Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Exploring the Pharmacological Mechanisms of *Tripterygium Wilfordii* Hook F against Cardiovascular Disease Using Network Pharmacology and Molecular Docking

Bingwu Huang
Wenzhou Medical University Second Affiliated Hospital https://orcid.org/0000-0001-8087-3554
Chengbin Huang
Wenzhou Medical University Second Affiliated Hospital
Liuyan Zhu
Wenzhou People's Hospital
Lina Xie
Wenzhou People's Hospital
Yi Wang
Wenzhou People's Hospital
Ning Zhu (zhuningccc@126.com)
Wenzhou People's Hospital https://orcid.org/0000-0002-7521-6266

Research

Keywords: CVD, Molecular Docking, Network Pharmacology, TwHF

Posted Date: January 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-151905/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Background

Tripterygium wilfordii Hook F (TwHF) has been used in traditional Chinese medicines (TCM) for treating cardiovascular disease (CVD). However, the underlying pharmacological mechanisms of the effects of TwHF against CVD remain elusive. This study revealed the pharmacological mechanisms of TwHF acting on CVD based on a pharmacology approach.

Materials and Methods

The active compounds were selected by Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) according to the absorption, distribution, metabolism, and excretion (ADME). The potential targets of TwHF were obtained by SwissTargetPrediction database. The CVD-related therapeutic targets were collected by the DrugBank, the GeneCards database and the OMIM database. Protein–protein interaction (PPI) network was generated by STITCH database. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed by R package. The network of drug-targets-diseases-pathways was constructed by Cytoscape software.

Results

The 51 effective ingredients of TwHF and the 178 common targets of TwHF and CVD-related were collected. Furthermore, AKT1, amyloid precursor protein (APP), Mitogen-activated protein kinase 1 (MAPK), phosphatidylinositol 3-kinase catalytic subunit alpha (PIK3CA) and cellular tumor antigen p53 (TP53) was identified the core targets involved in the mechanism of TwHF on CVD. Top ten GO (biological processes, cellular components and molecular functions) and KEGG pathways were screened with a P value ≤ 0.01. Finally, we constructed the network of TwHF-targets-CVD-GO-KEGG.

Conclusions

These findings demonstrate that the main active compound of TwHF, the core targets and pathways maybe provide new insights into the development of a natural therapy for the prevention and treatment of CVD.

Background

Cardiovascular disease (CVD) is a collective term for cardiovascular and cerebrovascular diseases, which is the first cause of death in the world[1]. The burden of CVD is on the rise globally, especially for low and middle income countries (LMIC)[2, 3]. In 2013, the World Health Organization (WHO) proposed that countries should reduce premature mortality that related to non-communicable diseases, including CVD,

by 25% by 2025[4]. Although Western medicine have made good progress in reducing the risk of cardiovascular events and total mortality, patients with long-term cardiovascular treatment still have difficult in adherence that might lead to discontinuation of these drugs. This can be attributed to the adverse reactions caused by multiple pharmacologic agents and some drugs that beyond the affordability of LMIC[3, 5]. Traditional Chinese Medicine (TCM), with thousands of years of history in China, has gained widespread clinical applications. In particular, TCM occupies a special position in their heart of the elderly. As a critical component of complementary and alternative medicine, TCM medications has been used for prevention and treatment of CVD[6].

Tripterygium wilfordii Hook F (TwHF), also known as Leigongteng and Thunder God Vine, has possesses many pharmacological activities such as anti-cancer, anti-inflammation, anti-fibrosis, anti-atherosclerosis and anti-autoimmune disorders[7–9]. Recently, several fundamental researches have indicated that low-dose TwHF can prevent cardiovascular diseases. Low-dose TwHF can improve the inflammatory reaction, reduce myocardial injury, and optimize acute coronary syndrome (ACS) rat's condition with inhibition of myocardial apoptosis[10]. TwHF extracts were shown to have cardioprotection effects by inducing the activation of Nrf2/HO-1 defense pathway, inhibiting the activation of NF-KB pathway and reducing the expression of NLRP3 inflammasome[11–13]. In addition, extracts can not only improve the vascular function in atherosclerosis, but may also help in the prevention of in-stent restenosis formation following endovascular treatment of lower-extremity artery disease[14, 15]. However, the underlying pharmacological mechanisms of the effects of TwHF against CVD remain elusive.

Network pharmacology is an innovative way to analyze the complicated relationship between drug and disease at the system level, which can provide clues for discovering new drug[16]. This approach integrates and constructs the complicated networks among drug targets, disease targets, and biological processes[17]. It is possible to reveal potential drug-target-disease interactions and realize novel therapeutic application beyond the traditional TCM application through network pharmacology [18]. In this study, target prediction, pharmacokinetic evaluation, molecular structure, biological function and pathway analysis using many available public databases and bioinformatics tools, have systematically elucidated the mechanisms of therapeutic effects of TwHF on CVD (Fig. 1).

Results

Active ingredients screening

Total of 51 effective ingredients of TwHF that satisfied DL ≥ 0.18 and OB ≥ 30% were screened from TCMSP. Among them, only 41 candidate compounds have the 2D structure, SMILES and PubChem ID (Table 1).

Table 1
A list of the final selected compounds from TwHF for network analysis.

Molecule ID	Molecule Name	Structure	OB(%)	DL
MOL003233	Triptofordin B2		107.71	0.76
MOL003209	Celallocinnine		83.47	0.59
MOL003188	Tripchlorolide		78.72	0.72
MOL003206	Canin		77.41	0.33
MOL003225	Hypodiolide A		76.13	0.49
MOL003279	99694-86-7		75.23	0.66
MOL003208	Celafurine		72.94	0.44
MOL003244	Triptonide		68.45	0.68
MOL005828	nobiletin		61.67	0.52
MOL002058	40957-99-1		57.2	0.62
MOL003217	Isoxanthohumol		56.81	0.39
MOL003224	Tripdiotolnide		56.4	0.67
MOL000211	Mairin		55.38	0.78
MOL003187	triptolide		51.29	0.68
MOL003280	TRIPTONOLIDE		49.51	0.49
MOL003185	(1R,4aR,10aS)-5-hydroxy-1-(hydroxymethyl)-7-isopropyl-8-methoxy-1,4a-dimethyl-4,9,10,10a-tetrahydro-3H-phenanthren-2-one		48.84	0.38
MOL003248	Triptonoterpene		48.57	0.28
MOL003196	Tryptophenolide		48.5	0.44
MOL003211	Celaxanthin		47.37	0.58
MOL003267	Wilformine		46.32	0.2
MOL003184	81827-74-9		45.42	0.53
MOL011169	Peroxyergosterol		44.39	0.82
MOL000449	Stigmasterol		43.83	0.76
MOL003245	Triptonoditerpenic acid		42.56	0.39
MOL000422	kaempferol		41.88	0.24
MOL003231	Triptoditerpenic acid B		40.02	0.36

Molecule ID	Molecule Name	Structure	OB(%)	DL
MOL003232	Triptofordin B1		39.55	0.84
MOL000296	hederagenin		36.91	0.75
MOL000358	beta-sitosterol		36.91	0.75
MOL003222	Salazinic acid		36.34	0.76
MOL003189	WILFORLIDE A		35.66	0.72
MOL003229	Triptinin B		34.73	0.32
MOL003266	21-Hydroxy-30-norhopan-22-one		34.11	0.77
MOL003238	Triptofordin F1		33.91	0.6
MOL003239	Triptofordin F2		33.62	0.67
MOL003278	salaspermic acid		32.19	0.63
MOL003235	Triptofordin D1		32	0.75
MOL003241	Triptofordin F4		31.37	0.67
MOL003242	Triptofordinine A2		30.78	0.47
MOL003236	Triptofordin D2		30.38	0.69
MOL003210	Celapanine		30.18	0.82

Targets Identification of TwHF and CVD

In total, 827 candidate targets for TwHF were identified using SwissTargetPrediction (Supplementary Table). 76 known CVD targets were found from the DrugBank database, 358 known CVD targets were collected from the GeneCards database, and 474 known CVD-related targets were obtained from the OMIM database (Supplementary Table). Finally, 802 CVD-related targets were identified by removing the repeated targets. The 178 potential targets were collected for subsequent analysis after comparing the targets of CVD and TwHF (Supplementary Table).

PPI Network Construction and Analysis

The PPI network was generated by uploading these 178 identified targets to the STITCH database (Figure 2). AKT1, amyloid precursor protein (APP), Mitogen-activated protein kinase 1 (MAPK), phosphatidylinositol 3-kinase catalytic subunit alpha (PIK3CA) and cellular tumor antigen p53 (TP53) was identified based on the highest interactive scores and the most interaction. These five genes were considered to be the key putative targets involved in the effects of TwHF on CVD.

GO and KEGG Pathway Enrichment Analyses

The 178 candidate targets were selected for GO and KEGG pathway enrichment analyses. The top ten GO analyses of biological process (BP), cellular component (CC), and molecular function categories (MF) were screened(Figure 3). As the results of GO enrichment, the enriched biological process categories were dominated by ERBB signaling pathway, regulation of generation of precursor metabolites and energy, peptidyl-serine phosphorylation, aging, peptidyl-serine modification, regulation of developmental growth, neuron death, regulation of DNA metabolic process, cellular response to peptide, and response to oxidative stress. Cell component analysis showed that spindle mainly accounted for the largest proportion. The enriched molecular function categories were dominated by phosphatase binding and protein serine/threonine kinase activity.

The KEGG pathway analysis showed that these targets were mainly associated with cancer, melanoma, platinum drug resistance, glioma, chronic myeloid leukemia, endocrine resistance, sphingolipid signaling pathway, neurotrophin signaling pathway, thyroid hormone signaling pathway, apoptosis, cellular senescence, hepatitis C, and hepatitis B (Figure 4).

Construction of network

The network visualization of TwHF-targets-CVD-GO-KEGG were generated by using Cytoscape software (Figure 5).

Molecular Docking

The crystal structures of potential targets, including AKT1 (PDB: 6CCY), APP (PDB:5BUO), MAPK1 (PDB:6SIG), PIK3CA (PDB:4TTU) and TP53 (PDB: 6RZ3) were collected from the RCSB Protein Data Bank (Figure 6). Figure 6 showed celaxanthin binds to AKT1 with a binding pocket consisting of SER-240 (2.9 Å); hypodiolide A fails to bind to APP without a binding pocket; triptofordin B2 binds to MAPK1 with a binding pocket consisting of SER-153 (3.3 Å) and ARG-155 (3.3 Å); triptofordin B2 binds to PIK3CA with a binding pocket consisting of GLN-582 (3.1 Å); Celallocinnine binds to TP53 with a binding pocket consisting of LEU-111 (3.2 and 3.0 Å), ASN-131 (3.1 Å) and TYR-126 (2.9 Å).

Discussion

There is an urgent need to promote new drugs for CVD treatment because of the heavy burden of CVD and the poor efficacy and side effects of the currently used medicines. Network pharmacology was applied to reveal the interaction between medicines and targets of diseases, and it can comprehensively describe the complexities between drugs and diseases[19, 20]. Therefore, the use of network pharmacology uncovering multiple drug-target interactions may contribute to novel drug discovery in complex disease such as CVD. TwHF exhibits therapeutic efficacy in preclinical models of CVD has been identified in several studies[21–23]. In the present study, the underlying mechanisms of protective effects of TwHF on CVD was uncovered by a network pharmacology strategy. Therapeutic targets and the signaling pathways were investigated by databases screening, PPI network construction, and pathway

enrichment analysis. Furthermore, to validate the specific interactions between core targets and CVD, molecular docking was conducted.

In this study, 51 active compounds of TwHF were determined based on ADME. Pharmacological analysis suggested that these active components may have protective effects on CVD. Nobiletin has been reported to attenuate hypoxia/reoxygenation-induced injury by the inhibition of oxidative stress and apoptosis in H9c2 cardiomyocytes, as well as myocardial ischemia and reperfusion injury in vivo[24, 25]. Triptonide ameliorates diabetic cardiomyopathy via mediating inflammation[26, 27]. Isoxanthohumol regulate vivo vascular proliferation in vivo the -inflammatory crosstalk of vascular cells, contributing to the treatment of angiogenesis and inflammation-related diseases[28]. Stigmasterol blocked Ang II-induced aortic smooth muscle cell proliferation by the arrest of the cell-cycle and promoting apoptosis and ROS production[29]. Kaempferol attenuates cardiac hypertrophy and isoproterenol-induced heart failure in diabetic rats[30, 31].

Subsequently, targets of TwHF and CVD were also identified. 178 common candidate targets between TwHF and CVD were selected. Finally, we screened 5 cores candidate genes for further analysis. The interactive values and interaction indicate that these targets are tightly contact with other targets in “CVD-target PPI network” and responsible for TwHF actiong on CVD and pathogenesis of CVD. As was well known,Akt signaling play an important role in many processes of CVD pathology such as atherosclerosis, vascular remodeling, and cardiac hypertrophy. Several Akt inhibitors have been proven to be potential novel therapeutics for the CVD[32]. PIK3R1, MAPK1 and PIK3CA may modulate platelet activation and be involved in CVD[33]. Class I phosphatidylinositol 3-kinases (PI3Ks) are composed of a regulatory subunit (p85 regulatory subunit) and a catalytic subunit (p110 catalytic subunit)[34, 35]. The catalytic subunit p110α of PI3K is encoded by the gene PIK3CA, which regulates doxorubicin-induced cardiotoxicity[36]. Indeed, the compounded cardiovascular risk of PI3Kα inhibitor use in breast cancer, is particularly relevant given the prevalence of p110α gain-of-function mutations[37]. p38 mitogen activated protein kinase (p38), extracellular signal-regulated kinase1/2 (ERK), and c-Jun NH2 terminal protein kinase (JNK), are major components of MAPK kinases, which control embryogenesis, differentiation, proliferation, and death[38]. The inactivation of JNK, p38MAPK, and ERK1/2 could block vascular smooth muscle cells proliferation and migration[39–41]. APP is associated with the adhesion of platelet to amyloid peptides and thrombus formation[42, 43]. It was reported TP53 can differentiate patient with left main coronary artery disease (CAD) from patients healthy participants[44].

Top ten GO of each category (BP, MF, CC) and KEGG pathway associated with TwHF acting on CVD were classified. The data showed that the major components were significantly related with multiple BPs, such as ERBB signaling pathway, regulation of generation of precursor metabolites and energy, peptidyl-serine phosphorylation, aging, peptidyl-serine modification, regulation of developmental growth, neuron death, regulation of DNA metabolic process, cellular response to peptide, and response to oxidative stress. KEGG pathway enrichments analysis showed TwHF may exert protective effects on CVD mainly by cancer pathways. Cancer and cardiovascular disease (CVD) share overlapping pathophysiology and risk factors as well as biological mechanisms[45]. Protein–protein interactions analysis have varied roles in driving

and maintaining the growth of cancer and CVD[46, 47]. TwHF was also determined as efficacy treatment of multiple cancers[48–50].

According to screening criteria of high OB, Celaxanthin, Hypodiolide A, Triptofordin B2, Triptofordin B2, Celallocinnine were chosen for the compound-ligand interaction analysis by molecular docking to validate the effects of TwHF acting on CVD. The results of molecular docking reflected that these active compounds possess suitable anti-CVD activity. However, to verify the active properties of TwHF and the molecular target genes of anti-CVD, further experimental studies need to be performed.

Conclusion

In summary, the network pharmacology method was performed to unveil the chemical basis and investigate the action mechanism of TwHF on CVD. Firstly, 51 active compounds of TwHF and 5 core target genes (AKT1, APP, MAPK, PIK3CA and TP53) of TwHF against CVD were identified. Then based on the analysis of GO and KEGG, cancer pathway was found to be closely associated with the protective effect of TwHF on CVD. It provides a theoretical basis and a clue for the pharmacological mechanism study of TwHF on CVD in this study. However, further experimental studies of these prediction results are needed to validate potential application.

Methods

Active Components Screening

Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, https://tcmspw.com/tcmsp.php) is an efficient pharmacology resource, which can be used to assess the pharmacokinetics of TCMs or related compounds[51]. It can provide the absorption, distribution, metabolism, and excretion (ADME) properties of compounds, the main indicators of which are oral bioavailability (OB) and drug similarity (DL). OB is a reliable indicator to evaluate the intrinsic quality of drugs objectively, which represents to the speed and degree of absorbing drugs into the circulatory system. And DL represents the sum of the pharmacokinetic properties and safety of compounds, which is calculated by comparing the functional or physical properties of compounds with those of the majority of known drugs[52]. In this paper, the compound name “leigongteng” was inputted to the TCMSP database and active ingredients with DL $\geq$ 0.18 and OB $\geq$ 30% were selected for subsequent analysis. Then SMILES and PubChem ID of candidate components were collected by using the Traditional Chinese Medicines Integrated Database (TCMID, http://www.megabionet.org/tcmid/)[53] and the PubChem (https://pubchem.ncbi.nlm.nih.gov/) database[54].

Targets Fishing

Identified and Predicted Targets of TwHF

The target of active components in TwHF were obtained from the SwissTargetPrediction (http://www.swisstargetprediction.ch), which is a free public resource used to accurately predict targets for bioactive molecules[55]. The therapeutic targets of active ingredients were predicted by inputting these components SMILES into SMILES string (s) and searching for their similar molecules. Within the range of “Homo sapiens”, high probability targets (probability $P < 0.05$) were collected after duplicate contents were removed.

Target Identification Of Known Therapeutic Targets Acting On Cvd

The CVD-related therapeutic targets were collected from the DrugBank (http://www.drugbank.ca)[56], the OMIM database (https://omim.org)[57], and the GeneCards database (https://www.genecards.org)[58]. DrugBank is a freely available network database, which provides molecular information about drugs, drug targets, drug effects, and drug interactions. OMIM database, a comprehensive web resource, is focusing on genes, genetic phenotypes, and their relationships. In addition, GeneCards is a public database that provides detailed information on annotated and predicted genes. With “cardiovascular disease” as the keyword, CVD-related targets were searched among three databases.

Protein–protein Interaction (ppi) Network Construction And Analysis

The identified targets were uploaded to the STITCH database v5.0 (http://stitch.embl.de/)[59] to build the protein–protein interaction network and clarify the functional and physical association between them. The protein interactions were limited to confidence score of 0.9 or higher. The core target genes were determined based on criterion of the highest interactive scores and the most interaction.

Construction Of Network Relationships

Cytoscape is a free application software, which can transform biomolecular interaction networks into a versatile and interactive visualization framework[62]. The core targets of TwHF against CVD were constructed for KEGG-GO enrichment visualization by Cytoscape (v3.7.1) software[63]. In interactive network, the nodes include TwHF, CVD and their core targets, GO and KEGG pathways. Then the edges represent the interaction between them.

Declarations

Acknowledgements

This study was supported by Zhejiang Provincial Natural Science Foundation of China under Grant no. LQ20H020011.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

Authors' contributions

Ning Zhu designed the study, and drafted and revised the manuscript. Bingwu Huang performed the research and wrote the manuscript. Chengbin Huang, Liuyan Zhu, Lina Xie and Yi Wang collected and analyzed the data. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors report no relationships that could be construed as a conflict of interest.

Author details

[1]Department of Cardiology, The Wenzhou Third Clinical Institute Affiliated To Wenzhou Medical University, The Third Affiliated Hospital of Shanghai University, Wenzhou People's Hospital, No. 299 Guan Road, Wenzhou 325000, Zhejiang Province, People's Republic of China. [2]Department of General Practice, The Wenzhou Third Clinical Institute Affiliated To Wenzhou Medical University, The Third Affiliated Hospital of Shanghai University, Wenzhou People's Hospital, No. 299 Guan Road, Wenzhou 325000, Zhejiang Province, People's Republic of China. [3]Department of Neurosurgery, The Wenzhou Third Clinical Institute Affiliated To Wenzhou Medical University, The Third Affiliated Hospital of Shanghai University, Wenzhou People's Hospital, No. 299 Guan Road, Wenzhou 325000, Zhejiang Province, People's Republic of China. [4]Department of Anesthesiology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, 109 Xueyuan West Road, Wenzhou 325000, Zhejiang Province, People's Republic of China. [5]Department of Orthopedic Surgery, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, 109 Xueyuan West Road, Wenzhou 325000, Zhejiang Province, People's Republic of China.

References

1. Olvera Lopez E, Ballard BD, Jan A. Cardiovascular Disease. StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2020, StatPearls Publishing LLC., 2020.
2. Dunbar SB, Khavjou OA, Bakas T et al. Projected Costs of Informal Caregiving for Cardiovascular Disease: 2015 to 2035: A Policy Statement From the American Heart Association, Circulation 2018;137:e558-e577.
3. Bansilal S, Castellano JM, Fuster V. Global burden of CVD: focus on secondary prevention of cardiovascular disease, Int J Cardiol 2015;201 Suppl 1:S1-7.
4. Joseph P, Leong D, McKee M et al. Reducing the Global Burden of Cardiovascular Disease, Part 1: The Epidemiology and Risk Factors, Circ Res 2017;121:677-694.
5. Leong DP, Joseph PG, McKee M et al. Reducing the Global Burden of Cardiovascular Disease, Part 2: Prevention and Treatment of Cardiovascular Disease, Circ Res 2017;121:695-710.
6. Hao P, Jiang F, Cheng J et al. Traditional Chinese Medicine for Cardiovascular Disease: Evidence and Potential Mechanisms, J Am Coll Cardiol 2017;69:2952-2966.
7. Zhao X, Liu Z, Ren Z et al. Triptolide inhibits pancreatic cancer cell proliferation and migration via down-regulating PLAU based on network pharmacology of Tripterygium wilfordii Hook F, Eur J Pharmacol 2020;880:173225.
8. Xue M, Jiang ZZ, Wu T et al. Anti-inflammatory effects and hepatotoxicity of Tripterygium-loaded solid lipid nanoparticles on adjuvant-induced arthritis in rats, Phytomedicine 2012;19:998-1006.
9. Choi BS, Sapkota K, Kim S et al. Antioxidant activity and protective effects of Tripterygium regelii extract on hydrogen peroxide-induced injury in human dopaminergic cells, SH-SY5Y, Neurochem Res 2010;35:1269-1280.
10. Peng WH, Chen GL, Zhou Y et al. Study of expression of circulating inflammatory factors in ACS rats with low dose of Tripterygium Wilfordii, Hellenic J Cardiol 2018;59:46-47.
11. Li X, Wu N, Zou L et al. Protective effect of celastrol on myocardial ischemia-reperfusion injury, Anatol J Cardiol 2017;18:384-390.
12. Li R, Lu K, Wang Y et al. Triptolide attenuates pressure overload-induced myocardial remodeling in mice via the inhibition of NLRP3 inflammasome expression, Biochem Biophys Res Commun 2017;485:69-75.
13. Yu H, Shi L, Zhao S et al. Triptolide Attenuates Myocardial Ischemia/Reperfusion Injuries in Rats by Inducing the Activation of Nrf2/HO-1 Defense Pathway, Cardiovasc Toxicol 2016;16:325-335.
14. Lu C, Yu X, Zuo K et al. Tripterine treatment improves endothelial progenitor cell function via integrin-linked kinase, Cell Physiol Biochem 2015;37:1089-1103.
15. Han B, Ge CQ, Zhang HG et al. Effects of tripterygium glycosides on restenosis following endovascular treatment, Mol Med Rep 2016;13:4959-4968.
16. Hopkins AL. Network pharmacology, Nat Biotechnol 2007;25:1110-1111.
17. Lu C, Bing Z, Bi Z et al. Top-100 Most Cited Publications Concerning Network Pharmacology: A Bibliometric Analysis, Evid Based Complement Alternat Med 2019;2019:1704816.

18. Li P, Chen J, Wang J et al. Systems pharmacology strategies for drug discovery and combination with applications to cardiovascular diseases, J Ethnopharmacol 2014;151:93-107.
19. Zhou W, Wang Y, Lu A et al. Systems Pharmacology in Small Molecular Drug Discovery, Int J Mol Sci 2016;17:246.
20. Song X, Zhang Y, Dai E et al. Prediction of triptolide targets in rheumatoid arthritis using network pharmacology and molecular docking, Int Immunopharmacol 2020;80:106179.
21. Ye S, Luo W, Khan ZA et al. Celastrol Attenuates Angiotensin II-Induced Cardiac Remodeling by Targeting STAT3, Circ Res 2020;126:1007-1023.
22. Der Sarkissian S, Cailhier JF, Borie M et al. Celastrol protects ischaemic myocardium through a heat shock response with up-regulation of haeme oxygenase-1, Br J Pharmacol 2014;171:5265-5279.
23. Cheng M, Wu G, Song Y et al. Celastrol-Induced Suppression of the MiR-21/ERK Signalling Pathway Attenuates Cardiac Fibrosis and Dysfunction, Cell Physiol Biochem 2016;38:1928-1938.
24. Liu F, Zhang H, Li Y et al. Nobiletin suppresses oxidative stress and apoptosis in H9c2 cardiomyocytes following hypoxia/reoxygenation injury, Eur J Pharmacol 2019;854:48-53.
25. Zhang BF, Jiang H, Chen J et al. Nobiletin ameliorates myocardial ischemia and reperfusion injury by attenuating endoplasmic reticulum stress-associated apoptosis through regulation of the PI3K/AKT signal pathway, Int Immunopharmacol 2019;73:98-107.
26. Guo X, Xue M, Li CJ et al. Protective effects of triptolide on TLR4 mediated autoimmune and inflammatory response induced myocardial fibrosis in diabetic cardiomyopathy, J Ethnopharmacol 2016;193:333-344.
27. Liang Z, Leo S, Wen H et al. Triptolide improves systolic function and myocardial energy metabolism of diabetic cardiomyopathy in streptozotocin-induced diabetic rats, BMC Cardiovasc Disord 2015;15:42.
28. Negrão R, Duarte D, Costa R et al. Isoxanthohumol modulates angiogenesis and inflammation via vascular endothelial growth factor receptor, tumor necrosis factor alpha and nuclear factor kappa B pathways, Biofactors 2013;39:608-622.
29. Li C, Liu Y, Xie Z et al. Stigmasterol protects against Ang II-induced proliferation of the A7r5 aortic smooth muscle cell-line, Food Funct 2015;6:2266-2272.
30. Feng H, Cao J, Zhang G et al. Kaempferol Attenuates Cardiac Hypertrophy via Regulation of ASK1/MAPK Signaling Pathway and Oxidative Stress, Planta Med 2017;83:837-845.
31. Zhang L, Guo Z, Wang Y et al. The protective effect of kaempferol on heart via the regulation of Nrf2, NF-κβ, and PI3K/Akt/GSK-3β signaling pathways in isoproterenol-induced heart failure in diabetic rats, Drug Dev Res 2019;80:294-309.
32. Abeyrathna P, Su Y. The critical role of Akt in cardiovascular function, Vascul Pharmacol 2015;74:38-48.
33. Yu G, Luo Z, Zhou Y et al. Uncovering the pharmacological mechanism of Carthamus tinctorius L. on cardiovascular disease by a systems pharmacology approach, Biomed Pharmacother

2019;117:109094.

34. Leevers SJ, Vanhaesebroeck B, Waterfield MD. Signalling through phosphoinositide 3-kinases: the lipids take centre stage, Curr Opin Cell Biol 1999;11:219-225.
35. Carpenter CL, Duckworth BC, Auger KR et al. Purification and characterization of phosphoinositide 3-kinase from rat liver, J Biol Chem 1990;265:19704-19711.
36. McLean BA, Patel VB, Zhabyeyev P et al. PI3Kα Pathway Inhibition With Doxorubicin Treatment Results in Distinct Biventricular Atrophy and Remodeling With Right Ventricular Dysfunction, J Am Heart Assoc 2019;8:e010961.
37. Network CGA. Comprehensive molecular portraits of human breast tumours, Nature 2012;490:61-70.
38. Pearson G, Robinson F, Beers Gibson T et al. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions, Endocr Rev 2001;22:153-183.
39. Wang Y, Zhang X, Gao L et al. Cortistatin exerts antiproliferation and antimigration effects in vascular smooth muscle cells stimulated by Ang II through suppressing ERK1/2, p38 MAPK, JNK and ERK5 signaling pathways, Ann Transl Med 2019;7:561.
40. Taniyama Y, Ushio-Fukai M, Hitomi H et al. Role of p38 MAPK and MAPKAPK-2 in angiotensin II-induced Akt activation in vascular smooth muscle cells, Am J Physiol Cell Physiol 2004;287:C494-499.
41. Kyotani Y, Zhao J, Tomita S et al. Olmesartan inhibits angiotensin II-Induced migration of vascular smooth muscle cells through Src and mitogen-activated protein kinase pathways, J Pharmacol Sci 2010;113:161-168.
42. Visconte C, Canino J, Guidetti GF et al. Amyloid precursor protein is required for in vitro platelet adhesion to amyloid peptides and potentiation of thrombus formation, Cell Signal 2018;52:95-102.
43. Canobbio I, Visconte C, Momi S et al. Platelet amyloid precursor protein is a modulator of venous thromboembolism in mice, Blood 2017;130:527-536.
44. Kolovou V, Tsipis A, Mihas C et al. Tumor Protein p53 (TP53) Gene and Left Main Coronary Artery Disease, Angiology 2018;69:730-735.
45. Tiwari S, Dwivedi UN. Discovering Innovative Drugs Targeting Both Cancer and Cardiovascular Disease by Shared Protein-Protein Interaction Network Analyses, Omics 2019;23:417-425.
46. Ivanov AA, Khuri FR, Fu H. Targeting protein-protein interactions as an anticancer strategy, Trends Pharmacol Sci 2013;34:393-400.
47. Sarajlić A, Janjić V, Stojković N et al. Network topology reveals key cardiovascular disease genes, PLoS One 2013;8:e71537.
48. Zhang C, He XJ, Li L et al. Effect of the Natural Product Triptolide on Pancreatic Cancer: A Systematic Review of Preclinical Studies, Front Pharmacol 2017;8:490.
49. Wen L, Chen Y, Zeng LL et al. Triptolide Induces Cell Apoptosis by Targeting H3K4me3 and Downstream Effector Proteins in KM3 Multiple Myeloma Cells, Curr Pharm Biotechnol 2015;17:147-160.

50. Fuchs O. Transcription factor NF-κB inhibitors as single therapeutic agents or in combination with classical chemotherapeutic agents for the treatment of hematologic malignancies, Curr Mol Pharmacol 2010;3:98-122.
51. Ru J, Li P, Wang J et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines, J Cheminform 2014;6:13.
52. Liu F, Li Y, Li M et al. Study on Mechanism of Iridoid Glycosides Derivatives from Fructus Gardeniae in Jiangxi Province by Network Pharmacology, Evid Based Complement Alternat Med 2020;2020:4062813.
53. Huang L, Xie D, Yu Y et al. TCMID 2.0: a comprehensive resource for TCM, Nucleic Acids Res 2018;46:D1117-d1120.
54. Xu J, Wang F, Guo J et al. Pharmacological Mechanisms Underlying the Neuroprotective Effects of Alpinia oxyphylla Miq. on Alzheimer's Disease, Int J Mol Sci 2020;21.
55. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules, Nucleic Acids Res 2019;47:W357-w364.
56. Wishart DS, Feunang YD, Guo AC et al. DrugBank 5.0: a major update to the DrugBank database for 2018, Nucleic Acids Res 2018;46:D1074-d1082.
57. Amberger JS, Hamosh A. Searching Online Mendelian Inheritance in Man (OMIM): A Knowledgebase of Human Genes and Genetic Phenotypes, Curr Protoc Bioinformatics 2017;58:1.2.1-1.2.12.
58. Stelzer G, Rosen N, Plaschkes I et al. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses, Curr Protoc Bioinformatics 2016;54:1.30.31-31.30.33.
59. Kuhn M, Szklarczyk D, Franceschini A et al. STITCH 2: an interaction network database for small molecules and proteins, Nucleic Acids Res 2010;38:D552-556.
60. Blake JA CK, Dolan ME, Drabkin HJ, Hill DP, Ni L, Sitnikov D, Burgess S, Buza T, Gresham C, McCarthy F. Gene Ontology Consortium: going forward, Nucleic Acids Res 2015;43:D1049-1056.
61. Kanehisa M, Furumichi M, Tanabe M et al. KEGG: new perspectives on genomes, pathways, diseases and drugs, Nucleic Acids Res 2017;45:D353-d361.
62. Shannon P, Markiel A, Ozier O et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks, Genome Res 2003;13:2498-2504.
63. Li R, Guo C, Li Y et al. Therapeutic targets and signaling mechanisms of vitamin C activity against sepsis: a bioinformatics study, Brief Bioinform 2020.
64. Zhang J, Li H, Zhang Y et al. Uncovering the Pharmacological Mechanism of Stemazole in the Treatment of Neurodegenerative Diseases Based on a Network Pharmacology Approach, Int J Mol Sci 2020;21.

Figures

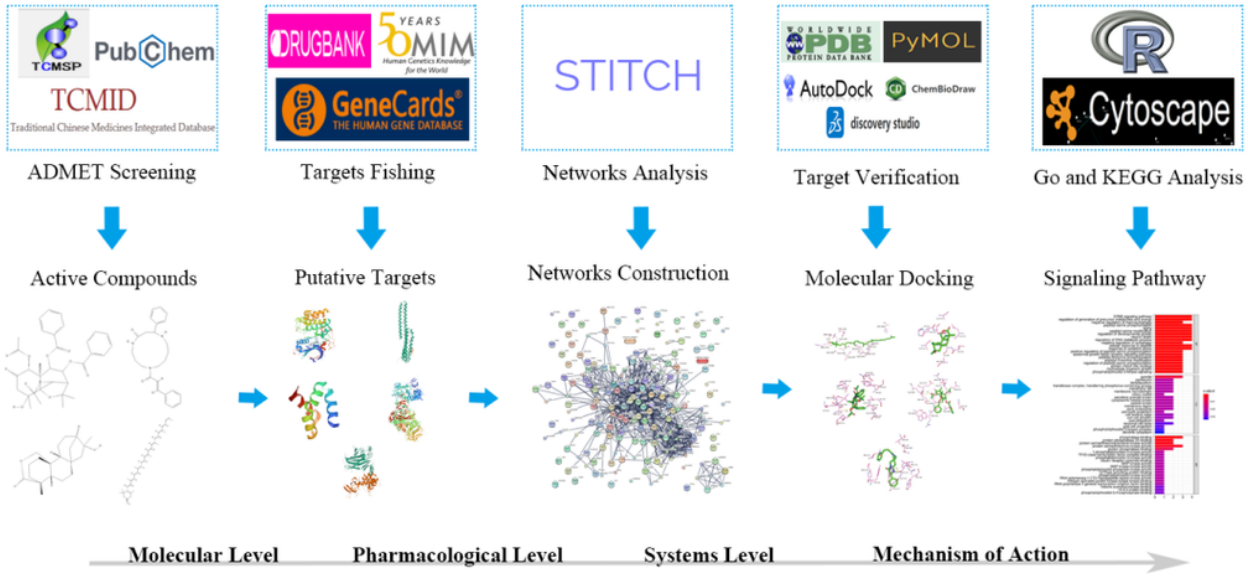


Figure 1

Network pharmacology for deciphering pharmacological mechanisms of Tripterygium wilfordii Hook F acting on cardiovascular disease.

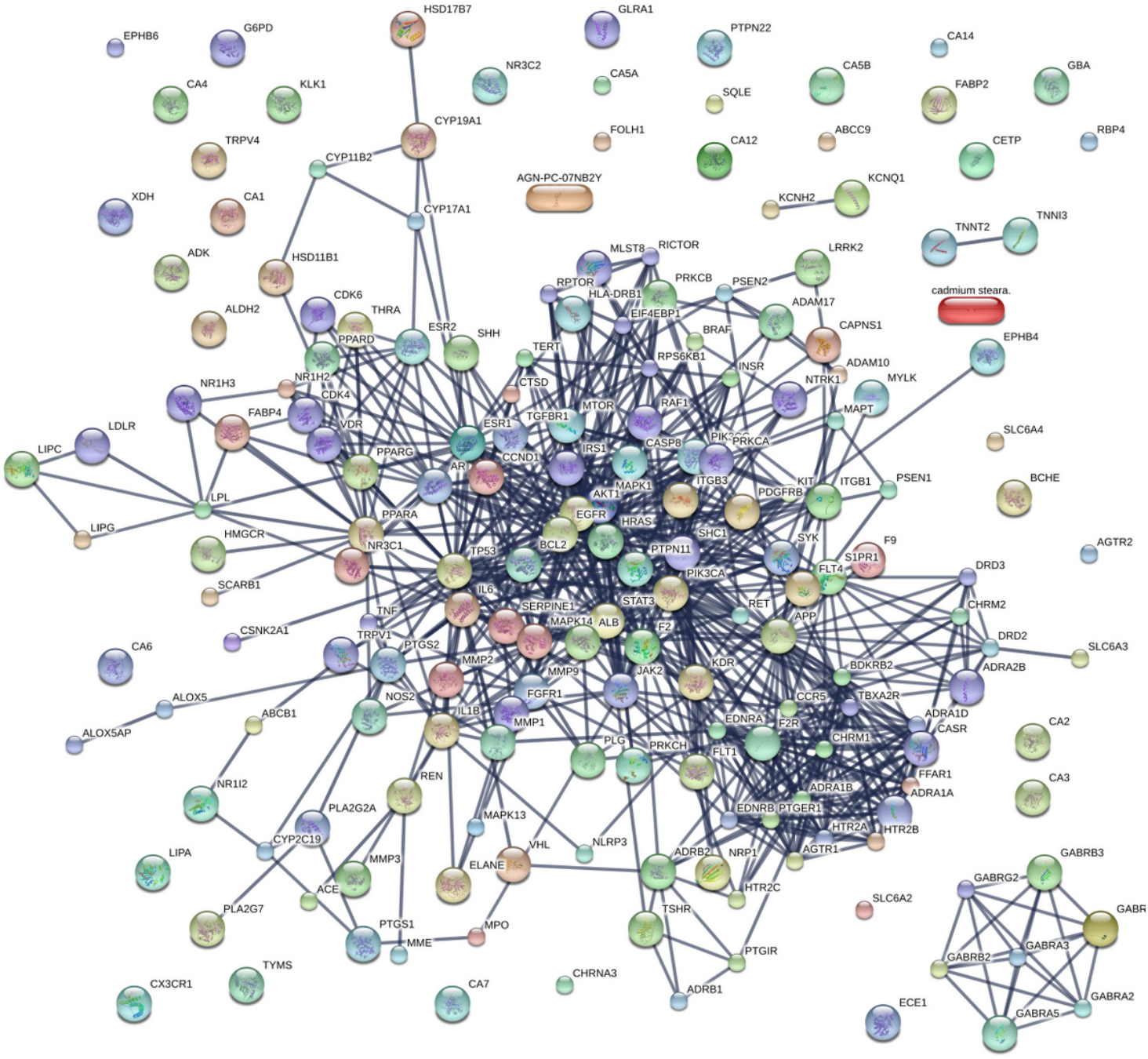


Figure 2

Protein–protein interaction (PPI) network of putative target genes.

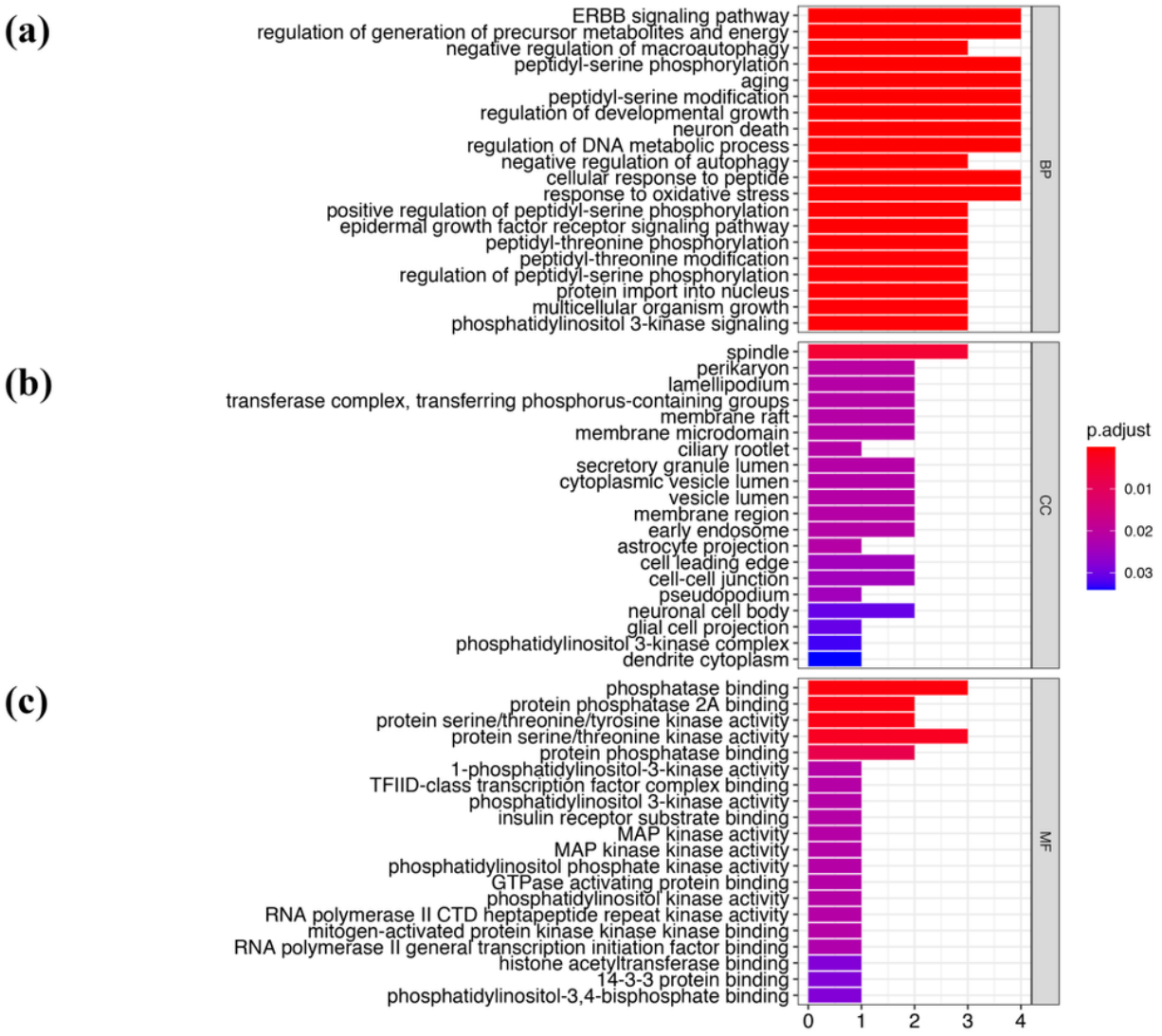


Figure 3

GO map of putative target genes. (a) Biological process categories. (b) Cellular component categories. (c) Molecular function categories.

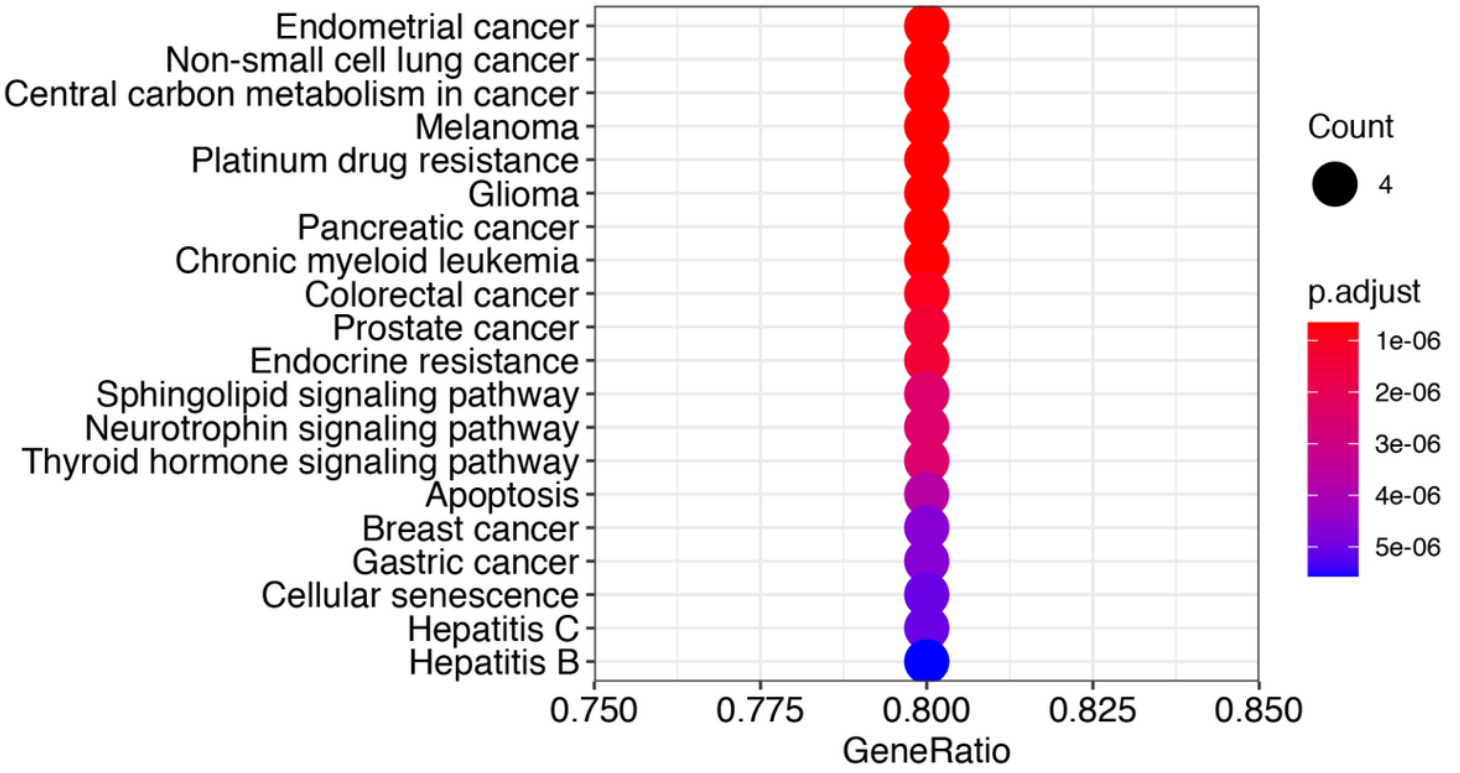


Figure 4

KEGG pathway analysis of putative target genes.

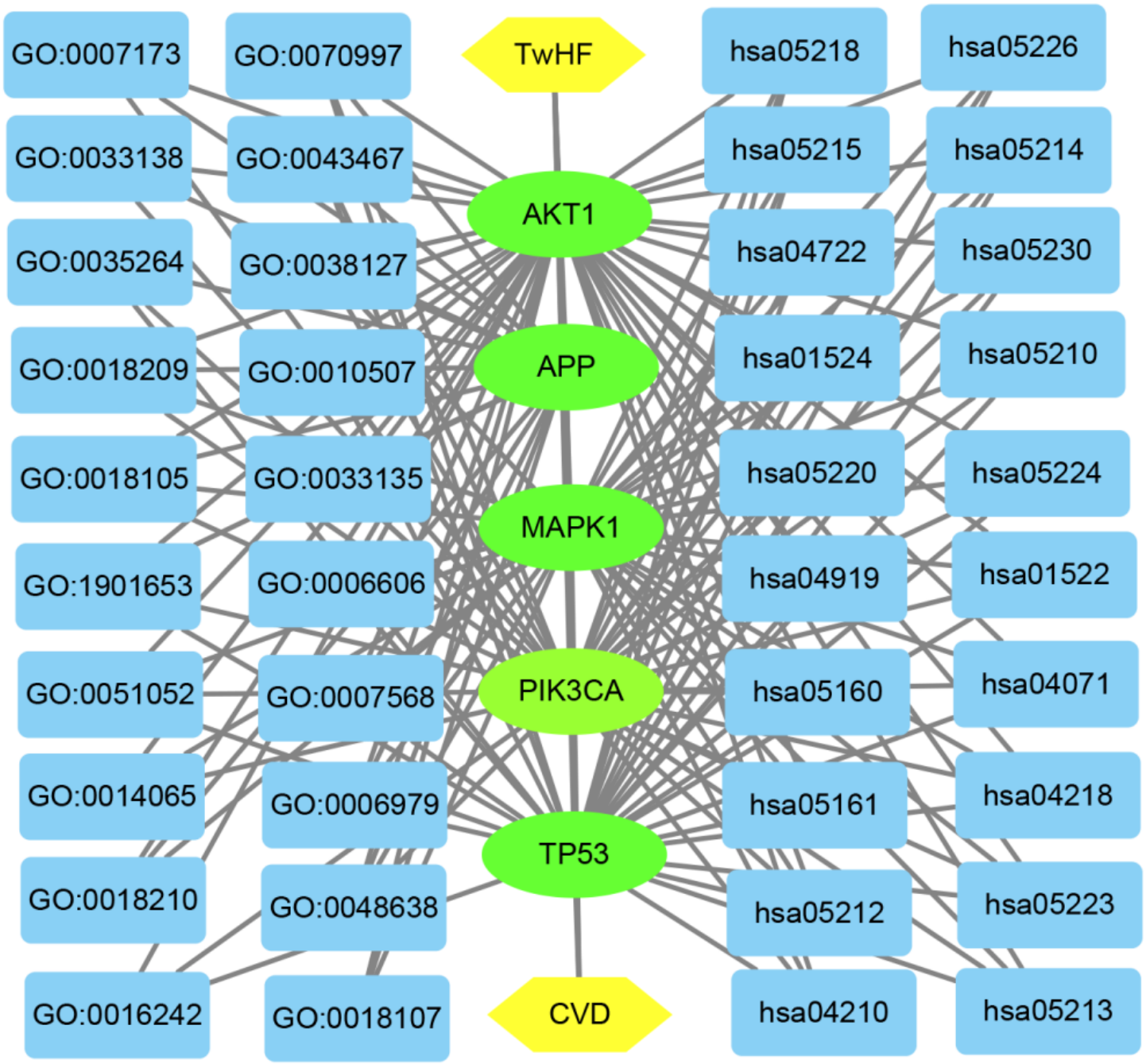


Figure 5

TwHF-targets-CVD-GO-KEGG network.

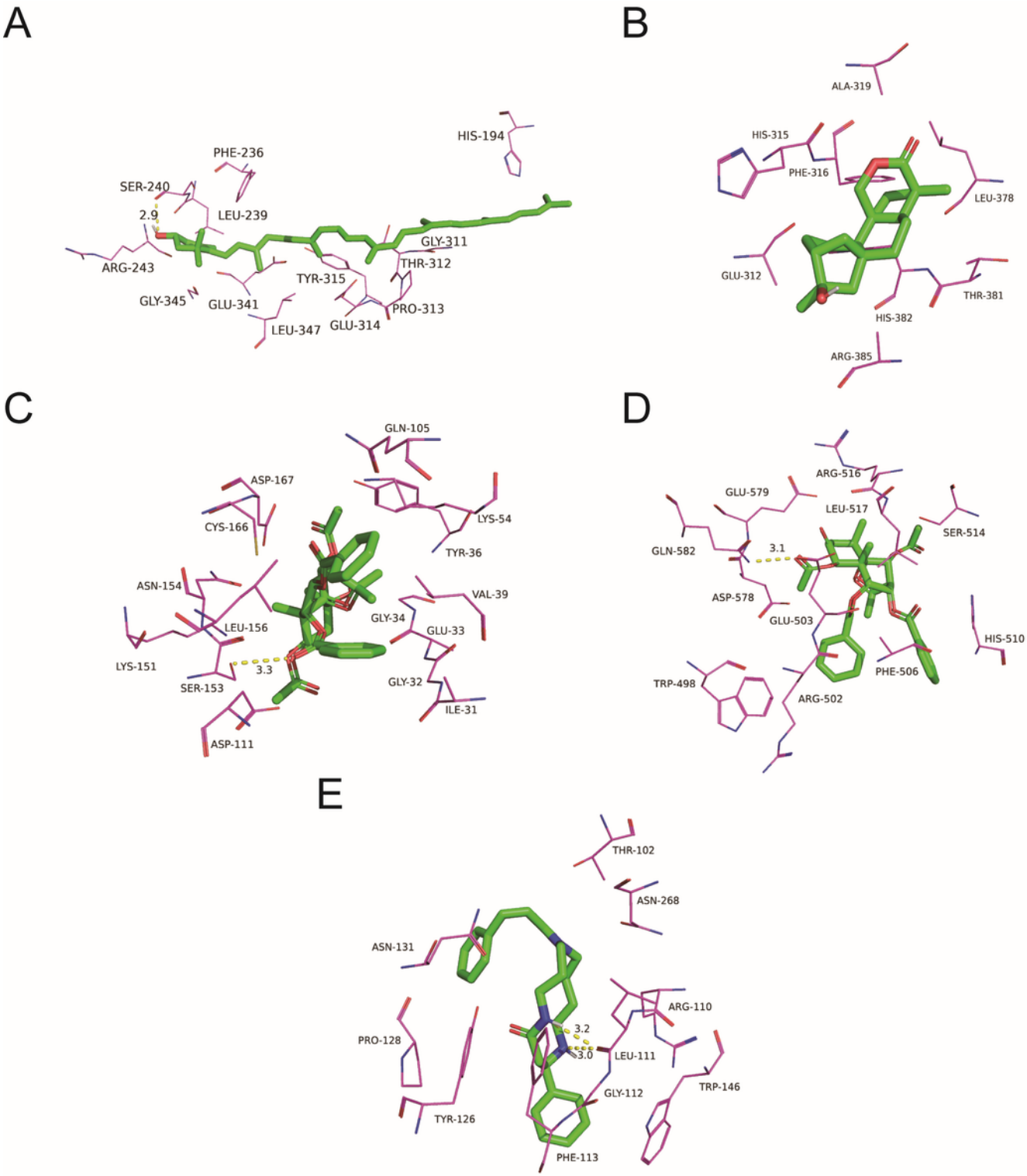


Figure 6

Molecular models of the binding of TwHF to the predicted targets (A) AKT1, (B) APP, (C) MAPK1, (D) PIK3CA, and (E) TP53 shown as 3D diagrams.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable.xlsx