Network Pharmacology-Based Prediction of Active Ingredients and Mechanisms of Tongxinluo Against Acute Myocardial Infarction

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Abstract

Purpose: The aim of this network pharmacology was to explore the potential active ingredients and mechanisms of Tongxinluo (TXL) against acute myocardial infarction (AMI).

Methods: We selected active ingredients and targets of TXL according to TCMSP database and converted protein targets into gene symbol by UniProt database. Therapeutic gene targets on AMI were collected from DisGeNET and GeneCards databases. The overlapping genes between ingredients and AMI were identified using Venn diagram. Then, the interaction network between ingredients and overlapping genes was constructed, visualized, and analyzed by Cytoscape software. Protein-protein interaction (PPI) was analyzed by String database. Finally, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of overlapping genes were carried out by metascape platform.

Results: A total of 111 active ingredients, 184 ingredient-related genes, and 1020 AMI-related genes were retrieved using public databases. Eventually, 79 overlapping genes between TXL and AMI were identified. Cytoscape and PPI results suggested that the active ingredients and genes of TXL against AMI consisted of 66 active ingredients and 79 genes, among them beta-sitosterol and IL-6 were the uppermost active ingredient and hub gene, respectively. Metascape results exhibited that the key mechanism of TXL against AMI might be reducing oxidative stress in cell membrane by inactivating pathways in cancer.

Conclusion: This network pharmacology study reveals potential mechanisms of multi-target and multi-component TXL in the treatment of AMI, providing scientific evidence for further expounding the active ingredients and mechanisms of TXL against AMI.

Introduction

Acute myocardial infarction (AMI) is a common cardiac emergency, with the potential for subsequent morbidity and mortality.[1] Early reperfusion and restoration of the blood flow in the occluded artery remain the cornerstone for preserving the viability of the ischemic myocardium and limiting infarct size.[2] Despite the management of AMI has improved dramatically over the past three decades and continues to evolve,[3] and a decrease in AMI-related death has also been seen in some countries,[4, 5] however, on the other hand, mortality seems to have plateaued or even elevated in some developing countries.[6, 7]Due to the rate of mortality and recurrent MI remain high after the acute phase of MI,[8] it is vital to improve medical care during AMI admission and integrate medical care into secondary prevention programs. As the cornerstone in anti-atherosclerotic regimen, statin has been demonstrated substantial efficacy in reducing cardiovascular events. However, the increased attention on the adverse events associated with statin such as potential harmful effects on muscle and liver has unfortunately led to statin therapy discontinuation, nonadherence to therapy or concerns about initiating statin therapy.[9] Thus, it is warranted to explore alternative anti-atherosclerotic medications with high efficacy as well as low side-effect.

Tongxinluo (TXL), a traditional Chinese medicine (TCM) formula composed of *Ginseng*, *Hirudo*, *Scorpio*, *Radix Paeoniae Rubra*, *Cicadidae*, *Eupolyphaga*, *Scolopendra*, *Lignum Dalbergiae Odoriferae*, *Santalaceae*, *Olibanum, Ziziphi Spinosae Semen*, and *Borneolum*, was registered with the China State Food and Drug Administration (CFDA) in 1996. Previous studies demonstrated protective effects of TXL in a substantial number of diseases, such as pulmonary hypertension,[10] angina pectoris,[11] acute stroke,[12] atherosclerosis.[13] For the past few years, TXL has been an increasingly important strategy for treatment of atherosclerosis in China due to its good therapeutic effect and low toxic side effects. However, the chemical and pharmacological foundations of TXL in inhibiting atherosclerosis, especially AMI, was not globally evaluated with appropriate approaches. Therefore, the studies on active ingredients and mechanisms of TXL against AMI should be strengthened to provide scientific evidence to support its clinical application in treating AMI.

Because of the complex composition of TCM, conventional pharmacological approaches to experimentally identify the unique action of mechanism may not be feasible for TCM research. In order to comprehensively evaluate the active chemical ingredients and pharmacological effects of TCM, the TCM network pharmacology based on big databases has become a promising and useful tool to characterize the action mechanisms of complicated drug system in detail, ranging from the molecular level to the pathway level.[14] Network pharmacology can decipher the mechanism of drugs action with a holistic perspective, which emphasizes the paradigm shift from “one target, one drug” to “network target, multicomponent therapeutics”.[15] The characteristic is paramount for TCM research, as the holistic theory has long been central to TCM treatments of various diseases.[16] Combined with pharmacology and pharmacodynamics, network pharmacology has been successfully applied to explain the mechanism of TCM at the molecular network level.[17]

In this study, we aim to utilize network pharmacology to systematically identify the active ingredients in TXL, construct a network pharmacological model of active ingredients, and analyze the potential anti-AMI mechanism of active ingredients in TXL. First, active ingredients from TXL and genes related to selected active ingredients were screened. Then, genes related to AMI were identified, and the overlapping genes between ingredients and AMI gene targets were identified. Third, interaction network between overlapping genes and related ingredients were analyzed. Fourth, protein–protein interaction (PPI) network analysis of the overlapping gene targets was conducted to investigate the interaction between overlapping gene targets. Finally, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of overlapping genes were carried out to explore the molecular mechanisms of TXL against AMI. The workflow is shown in Fig. 1.

Material And Methods

Identification and Screening of Active Ingredients and Corresponding Targets in Tongxinluo

To collect the constituent data of TXL, we primarily used the Traditional Chinese Medicine Systems Pharmacology Database[18] (TCMSP, https://tcmspw.com/tcmsp.php), a unique systems pharmacology platform of Chinese herbal medicines that captures the relationships between drugs, targets and diseases. Meanwhile, the Traditional Chinese Medicine Integrated Database[19] (TCMID, http://www.megabionet.org/tcmid/) and the Traditional Chinese Medicine Information Database[20] (TCM-ID, http://bidd.group/TCMID/) were used as supplementary databases in case data were not available in TCMSP. The effective ingredients of TXL were mainly filtered by two criteria: oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18, which are the most important indicators for evaluating the characteristics of ADME (absorption, distribution, metabolism and excretion).[21] TCM is often administered orally, and its OB is determined by body absorption, distribution, and liver metabolism. Therefore, the degree of OB largely determines the effect of the ingredient on disease. DL is used to screen out first-rank ingredients and improve candidate ingredients in the early period of drug development.

The protein targets associated with active ingredients were retrieved from the TCMSP database. Full names of the protein targets were converted into gene symbol on the basis of UniProt (https://www.uniprot.org/) for following analysis.

AMI-Related Targets

The different genes associated with AMI were gathered from DisGeNET[22] (http://www.disgenet.org/), a discovery platform contains one of the largest publicly available collections of genes and variants associated with human diseases; and GeneCards[23] (https://www.genecards.org/), an integrative database that provides comprehensive information on all annotated and predicted human genes with keywords “acute myocardial infarction”. Genes met Score > 0.1 in DisGeNET or Score > 10 in GeneCards were selected.

The overlapping genes between active ingredients and AMI were visualized by Venn diagram, plotted using the Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/), a free online platform. The overlapping gene targets of TXL and AMI were then regarded as the core targets of TXL for AMI.

Protein-Protein Interaction (PPI) Network Construction of Overlapping Genes

To explain the interaction between overlapping gene targets, PPI coming from String[24] (https://string-db.org/cgi/input.pl) with the species limited to “Homo sapiens” was constructed. The associations in STRING include direct (physical) interactions as well as indirect (functional) interactions. The website generates a score for each protein mutual information, with low confidence defined as scores < 0.4, medium confidence defined as scores 0.4 to 0.7, and high confidence defined as scores > 0.7, respectively. In our analysis, PPI with direct interactions, high confidence, and hiding disconnected nodes were reserved.

Network Construction

Network construction was performed as follows: (1) herb-ingredient-target network was built by connecting herbs, chemical ingredients and corresponding targets; (2) herb-ingredient-overlapping target network was built by connecting herbs, chemical ingredients and overlapping gene targets; (3) target-pathway network was built by connecting targets and top 20 pathways; (4) ingredient-target-pathway network was built by connecting ingredients, targets, and top 20 pathways. The network of the interactions was constructed, visualized, and analyzed through Cytoscape 3.8.0[25] (http://www.cytoscape.org/), an open-source software platform which integrates biomolecular interaction networks with different types of attribute data into a unified conceptual framework. Nodes in network indicate herbs, ingredients, gene targets, or pathways, while edges between nodes suggest interactions between herbs, ingredients, gene targets, or pathways.

The key active ingredients of TXL against AMI were selected out by setting “Degree value”, which represents the edge numbers of ingredients in network. The larger the value of ingredients is, the more important they are for the therapeutic effect of TXL on AMI.

Gene Ontology and Pathway Enrichment of Overlapping Genes

To elucidate the role of overlapping gene targets in gene function and signaling pathway, Metascape[26] (https://metascape.org/), a web-based portal designed to provide a comprehensive gene list annotation and analysis resource, was applied to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Enriched GO terms and pathways were defined as those with False Discovery Rate (FDR) < 0.05. In GO enrichment analysis, the bubble chart was plotted by using the Rpackage “clusterProfiler”.

Results

Active Ingredients and Corresponding Gene Targets in TXL

All chemicals that meet the screening criteria: OB > 30% and DL > 0.18 were considered as candidate active ingredients. Eventually, a total of 111 active ingredients were retrieved in TCMSP, TCMID, and TCM-ID databases after screening ADME parameters, including 37 ingredients from *Lignum Dalbergiae Odoriferae*, 29 ingredients from *Radix Paeoniae Rubra*, 22 ingredients from *Ginseng*, 9 ingredients from *Ziziphi Spinosae Semen*, 8 ingredients from *Olibanum*, 3 ingredients from *Borneolum*, 3 ingredients from *Santalaceae*, 2 ingredients from *Eupolyphaga*, 2 ingredients from *Scorpio*, 1 ingredient from *Scolopendra*, and 1 ingredient from *Hirudo*, respectively. Notably, MOL000358(beta-sitosterol) was the common active ingredient among *Radix Paeoniae Rubra*, *Ginseng*, and *Lignum Dalbergiae Odoriferae*; MOL000359(sitosterol) was the common active ingredient between *Lignum Dalbergiae Odoriferae* and *Radix Paeoniae Rubra*; MOL000422(kaempferol) was the common active ingredient between *Ginseng* and *Eupolyphaga*; MOL000449(Stigmasterol) was the common active ingredient between *Ginseng* and

Radix Paeoniae Rubra; MOL000953(cholesterol) was the common active ingredient between *Scorpio* and *Scolopendra*. The main active ingredients of TXL are shown in Table 1.

Table 1
Basic information of active ingredients of TXL

Herb	Mol ID	Molecule Name	OB (%)	DL
Ginseng	MOL002879	Diop	43.59	0.39
	MOL000449	Stigmasterol	43.83	0.76
	MOL000358	beta-sitosterol	36.91	0.75
	MOL003648	Inermin	65.83	0.54
	MOL000422	kaempferol	41.88	0.24
	MOL004492	Chrysanthemaxanthin	38.72	0.58
	MOL005308	Aposiopolamine	66.65	0.22
	MOL005314	Celabenzine	101.88	0.49
	MOL005317	Deoxyharringtonine	39.27	0.81
	MOL005318	Dianthramine	40.45	0.2
	MOL005320	arachidonate	45.57	0.2
	MOL005321	Frutinone A	65.9	0.34
	MOL005344	ginsenoside rh2	36.32	0.56
	MOL005348	Ginsenoside-Rh4_qt	31.11	0.78
	MOL005356	Girinimbin	61.22	0.31
	MOL005357	Gomisin B	31.99	0.83
	MOL005360	malkangunin	57.71	0.63
	MOL005376	Panaxadiol	33.09	0.79
	MOL005384	suchilactone	57.52	0.56
	MOL005399	alexandrin_qt	36.91	0.75
	MOL005401	ginsenoside Rg5_qt	39.56	0.79
	MOL000787	Fumarine	59.26	0.83
Hirudo	MOL001406	crocetin	35.3	0.26
Scorpio	MOL011455	20-hexadecanoylingenol	32.7	0.65
	MOL000953	cholesterol	37.87	0.68
Radix Paeoniae Rubra	MOL001002	ellagic acid	43.06	0.43

Herb	Mol ID	Molecule Name	OB (%)	DL
	MOL001918	paeoniflorgenone	87.59	0.37
	MOL001921	Lactiflorin	49.12	0.8
	MOL001924	paeoniflorin	53.87	0.79
	MOL001925	paeoniflorin_qt	68.18	0.4
	MOL002714	baicalein	33.52	0.21
	MOL002776	Baicalin	40.12	0.75
	MOL000358	beta-sitosterol	36.91	0.75
	MOL000359	sitosterol	36.91	0.75
	MOL004355	Spinasterol	42.98	0.76
	MOL000449	Stigmasterol	43.83	0.76
	MOL000492	(+)-catechin	54.83	0.24
	MOL006990	(1S,2S,4R)-trans-2-hydroxy-1,8-cineole-B-D-glucopyranoside	30.25	0.27
	MOL006992	(2R,3R)-4-methoxyl-distylin	59.98	0.3
	MOL006994	1-o-beta-d-glucopyranosyl-8-o-benzoylpaeonisuffrone_qt	36.01	0.3
	MOL006996	1-o-beta-d-glucopyranosylpaeonisuffrone_qt	65.08	0.35
	MOL006999	stigmast-7-en-3-ol	37.42	0.75
	MOL007003	benzoyl paeoniflorin	31.14	0.54
	MOL007004	Albiflorin	30.25	0.77
	MOL007005	Albiflorin_qt	48.7	0.33
	MOL007008	4-ethyl-paeoniflorin_qt	56.87	0.44
	MOL007012	4-o-methyl-paeoniflorin_qt	56.7	0.43
	MOL007014	8-debenzoylpaeonidanin	31.74	0.45
	MOL007016	Paeoniflorigenone	65.33	0.37
	MOL007018	9-ethyl-neo-paeoniaflorin A_qt	64.42	0.3
	MOL007022	evofolinB	64.74	0.22
	MOL007025	isobenzoylpaeoniflorin	31.14	0.54
	MOL002883	Ethyl oleate (NF)	32.4	0.19

Herb	Mol ID	Molecule Name	OB (%)	DL
	MOL005043	campest-5-en-3beta-ol	37.58	0.71
Eupolyphaga	MOL000422	kaempferol	41.88	0.24
	MOL000098	quercetin	46.43	0.28
Scolopendra	MOL000953	cholesterol	37.87	0.68
Lignum Dalbergiae Odoriferae	MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	42.36	0.21
	MOL001792	DFV	32.76	0.18
	MOL000228	(2R)-7-hydroxy-5-methoxy-2-phenylchroman-4-one	55.23	0.2
	MOL002565	Medicarpin	49.22	0.34
	MOL002914	Eriodyctiol (flavanone)	41.35	0.24
	MOL002938	(3R)-4'-Methoxy-2',3,7-trihydroxyisoflavanone	68.86	0.27
	MOL002939	(3R)-5'-Methoxyvestitol	83.06	0.26
	MOL002940	(3R)-3-(2,3-dihydroxy-4-methoxyphenyl)-7-hydroxychroman-4-one	52.06	0.27
	MOL002941	(3R)-3-(2,3-dihydroxy-4-methoxyphenyl)chroman-7,8-diol	82.35	0.27
	MOL002950	(3R)-7,2',3'-trihydroxy-4-methoxyisoflavan	69.65	0.24
	MOL002957	9-O-Methylcoumestrol	33.73	0.38
	MOL002958	3'-Hydroxymelanettin	30.69	0.27
	MOL002959	3'-Methoxydaidzein	48.57	0.24
	MOL002961	(-)-Vestitol	70.29	0.21
	MOL002962	(3S)-7-hydroxy-3-(2,3,4-trimethoxyphenyl)chroman-4-one	48.23	0.33
	MOL002963	4',5',7-trimethyl-3-methoxyflavone	40.66	0.25
	MOL002966	Dalbergin	78.18	0.2
	MOL002967	7-hydroxy-4'-methoxy-2',5'-dioxo-4-[(3R)-2',7-dihydroxy-4'-methoxyisoflavan-5'-yl]isoflavane	34.78	0.7
	MOL002973	Bowdichione	55.78	0.28
	MOL002975	butin	69.94	0.21

Herb	Mol ID	Molecule Name	OB (%)	DL
	MOL002981	Duartin	70.63	0.34
	MOL002982	(3R,4R)-3',7-dihydroxy-2',4'-dimethoxy-4-[(2S)-4',5,7-trihydroxyflavanone-6-yl]isoflavan	33.96	0.63
	MOL002985	isoduartin	74.11	0.34
	MOL002989	4-Hydroxyhomopterocarpin	48.41	0.43
	MOL002990	(6aR,11aR)-3,9,10-trimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-4-ol	66.86	0.53
	MOL002991	(6aR,11aR)-3,9-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromene-4,10-diol	38.96	0.48
	MOL002996	odoricarpin	55.02	0.53
	MOL002997	3-(2-hydroxy-3,4-dimethoxyphenyl)-2H-chromen-7-ol	86.18	0.27
	MOL002999	Sativanone	85.63	0.27
	MOL003000	Stevein	36.54	0.24
	MOL003001	Vestitone	52.83	0.24
	MOL003002	violanone	80.24	0.3
	MOL003003	Xenognosin B	72.71	0.24
	MOL000358	beta-sitosterol	36.91	0.75
	MOL000359	sitosterol	36.91	0.75
	MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.42
	MOL000392	formononetin	69.67	0.21
Santalaceae	MOL000354	isorhamnetin	49.6	0.31
	MOL000006	luteolin	36.16	0.25
	MOL002322	isovitexin	31.29	0.72
Olibanum	MOL001215	tirucallol	42.12	0.75
	MOL001241	O-acetyl-α-boswellic acid	42.73	0.7
	MOL001243	3alpha-Hydroxy-olean-12-en-24-oic-acid	39.32	0.75
	MOL001255	Boswellic acid	39.55	0.75
	MOL001263	3-oxo-tirucallic,acid	42.86	0.81

Herb	Mol ID	Molecule Name	OB (%)	DL
	MOL001265	acetyl-alpha-boswellic,acid	42.73	0.7
	MOL001272	incensole	45.59	0.22
	MOL001295	phyllocladene	33.4	0.27
Ziziphi Spinosae Semen	MOL001521	ceanothic acid	33.41	0.77
	MOL001522	(S)-Coclaurine	42.35	0.24
	MOL001525	Daucosterol	36.91	0.75
	MOL001527	jujuboside A_qt	34.96	0.62
	MOL001532	phytosterol	36.91	0.75
	MOL001539	sanjoinenine	67.28	0.79
	MOL001542	swertisin	31.83	0.75
	MOL001546	zizyphusine	41.53	0.55
	MOL000211	Mairin	55.38	0.78
Borneolum	MOL006861	asiatic acid	41.38	0.71
	MOL006862	bronyl acetate	59.3	0.51
	MOL006865	dipterocarpol	41.71	0.76
Cicadidae				

To further study the potential mechanism of anti-AMI, it is of paramount importance to understand the gene targets on which these ingredients act. According to the target screening of the ingredients based on TCMSP, we found that there were 184 protein targets among 10 Chinese herbs in TXL. There were 109 targets in *Ginseng*, 88 targets in *Radix Paeoniae Rubra*, 79 targets in *Santalaceae*, 78 targets in *Lignum Dalbergiae Odoriferae*, 29 targets in *Ziziphi Spinosae Semen*, 15 targets in *Hirudo*, 14 targets in *Olibanum*, 11 targets in *Eupolyphaga*, 4 targets in *Scorpio*, and 4 targets in *Scolopendra*, respectively. The targets of *Borneolum* and the herb *Cicadidae* were not found in TCMSP or alternative databases. The protein targets were converted into gene targets by UniProt. The analysis of herb-ingredient-target network relationship among 11 Chinese herbs, 111 ingredients, and 184 ingredient-related targets was shown in Fig. 2, which revealed that the interaction contained 306 nodes and 1234 edges.

Identification of AMI-Related Genes

After administration of search queries in GeneCards and DisGeNET databases, a total of 1020 AMI-relevant genes were obtained. Furthermore, intersection between ingredient-related and AMI-relevant gene

targets were performed by Venn diagram. Eventually, a total of 79 overlapping genes were obtained. The Venn diagram of overlapping genes was displayed in Fig. 3.

Key Active Ingredients of TXL Against AMI

The interactions between 79 overlapping genes and corresponding ingredients and herbs were visualized by network in Fig. 4, which includes 155 nodes and 627 edges. The results suggested that the therapeutic effect of TXL on AMI was directly related to 10 herbs, 66 active ingredients, and 79 genes. *Lignum Dalbergiae Odoriferae* had 29 active ingredients, which was the largest number among the 10 herbs. Based on the degree value of each ingredient, it was very easy to distinguish the contribution difference of 66 active ingredients to TXL against AMI. Beta-sitosterol (Table 2), which connected to 53 genes, was considered as the uppermost active ingredient of TXL against AMI.

Table 2
A list of the main ingredients linking to overlapping gene targets

No.	Mol ID	Ingredient	Gene Number	Herb
1	MOL000358	beta-sitosterol	53	Radix Paeoniae Rubra
2	MOL000422	Diop	38	Ginseng
3	MOL000006	luteolin	30	Santalaceae
4	MOL000449	Stigmasterol	27	Ginseng
5	MOL000392	formononetin	20	Lignum Dalbergiae Odoriferae
6	MOL002961	(-)-Vestitol	19	Lignum Dalbergiae Odoriferae
6	MOL003003	Xenognosin B	19	Lignum Dalbergiae Odoriferae
8	MOL002714	baicalein	18	Radix Paeoniae Rubra
9	MOL000354	isorhamnetin	17	Santalaceae
10	MOL002985	isoduartin	16	Lignum Dalbergiae Odoriferae
11	MOL002981	Duartin	15	Lignum Dalbergiae Odoriferae
12	MOL002565	Medicarpin	14	Lignum Dalbergiae Odoriferae
12	MOL002963	4',5',7-trimethyl-3-methoxyflavone	14	Lignum Dalbergiae Odoriferae
14	MOL000787	Fumarine	12	Ginseng
14	MOL002950	(3R)-7,2',3'-trihydroxy-4-methoxyisoflavan	12	Lignum Dalbergiae Odoriferae
14	MOL002962	(3S)-7-hydroxy-3-(2,3,4-trimethoxyphenyl)chroman-4-one	12	Lignum Dalbergiae Odoriferae

No.	Mol ID	Ingredient	Gene Number	Herb
14	MOL002999	Sativanone	12	Lignum Dalbergiae Odoriferae
14	MOL003001	Vestitone	12	Lignum Dalbergiae Odoriferae
19	MOL001002	ellagic acid	11	Radix Paeoniae Rubra
19	MOL002941	(3R)-3-(2,3-dihydroxy-4-methoxyphenyl)chroman-7,8-diol	11	Lignum Dalbergiae Odoriferae
19	MOL002990	(6aR,11aR)-3,9,10-trimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-4-ol	11	Lignum Dalbergiae Odoriferae
22	MOL005321	Frutinone A	10	Ginseng
22	MOL002940	(3R)-3-(2,3-dihydroxy-4-methoxyphenyl)-7-hydroxychroman-4-one	10	Lignum Dalbergiae Odoriferae
22	MOL002996	odoricarpin	10	Lignum Dalbergiae Odoriferae

Analysis of PPI Network

Overlapping genes were submitted to STRING for PPI network construction, and high confidence of interaction data with a score > 0.7 was selected (Fig. 4A). PPI was displayed by a total of 79 nodes and 416 edges with average node degree of 10.5, of which nodes represented the gene targets and the edges represented the interactions between the genes. In this network interaction (Fig. 4B), the darker the color, the stronger the gene corresponding to the node, which indicates that the gene plays a key role in the whole interaction network and is an important gene target. Figure 4B showed that *IL6*, *AKT1*, *TNF*, *JUN*, *TP53*, *MAPK8*, *VEGF*, *MMP9*, and *NOS3* were centrally located in the PPI network, indicating that these genes were involved in the main pathogenesis of AMI. *IL6* showed the highest degree in interaction with 36 times, followed by *AKT1* (34 times), *TNF* (32 times), *JUN* (31 times), *TP53* (30 times), and *MAPK8* (30 times), respectively. Top 20 genes with higher interaction times were shown in Fig. 4C.

GO Analysis of Overlapping Gene targets

The top 20 significantly enriched terms in biological process (BP), cellular component (CC), and molecular function (MF) categories were selected, according to $P < 0.05$. As shown in Fig. 5A, in the BP category, four terms were involved in oxidative stress or oxidative stress-related progress: response to oxidative stress, response to reactive oxygen species, cellular response to oxidative stress, and cellular

response to reactive oxygen species. In the CC category, the gene targets were mainly classified into membrane raft, membrane microdomain, and membrane region (Fig. 5B). Simultaneously, MF terms mainly contained receptor ligand activity, signaling receptor activator activity, and endopeptidase activity (Fig. 5C).

KEGG enrichment analysis

To further clarify the relationship between overlapping gene targets and the pathways, the top 20 pathways involving 61 gene targets were screened according to the KEGG analysis with $P < 0.05$ (Fig. 7A-7C). The results showed that these target proteins were mainly involved in pathways in cancer, AGE-RAGE signaling pathway in diabetic complications, and Fluid shear stress and atherosclerosis, and so on. The gene targets involved in cancer pathway included *AKT1, AR, BAX, BCL2, CASP3, CASP7, CASP9, CCND1, CXCL8, EGLN1, ESR1, ESR2, F2, GSK3B, GSTM1, GSTP1, HMOX1, IFNG, IGF2, IL4, IL6, JUN, MAPK1, MAPK8, MMP1, MMP2, MMP9, PIM1, PPARG, PTGS2, RELA, TGFB1, TP53*, and *VEGFA*. Meanwhile, *AKT1, BAX, BCL2, CASP3, CCND1, CXCL8, ICAM1, IL1B, IL6, JUN, MAPK1, MAPK14, MAPK8, MMP2, NOS3, PIM1, RELA, TGFB1, TNF, VCAM1*, and *VEGFA* involved in the AGE-RAGE signaling pathway in diabetic complications. The gene targets involved in Fluid shear stress and atherosclerosis were *AKT1*, *BCL2*, *GSTM1*, *GSTP1*, *HMOX1*, *ICAM1*, *IFNG*, *IL1B*, *JUN*, *KDR*, *MAPK14*, *MAPK8*, *MMP2*, *MMP9*, *NCF1*, *NOS3*, *RELA*, *TNF*, *TP53*, *VCAM1*, and *VEGFA*. Obviously, there are multiple gene targets in one pathway, and the same gene target exists in multiple pathways (Fig. 7D).

Discussion

TCM has a long history in the treatment of chronic diseases such as coronary artery disease and various complications. In the pharmacological research of active ingredients, due to the complex composition of TCM, conventional pharmacological approaches to identify the unique ingredients and mechanisms may not be feasible. However, the active ingredients and gene targets are important in uncovering the pharmacological mechanisms of TCM, which can provide a theoretical basis for drug development and design.[27] Therefore, the network pharmacology provides a unique and innovative way to study the active ingredients and understand the mechanisms of multi-ingredient–multi-target–multi-pathway mode of TCM.[28]

In this study, 111 active ingredients and 184 gene targets in TXL were determined by TCMSP database. Subsequently, 79 overlapping genes related to AMI were obtained. Herb-ingredient-overlapping target network suggested that the therapeutic effect of TXL on AMI was directly related to 66 active ingredients. Based on the degree value of each ingredient, beta-sitosterol was considered as the uppermost active ingredient of TXL against AMI. PPI analysis showed that the hub genes were *IL6*, *AKT1*, *TNF*, *JUN*, *TP53*, *MAPK8*, *VEGF*, *MMP9*, and *NOS3.* GO analysis results showed that the overlapping genes were mainly involved in oxidative stress process, the main cellular component was membrane, and the main molecular function was receptor ligand activity. The main pathways were pathways in cancer, AGE-RAGE signaling pathway in diabetic complications, and Fluid shear stress and atherosclerosis.

AMI is usually initiated by myocardial ischemia due to coronary artery obstruction. In the ischemic myocardium, reactive oxygen species (ROS) are generated, which can directly injure cell membrane and induce cell death. Meanwhile, in the ischemic and surrounding myocardium, inflammatory cytokines, e.g., tumor necrosis factor (TNF)-alpha can be produced via the generation of ROS in cardiac myocytes. TNF-alpha can regulate cell survival and cell death, and act as a trigger of another inflammatory reaction. Inversely, inflammatory cytokines can also stimulate ROS formation.[29] In chronic stage, ROS and inflammatory cytokines activate the matrix metalloproteinase (MMP), which elicits the degradation of collagens and may cause a slippage in myofibrillar alignment and left ventricular dilatation. It is reported that in transgenic mice with cardiac overexpression of TNF-alpha, when MMP-2 and MMP-9 expressions were attenuated by inhibition of TNF-alpha, further collagen synthesis, deposition and denaturation were prevented and left ventricular diastolic function was improved. Furthermore, a clinical study also demonstrated a positive correlation between oxidative stress and relative level of MMP-2 and MMP-9 in patients with coronary artery disease.[30]

In our analysis, beta-sitosterol, the uppermost active ingredient of TXL against AMI, is a natural ingredient widely found in many vegetable oils, nuts and plant medicines, and resembles cholesterol structurally, which is considered as a therapeutic agent to reduce serum cholesterol levels and suppress intestinal cholesterol absorption.[31] Meanwhile, several studies also reported that beta-sitosterol functioned as important molecules in stabilizing the phospholipid bilayers of cell membranes and had the potential to suppress obesity-related chronic inflammations, revert immune abnormalities, and regulate the production of nitric oxide.[32] [33] Beta-sitosterol was effective in cardiovascular protection by enhancing cellular glutathione redox cycling, which led to reduced oxidant injury in rat cardiomyocytes.[34] Meanwhile, beta-sitosterol isolated from various plants can modulate antioxidant enzyme levels in pathogenesis and decrease free radical generation and act as scavengers of free radicals *in vitro*.[35] In the recent experiment conducted by Lin et al,[36] the protective effects of beta-sitosterol on myocardial ischemia/reperfusion (I/R) injury were confirmed in the *in vivo* animal model. The experiment suggested that I/R injury suppressed cell viability and induced cell apoptosis and ROS production, yet beta-sitosterol treatment concentration dependently increased the cell viability, reduced cell apoptotic rates and ROS production of I/R-stimulated H9c2 cells. Moreover, increased infarcted area and cell apoptosis in the heart tissues were also observed in the I/R mouse model, whereas beta-sitosterol treatment could alleviate infarction and cell apoptosis. Therefore, these results above implied that beta-sitosterol was effective in exerting protective actions against oxidative stress and myocardial I/R injury.

Interleukin (IL)-6 is a cytokine with both proinflammatory and anti-inflammatory effects on many cell types, affecting B-cell immunoglobulin production, T-cell cytotoxic activity, platelet production and reactivity, and endothelial function. [37] Meta-analysis performed by the Emerging Risk Factors Collaboration demonstrated that for each SD increase in log IL-6, there was a 25% increase in risk of future vascular events.[38] Moreover, in a randomized trial including 3,489 patients, circulating IL-6 was a strong independent marker of increased risk for mortality in patients with unstable coronary artery disease. TNF is one of the most important cytokines produced by macrophages and released very rapidly after all types of injuries and stimuli, which plays a very important role in host defense. However,

prolonged TNF production is also associated with pathology.[39] Therefore, removal of a upregulated cytokine can make a clinical difference. A retrospective cohort study including 8,845 patients suggested that patients treated with TNF inhibitors had approximately half the risk of developing MI (hazard ratio [HR], 0.50; 95% CI, 0.32–0.81) compared with psoriasis patients treated with topical agents.[40] Another protein, MMP-9 is a collagenase, which upregulated in the diabetic heart, and ablation of MMP-9 decreased infarct size in the non-diabetic MI heart, which provided a novel intracellular role of MMP-9 in mediating cell death via apoptosis and pyroptosis.[41] Consistently, the results based on mouse model fed with high fat diet suggested that TXL treatment could lower the expressions of inflammatory cytokines including IL-6, TNF-alpha and MMP-2 in a dose-dependent manner when compared with the control group.[13] AKT1 is the predominant isoform in vascular endothelial cells and plays a crucial role in physiological and pathological angiogenesis.[42] Many of the angiogenic functions attributed to vascular endothelial growth factor (VEGF) are mediated by intracellular activation of the phosphoinositide 3-kinase–Akt signaling pathway. Similarly, VEGF is important in vasculogenesis, which defined as the formation of blood vessels from de novo generation of endothelial cells and angiogenesis. Again, the effect of TXL on VEGF had been explained by previous research, which indicated that the VEGF expression in TXL treated bone marrow mesenchymal stem cells was increased compared to the control group.[43]

Most importantly, activation of endothelial nitric oxide synthase (eNOS) in cardiac microvascular endothelial cells, whose gene name is NOS3, plays a critical role in the protection against myocardial I/R injury. In our recent experiment where *ex vivo*, *in vivo*, and *in vitro* settings of I/R were used, we identified the signals produced by cardiomyocytes that can regulate cardiac microvascular endothelial cells biology during myocardial I/R injury. We found that cardiac microvascular endothelial cells-derived eNOS activity was required for the cardioprotection of TXL,[44] which again confirmed the mechanism of TXL in the treatment of AMI.

KEGG enrichment analysis suggested that the main pathway was pathways in cancer. Similar with cancer cells, ischemic myocardium are also characterized by high levels of oxidative stress.[45] At low levels, ROS increase cell proliferation and survival through the post-translational modification of kinases and phosphatases, which is required for homeostatic signaling events. At moderate levels, ROS induce the expression of stress-responsive genes, which in turn trigger the expression of proteins providing prosurvival signals, such as VEGF.[46] At high levels, however, ROS can result in damage to macromolecules, including DNA, and cause permeabilization of the mitochondria, leading to the release of cytochrome c and apoptosis.[47] The abovementioned process was similar to that of I/R injury in patients with myocardial infarction.[48] Consistent with our KEGG pathway analysis, in a study investigating the anti-oxidation properties of TXL, the authors found that the remarkably up-regulated expression of NADPH oxidase subunits $p22^{phox}$, $p47^{phox}$ and inflammatory factors TNF-alpha, IL-1β and NF-κB induced by C16 could be obviously decreased following pretreatment with TXL,[49] which again highlighted the important role of TXL in the process of oxidative stress. Meanwhile, the main pathway of TXL against AMI was also involved in AGE-RAGE signaling pathway in diabetic complications, which

indicated that TXL may be effective in decreasing diabetic complication. The Fluid shear stress and atherosclerosis pathway has been confirmed.[13]

In our China Tongxinluo Study for myocardial protection in patients with Acute Myocardial Infarction (CTS-AMI) trial,[50] we are going to recruit 3,796 eligible patients with ST-segment elevation myocardial infarction (STEMI) from 120 centers and randomize them in a 1:1 ratio to TXL or placebo groups. The primary endpoint is 30-day major adverse cardiovascular and cerebrovascular events composed of cardiac death, myocardial reinfarction, emergency coronary revascularization, and stroke. Based on the results of CTS-AMI trial, we sought to determine the clinical efficacy, safety, and mechanisms of TXL in the treatment of STEMI patients in the reperfusion era.

There are several limitations in our study. First, our results need to be further verified by experiments. However, findings from our study provided insight into the research of TXL. Meanwhile, in our CTS-AMI trial, we are going to systematically elucidate the efficacy and mechanisms of TXL in the treatment of AMI, which is helpful in explaining the role of TXL. Second, the TCMSP database had not been updated since 2014,[18] therefore, we still could not completely understand the accurate therapeutic mechanism of TXL on AMI despite the results of network pharmacology. More comprehensive and timely updated TCM databases are needed to make the results of network pharmacology analysis more reliable. A comprehensive understanding of the effect of TXL on AMI depends on the common development of multi-disciplines and further invalidation both in experimental and clinical situation.

Conclusion

In conclusion, the findings of this work suggested that the active ingredients and genes of TXL against AMI consisted of 66 active ingredients and 79 genes, beta-sitosterol and IL-6 were the uppermost active ingredient and hub gene respectively in the course of against AMI. The key mechanism might be related to oxidative stress in the membrane. This work provides scientific evidence to support the clinical effect of TXL on AMI. However, deep analysis of anti-AMI pharmacological effects of TXL, as well as the targets and pathways acting with the active ingredients still need to be further validated.

Declarations

Acknowledgments

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Disclosure

The author reports no conflicts of interest in this work.

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Figures

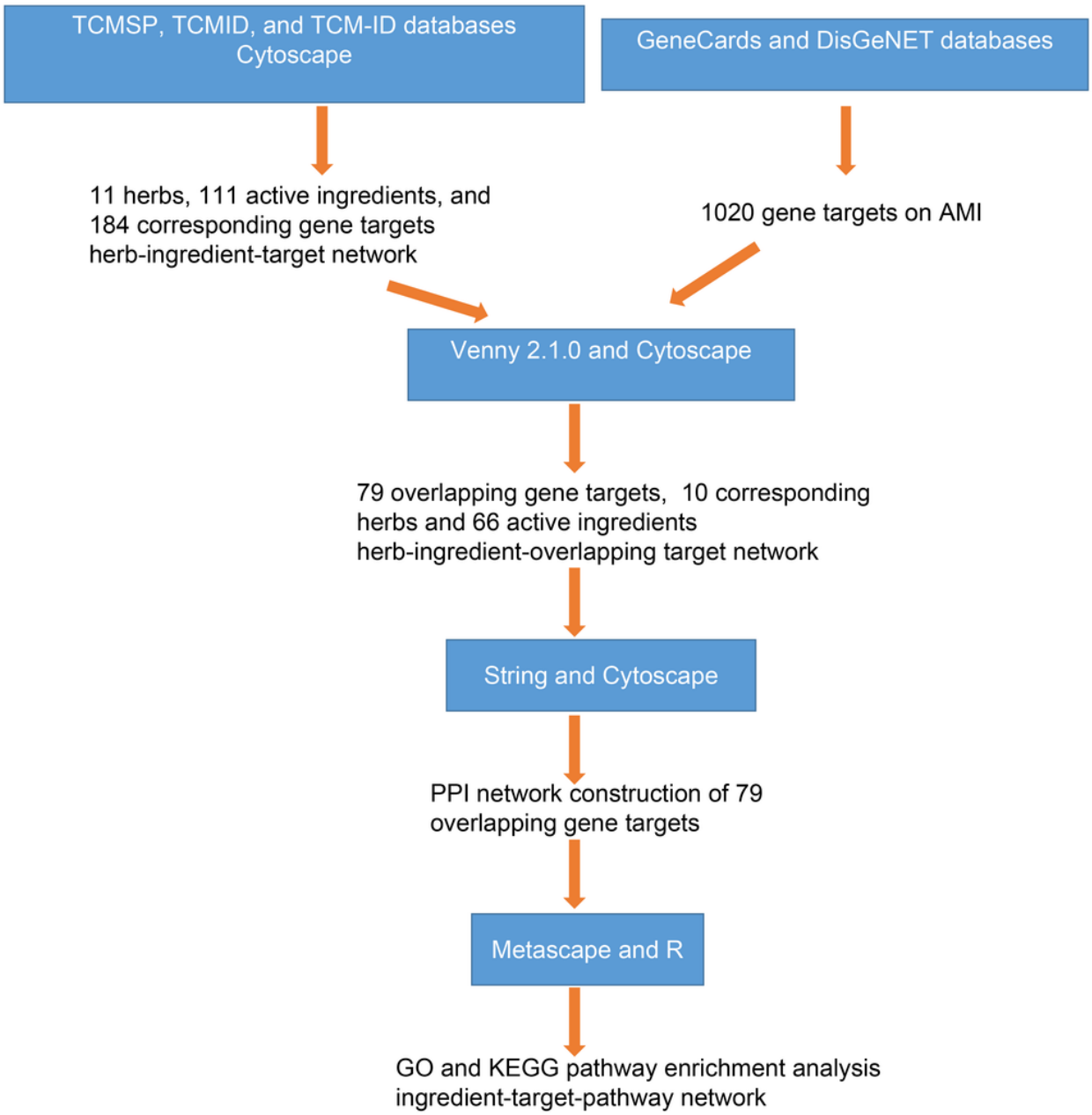

Figure 1

The flowchart of the network pharmacological analysis approach. Abbreviations: AMI, acute myocardial infarction, PPI, protein–protein interaction, GO, Gene Ontology, KEGG, Kyoto Encyclopedia of Genes and Genomes

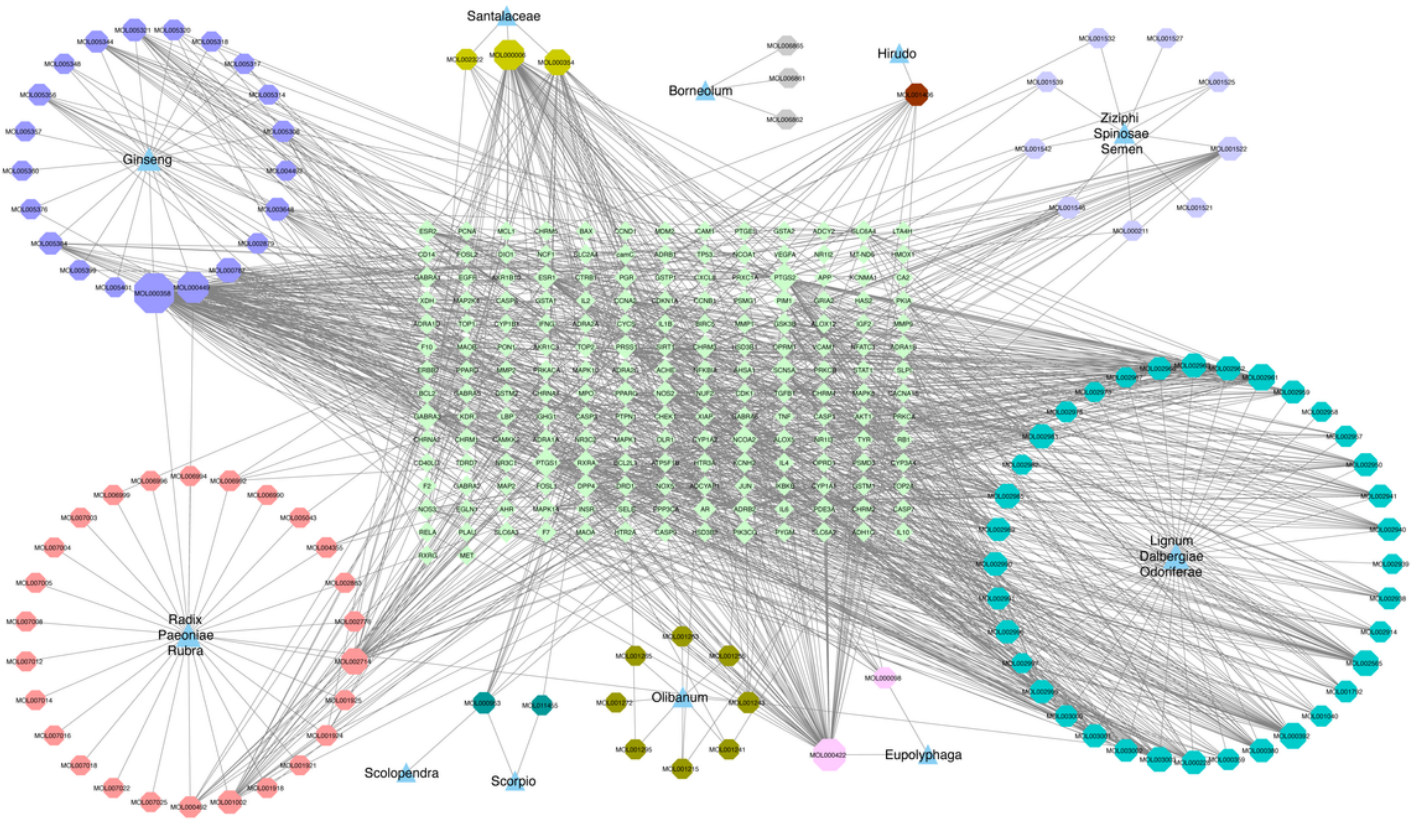

Figure 2

Herb-ingredient–target interaction network. Notes: The cyan triangles represent the 11 herbs, the octagons with different color represent the corresponding active ingredients, the aqua diamonds in the middle represent the corresponding gene targets.

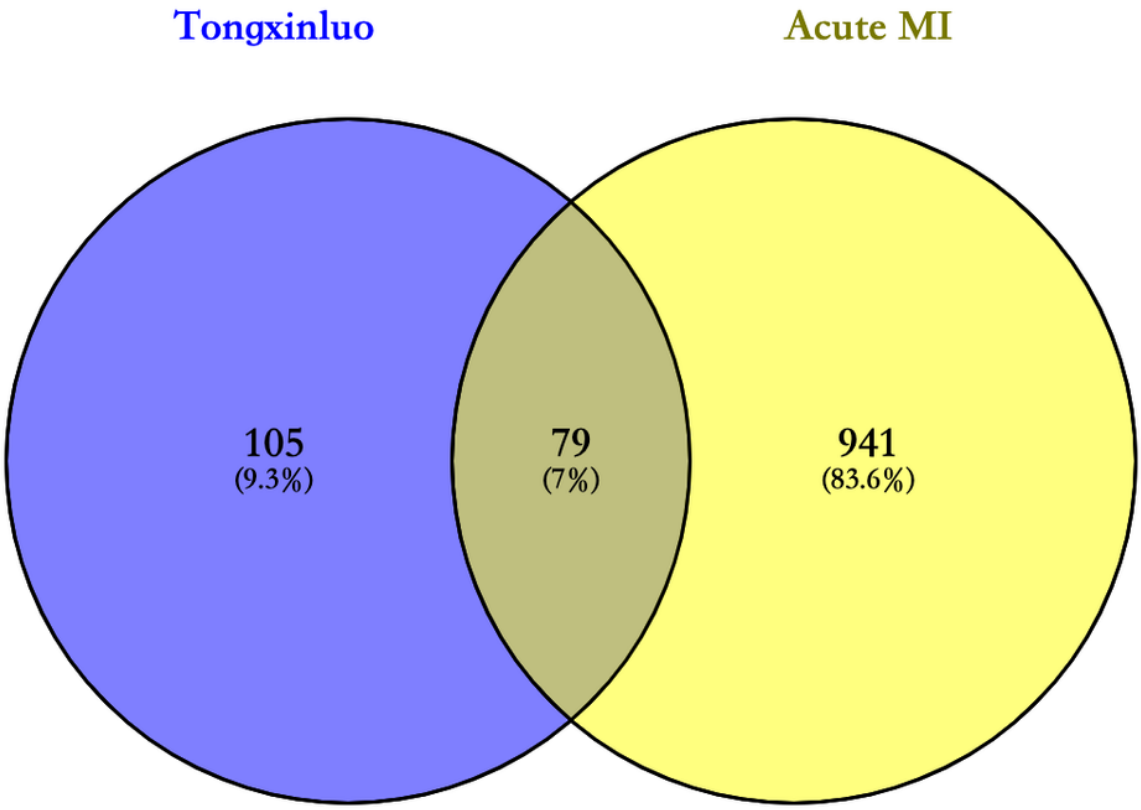

Figure 3

Venn diagram of the overlapping genes between Tongxinluo and AMI.

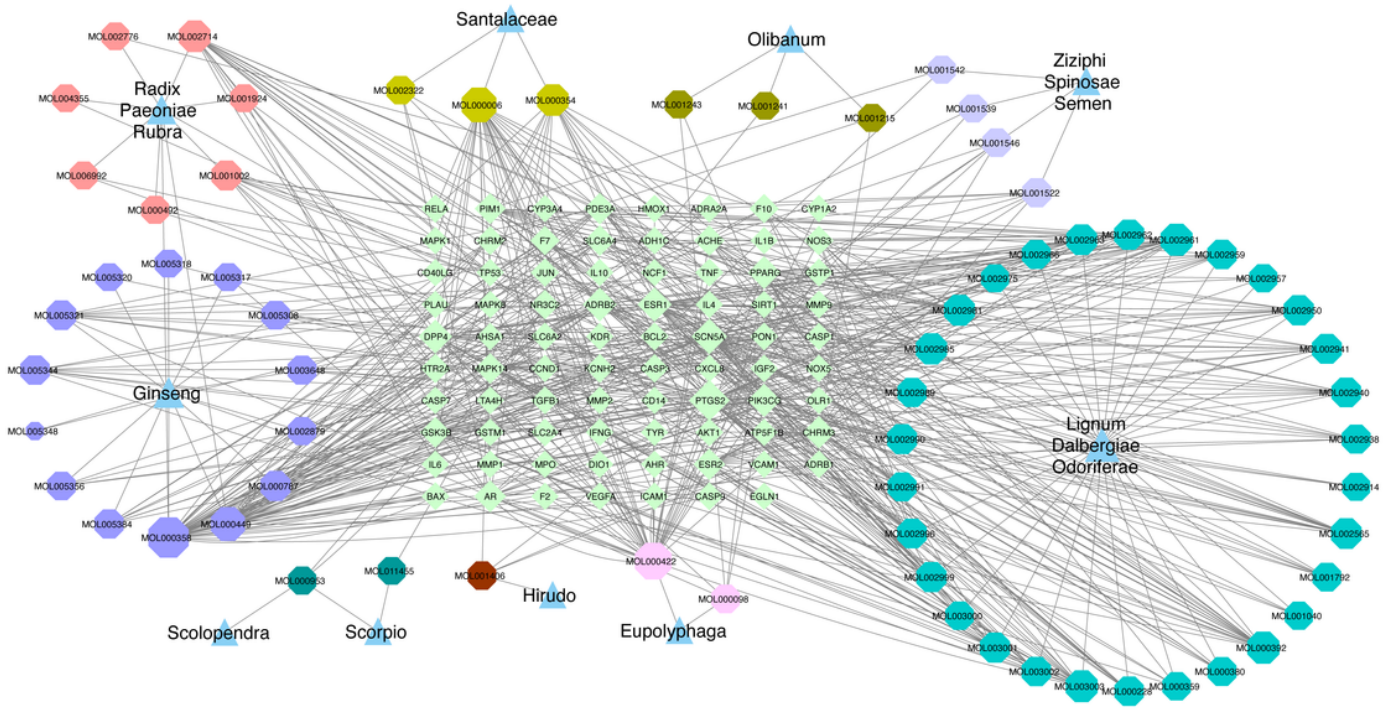

Figure 4

Herb-ingredient–target interaction network. Notes: The cyan triangles represent the 10 herbs, the octagons with different color represent the corresponding active ingredients, the aqua diamonds in the middle represent the corresponding gene targets.

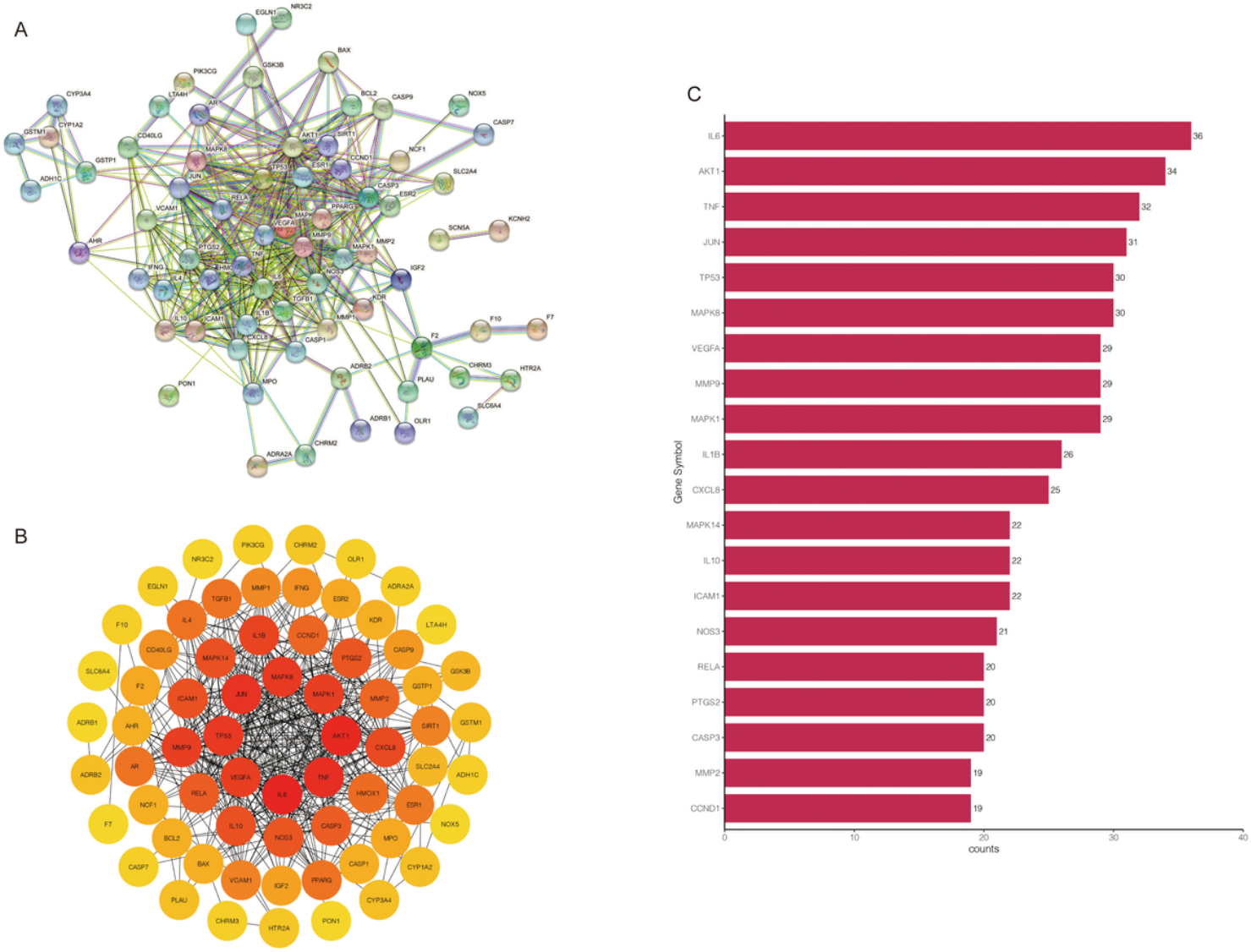

Figure 5

PPI network analysis of the overlapping gene targets.

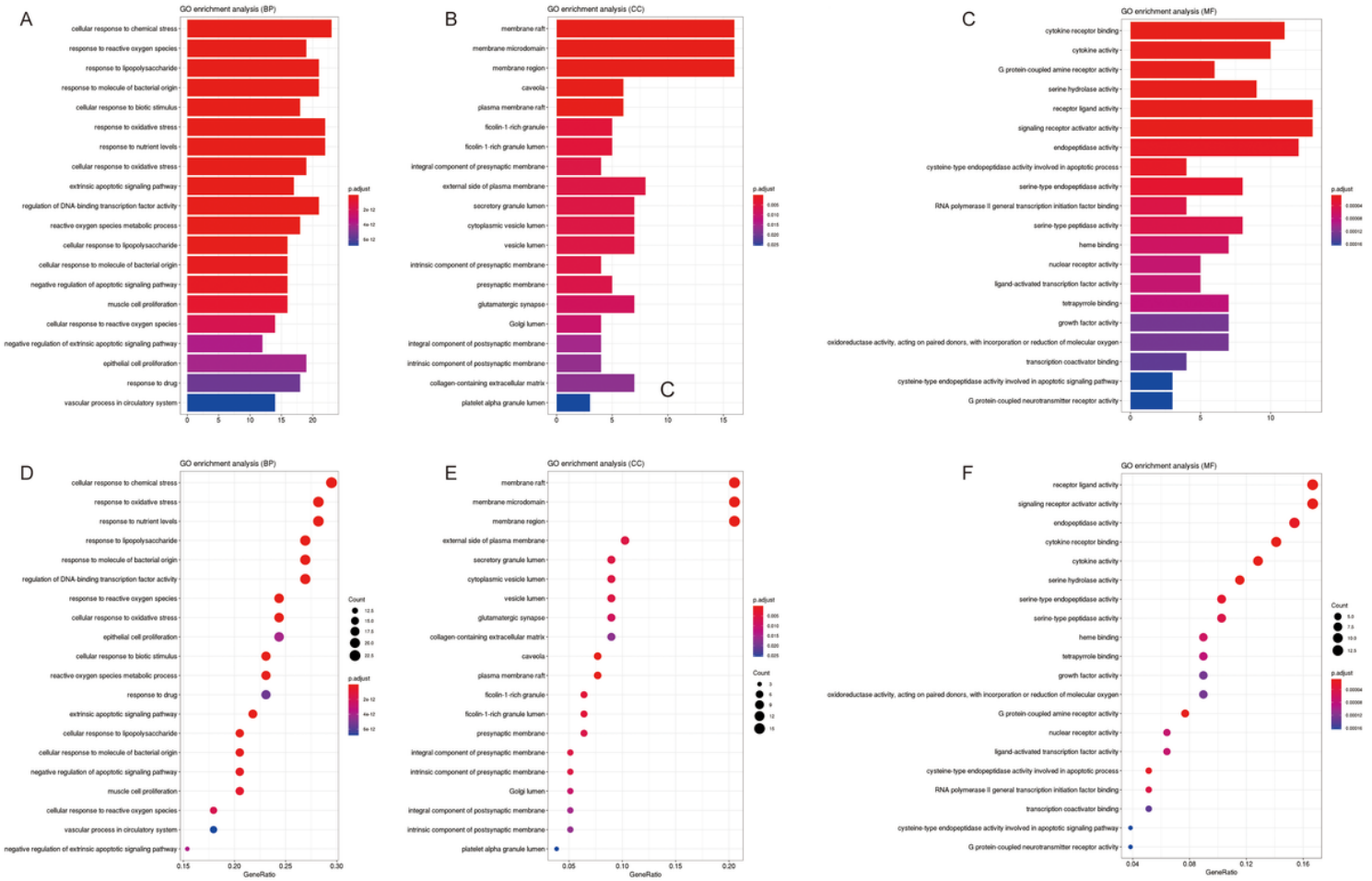

Figure 6

GO enrichment analysis of the overlapping gene targets. Notes: A, Bar graph of BP analyses. B, Bar graph of CC analyses. C, Bar graph of MF analyses. D, Dot plot of BP analyses. E, Dot plot of CC analyses. F, Dot plot of MF analyses. GO: gene ontology, BP: biological process, CC: cellular component, MF: molecular function.

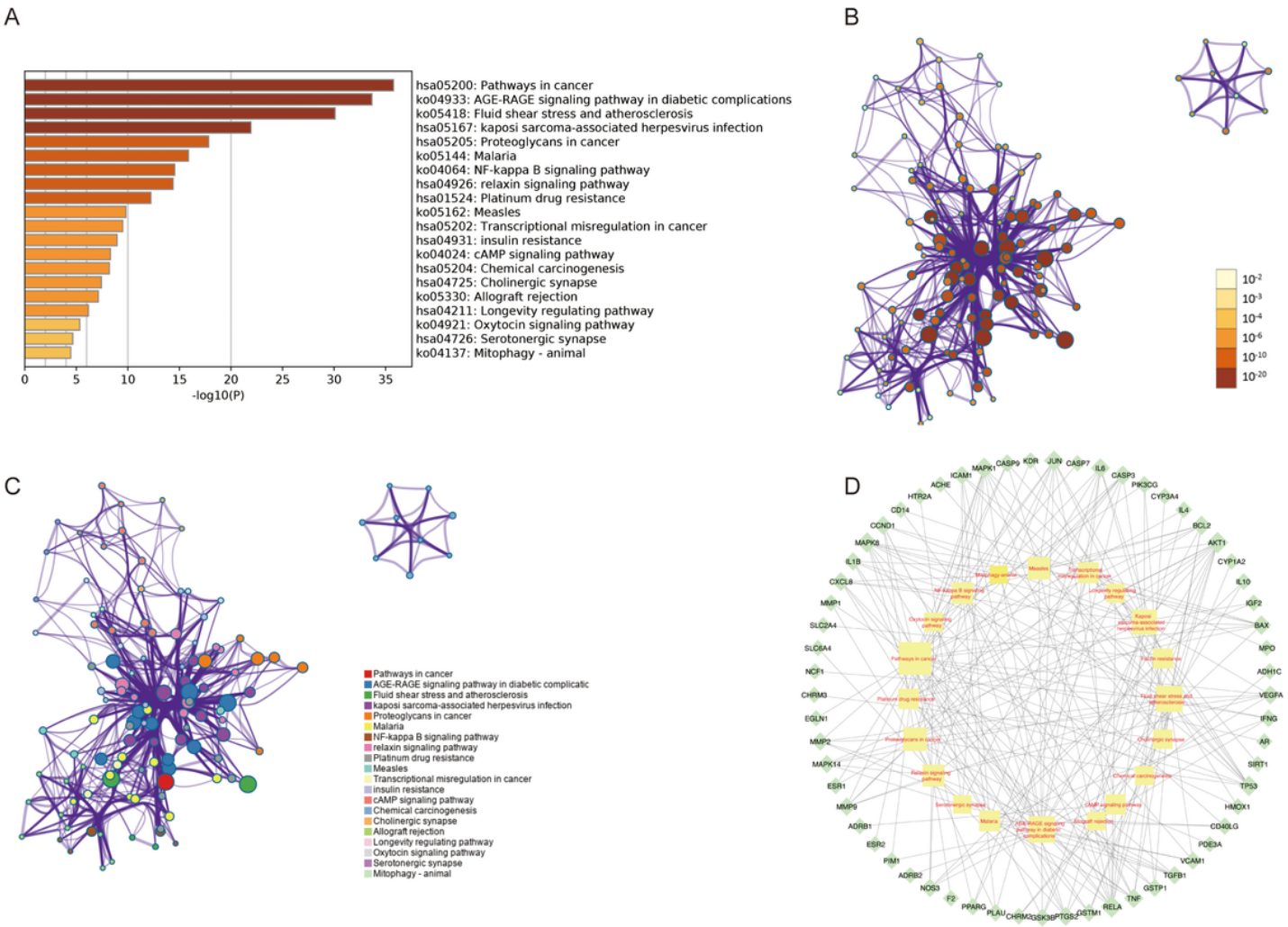

Figure 7

KEGG analysis of gene targets (A-C) and target–pathway interaction network (D). Notes: The aqua diamonds represent the gene targets, and the yellow rectangles represent the top 20 pathways.

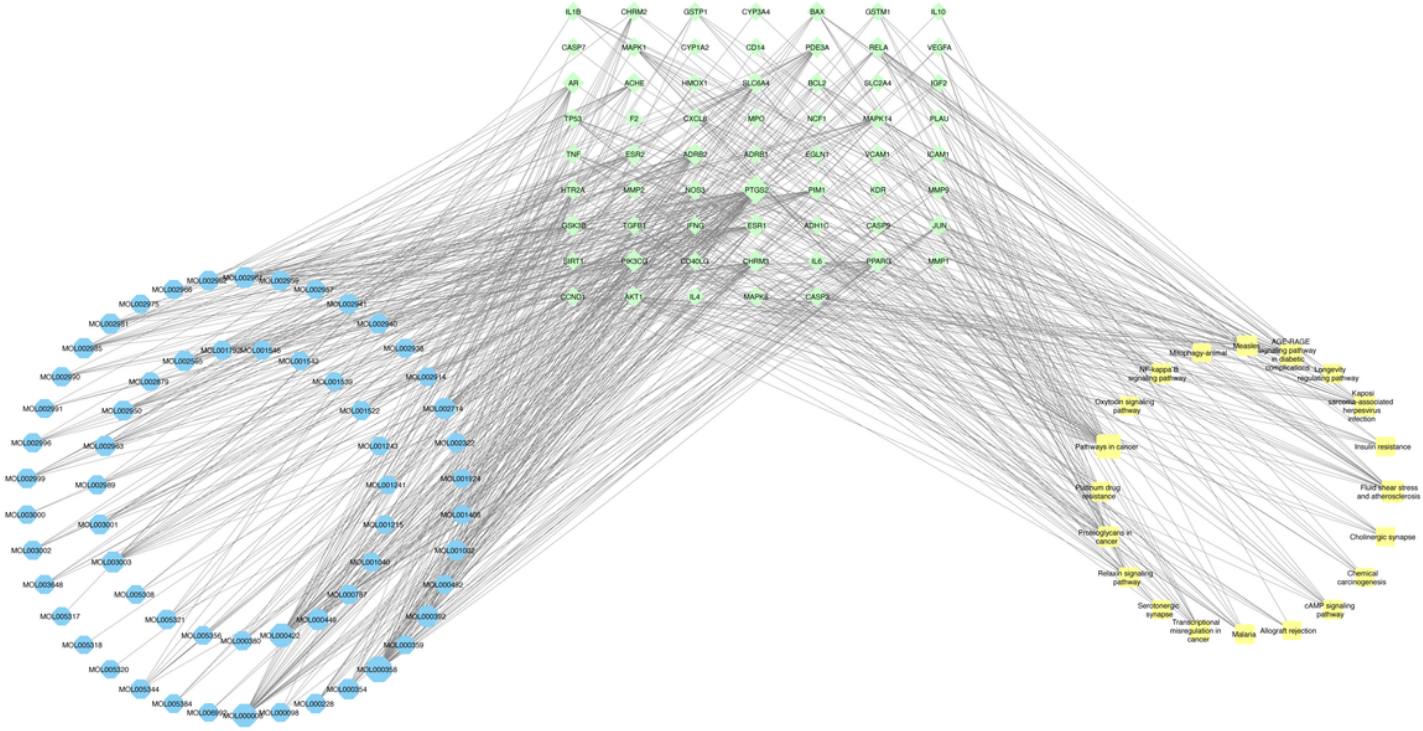

Figure 8

Ingredient–target-pathway interaction network. Notes: The blue octagons represent the active ingredients, the aqua diamonds in the middle represent the gene targets, and the yellow rectangles represent the top 20 pathways.