activator of transcription 3 (STAT3), protein kinase B1 (AKT1), mitogen activated protein kinase 3, cellular tumor antigen p53 (TP53), vascular endothelial growth factor A (VEGFA), transcription factor p65 (RELA) and proto-oncogene c-Fos, and so on. Eight of the 30 key targets, that is estrogen receptor, thrombin, retinoic acid receptor RXR-alpha, RELA, AKT1, TP53, matrix metalloproteinase-9 (MMP9), and VEGFA, were regulated by more than 3 compounds and expressed in at least 4 herbs.

Enriched KEGG pathway analysis of these 30 targets was carried out by using ClueGO, a plug-in of Cytoscape. As shown in Figure 5, the results clarified that these key targets were significantly correlated with 79 signaling pathways such as proteoglycans in cancer, mitogen activated protein kinase signaling pathway, th17 cell differentiation, inflammatory bowel disease, non-small cell lung cancer, endometrial cancer, interleukin-17

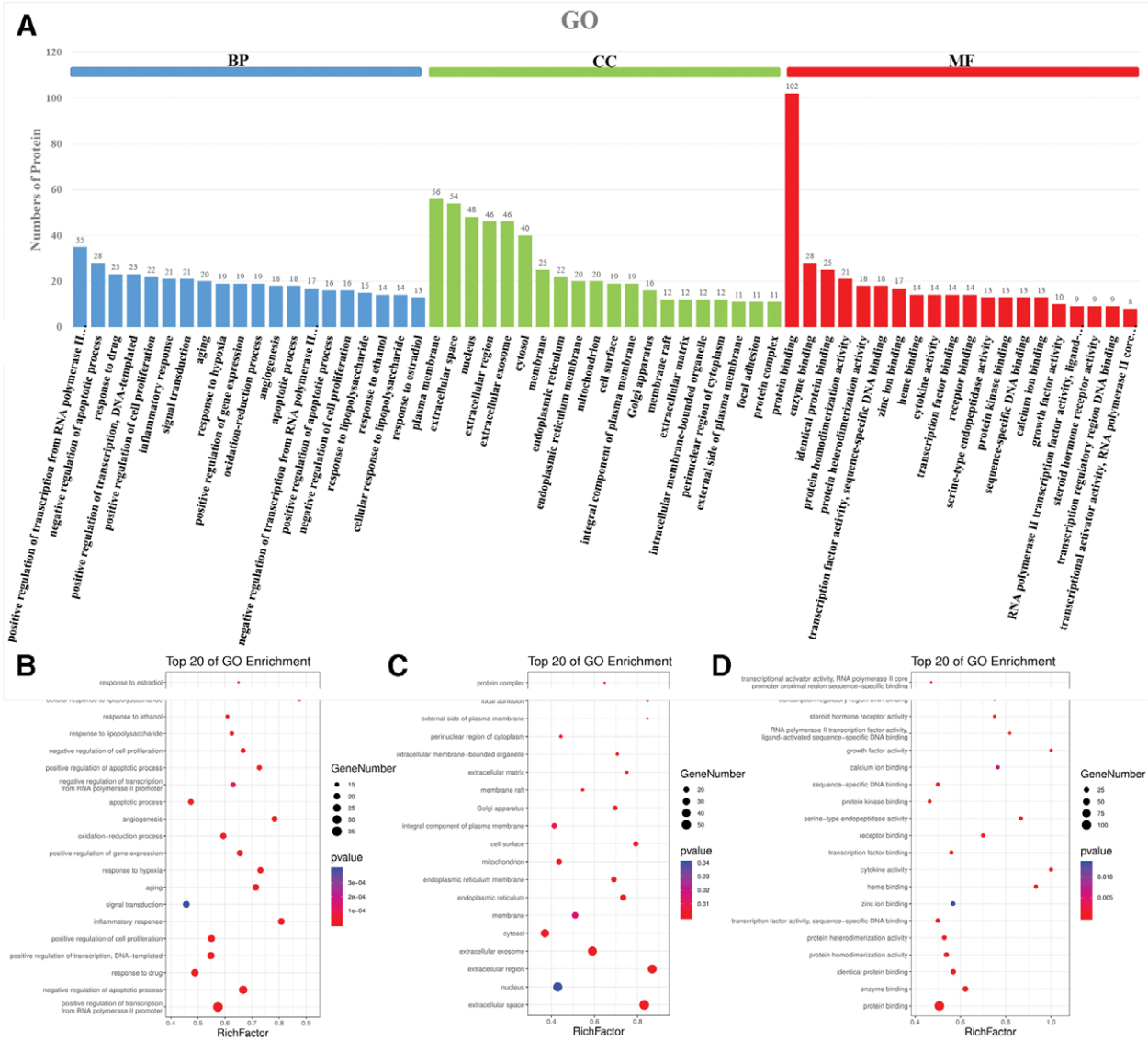

Figure 7. Gene Ontology enrichment analysis. (A) The overview bars for the first 20 terms of GO enrichment statistics. (B) The first 20 terms in BP item. (C) The first 20 terms in CC item. (D) The first 20 terms in MF item. Each bubble signals a GO term, and its size represents the gene number. The color of the bubble indicates the *P*-value.

signaling pathway, hypoxia-inducible factor-1 (HIF-1) signaling pathway, tumor necrosis factor signaling pathway, and relaxin signaling pathway. Sorted by degree value in descending order, mitogen activated protein kinases 3 were the targets that could regulated the largest number of signaling pathways in this networks.

All the above results indicated that XFZYC may play a targeted therapeutic role on CHD mainly through these hub targets by regulating numerous signaling pathways.

3.4. Network construction and network analysis

Eleven herbs of XFZYC, 139 compounds, 275 compound-related targets, 1 disease, 1593 disease-related targets, and 127 intersection targets were imported into Cytoscape_v3.7.1 Software, and drug–component–target–disease network of XFZYC was obtained (Fig. 6). The network topology analysis showed 1892 nodes and 7415 edges. The average degree centrality value of the nodes was 7.83827 and there were 198 nodes with degree centrality value higher than that. Similarly, the average betweenness centrality value of the nodes was 0.00078 and there were 111 nodes with the network, including degree centrality, betweenness centrality value higher than that. With degree centrality ≥ 4, drug–component–target–disease network was constructed with 337 nodes (10 herbs, 120 compounds, 79 compound-related targets, 1 diseases, and 127 intersection targets), indicating that the essence of disease is the imbalance of complex biological networks, and multiple components have synergistic relationship when acting on the same target. The intervention of TCM on disease has the characteristics of complex chemical composition and multi-target integrative regulation.

3.5. Pathway enrichment analysis of potential target genes

To clarify the diverse mechanisms of XFZYC from a systematic level, GO enrichment analysis for 127 common targets was performed. Figure 7A showed the top 20 terms of BP (blue bars), CC (green bars), and MF (red bars) in 1 column. These top 20 terms in the 3 categories were also illustrated in bubble chart forms respectively (Fig. 7B–D). As regards BP item, the targets of XFZYC related to CHD were significantly enriched in the response to positive regulation of transcription from RNA polymerase II promoter, response to negative

regulation of apoptotic process, response to drug, response to positive regulation of transcription, response to DNA-templated, response to positive regulation of cell proliferation, response to inflammatory response, response to signal transduction, and response to aging. As a key BP, positive regulation of transcription from RNA polymerase II promoter was simultaneously regulated by 35 direct targets. These results are helpful to elucidate the BP changes of the body after XFZYC treatment.

To further reveal the potential therapeutic mechanisms of XFZYC on CHD, KEGG pathway enrichment analysis on the 127 active targets was conducted and 172 signaling pathways were then screened with KappaScore ≥ 0.4 (Fig. 8). The overview diagram of the KEGG results was displayed in Figure 9A. The most significant KEGG items of the targets were shown in Figure 9B, including pathways in cancer, programmed death-ligand 1 (PD-L1) expression and programmed death cell receptor-1 (PD-1) checkpoint pathway in cancer, T cell receptor signaling pathway, FOXO signaling pathway, Ras signaling pathway, interleukin-17 signaling pathway, and VEGF signaling pathway in turn.

3.6. Molecular docking validation results

To verify the accuracy of the network analysis results, molecular docking models of the top 6 of the 30 key target proteins, which were obtained from drug–compound–target network mentioned in Section 3.3, were set up respectively. The root-mean-square deviation and -CDOCKER ENERGY value were calculated after the primitive ligand was extracted from the active pocket and the model was re-docked with the key compounds. As is shown in Table 2, the root-mean-square deviation values of STAT3, IL-6, AKT1, mitogen activated protein kinases 3, VEGFA, and TP53 were all <2Å, meaning that the binding mode between the primitive ligand and the protein could be well duplicated, and the docking result had high reliability (Table S3, Supplemental Digital Content, http://links.lww.com/MD/O259). Figure 10 showed the interaction diagrams of molecular docking models between the core target proteins and the active compounds with the best docking effect. Further analysis showed that among the 19 potential core compounds, 42.11 % had higher docking activity with IL-6, and 36.84 % had higher docking activity with AKT1. Similarly, 31.58 % had higher docking activity with STAT3 and TP53, and 26.32 % had higher docking activity with mitogen activated protein kinases 3. VEGFA held the least number of compounds, accounting for 21.05 %. Thus, the composition of XFZYC has good binding activity with the potential core targets, which suggests that XFZYC may have anti-AS effect by acting on the potential core targets.

4. Discussion

By studying the effects of TCM on diseases, TCM network pharmacological analysis is being a research method to reveal the relationship between diseases and complex components. Although XFZYC has been used for a long time in the treatment of CHD in China, the functional mechanism is still unclear. This study was designed to study the components and targets of XFZYC on CHD by using the method of network pharmacology.

Our network pharmacology study showed that XFZYC with 11 herbs contained 139 compounds and 275 genes, 127 of which were the potential genes related to CHD. Further network topology analysis and KEGG pathway enrichment analysis of these 127 overlapped genes indicated that XFZYC had 30 key targets that regulated 79 major signaling pathways, such as proteoglycans in cancer, mitogen activated protein kinase signaling pathway, th17 cell differentiation, inflammatory bowel disease, non-small cell lung cancer, endometrial cancer, interleukin-17 signaling pathway, HIF-1 signaling pathway, tumor necrosis factor signaling pathway, and relaxin signaling pathway. The 30 key targets included STAT3, IL-6, AKT1,

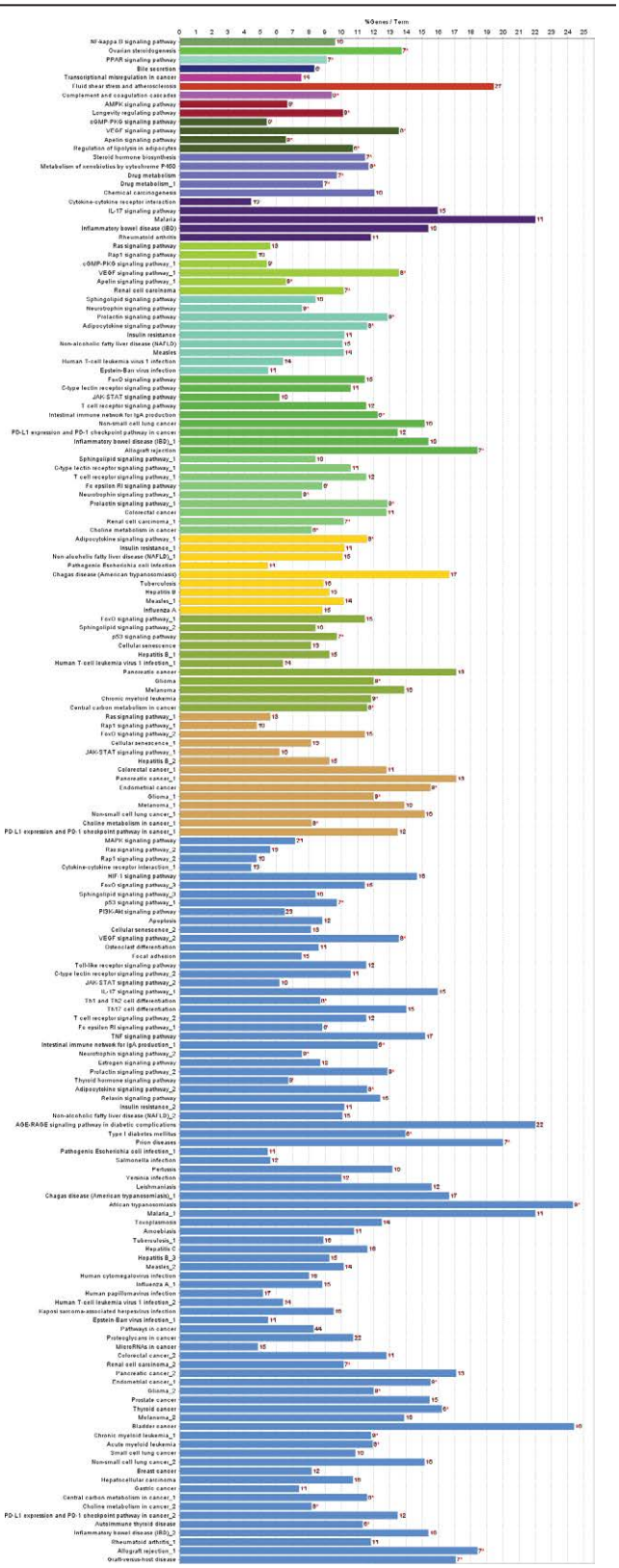

Figure 8. KEGG enrichment analysis. The bar's length represents the percentage of targets in the pathway terms. *$P < .05$, **$P < .01$. KEGG = Kyoto Encyclopedia of Genes and Genomes.

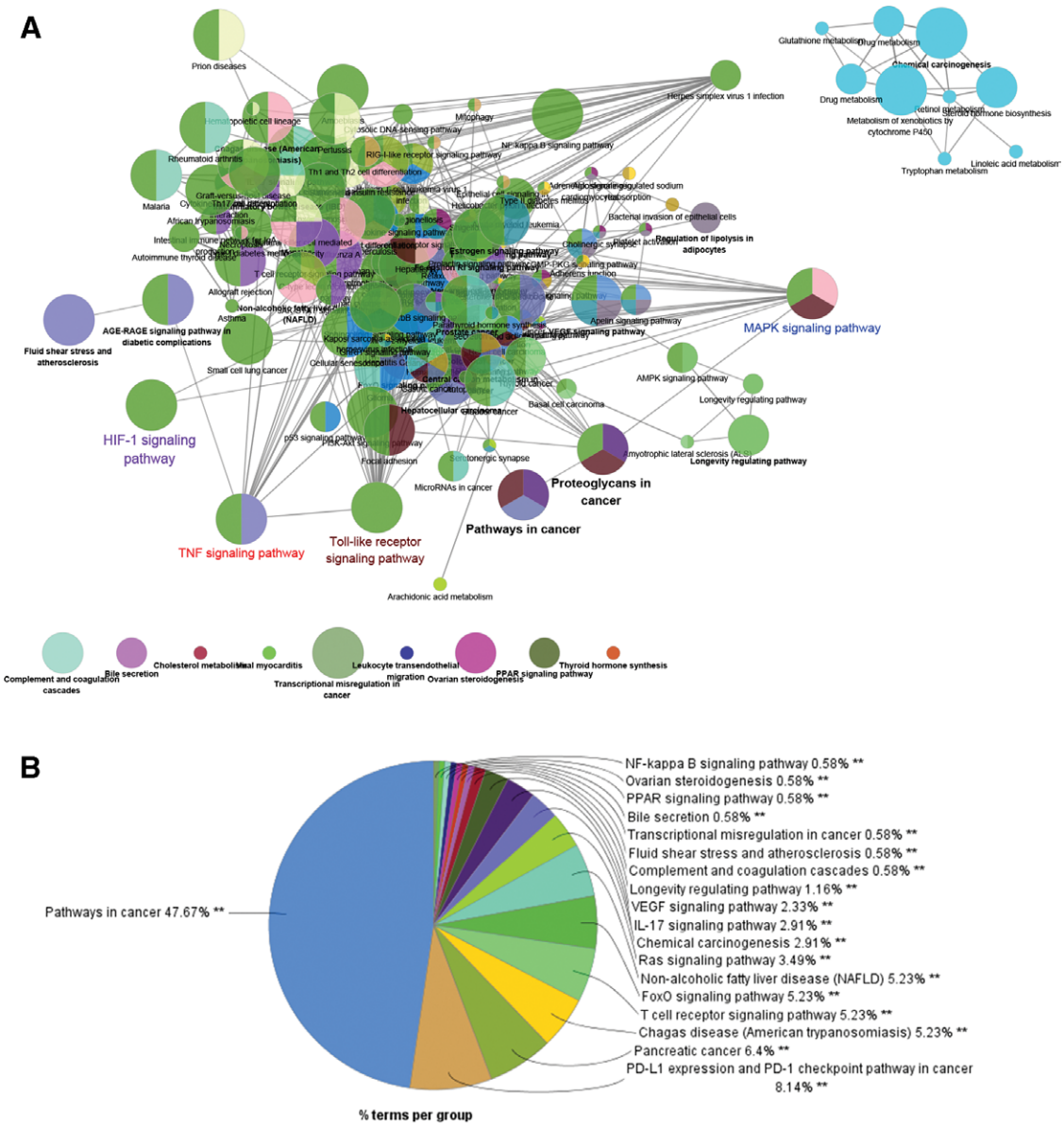

Figure 9. KEGG pathway mapping. (A) The specific clusters were generated by the functional groups of the target genes with the tools of ClueGO and CluePedia. Nodes represent the enrichment pathways, the connecting lines between nodes represent the common gene number between pathways, and the node colors represent the grouping information. (B) The pie chart reveals the proportion of each group related to the 127 targets. KEGG = Kyoto Encyclopedia of Genes and Genomes.

mitogen activated protein kinases 3, VEGFA, TP53, RELA, and so on. In this complex network, mitogen activated protein kinases 3, AKT1, RELA, mitogen activated protein kinases 8, IL-6, and TP53 involved in more regulatory pathways. After data association analysis of herbs, compounds and targets, we concluded that 8 of the 30 key targets (estrogen receptor, thrombin, RXR-alpha, RELA, AKT1, TP53, MMP9, and VEGFA) were controled by more than 3 compounds and expressed in at least 4 herbs, respectively. Among the 8 genes, AKT1, RELA, and TP53 were the overlapped ones in the above 2 situations. Hence, we maintained that the 3 genes may be the most critical genes for XFZYC to play a targeted therapeutic role on CHD. The success of the simulated molecular docking test also showed that the active compounds had perfect binding activity with the target, indicating that the data obtained in our study was more accurate and had strong clinical guiding significance.

GO enrichment analysis described the targets of XFZYC in BP, CC, and MF. When it refers to CC, more related targets of XFZYC were located in the plasma membrane, which could also be called the cell membrane. When the product of a gene is secreted from a cell into the tissue fluid or blood, it must be transmitted outward through the plasma membrane under the action of a membrane receptor, which is usually annotated as the plasma membrane. For instance, phosphorylation is the most significant posttranslational modification of its activity in the physiological process of CHD, and the key to AKT activation is to transport it to the plasma membrane. This translocation requires a pH domain and it is influenced by wortmannin. After activation, AKT is separated from the plasma membrane and transferred to the cytoplasm and nucleus.[30] The above process is known as the classic PI3K-dependent AKT activation. Once activated by insulin, platelet-derived growth factor, VEGF, and epidermal growth factor in cardiovascular system, these growth factors could regulate AKT activity through transcription or translation mechanism, thus affecting the structure and function of heart, promoting cell survival, regulating vasomotor tension, and endothelial cell proliferation.[31,32] CHD is an inflammatory process in which many inflammatory cytokines

Table 2

Related information of molecular docking models.

Target name	PDB ID	RMSD (Å)	The number of successful components in active components	The informations of the successful components	
				Name	-CDOCKER ENERGY
STAT3	1Q1M	0.3946	6 (31.58 %)	2,3-DidehydroGA70	-74.0923
				Beta-carotene	-67.0335
				Stigmasterol	-47.7636
				Beta-sitosterol	-36.4334
				Licoagrocarpin	-14.1378
				Shinpterocarpin	-2.43328
IL-6	1ALU	1.1916	8 (42.11 %)	Beta-carotene	-98.301
				2,3-DidehydroGA70	-73.5741
				Stigmasterol	-57.6296
				Beta-sitosterol	-49.9751
				Licoagrocarpin	-22.4029
				Shinpterocarpin	-12.4906
				Nobiletin	-5.16498
				Medicarpin	-4.68937
AKT1	1UQN	0.9147	7 (36.84 %)	Beta-carotene	-77.0616
				2,3-DidehydroGA70	-65.1501
				Stigmasterol	-55.909
				Beta-sitosterol	-41.1766
				Licoagrocarpin	-17.1256
				Shinpterocarpin	-6.66162
				Medicarpin	-0.657855
MAPK3	4QTB	0.7291	5 (26.32 %)	2,3-DidehydroGA70	-67.6397
				Beta-carotene	-61.0223
				Stigmasterol	-40.1457
				Beta-sitosterol	-33.0366
				Licoagrocarpin	-5.9953
VEGFA	3BDY	1.2769	4 (21.05 %)	2,3-DidehydroGA70	-62.3588
				Licoagrocarpin	-18.9543
				Nobiletin	-13.3043
				Shinpterocarpin	-3.44626
TP53	6GGA	0.4144	6 (31.58 %)	2,3-DidehydroGA70	-71.1819
				Beta-carotene	-69.989
				Stigmasterol	-52.8912
				Beta-sitosterol	-43.185
				Licoagrocarpin	-12.2798
				Shinpterocarpin	-3.61156

AKT1 = protein kinase B1, IL-6 = interleukin-6, MAPK3 = mitogen activated protein kinases 3, STAT3 = signal transducer and activator of transcription 3, TP53 = cellular tumor antigen p53, VEGFA = vascular endothelial growth factor A.

are secreted from cells into the blood and divide these target areas into plasma membranes. The results of our GO analysis indicated that XFZYC might have the function of regulating these processes.

By analyzing the MF item of the target genes, we further learned that the BP item of its main components. The main MF item of XFZYC was to regulate protein binding and enzyme binding, and thus affect cell apoptosis and alleviating inflammatory response. This result was consistent with the BP item. The transcription process in organisms was initiated primarily by the combination of transcription factors and RNA polymerase II with downstream DNA to form transcriptional initiation complex.

KEGG results showed that PD-L1 expression and PD-1 checkpoint pathway in cancer, T cell receptor signaling pathway, FOXO signaling pathway, Ras signaling pathway, interleukin-17 signaling pathway, and VEGF signaling pathway were the main signaling pathways regulated by the target genes. PD-L1 is one of the ligands of PD-1, a member of the immune receptor superfamily. Studies have shown that T cells regulates the progression of AS, during which down-regulation of PD-L1 expression can reduce the immunosuppressive effect of the PD-1/PD-L1 signaling pathway and enhance the function of T cells to the patients with AS. The immune activation state of the body was induced and PD-L1 gradually played a certain role in promoting plaque progress, inducing the stable condition of the plaque.[33] T cell receptor signaling pathway can influence the formation of T cell immune pool by changing the level of ox-LDL, making T cell clone expanded and activated, causing a series of immune responses, and eventually leading to persistent chronic inflammation.[34] FOXO signaling pathway can regulate lipid metabolism and affect vascular endothelial matrix remodeling.[35] Reactive oxygen species signaling pathway is able to induce the increase of intracellular reactive oxygen species and regulate the function of gap junction proteins in vascular endothelial cells.[36,37] Interleukin-17 signaling pathway may reduce plaque area and stabilize plaque.[38,39] VEGF signaling pathway plays a role in initiating angiogenesis and mediating cascade signaling and autophagy system.[40,41] Therefore, our results have led the perspective to consider the possibility that the intervention effect of XFZYC on CHD could be through regulating the above signaling pathways.

The results of KEGG enrichment also showed that P13K–AKT signaling pathway, nuclear factor kappa-B signaling pathway, HIF-1 signaling pathway and other pathways associated with immune response and inflammation were significantly abundant. Inflammatory reaction is an important mechanism for the occurrence of AS, which is the basis of the formation of CHD, and vascular endothelial injury is a key initiating step in the occurrence and development of CHD.[42–44] When vascular

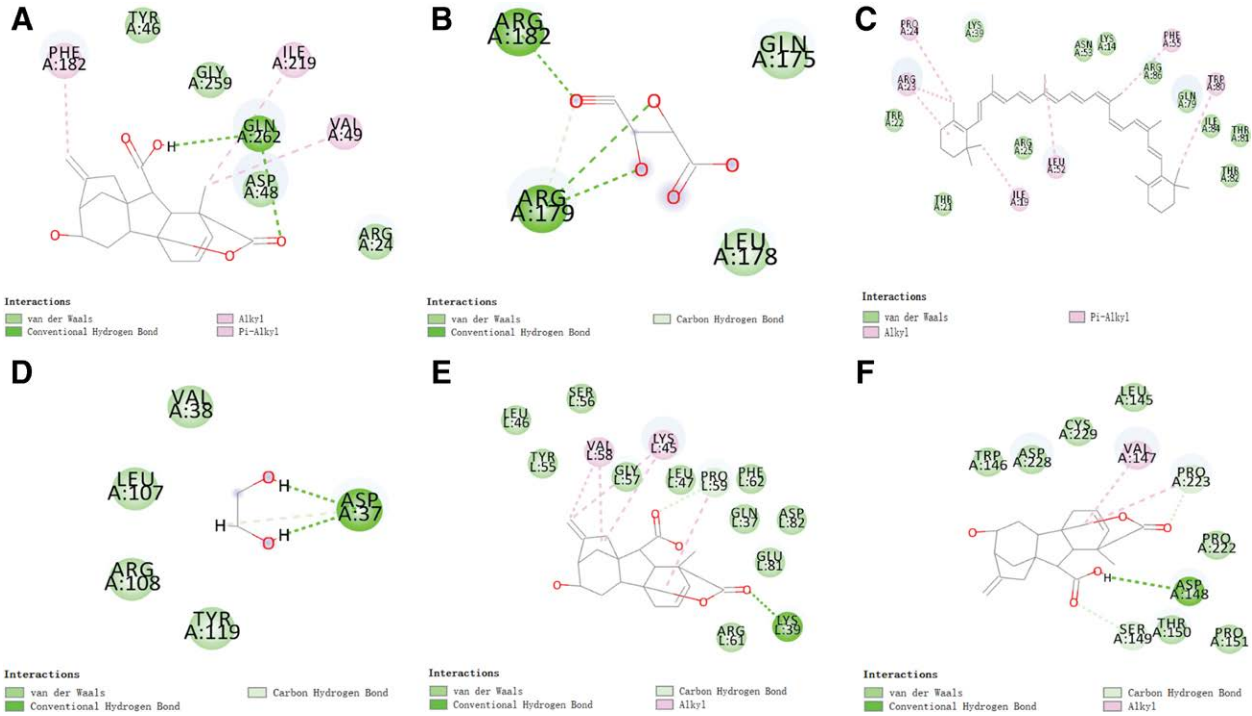

Figure 10. 3D structures of the molecular docking models and target verification with the best docking score. (A) High-resolution crystal structure of STAT3 (PDB_ID: 1Q1M) and the interaction diagram of STAT3 and 2,3-didehydro GA70. (B) High-resolution crystal structure of IL-6 (PDB_ID: 1ALU) and the interaction diagram of IL-6 and beta-carotene. (C) High-resolution crystal structure of AKT1 (PDB_ID: 1UNQ) and the interaction diagram of AKT1 and beta-carotene. (D) High-resolution crystal structure of MAPK3 (PDB_ID: 4QTB) and the interaction diagram of MAPK3 and 2,3-didehydro GA70. (E) High-resolution crystal structure of VEGFA (PDB_ID: 3BDY) and the interaction diagram of VEGFA and 2,3-didehydro GA70. (F) High-resolution crystal structure of TP53 (PDB_ID: 6GGA) and the interaction diagram of TP53 and 2,3-didehydro GA70. The circle in each picture on the left of each group is the position of the active pocket.

endothelial cells are damaged by inflammatory factors, the lipid migration and deposition would be promoted by the destruction of endothelial integrity, which may transform macrophages into foam cells. The above factors, together with lipid deposition in the vascular wall, lead to AS and further enhance the expression of inflammatory cytokines, such as the release of IL-6 and the intervention of smooth muscle cells migration to the intima. AS was thus formed and it was gradually developed into CHD. These pathological processes may promote the progression of AS and eventually lead to CHD.

Finally, when combined the results of the above network pharmacology prediction and the virtual verification results of molecular docking, it can be seen that beta-sitosterol has different expression levels in 6 herbs of XFZYC, that is TR, HH, CS, DG, NX, and ZQ. When absorbed into the body, beta-sitosterol can intervene the signaling pathways by regulating the expression levels of AKT1 and MMP9 genes so as to exert the therapeutic effect of CHD (Fig. 11). The signaling pathways included PI3K–AKT signaling pathway, AMPK signaling pathway, HIF-1 signaling pathway, janus kinase (JAK)–STAT signaling pathway, VEGF signaling pathway, and so on.

AKT1 is 1 of the 3 isoforms of AKT and the main medium of downstream action of PI3K, playing a key role in various BPs such as glucose metabolism, cell cycle regulation, transcriptional regulation, and so on.[30,45] AKT1 has been proved to be involved in the occurrence and development of inflammation, cancer, diabetes, and cardiovascular diseases.[46–50] MMP9 is an important member of the matrix metallo proteinases family, a zinc-dependent endopeptidase family that can degrade extracellular matrix components. It has been manifested that MMP9 is involved in the synthesis and degradation of extracellular matrix and the regulation of inflammatory mediators, resulting in myocardial remodeling and thus leading MMP9 a therapeutic target to stabilize AS plaque.[51] PI3K is an intracellular signaling protein with catalytic activity, which can phosphorylate the downstream signaling protein AKT after binding to it. These 2 signaling factors together constitute the PI3K–AKT signaling pathway, which can influence the formation and development of AS by regulating the migration, transformation and adhesion of vascular endothelial cells, monocytes, and macrophages.[52–55] Phosphorylated AKT can also up-regulate the expression level of MMP9 and promote endothelial cell injury and migration. MMP9 may consequently be a much crucial molecular target of the PI3K–AKT signaling pathway. The expression level of MMP9 is promoted by the abnormal activation of PI3K–AKT signaling pathway. Nuclear factor kappa-B is activated and inflammatory cytokines are released later, inducing discomfort such as chest pain.[56] It can be demonstrated that PI3K–AKT signaling pathway plays a critical role in the treatment of CHD and XFZYC can influence PI3K–AKT signaling pathway by regulating the core targets (such as AKT1 and MMP9) in this progression.

In our study, the possible bioactive components in XFZYC and the potential pharmacological mechanism of XFZYC in treating CHD were systematically investigated by means of network pharmacology. As XFZYC a Chinese patent medicine that has a long history of safe use in Chinese clinical practice, its ingredients are either medicinal plants or edible diets. The potential advantages of TCM network pharmacology research strategies are obvious. However, the required data information from the databases is not so extensive and this research strategy only can be used for predictions, which makes it have certain limitations. Hence, relevant experimental studies from the level of genes and proteins will be confirmed in the future to further confirm the results of our study.

5. Conclusions

This study manifested that the network pharmacological analysis does have the advantage in systematically exploring the interventions of Chinese herbal medicines in the treatment of

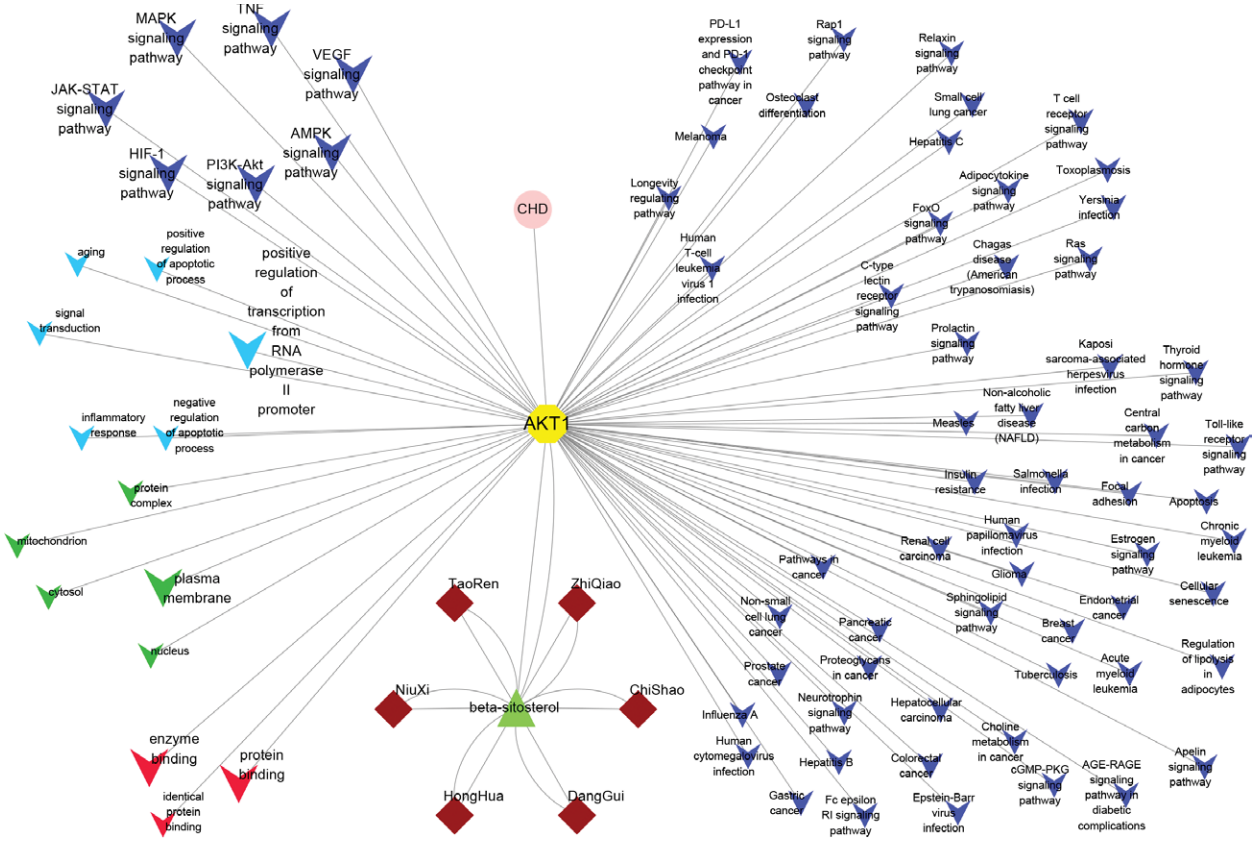

Figure 11. Related component and enrichment pathways network of AKT1. Brown nodes represent drugs, green node represents component, yellow node represents AKT1, pink node represents disease, blue nodes represent biological process of GO enrichment, dark green nodes represent cellular component of GO enrichment, red nodes represent molecular function of GO enrichment, and dark blue nodes represent KEGG enrichment pathways. Abbreviation: GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes.

complex diseases such as CHD. In this study, we screened the potential targets and the key compounds for XFZYC to CHD by using network topology analysis, and the core targets related to CHD were stood out in PPI network. A good pairing relationship between the receptor (the core compounds) and the ligand (the core targets) was then verified by using molecular docking technique. GO and KEGG enrichment analysis were then used to analyze the multi-target mechanism of XFZYC in the treatment of CHD. This network pharmacological study can provide a scientific basis for further study on the therapeutic effect of XFZYC on CHD.

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