

Research Article

Exploration of the molecular targets and mechanisms of suxiao xintong dropping pills for myocardial infarction by network pharmacology method

Daqiu Chen*, Yanqing Wu*, Yixing Chen, Qiaoxing Chen, Xianhua Ye,  Shanghua Xu and Shunxiang Luo

Department of Cardiology, Affiliated Nanping First Hospital, Fujian Medical University, Nanping 353000, Fujian Province, China

Correspondence: Shanghua Xu (13365998866@163.com) or Shunxiang Luo (15959755605@139.com)

Background: Suxiao Xintong dropping pills (SXXTDP), a traditional Chinese medicine, is widely applied for treating myocardial infarction (MI). However, its therapy mechanisms are still unclear. Therefore, this research is designed to explore the molecular mechanisms of SXXTDP in treating MI.

Methods: The active ingredients of SXXTDP and their corresponding genes of the active ingredients were retrieved from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database. MI-related genes were identified via analyzing the expression profiling data (accession number: GSE97320). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed to study the shared genes of drug and disease. Through protein–protein interaction (PPI) network and the Cytoscape plugin cytoHubba, the hub genes were screened out. The compounds and hub targets binding were simulated through molecular docking method.

Results: We obtained 21 active compounds and 253 corresponding target genes from TCMSP database. 1833 MI-related genes were identified according to $P < 0.05$ and $|\log_2 FC| \geq 0.5$. 27 overlapping genes between drug and disease were acquired. GO analysis indicated that overlapping genes were mainly enriched in MAP kinase activity and antioxidant activity. KEGG analysis indicated that overlapping genes were mainly enriched in IL-17 signaling pathway and TNF signaling pathway. We obtained 10 hub genes via cytoHubba plugin. Six of the 10 hub genes, including *PTGS2*, *MAPK14*, *MMP9*, *MAPK1*, *NFKBIA*, and *CASP8*, were acted on molecular docking verification with their corresponding compounds of SXXTDP.

Conclusion: SXXTDP may exert cardioprotection effect through regulating multiple targets and multiple pathways in MI.

*These authors contributed equally to this work.

Received: 09 December 2020

Revised: 17 July 2021

Accepted: 19 July 2021

Accepted Manuscript Online:
21 July 2021Version of Record published:
05 August 2021

Introduction

Myocardial infarction (MI) is a class of cardiovascular diseases that possess high mortality all over the world [1]. Percutaneous coronary intervention (PCI) and thrombolytic therapy are considered as the best treatment strategies to rescue the endangered myocardium in MI. Nevertheless, the ischemic but still viable cardiomyocytes don't always be saved after reperfusion. Inflammatory and oxidative stress responses will lead to further cardiomyocyte necrosis after MI. Hence, it is clinically significant to seek for an approach to inhibit inflammatory and oxidative stress responses after MI, which will protect the ischemic but still viable cardiomyocytes.

Some Traditional Chinese Medicine (TCM) can improve blood circulation. Therefore, TCM has been employed to treat ischemia cardiovascular diseases in China for a long time. SXXTDP comprising Chuanxiong (Rhizoma Chuanxiong), Mudanpi (Cortex Moutan), and Bingpian (Borneolum Syntheticum). SXXTDP has broad pharmacological activities, including anti-inflammation, anti-oxidant, anti-arrhythmic activity, and so on [2,3]. As is known to all, western medicine is single target therapy, which sometimes can not exert optimal effects on the treatment of complicated diseases. In contrast, TCM has the characteristics of multiple targets and multiple pathways in the treatment of diseases. At present, it remains unclear what are the potential molecular mechanisms of SXXTDP in treating MI.

Network pharmacology is an effective method to discover novel drugs and mechanisms. So far, this method has been successfully used to clarify the multi-target regulation of TCM in treating diseases. For example, Li et al. explored the potential mechanisms of Tongxinluo capsule in the treatment of coronary heart disease [4]. Yu et al. found that the mechanisms of XiaoLuoWan in treating uterine fibroids by the network pharmacology method [5]. The ultimate goal is to raise the treatment effect, reduce the side effect, and accelerate the development of novel drugs.

In the present study, we find the active ingredients and potential molecular mechanisms of SXXTDP in treating MI by utilizing network pharmacology method. These findings indicate that SXXTDP is effective in anti-inflammation, anti-oxidant, anti-arrhythmic activity, and anti-apoptosis, and thus can be applied to the treatment of MI.

Methods

Identification of the active compounds of SXXTDP and corresponding to target genes

All chemical constituents of SXXTDP were retrieved from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (<https://tcmssp.com/tcmssp.php>) [6]. We selected the oral bioavailability (OB) $\geq 30\%$ and drug-likeness (DL) ≥ 0.18 as the screening criteria [7,8]. The target genes corresponding to active compounds of SXXTDP were screened out from TCMSP database. Obtained target genes were imported into UniProt (<https://www.uniprot.org/>) [9] to search for their information, including the gene symbol, gene ID, and so on [10].

Identification of target genes related to MI

The expression profiling data of GSE97320 and annotation information of microarray platform GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array) were acquired from the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>), including 3 samples from healthy individuals and 3 MI samples. On the basis of the annotation information of platform GPL570, probe IDs were converted to the corresponding genes. According to $P < 0.05$ and $|\log_2FC| \geq 0.5$, differential expression genes (DEGs) between healthy individuals and MI were obtained by using the package limma of R language [11] and were visualized by a volcano plot.

Acquisition of overlapping genes between drug and disease, construction of a drug-compound-target genes network

Using the Perl language, we acquired the overlapping target genes of drug and disease. By using Cytoscape 3.8.0 software [12], 'drug-compound-target' network was established. In the network diagram, the nodes of triangle and ellipse represent active compounds of drug and target genes, respectively, and they are connected by edges.

Establishing PPI network of overlapping genes and selection of hub genes

The overlapping genes of drug and disease were imported into the STRING database (<https://string-db.org/>) [13] and a protein-protein interaction (PPI) network was constructed. The screening conditions were the species as "Homo sapiens" and the combined score > 0.4 . In the PPI diagram, each node represents a gene and the nodes are connected by lines. By using the Cytoscape plugin cytoHubba [14], the key genes were identified by Maximal Clique Centrality (MCC) method.

GO enrichment analysis

Gene Ontology (GO) analysis is an important method that describes the features of candidate targets. By using ClusterProfiler package [15], a bioconductor package [16], the shared target genes of drug and disease were analyzed with the GO enrichment analysis tool. The screening criteria was $P < 0.05$.

Table 1 Compounds in Suxiao Xintong Dropping Pills

MolID	Molecule name	OB(%)	DL
MOL006861	Asiatic acid	41.38	0.71
MOL006862	Bronyl acetate	59.3	0.51
MOL006865	Dipterocarpol	41.71	0.76
MOL001494	Mandenol	42	0.19
MOL002135	Myricanone	40.6	0.51
MOL002140	Perlolyrine	65.95	0.27
MOL002151	Senkyunone	47.66	0.24
MOL002157	Wallichilide	42.31	0.71
MOL000359	Sitosterol	36.91	0.75
MOL000433	FA	68.96	0.71
MOL001925	paeoniflorin.qt	68.18	0.4
MOL000211	Mairin	55.38	0.78
MOL000359	Sitosterol	36.91	0.75
MOL000422	Kaempferol	41.88	0.24
MOL000492	(+)-catechin	54.83	0.24
MOL007003	Benzoyl paeoniflorin	31.14	0.54
MOL007369	4-O-methylpaeoniflorin.qt	67.24	0.43
MOL007374	5-[[5-(4-methoxyphenyl)- 2-furyl]methylene] barbituric acid	43.44	0.3
MOL007382	Mudanpioside-h.qt 2	42.36	0.37
MOL007384	Paeonidanin.qt	65.31	0.35
MOL000098	Quercetin	46.43	0.28

KEGG Pathway Enrichment Analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis is an important method that describes enrichment of signal pathways of candidate targets. By using ClusterProfiler package, the shared target genes of drug and disease were analyzed with the KEGG pathways analysis tool. The screening criteria was $P < 0.05$.

Molecular docking of the main active ingredients of SXXTDP and core proteins

According to the results of GO analysis and KEGG pathway, we selected the key protein receptor and ligand associated with protein receptor. The 2D chemical structure of small molecular ligands were acquired from PubChem (<https://pubchem.ncbi.nlm.gov/>) [17]. The 3D chemical structures of small molecular ligands were constructed by using ChemOffice software [18]. The 3D chemical structure of protein receptor was acquired from PDB (<http://www.rcsb.org/>) [19]. PyMol 2.4.0 software (<https://pymol.org/>) [20] was used to remove molecular ligands and water molecules of the protein receptor. After installing AutoDock Vina and AutoDockTools-1.5.6 software [21], the format of protein receptor and small molecular ligands was transformed into pdbqt format. The active pocket was subsequently determined. By using Perl language, molecular docking was generated through AutoDock Vina [22]. Based on the binding energy value, the lowest the binding energy value was selected as the docking affinity. Finally, the visualizing 3D structures of molecular ligand and protein receptor bonding were constructed using PyMol software.

Results

Identification of the active compounds and corresponding to target genes

According to the screening criteria of the $OB \geq 30\%$ and $DL \geq 0.18$, 21 active compounds of SXXTDP were obtained from the TCMSP database, including 3 ingredients of Bingpian, 7 ingredients of Chuanxiong, and 11 ingredients of Mudanpi, as shown in Table 1. From the TCMSP database, we obtained 279 target genes with corresponding to active compounds, including 42 target genes of Chuanxiong and 237 target genes of Mudanpi. 279 full names of genes were converted to gene symbols through Uniprot database. Finally, we obtained 253 target genes after removing duplications. The details of the above data see the supplementary materials (Document 1).

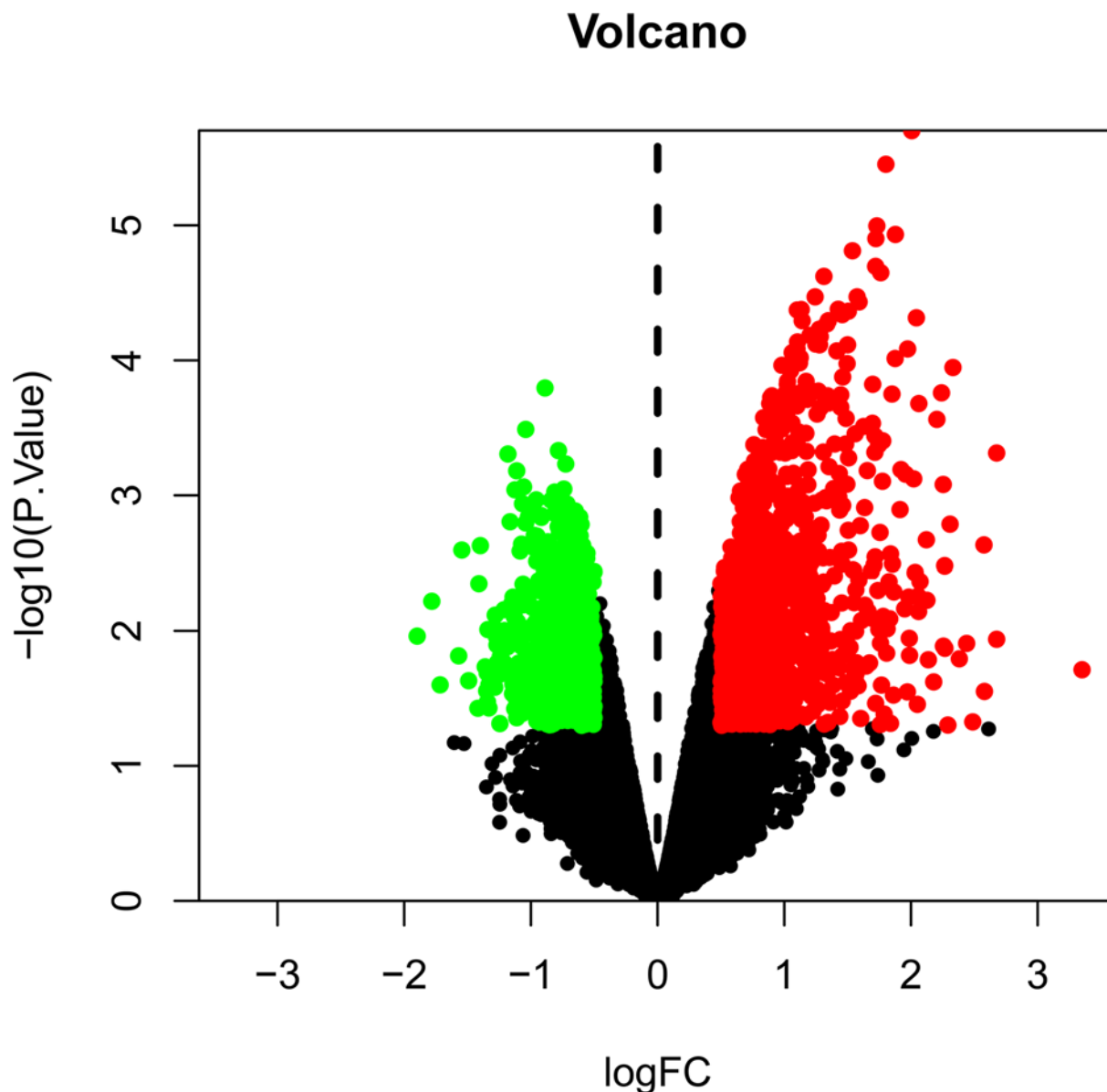


Figure 1. Volcano plot of DEGs of MI

The red and green dots represent up-regulated and down-regulated genes, respectively.

Identification of target genes related to MI

According to the criteria of $|\text{Log}_2\text{FC}| \geq 0.5$ and $P < 0.05$, 1833 DEGs, including 1117 up-regulated genes and 716 down-regulated genes, were identified by analyzing the expression profiling data (accession number: GSE97320). A volcano plot of DEGs was shown in Figure 1. The red and green dots represent up-regulated and down-regulated genes, respectively. The details of the above data are shown in the supplementary materials (Document 2).

Construction of a drug-compound-target genes network

About 27 overlapping genes related with MI and SXXTDP were obtained through the Perl language, including *PTGS2*, *NCOA2*, *PTGS1*, *MAPK14*, *NR3C2*, *CALM1*, *STAT1*, *ALOX5*, *SLPI*, *CAT*, *AKR1B1*, *CCND1*, *BCL2L1*, *MMP9*, *MAPK1*, *NFKBIA*, *CASP8*, *HSPA5*, *PRKCB*, *MGAM*, *THBD*, *COL1A1*, *IFNGR1*, *TOP2A*, *NFE2L2*, *RUNX2*, and *E2F2*. Next, the network of 'drug-compounds-targets' was constructed by utilizing Cytoscape 3.8.0 software, as shown in Figure 2. This network explained that compounds of SXXTDP could interfere with MI via binding multiple target genes.

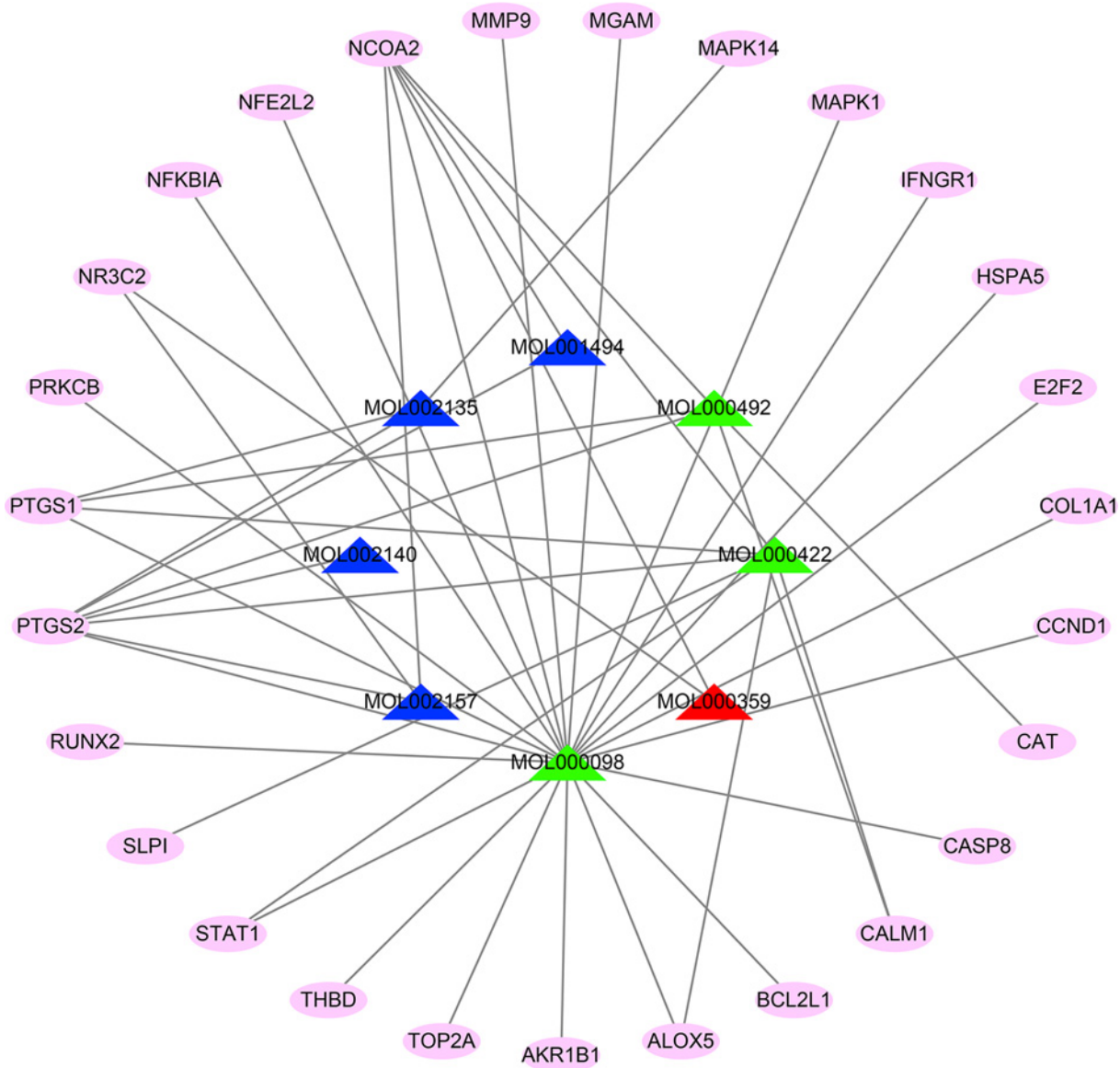


Figure 2. Compound-target network of SXXTDP

The blue triangles represent the compounds coming from Chuanxiong. The green triangles represent the compounds coming from Mudanpi. The red triangle represents the compound coming from Chuanxiong and Mudanpi. The node of pink ellipse represents target genes.

Establishing PPI network of overlapping genes and selection of hub genes

About 27 overlapping genes associated with disease and drug were inputted into STRING database. We obtained a PPI network after selecting “Homo sapiens” and the medium confidence >0.4, as shown in Figure 3A. There were 27 nodes and 89 edges in this network. Using the Cytoscape plugin cytoHubba, we obtained the top 10 key genes by MCC method, including *MAPK1*, *MAPK14*, *CCND1*, *CASP8*, *BCL2L1*, *MMP9*, *PTGS2*, *STAT1*, *NFKBIA*, and *CAT*, as shown in Figure 3B.

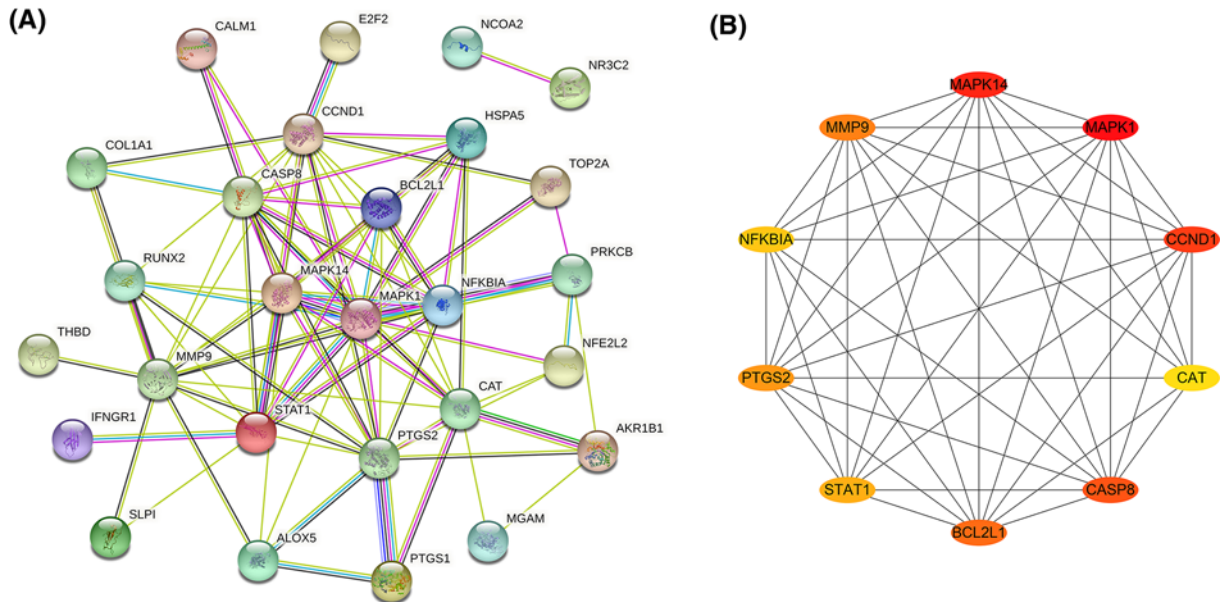


Figure 3. Protein-protein interaction network

(A) PPI network of MI. Each node represents one target gene. The lines of different colors represent different sources of the evidence for protein-protein interaction. (B) The core genes are sorted by MCC method. The ellipse nodes represent genes. The more lines are there in the network, the more important the genes are.



Figure 4. Compound-target network of SXSTD

On the basis of *P* value, the top 20 GO functional terms were selected. The color represents *P* value and the size of the dot represents the number of genes.

GO enrichment analysis

In order to further analyze the function of 27 overlapping genes, GO enrichment analysis was conducted by the clusterProfiler package in R language. The top 20 GO enrichment terms (adjusted, *P* < 0.05) were presented in Figure 4. The top 10 GO terms were listed in Table 2.

Table 2 The top 10 GO terms of 27 overlapping genes

GO terms	Description	Adjusted <i>P</i> -value	Corresponding genes
GO:0004601	Peroxidase activity	0.00499534	<i>PTGS2, PTGS1, CAT</i>
GO:0016684	Oxidoreductase activity, acting on peroxide as acceptor	0.00499534	<i>PTGS2, PTGS1, CAT</i>
GO:0004707	MAP kinase activity	0.009969697	<i>MAPK14, MAPK1</i>
GO:0004708	MAP kinase kinase activity	0.009969697	<i>MAPK14, MAPK1</i>
GO:0016209	Antioxidant activity	0.009969697	<i>PTGS2, PTGS1, CAT</i>
GO:0051213	Dioxygenase activity	0.010516935	<i>PTGS2, PTGS1, ALOX5</i>
GO:0016702	Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen	0.017094371	<i>PTGS2, ALOX5</i>
GO:0016701	Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen	0.017094371	<i>PTGS2, ALOX5</i>
GO:0020037	Heme binding	0.018048512	<i>PTGS2, PTGS1, CAT</i>
GO:0005164	Tumor necrosis factor receptor binding	0.018048512	<i>STAT1, CASP8</i>

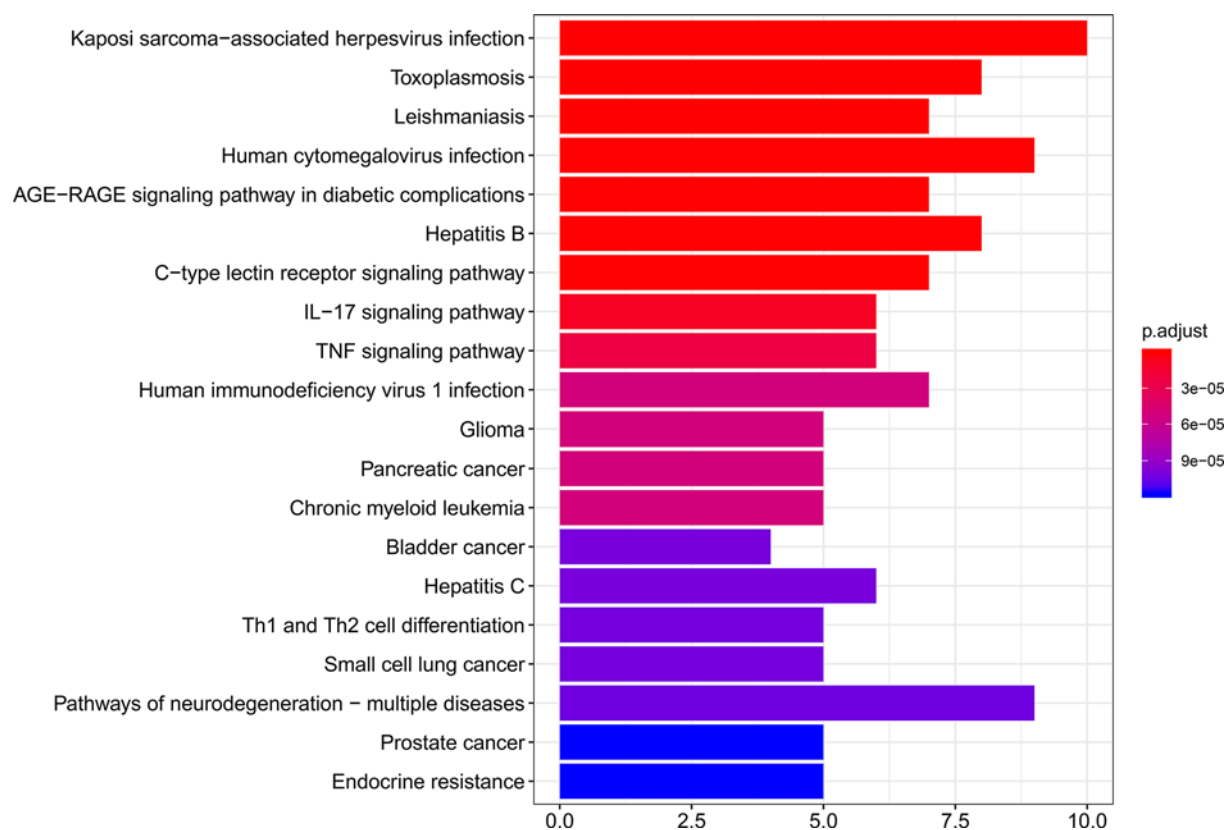


Figure 5. Compound-target network of SXXTDP

On the basis of *P* value, the top 20 pathways are shown. The color represents *P* value and the length of the column represents the number of genes.

KEGG pathway enrichment analysis

KEGG enrichment analysis was conducted by the clusterProfiler package in R language. The top 20 KEGG enrichment terms (adjusted, $P < 0.05$) were presented in Figure 5. The top 10 KEGG pathways were listed in Table 3.

Table 3 The top 10 KEGG pathways of 27 overlapping genes

ID	Pathway name	Adjusted <i>P</i> <0.05	Corresponding genes
hsa05167	Kaposi sarcoma-associated herpesvirus infection	3.48E-08	<i>PTGS2, MAPK14, CALM1, STAT1, CCND1, MAPK1, NFKBIA, CASP8, IFNGR1, E2F2</i>
hsa05145	Toxoplasmosis	1.27E-07	<i>MAPK14, STAT1, ALOX5, BCL2L1, MAPK1, NFKBIA, CASP8, IFNGR1</i>
hsa05140	Leishmaniasis	1.92E-07	<i>PTGS2, MAPK14, STAT1, MAPK1, NFKBIA, PRKCB, IFNGR1</i>
hsa05163	Human cytomegalovirus infection	6.88E-07	<i>PTGS2, MAPK14, CALM1, CCND1, MAPK1, NFKBIA, CASP8, PRKCB, E2F2</i>
hsa04933	AGE-RAGE signaling pathway in diabetic complications	6.88E-07	<i>MAPK14, STAT1, CCND1, MAPK1, PRKCB, THBD, COL1A1</i>
hsa05161	Hepatitis B	6.88E-07	<i>MAPK14, STAT1, MMP9, MAPK1, NFKBIA, CASP8, PRKCB, E2F2</i>
hsa04625	C-type lectin receptor signaling pathway	6.88E-07	<i>PTGS2, MAPK14, CALM1, STAT1, MAPK1, NFKBIA, CASP8</i>
hsa04657	IL-17 signaling pathway	9.38E-06	<i>PTGS2, MAPK14, MMP9, MAPK1, NFKBIA, CASP8</i>
hsa04668	TNF signaling pathway	2.36E-05	<i>PTGS2, MAPK14, MMP9, MAPK1, NFKBIA, CASP8</i>
hsa05170	Human immunodeficiency virus 1 infection	5.18E-05	<i>MAPK14, CALM1, BCL2L1, MAPK1, NFKBIA, CASP8, PRKCB</i>

Molecular docking of the main active ingredients of SXXTDP and core proteins

Using molecular docking approach, we verified the binding sites of the target genes and their corresponding compounds of SXXTDP. According to IL-17 signaling pathway and TNF signaling pathway from the results of KEGG pathway enrichment analysis, we determined six key genes, including *PTGS2, MAPK14, MMP9, MAPK1, NFKBIA*, and *CASP8*. We identified that quercetin was the ligand of *MMP9, MAPK1, NFKBIA*, and *CASP8* protein receptors. The 2D structure of quercetin was obtained by using the PubChem database, and then the 2D structure of quercetin was converted to 3D structure through ChemOffice software. We acquired the 3D chemical structures of *MMP9, MAPK1, NFKBIA*, and *CASP8* protein receptors from PDB. The water molecules and molecule ligands of *MMP9, MAPK1, NFKBIA*, and *CASP8* protein receptors were removed by utilizing PyMol 2.4.0 software. We obtained the visualizing 3D structures of quercetin, *MMP9, MAPK1, NFKBIA*, and *CASP8* protein receptors bonding by using AutoDockTools and AutoDock Vina. The greater the absolute value of the docking affinity, the more powerful the binding ability between the active site of the protein receptor and the compound. In accordance with the above method, *MAPK14-Myricanone, PTGS2-Perlolryrine, PTGS2-Myricanone, PTGS2-Mandenol, PTGS2-kaempferol, PTGS2-(+)-catechin*, and *PTGS2-wallichilide* were verified by molecular docking, as shown in Figure 6. A total of eleven pairs entered into the docking simulation, including *MAPK1-quercetin* docking (-8.4 kcal/mol), *MAPK14-Myricanone* docking (-7.2 kcal/mol), *MMP9-quercetin* docking (-7.9 kcal/mol), *NFKBIA-quercetin* docking (-7.7 kcal/mol), *CASP8-quercetin* docking (-7.7 kcal/mol), *PTGS2-Perlolryrine* docking (-8.0 kcal/mol), *PTGS2-Myricanone* docking (-7.6 kcal/mol), *PTGS2-Mandenol* docking (-6.1 kcal/mol), *PTGS2-kaempferol* docking (-9.3 kcal/mol), *PTGS2-(+)-catechin* docking (-8.5 kcal/mol), and *PTGS2-wallichilide* docking (-6.2 kcal/mol), as shown in Table 4. From the docking result, most binding complexes possessed high binding affinity. Next, simvastatin was set as a positive control. The 2D structure of simvastatin was obtained by using the PubChem database, and then the 2D structure of simvastatin was converted to 3D structure through ChemOffice software. We docked simvastatin with the above 6 key genes and found simvastatin had certain binding activity with *MAPK1, MAPK14, MMP9, NFKBIA, CASP8*, and *PTGS2* (see Table 5). *MAPK1-simvastatin, MAPK14-simvastatin, MMP9-simvastatin, NFKBIA-simvastatin, CAPS8-simvastatin*, and *PTGS2-simvastatin* were verified by molecular docking, as shown in Figure 7.

Discussion

The leading causes of death in rural and urban residents were cardiovascular diseases according to China Cardiovascular Diseases Report 2017. Despite advances in medical technology in recent years, myocardial infarction (MI) continues to be regarded as a pivotal risk factor of death [23]. TCM has been employed to treat ischemia cardiovascular diseases in China for a long time. Therefore, it is imperative to develop novel drugs for MI treatment. Due to

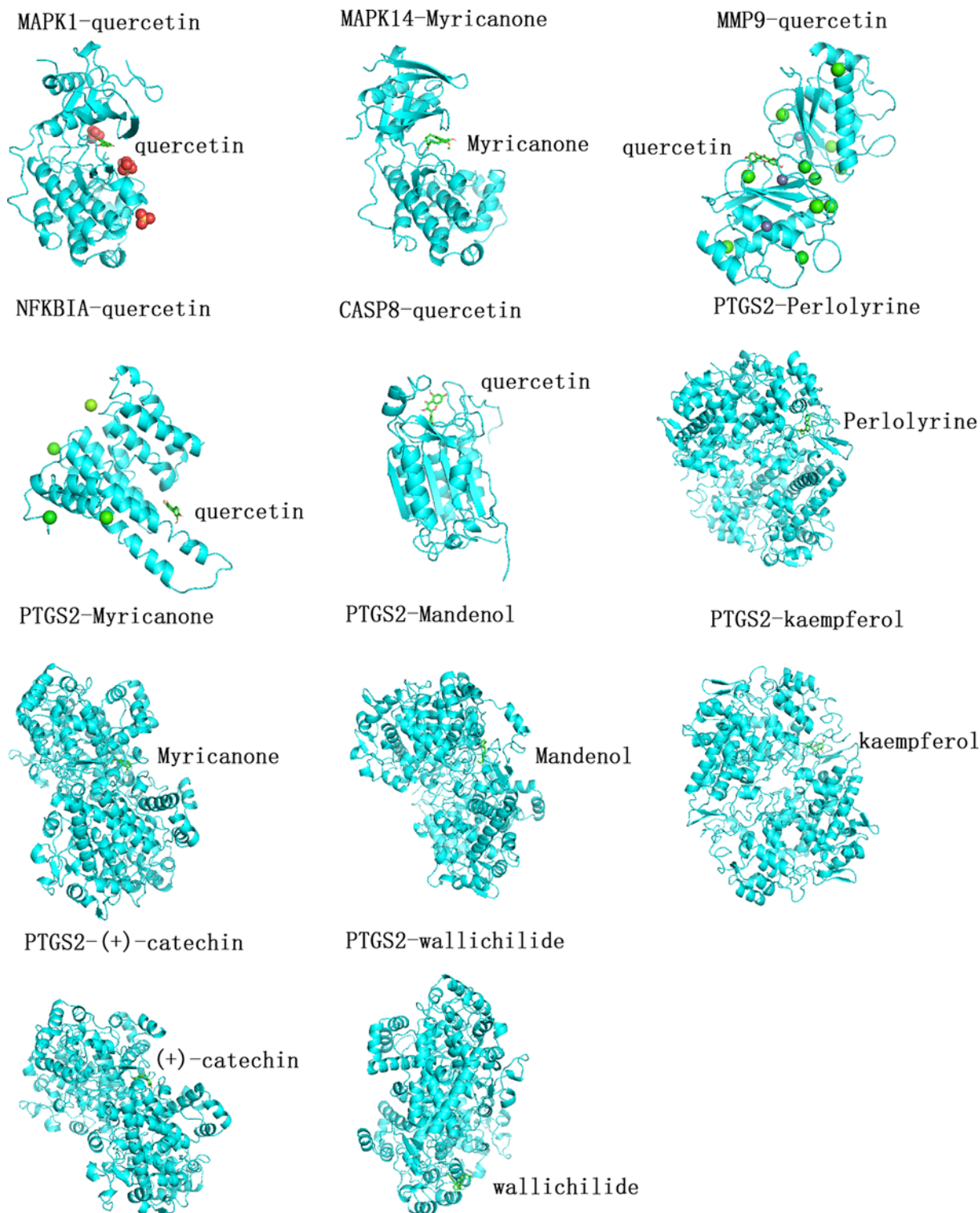


Figure 6. Molecular docking models

Molecular model of the ingredient is in the binding pocket of the protein, which is displayed via 3D-map technology. The ingredients are displayed in a ringlike structure colored green.

Table 4 Results of the molecular docking of the six core genes with compounds of SXXTDP

Number	Core genes	PDB ID	Compound	Docking affinity (kcal/mol)
1	MAPK1	5lck	Quercetin	-8.4
2	MAPK14	2qd9	Myricanone	-7.2
3	MMP9	2ow1	Quercetin	-7.9
4	NFKBIA	6y1j	Quercetin	-7.7
5	CASP8	3kjq	Quercetin	-7.7
6	PTGS2	5f19	Perlolryrine	-8.0
			Myricanone	-7.6
			Mandenol	-6.1
			Kaempferol	-9.3
			(+)-catechin	-8.5
			Wallichilide	-6.2

Table 5 Results of the molecular docking of the six core genes with simvastatin

Number	Core genes	PDB ID	Compound	Docking affinity (kcal/mol)
1	MAPK1	5lck	Simvastatin	-8.2
2	MAPK14	2qd9	Simvastatin	-8.0
3	MMP9	2ow1	Simvastatin	-7.3
4	NFKBIA	6y1j	Simvastatin	-8.1
5	CASP8	3kjq	Simvastatin	-7.4
6	PTGS2	5f19	Simvastatin	-9.2

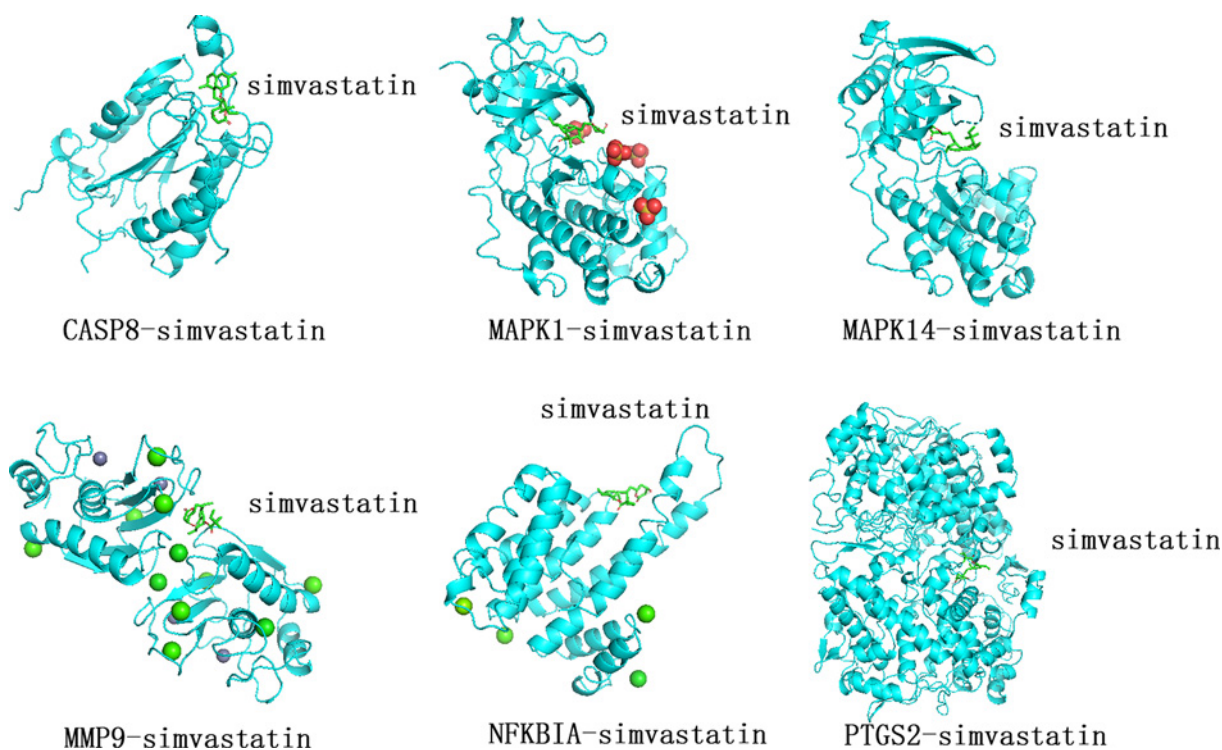


Figure 7. Molecular docking models

Molecular model of simvastatin is in the binding pocket of the protein, which is displayed via 3D-map technology. Simvastatin is displayed in a ringlike structure colored green.

the multi-target treatment effects of TCM, it can serve as a significant repository to develop drugs for the treatment of MI.

The present study used network pharmacology and molecular docking simulation to research the molecular mechanisms of SXXTDP in the treatment of MI. It was found that SXXTDP exerted a potential role in treating MI through regulating multiple target genes, including *MAPK1*, *MAPK14*, *CCND1*, *CASP8*, *BCL2L1*, *MMP9*, *PTGS2*, *STAT1*, *NFKBIA*, and *CAT*. SXXTDP is comprised of Chuanxiong, Mudanpi, and Bingpian. Those ingredients have broad pharmacological activities, including anti-inflammation, anti-oxidant, anti-arrhythmic activity, and so on [2,3]. The active compounds and target genes of SXXTDP were predicted by using the TCMSP database. We obtained 21 active ingredients of SXXTDP and 253 corresponding target genes in total. About 1833 MI-related genes were identified according to $P < 0.05$ and $|\log_2FC| \geq 0.5$. A total of 27 overlapping genes between drug and disease were acquired. GO analysis indicated that overlapping genes were mainly enriched in MAP kinase activity, antioxidant activity. KEGG analysis indicated that overlapping genes were mainly enriched in IL-17 signaling pathway and TNF signaling pathway. We obtained 10 hub genes via cytoHubba plugin. Six of the 10 hub genes, including *PTGS2*, *MAPK14*, *MMP9*, *MAPK1*, *NFKBIA*, and *CASP8*, were acted on molecular docking verification with their corresponding compounds of SXXTDP. Study finds that IL-17 signaling pathway is involved in immune responses [24]. Inhibition of IL-17 signaling pathway can improve immune response balance and attain cardioprotection in rats with heart failure [25]. MIR-324/SOCS3 axis can regulate TNF signaling pathway and further improve the hypoxia/reoxygenation-induced myocardial injury [26]. Study finds that d-Limonene alleviates myocardial infarction injury via antioxidant effect [27]. Rosuvastatin can improve cardiac function via reducing p38 MAP kinase activity in rats after myocardial infarction [28].

Mitogen-activated protein kinase 1 (also known as MAPK1) and mitogen-activated protein kinase 14 (also known as MAPK14) are two members of MAP kinase family. MAP kinases are involved in many cellular processes such as transcription regulation and proliferation. Cardiomyocyte-specific deletion of the *Mapk14* and *Mapk11* genes reduce myocardial cells apoptosis and increase cardiomyocytes proliferation via inactivation of p38 activity [29]. Matrix metalloproteinase 9 (also named as MMP9) belongs to proteins of the matrix metalloproteinase family, which participates the breakdown of extracellular matrix. Many studies report that MMPs exert a main role in atherosclerotic plaque disruption and result in myocardial infarction. Study finds that Kai-Xin-San can protect cardiomyocytes effect via regulating MMPs [30]. NFKB inhibitor alpha (also named as NFKBIA) participates in inflammatory responses. Study finds that blocking NFKBIA-mediated NF- κ B signalling pathway can protect against myocardial infarction in mice [31]. Caspase 8 (*CASP8*) is one of the members of the cysteine-aspartic acid protease (caspase) family, which plays an important role in the execution phase of cell apoptosis. Study finds that Ebselen can inhibit myocardial apoptosis by reducing the expression of caspase-8 and caspase-3 [32]. Prostaglandin-endoperoxide synthase 2 (also named as *PTGS2*) acts both as a peroxidase and as a dioxygenase. Study finds that miR-26b can improve myocardial remodel and reduce the inflammatory response in mice with myocardial infarction via inhibiting *PTGS2* to activate the MAPK pathway [33].

Quercetin, a flavonoid, has special biological functions, including antioxidant, anti-inflammatory, anti-platelet aggregation, and so on [34]. A study reports that quercetin has evidently antioxidant, anti-apoptotic, and anti-inflammatory effects on rat with MI and can protect against cardiomyocytes injury [35]. Myricanone has anti-oxidant and anti-inflammatory [36]. Perlolryrine has antiproliferative activities [37], which may improve myocardial remodel. Kampeferol, a dietary flavonoid, has the characteristic of antioxidant activities, anti-inflammatory, and anti-apoptotic. Study finds that Kampeferol protects against apoptosis and oxidative stress damage of myocardial cells in rats with isoproterenol-induced cardiac toxicity [38]. (+)-catechin, a bioactive polyphenol, has antioxidant property. Study reports that catechin can alleviate hypoxia/reoxygenation-induced cardiomyocytes apoptosis by down-regulating lncRNA MIAT [39]. At present, there are very limited researches about Wallichilide and Mandenol.

Simvastatin, HMG-CoA reductase inhibitor, is effective in antioxidant activity [40], anti-apoptosis [41], and anti-inflammatory effect [42]. Simvastatin can improve reparative fibrosis post-myocardial infarction [43] and cardiac function after myocardial infarction and decrease myocardial apoptosis [44]. Therefore, simvastatin was set as a positive control and its values were compared with the active ingredients of SXXTDP. Interestingly, the affinity of key genes binding with quercetin, myricanone, and kaempferol is similar to that of simvastatin. Having referred to lots of documents, we found that simvastatin inhibited the expression of *CASP8* [43], *MAPK1* [45], *MMP9* [46], and *PTGS2* [47]. These results indicated that *MAPK1*, *MAPK14*, *MMP9*, *NFKBIA*, *CASP8*, and *PTGS2* may be the key targets for the pharmacological action of SXXTDP in treating myocardial infarction.

The results of this research showed that the active ingredients of SXXTDP played an active role in anti-apoptosis, anti-oxidation, anti-inflammation, and improvement of cardiac remodeling roles. According to the molecular docking simulation, we found that *MAPK1*-quercetin, *MMP9*-quercetin, *NFKBIA*-quercetin, *CASP8*-quercetin,

MAPK14-Myricanone, PTGS2-Perlolryrine, PTGS2-Myricanone, PTGS2-kaempferol, and PTGS2-catechin might exert important roles in treating MI.

Conclusion

The present study reveals that SXXTDP exert cardioprotection effect via regulating multiple targets and multiple pathways in MI. SXXTDP may be a promising drug in treating MI. Meanwhile, our study will provide a scientific basis for the further lab studies.

Data Availability

The data used to support the findings of this study are included in the article and Supplementary Materials.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

This project was funded by the Natural Science Foundation of Fujian Province, China [grant number 2019J01610]. Thanks to Sijiang Li, who corrects English grammar for this article.

CRedit Author Contribution

Daqiu Chen: Data curation, Software, Methodology, Writing—original draft. **Yanqing Wu:** Supervision. **Yixing Chen:** Validation. **Qiaoxing Chen:** Formal analysis. **Xianhua Ye:** Visualization. **Shanghua Xu:** Methodology, Funding. **Shunxiang Luo:** Methodology.

Consent for Publication

All the authors of the article gave their consent to publishing the current research.

Abbreviations

DEG, differential expression genes; DL, drug-likeness; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MI, myocardial infarction; OB, oral bioavailability; PCI, percutaneous coronary intervention; PPI, protein–protein interaction; TCM, Traditional Chinese Medicine; TCMSp, Traditional Chinese Medicine System Pharmacology.

References

- 1 Heusch, G. and Gersh, B.J. (2017) The pathophysiology of acute myocardial infarction and strategies of protection beyond reperfusion: a continual challenge. *Eur. Heart J.* **38**, 774–784, published online EpubMar 14, <https://doi.org/10.1093/eurheartj/ehw224>
- 2 Li, Y., Zhu, Z., Zhang, T. and Zhou, Y. (2019) Ligustrazine attenuates inflammation and oxidative stress in a rat model of arthritis via the Sirt1/NF- κ B and Nrf-2/HO-1 pathways. *Arch. Pharm. Res.* **42**, 824–831, published online EpubSep, <https://doi.org/10.1007/s12272-018-1089-0>
- 3 Ma, Y.L., Bates, S. and Gurney, A.M. (2006) The effects of paeonol on the electrophysiological properties of cardiac ventricular myocytes. *Eur. J. Pharmacol.* **545**, 87–92, published online EpubSep 18, <https://doi.org/10.1016/j.ejphar.2006.06.064>
- 4 Li, G., Xu, Q., Han, K., Yan, W. and Huang, C. (2020) Experimental evidence and network pharmacology-based analysis reveal the molecular mechanism of Tongxinluo capsule administered in coronary heart diseases. *Biosci. Rep.* **40**, BSR20201349, published online EpubOct 30
- 5 Yu, Y., Yang, F. and Liu, H. (2020) Network pharmacology evaluation of the active ingredients and potential targets of XiaoLuoWan for application to uterine fibroids. *Biosci. Rep.* **40**, BSR20202342, published online EpubDec 23, <https://doi.org/10.1042/BSR20202342>
- 6 Ru, J., Li, P., Wang, J., Zhou, W., Li, B., Huang, C. et al. (2014) TCMSp: a database of systems pharmacology for drug discovery from herbal medicines. *J. Cheminform.* **6**, 13, <https://doi.org/10.1186/1758-2946-6-13>
- 7 Liu, H., Wang, J., Zhou, W., Wang, Y. and Yang, L. (2013) Systems approaches and polypharmacology for drug discovery from herbal medicines: an example using licorice. *J. Ethnopharmacol.* **146**, 773–793, published online EpubApr 19, <https://doi.org/10.1016/j.jep.2013.02.004>
- 8 Xu, X., Zhang, W., Huang, C., Li, Y., Yu, H., Wang, Y. et al. (2012) A novel chemometric method for the prediction of human oral bioavailability. *Int. J. Mol. Sci.* **13**, 6964–6982, <https://doi.org/10.3390/ijms13066964>
- 9 Pundir, S., Martin, M.J. and O'Donovan, C. (2016) UniProt Tools. *Curr. Protoc. Bioinformatics* **53**, 1.29.1–1.29.15, published online EpubMar 24, <https://doi.org/10.1002/0471250953.bi0129s53>
- 10 Wu, L.H., Lin, C., Lin, H.Y., Liu, Y.S., Wu, C.Y., Tsai, C.F. et al. (2016) Naringenin Suppresses Neuroinflammatory Responses Through Inducing Suppressor of Cytokine Signaling 3 Expression. *Mol. Neurobiol.* **53**, 1080–1091, published online EpubMar, <https://doi.org/10.1007/s12035-014-9042-9>
- 11 Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W. et al. (2015) limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic. Acids. Res.* **43**, e47, published online EpubApr 20, <https://doi.org/10.1093/nar/gkv007>

- 12 Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D. et al. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* **13**, 2498–2504, published online EpubNov, <https://doi.org/10.1101/gr.1239303>
- 13 Szklarczyk, D., Morris, J.H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M. et al. (2017) The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. **45**, D362–D368, published online EpubJan 4, <https://doi.org/10.1093/nar/gkw937>
- 14 Chin, C.H., Chen, S.H., Wu, H.H., Ho, C.W., Ko, M.T. and Lin, C.Y. (2014) cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst. Biol.* **8**, S11, <https://doi.org/10.1186/1752-0509-8-S4-S11>
- 15 Yu, G., Wang, L.G., Han, Y. and He, Q.Y. (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* **16**, 284–287, published online EpubMay, <https://doi.org/10.1089/omi.2011.0118>
- 16 Robinson, M.D., McCarthy, D.J. and Smyth, G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139–140, published online EpubJan 1, <https://doi.org/10.1093/bioinformatics/btp616>
- 17 Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S. et al. (2019) PubChem 2019 update: improved access to chemical data. *Nucleic Acids Res.* **47**, D1102–D1109, published online EpubJan 8, <https://doi.org/10.1093/nar/gky1033>
- 18 Buntrock, R.E. (2002) ChemOffice Ultra 7.0. *J. Chem. Inf. Comput. Sci.* **42**, 1505–1506, published online EpubNov-Dec, <https://doi.org/10.1021/ci025575p>
- 19 Burley, S.K., Berman, H.M., Kleywegt, G.J., Markley, J.L., Nakamura, H. and Velankar, S. (2017) Protein Data Bank (PDB): The Single Global Macromolecular Structure Archive. *Methods Mol. Biol.* **1607**, 627–641, https://doi.org/10.1007/978-1-4939-7000-1_26
- 20 Gasanoff, E.S., Li, F., George, E.M. and Dagda, R.K. (2019) A Pilot STEM curriculum designed to teach high school students concepts in biochemical engineering and pharmacology. *EC Pharmacol. Toxicol.* **7**, 846–877, published online EpubAug
- 21 Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S. et al. (2009) AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* **30**, 2785–2791, published online EpubDec, <https://doi.org/10.1002/jcc.21256>
- 22 Trott, O. and Olson, A.J. (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **31**, 455–461, published online EpubJan 30, <https://doi.org/10.1002/jcc.21334>
- 23 Roth, G.A., Johnson, C., Abajobir, A., Abd-Allah, F., Abera, S.F., Abyu, G. et al. (2017) Global, regional, and national burden of cardiovascular diseases for 10 Causes, 1990 to 2015. *J. Am. Coll. Cardiol.* **70**, 1–25, published online EpubJul 4, <https://doi.org/10.1016/j.jacc.2017.04.052>
- 24 Geijtenbeek, T.B., van Vliet, S.J., Engering, A., Hart, B.A. and van Kooyk, Y. (2004) Self- and nonself-recognition by C-type lectins on dendritic cells. *Annu. Rev. Immunol.* **22**, 33–54, <https://doi.org/10.1146/annurev.immunol.22.012703.104558>
- 25 Chen, Z., Yan, W., Mao, Y., Ni, Y., Zhou, L., Song, H. et al. (2018) Effect of aerobic exercise on Treg and Th17 of rats with ischemic cardiomyopathy. *J. Cardiovasc. Transl. Res.* **11**, 230–235, published online EpubJun, <https://doi.org/10.1007/s12265-018-9794-0>
- 26 Han, X., Chen, X., Han, J., Zhong, Y., Li, Q. and An, Y. (2020) MiR-324/SOCS3 Axis protects against hypoxia/reoxygenation-induced cardiomyocyte injury and regulates myocardial ischemia via TNF/NF- κ B signaling pathway. *Int. Heart J.* **61**, 1258–1269, published online EpubNov 13
- 27 Durço, A.O., de Souza, D.S., Heimfarth, L., Miguel-Dos-Santos, R., Rabelo, T.K., Oliveira Barreto, T. et al. (2019) d-limonene ameliorates myocardial infarction injury by reducing reactive oxygen species and cell apoptosis in a murine model. **82**, 3010–3019, published online EpubNov 22, <https://doi.org/10.1021/acs.jnatprod.9b00523>
- 28 Xu, Y., Tang, T., Ding, Y., Yao, R., Xie, J., Liao, M. et al. (2010) Improved cardiac performance by rosuvastatin is associated with attenuations in both myocardial tumor necrosis factor- α and p38 MAP kinase activity in rats after myocardial infarction. *Am. J. Med. Sci.* **340**, 121–127, published online EpubAug, <https://doi.org/10.1097/MAJ.0b013e3181e2b353>
- 29 Yokota, T., Li, J., Huang, J., Xiong, Z., Zhang, Q., Chan, T. et al. (2020) p38 Mitogen-activated protein kinase regulates chamber-specific perinatal growth in heart. *J. Clin. Invest.* **130**, 5287–5301, published online EpubOct 1, <https://doi.org/10.1172/JCI135859>
- 30 Hu, Y., Dong, X., Zhang, T., Ma, H., Yang, W., Wang, Y. et al. (2020) Kai-Xin-San suppresses matrix metalloproteinases and myocardial apoptosis in rats with myocardial infarction and depression. *Mol. Med. Rep.* **21**, 508–516, published online EpubJan, <https://doi.org/10.3892/mmr.2019.10807>
- 31 Song, K.Y., Zhang, X.Z., Li, F. and Ji, Q.R. (2020) Silencing of ATP2B1-AS1 contributes to protection against myocardial infarction in mouse via blocking NFKBIA-mediated NF- κ B signalling pathway. **24**, 4466–4479, published online EpubApr, <https://doi.org/10.1111/jcmm.15105>
- 32 Cheng, B., Zhong, J.P., Wu, F.X., Li, G.L., Ruan, Q.X., Luo, G. et al. (2019) Ebselen protects rat hearts against myocardial ischemia-reperfusion injury. *Exp. Ther. Med.* **17**, 1412–1419, published online EpubFeb, <https://doi.org/10.3892/etm.2018.7089>
- 33 Ge, Z.W., Zhu, X.L., Wang, B.C., Hu, J.L., Sun, J.J., Wang, S. et al. (2019) MicroRNA-26b relieves inflammatory response and myocardial remodeling of mice with myocardial infarction by suppression of MAPK pathway through binding to PTGS2. *Int. J. Cardiol.* **280**, 152–159, published online EpubApr 1, <https://doi.org/10.1016/j.ijcard.2018.12.077>
- 34 Li, Y., Yao, J., Han, C., Yang, J., Chaudhry, M.T., Wang, S. et al. (2016) Quercetin, inflammation and immunity. *Nutrients* **8**, 167, published online EpubMar 15, <https://doi.org/10.3390/nu8030167>
- 35 Li, B., Yang, M., Liu, J.W. and Yin, G.T. (2016) Protective mechanism of quercetin on acute myocardial infarction in rats. *Genet. Mol. Res.* **15**, 15017117, published online EpubMar 11, <https://doi.org/10.4238/gmr.15017117>
- 36 Ibrahim, S.R.M., Mohamed, G.A., Khedr, A.I.M. and Aljaeid, B.M. (2017) Anti-oxidant and anti-inflammatory cyclic diarylheptanoids from alnus japonica stem Bark. *Iran J. Pharm. Res.* **16**, 83–91, published online EpubWinter
- 37 Lee, S.H., Jeong, S.J., Jang, G.Y., Kim, M.Y., Hwang, I.G., Kim, H.Y. et al. (2016) Isolation and identification of an antiproliferative compound from fructose-tryptophan maillard reaction products. *J. Agric. Food Chem.* **64**, 3041–3047, published online EpubApr 20, <https://doi.org/10.1021/acs.jafc.6b00157>
- 38 Suchal, K., Malik, S., Gamad, N., Malhotra, R.K., Goyal, S.N., Bhatia, J. et al. (2016) Kampeferol protects against oxidative stress and apoptotic damage in experimental model of isoproterenol-induced cardiac toxicity in rats. *Phytomedicine* **23**, 1401–1408, published online EpubNov 15, <https://doi.org/10.1016/j.phymed.2016.07.015>

- 39 Cong, L., Su, Y., Wei, D., Qian, L., Xing, D., Pan, J. et al. (2020) Catechin relieves hypoxia/reoxygenation-induced myocardial cell apoptosis via down-regulating lncRNA MIAT. *24*, 2356–2368, published online EpubFeb, <https://doi.org/10.1111/jcmm.14919>
- 40 Chartoumpakis, D., Ziros, P.G., Psyrogiannis, A., Kyriazopoulou, V., Papavassiliou, A.G. and Habeos, I.G. (2010) Simvastatin lowers reactive oxygen species level by Nrf2 activation via PI3K/Akt pathway. *Biochem. Biophys. Res. Commun.* **396**, 463–466, published online EpubMay 28, <https://doi.org/10.1016/j.bbrc.2010.04.117>
- 41 Sun, W., Pan, R., Song, J. and Sun, H. (2019) The effects of simvastatin preconditioning on the expression of caspase-3 after myocardial ischemia reperfusion injury in rats. *Exp. Ther. Med.* **17**, 2230–2234, published online EpubMar, <https://doi.org/10.3892/etm.2019.7164>
- 42 Zhang, J., Cheng, X., Liao, Y.H., Lu, B., Yang, Y., Li, B. et al. (2005) Simvastatin regulates myocardial cytokine expression and improves ventricular remodeling in rats after acute myocardial infarction. *Cardiovasc. Drugs Ther.* **19**, 13–21, published online EpubJan, <https://doi.org/10.1007/s10557-005-6893-3>
- 43 Vilahur, G., Casani, L., Peña, E., Juan-Babot, O., Mendieta, G., Crespo, J. et al. (2014) HMG-CoA reductase inhibition prior reperfusion improves reparative fibrosis post-myocardial infarction in a preclinical experimental model. *Int. J. Cardiol.* **175**, 528–538, published online EpubAug 20, <https://doi.org/10.1016/j.ijcard.2014.06.040>
- 44 Luo, K.Q., Long, H.B. and Xu, B.C. (2015) Reduced apoptosis after acute myocardial infarction by simvastatin. *Cell Biochem. Biophys.* **71**, 735–740, published online EpubMar, <https://doi.org/10.1007/s12013-014-0257-1>
- 45 Fliedner, S.M., Engel, T., Lendvai, N.K., Shankavaram, U., Nölting, S., Wesley, R. et al. (2014) Anti-cancer potential of MAPK pathway inhibition in paragangliomas-effect of different statins on mouse pheochromocytoma cells. *PLoS ONE* **9**, e97712, <https://doi.org/10.1371/journal.pone.0097712>
- 46 Andrade, V.L., do Valle, I.B. and Sandrim, V.C. (2013) Simvastatin therapy decreases MMP-9 levels in obese women. *J. Clin. Pharmacol.* **53**, 1072–1077, published online EpubOct, <https://doi.org/10.1002/jcph.146>
- 47 Shahida, B., Johnson, P.S., Jain, R., Brorson, H., Åsman, P., Lantz, M. et al. (2019) Simvastatin downregulates adipogenesis in 3T3-L1 preadipocytes and orbital fibroblasts from Graves' ophthalmopathy patients. *Endocr. Connect.* **8**, 1230–1239, published online EpubSep, <https://doi.org/10.1530/EC-19-0319>