Integrating Network Pharmacology and Pharmacological Validation for Deciphering the Mechanism of Qishen Yixin Granules in Suppressing myocardial fibrosis

Hugang Jiang
Gansu University of Chinese Medicine
Xinqiang Wang
Affiliated Hospital of Gansu University of Chinese Medicine
Wenyan Lin
Affiliated Hospital of Gansu University of Chinese Medicine
Kai Liu
Gansu University of Chinese Medicine
Yingdong Li
Gansu University of Chinese Medicine
Xinke Zhao (zxkd412@163.com)
Affiliated Hospital of Gansu University of Chinese Medicine

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Abstract

The present study aimed to reveal the bioactive compounds and molecular mechanisms of Qishen Yixin Granules (QSYXG) in the treatment of MF using an integrated network pharmacology and pharmacological validation approach. All active ingredients, drug targets, MF genes were screened from the TCMSP database, drug-target databases, GeneCard database, respectively. Functional and pathway enrichment analyses were operated through the clusterProfiler package in R programming language. Experimental validation was performed using haematoxylin-eosin staining, Masson's trichrome staining and immunohistochemistry in isoproterenol-induced MF rats, and western blot analysis, phalloidin staining and immunofluorescence staining were used to elucidate the predicted mechanism on H9C2 cells. In this study, 55 bioactive components and 59 putative targets were collected. Functional enrichment analysis revealed that responses to lipopolysaccharides, oxidative stress and hypoxia were the key biological processes and were regulated simultaneously by six direct targets, including PTGS2, MAPK14, AKT1, MAPK8, IL-6 and IL-1β. Five pathways were determined by KEGG pathway enrichment analysis. The experiment results indicate QSYXG could down-regulated expression of PTGS2, MAPK14 and MAPK8 and up-regulated expression of AKT1 in the treatment of MF. This study revealed QSYXG could alleviate MF based on multiple components, targets and pathways.

Background

Myocardial fibrosis (MF), which is a pre-lesion stage of heart failure (HF)[1, 2], is frequently diagnosed in adults aged 60–80years in China [3, 4]. Many MF patients have various risk factors, such as hypertension, ischemic cardiomyopathy, abuse of isoproterenol and long-term thoracic radiation therapy[5–7]. Previous researches have indicated that the predominant mechanism of MF is via various cytokines causing fibroblasts to become activated and transdifferentiate into myofibroblasts; these increase the production of collagen, which is then deposited in the extracellular matrix [8–11]. The formation of MF is intimately to HF, myocardial Stiffening caused by severe collagen deposition that are more likely to have progressive HF[12, 13]. Therefore, MF treatment and its corresponding mechanism have recruited a matter of researches attention.

Traditional Chinese medicine (TCM) has unique anti-MF advantages due to its syndrome differentiation, treatment and holistic view[14, 15]. Hence, TCM has been gradually approbated by non-Chinese and applied in the treatment of MF due to its renowned efficacy, the rich resources available, and fewer adverse reactions[16, 17]. Qishen Yixin Granules (QSYXG) is one of the important Chinese patent medicine, which invented and produced by Affiliated Hospital of Gansu University of Traditional Chinese Medicine. QSYXG has been widely applied as a synergistic therapy in the treatment of chronic heart failure (CHF), and this combination therapy could alleviates clinical symptoms and complications of CHF patients that was proved by physicians[18–20]. QSYXG is composed of *Angelica sinensis*, *Astragali radix*, *Lycii fructus*, *Polygonati rhizoma* and *Ginseng radix et rhizoma* (at a ratio of 20:30:10:10:15, respectively) and functions to reinforce the qi and activate the blood. Previous experiments researches have illustrated that QSYXG can attenuate the deposition of collagen fibres between myocardiocytes and down-regulate

the expression of IL-6 and IL-1β in myocardial tissue. Experimental data has also shown that QSYXG has a potential anti-MF effect and can markedly improve a patient's condition. However, the molecular mechanism of QSYXG in the treatment of CHF remains unknown. The multi-component, multi-target and multi-effect properties of Chinese herbal formulae such as QSYXG, and their synergistic and antagonistic interactions, makes it difficult to identify their effective mechanisms through conventional pharmacological approaches[21, 22]. Furthermore, the complex components and indistinct mechanism of QSYXG restrict its clinical application.

Network pharmacology approach is a newly emerging method of researching TCM, which is based on large databases and has become a potential tool to demonstrate the intricate mechanisms of TCM in detail from the molecular level to the pathway level through establishing ingredient-target, target–pathway and target–disease networks[23]. In the present study, network pharmacology approach were used to construct the pharmacological network of QSYXG to speculate the active ingredients, prominent targets and pathways relating to MF. Additionally, the hypotheses were confirmed *in vivo* and *vitro* experiments. A flowchart of the procedures used in this study is shown in Fig. 1.

Materials And Methods

Data preparation

Data on the components of *Angelica sinensis*, *Astragali radix*, *Lycii fructus*, *Polygonati rhizoma* and *Ginseng radix et rhizoma* were retrieved from the TCMSP database (http://lsp.nwu.edu.cn/tcmsp.php) and screened for oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. All of the compounds were opted as medicinal ingredients in the present study. The protein targets of the bioactive compounds in QSYXG were retrieved from the TCMSP (http://lsp.nwu.edu.cn/tcmsp.php), BATMAN (http://bionet.ncpsb.org/batman-tcm/) and TTD (http://bidd.nus.edu.sg/group/cjttd/) databases. The potential targets related to MF were obtained from Genecards, a publicly accessible database where could freely acquired target information of human diseases, using the keyword 'Myocardial fibrosis'.

Network construction and analysis

An interaction network of bioactive QSYXG ingredients and putative targets for the treatment of MF was constructed based on their interaction data. Cytoscape (Version 3.7.2) was used to visualise the interaction network. The protein-protein interaction (PPI) data in this study were retrieved from six current PPI datasets, including the Biological General Repository for Interaction Datasets (BioGRID), Database of Interacting Proteins (DIP), Biomolecular Interaction Network Database (BIND), Molecular INTeraction Database (MID), Human Protein Reference Database (HPRD) and InAct. The BisoGenet 3.0 plugin in Cytoscape was then used to construct a PPI network, and the degree and betweenness centrality were analysed by the CytoNCA plugin in Cytoscape to determine the topological importance of nodes in the network. The clusterProfiler package in R programming language was used to annotate and visualise KEGG pathway and GO terms associated with biological processes (BP), molecular functions (MF) and cellular components (CC).

Herbs and reagents

Angelica sinensis, *Astragali radix*, *Lycii fructus*, *Polygonati rhizoma* and *Ginseng radix et rhizoma* were purchased from the Affiliated Hospital of Gansu University of Chinese Medicine (Lan-zhou, China). The origin and quality of these herbs were consistent with the standards in Chinese Pharmacopeia (2015 Edition). Prostaglandin-endoperoxide synthase 2 (1:1,000; PTGS2; cat. no. ab54629), mitogen-activated protein kinase 14 (1:2,000; MAPK14; cat. no. ab33624), protein kinase 1 (1:2,000; AKT1; cat. no. ab15373) and mitogen-activated protein kinase 8 (1:2,000; MAPK8; cat. no. ab15028) were purchased from Abcepta, Inc. (San Diego, United States).

Animal experiments

Male Wistar rats (200 ± 20 g; licence number: SCXK-(A) 2019-014) were obtained from the laboratory animal centre of Gansu University of Chinese Medicine. The Wistar rats were fed in the feeding environment (12:12 h light:dark cycle; temperature: 24 ± 1 °C; humidity: 40 ± 5%). After adaptation for 5 days, 30 Wistar rats were stochastically grouped into 3 groups of ten rats each. Rats in the negative control group received subcutaneous saline injection (2 $mL \cdot kg^{-1} \cdot day^{-1}$) for 10 days. Rats in the model and QSYXG groups were injected subcutaneously with isoproterenol (30 $mg \cdot kg^{-1} \cdot day^{-1}$) to induce the MF animal model for 10 days. Subsequently, the normal and model groups received normal saline each day, while rats in the QSYXG group received intragastric administration of QSYXG at 7 $g \cdot kg^{-1} \cdot day^{-1}$ for 15 days. All of the above experimental procedures were performed according to the Guiding Principles for the Care and Use of Laboratory Animals of China. All animal studies were approbated by the Ethical Committee of the Animal Experiment Centre of Gansu University of Chinese Medicine.

Histopathological and immunohistochemical assays

Myocardial tissues were isolated, fixed in 10% formalin for 24 h, transferred to 70% ethanol and embedded in paraffin before sectioning into 4 μm thicknesses. Tissue sections deposed on slides were stained with haematoxylin and eosin (HE) for morphology and used a Masson's staining kit (cat. no. SBJ-0288), both from Nanjing Senbeijia Biological Technology Co., Ltd., Nanjing, China, according to the manufacturer's instructions. The sections to be assessed by immunohistochemistry were stained with antibodies to PTGS2, MAPK14, AKT1 and MAPK8. The slides were incubated with 3,3’-diaminobenzidine substrate (Vector Labs, Burlingame, CA) and counterstained with Harris haematoxylin (Sigma, St Louis, MO). The histopathology and immunohistochemistry were examined under a light microscope (Motic BA210; Motic Medical Diagnostic Systems Co., Ltd., Xiamen, China).

Preparation of medicated sera

Five male mature Wistar rats were afforded intragastric administration of QSYXG (7 $g \cdot kg^{-1} \cdot day^{-1}$), once a day for consequent 3 days. One hour after the terminal medication, all of the rats were intraperitoneally anaesthetised using pentobarbital sodium (2% Sodium Pentobarbital 0.3mL/100g). Each rat was pumped 10ml fresh blood by disposable syringe from the abdominal aorta, and that blood were

centrifuged in 5 min with 3000r/min. A 0.22 μm filter membrane were adopted to filter out the impurities in the these serum, heat-inactivated at 56 °C for 30 min and stored at −80 °C until use.

H9C2 cell culture

The H_9C_2 cell line was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle medium (DMEM; Gibco Laboratories, USA) supplemented with 10% foetal bovine serum (FBS; Gibco Laboratories, USA) and 100 $U{\cdot}mL^{-1}$ penicillin and 100 $mg{\cdot}mL^{-1}$ streptomycin in an atmosphere of 90% air and 10% CO_2 at 37 °C . The medium was replaced every 2 days and the cells were digested with 0.05% trypsin when the cell density reached 80–90%. H9C2 cells were seeded in six-well plates or 96-well plates and treated as appropriate for the following experiments. The ISO model of H9C2 cells was administrated to imitate myocardial injury *in vivo*. Briefly, when the H9C2 cells density conformed to the experimental requirements, that will be rinsed by three times with 1% PBS, and be intervened by isoproterenol (80 μM) for 48 h using 1% FBS DMEM to establish the cell model. After cell injury, the QSYXG group received 5 mL of QSYXG-medicated sera for 3 h and the model groups received 5 mL of PBS.

Western blot analysis.

After the different treatments, the cells were collected and lysed in ice-cold radioimmunoprecipitation assay (RIPA; Beyotime, China) buffer containing protease inhibitors phenylmethylsulfonyl fluoride (PMSF; Beyotime, China) and protease inhibitor cocktail (Beyotime, China) and then centrifuged at 12,000 rpm at 4 °C for 15 min to obtain the supernatant. Equal amounts of protein lysates were loaded onto a 5%-10-15% SDS-PAGE gel and transferred to polyvinylidene difluoride (PVDF) membrane. The membranes were blocked in 5% non-fat milk for 1 h at room temperature and then incubated overnight with primary antibodies against MAPK14 (1:800), MAPK8 (1:600) and GAPDH (1:1,000). The membranes were subsequently incubated with fluorescent secondary antibody (1:15,000) for 4 h at room temperature. The membranes were then washed three times with TBST for 5 minutes each time. The protein bands were detected with the Gel Doc XR system (BIO-RAD, USA).

Phalloidin staining

Cells were cultured for 24 h, fixed in 4% formalin for 20 min, washed three times with PBS, and stained with 5 $\mu g{\cdot}mL^{-1}$ of phalloidin conjugate solution (P5282, Sigma, Carlsbad, CA, USA) in PBS for 40 min at 37 °C. The cells were washed three times with PBS to remove the unbound phalloidin conjugate and imaged by confocal laser scanning microscopy (Fluoview1000; Olympus, Tokyo, Japan).

Immunofluorescence staining

After different interventions, H9C2 cells were fixed with 4% paraformaldehyde for 30 min, washed three times with PBS and then treated with 0.2% Triton X-100 (Solarbio, China) at room temperature for 20 min. After washing three times with PBS, cells were blocked in 5% BSA (Servicebio, China) for 30 min at room

temperature. Subsequently, cells were incubated with primary antibodies against PTGS2 (1:100) and AKT1(1:100) overnight at 4 °C. Samples were then incubated with anti-mouse secondary antibody (1:300) at 37 °C for 1 h and then cells were stained with DAPI (Wuhan Antgene, China) for 5 min. Images were obtained by confocal laser scanning microscopy.

Results

Screening out active components of QSYXG

As shown in *Supplement 1*, 55 unique bioactive components of QSYXG were screened out from the 628 chemical components. Respectively, there were two bioactive ingredients in *Angelica sinensis*, 13 bioactive components in *Astragali radix*, 23 bioactive components in *Lycii fructus*, 8 bioactive components in *Polygonati rhizoma*, 15 bioactive components in *Ginseng radix et rhizoma*. Among these, β-sitosterol was a common component *of Angelica sinensis*, *Lycii fructus*, *Polygonati rhizoma* and *Ginseng radix et rhizoma*, Stigmasterol was shared by *Angelica sinensis*, *Lycii fructus* and *Ginseng radix et rhizoma,* and kaempferol is simultaneously exist in *Astragali radix* and *Ginseng radix et rhizoma*.

Potential targets of QSYXG for the treatment of MF

Molecular similarity matching and database searching were used to obtain the targets of the active ingredients in QSYXG. A total of 224 potential targets were found after eliminating the overlaps. These targets of active compounds in QSYXG were mapped with 399 candidate targets relating to MF from the GeneCard database with 'score' ≥ 10.0, as shown in *Supplement 2~3*. This identified 59 targets of 55 components in QSYXG that were associated with MF, as shown in *Figure 2-1*, *Supplement 4*. These targets were considered to be the potential targets of QSYXG for the treatment of MF and used to establish a component-target network comprising 114 nodes (55 active ingredients and 59 potential targets) and 250 edges, as shown in *Figure 2-2*.

The candidate targets of QSYXG against MF

As demonstrated by network biology, multiple disease-related genes and proteins could synergistic effects in MF. To elucidate the pharmacological mechanism by which QSYXG alleviates MF, a protein-protein network (PPI) was constructed with 1,387 nodes and 26,914 edges, which may embody the behaviour and characteristic of the biomolecules as shown in *Figure 2-3A, Supplement 5.* Subsequently, a topological analysis of the PPI was conducted. Nodes with topological features exceeding the intermedy of all nodes were reputed as hubs in the network, and therefore candidate targets in the present study. These candidate targets were determined in the light of a widely used plugin CytoNCA, based on targets with higher values of the two topological features 'Degree' and 'Betweenness centrality'. Following the construction of the PPI network, calculation of these two topological parameters for all targets identified that targets with 'Degree' > 61 and 'Betweenness centrality' > 600 were the candidate targets of QSYXG for the treatment of MF. Ultimately, 15 direct targets were identified, as shown in *Figure 2-3B~2-3C, Supplement 6~7*.

Enrichment analysis of GO and KEGG pathway

To illuminate the biological properties of the 59 targets of QSYXG for MF, the GO and pathway enrichment analyses were conducted via colorspace, stringi, ggplot2, DOSE, clusterProfiler and enrichplot in the R programming language package. These enable the comparison of biological subjects among gene clusters that support humans via the implementation of methods to statistically analyse and visualise functional profiles (GO and KEGG) of gene and gene clusters. There were 478 biological process (BP), 20 cellular component (CC) and 22 molecular function (MF) terms in total, which fulfill the requirements of count ≥ 2. The detailed GO information is shown in *Supplement 8-10*. The top 20 significantly enriched terms in the BP, CC and MF categories are shown in *Figure 5A–C*, which enunciated that QSYXG may generate its therapeutic effects on MF via responses to lipopolysaccharides and hypoxia, cytokine receptor binding, and tetrapyrrole binding in the cytosol, membrane raft and membrane regions.

To explore the signal pathways of QSYXG that were potentially associated with MF, KEGG pathway analysis of related targets was enforced. The detailed results indicated that the alleviation of MF by QSYXG was closely related to 20 pathways, including several significant signalling pathways including the TNF signalling pathway, IL−17 signalling pathway, MAPK signalling pathway and C-type lectin receptor signalling pathway, as shown in *Figure 6, Supplement 11*.

QSYXG suppresses myocardial fibrosis

Isoproterenol-induced myocardial injury lesions are featured by necrotic myocytes, inflammation and consecutive restorative fibrosis. Myocyte necrosis and obvious infiltration of inflammatory cells—mainly macrophages—into the myocardial tissue were seen in the model group. In the QSYXG group, the myocardial trauma was almost renovated, but sporadic scars in the injured areas were observed. Slight collagen deposition was seen in the myocardium of the normal and negative control groups. Additionally, obvious collagen fibre deposition around the arterioles and metarterioles was present in the model group and thick collagen fibres were present between myocardiocytes. In most cases, the main feature was collagen interstitial fibrosis, giving a ‘brindled’ look to the myocardium through the alternation of areas with fibrosis and areas of myocardial cells. The extent of myocardial fibrosis in the QSYXG group was evidently smaller than in the model group, suggesting that QSYXG could alleviate MF.

QSYXG regulates the expression of PTGS2, MAPK14, AKT1 and MAPK8

The KEGG pathway enrichment results indicated that the TNF, IL−17, C-type lectin receptor, Toll-like receptor and VEGF signalling pathways were intimately associated with MF treatment by QSYXG. The primary targets, including PTGS2, MAPK14, AKT1, MAPK8, were predicted in the enrichment of these five pathways. The effect of QSYXG on AKT1, MAPK8, MAPK14 and PTGS2 protein expression was explored by immunohistochemistry analysis. The results show a conspicuously increased in AKT1 and the down-regulated expression of MAPK8, MAPK14 and PTGS2, as shown in *Figure 4*.

MAPK8 and MAPK14 are potential myocardial fibrosis targets

To confirm that the expression of MAPK8 and MAPK14 was affected in isoproterenol-induced H9C2 cells, western blot assay was used to determine the effect on protein levels. A significant reduction in the expression of MAPK8 and MAPK14 was observed in QSYXG-treated cells compared to model group cells. There was no significant difference in the expression of MAPK8 and MAPK14 at the protein level in QSYXG-treated cells compared to control group cells.

QSYXG alleviates extensive changes in cytoskeleton structure

The F-actin organisation in H9C2 cells was investigated by fluorescent phalloidin staining. Cardiomyocytes in the control group presented regular and well-defined actin organisation, while cardiomyocytes in the model group showed more interspersed and aberrant F-actin disposition. The differences could be visualised in the typical cardiomyocytes. However, treatment by QSYXG improved F-actin organisation in cardiomyocytes contrasted with the model group and produced a marked remission in isoproterenol-induced cardiac hypertrophy and cardiac fibrosis.

QSYXG ameliorates isoproterenol-aggravated injury

The function of QSYXG on the expression of AKT1 and PTGS2 in isoproterenol-induced H9C2 cells was investigated by Immunofluorescence staining. The expression of AKT1 was lower in the model group than the control group, and QSYXG could enhanced its expression. The expression of PTGS2 was higher in the model group compared with the control group, but the expression was lower in the QSYXG group than the model group, as shown in *Figure 5*.

Discussion

MF is closely associated with increased myocardial stiffness, restricted myocardial relaxation, arrhythmia, and plays a role in the remodelling process that leads to HF[2, 24–26]. Previous research has shown that MF is caused by the deposition of fibrillar connective tissue between cardiomyocytes and cardiac fibroblasts[27–29]. Current Western medicine does not provide adequate therapeutic regimens for the treatment of MF. Some experimental research has demonstrated that transforming growth factor-β (TGF-β) and galectin-3 play key roles in the development of MF and that angiotensin II and aldosterone are essential to promoting the deposition of collagen in the myocardium as a response to hypoxia, oxidative stress and lipopolysaccharides[30–34]. Thus, further exploration of the potential mechanism of MF is prominent in the development of more specific, effective and less toxic MF therapies.

Previous studies have shown that QSYXG can markedly suppress the deposition of collagen in the myocardium and reduce ventricular premature beats in HF. The overall efficacy of QSYXG combined with sacubitril/valsartan in the treatment of chronic HF is as high as 70%. The animal experiments in this study also showed that QSYXG could alleviate the pathological injury of isoproterenol-induced MF rats.

In the present study, network construction approaches were adopted to screen the potential bioactive compounds and targets, and to explore the sophisticated mechanisms of QSYXG in the treatment of MF[35, 36]. The results of network pharmacological analysis indicated that QSYXG ameliorated MF by multi-target, multi-component regulation, manifesting the idiographic advantages of QSYXG in the treatment of MF. Gene ontology (GO) enrichment analysis was employed to evaluate gene function from biological process (BP), molecular function (MF) and cell composition (CC) perspectives. BP function was governed to clarify the biological functions in which the direct targets of QSYXG were involved in the treatment of MF. The results of GO enrichment analysis showed that the eventful targets of QSYXG alleviated MF predominantly by partaking in biological processes in response to lipopolysaccharides, oxidative stress, hypoxia, nutrient levels and mechanical stimulus. This indicated that the bioactive ingredients of QSYXG could directly suppress oxidative stress, improve myocardial tolerance to hypoxia and promote myocardial energy metabolism by supplementing qi and activating blood circulation.

The putative targets of QSYXG were MF, PTGS2, MAPK14, AKT1, MAPK8, as predicted by their p-values. These targets were related to the TNF, IL-17, C-type lectin receptor, Toll-like receptor and VEGF signalling pathways. Accordingly4, the biological mechanism of QSYXG in MF treatment was explored from the different perspectives of KEGG and GO enrichment analysis. However, the results predicted by network pharmacological analysis required experimental validation. The direct targets IL-6 and IL-1β were verified in our previous study on the treatment of MF by QSYXG. Therefore, in the present study, the roles of PTGS2, MAPK14, AKT1 and MAPK8 in the treatment of MF were explored. In the present study, an MF rat model revealed that QSYXG may inhibit MF progression mainly via significantly down-regulation of the expression of PTGS2, MAPK14 and MAPK8, and marked up-regulation of the expression of AKT1. Meanwhile, QSYXG treatment significantly decreased the deposition of collagen in myocardial tissue. These results were in accordance with predictions based on the network pharmacology results; that is, that QSYXG exerted its anti-MF efficacy via regulating the expression of PTGS2, MAPK14, AKT1 and MAPK8.

All the above findings demonstrate a triumphant application of the network pharmacology approach in discerning the mechanism of action of TCM. The comprehensive pharmacological mechanisms by which QSYXG ameliorates MF will be elucidated in a future study. In summary, this study elaborately illustrated the pharmacological mechanisms of QSYXG in the treatment of MF via network pharmacology and animal experiments. It is also show solicitude for that network pharmacology has dominant superiority in demonstrating the therapeutic mechanisms of TCM, but there is also the need for further animal experimental research.

Conclusion

The network pharmacology approach was utilized to spot 55 active components and 59 corresponding targets of QSYXG in the alleviation of MF. Combined with experimental verification both *in vitro* and *in vivo*, it was certificated that QSYXG may partially mitigate MF by regulating that targets of PTGS2, MAPK14, AKT1 and MAPK8, and subsequently lessening the deposition of collagen in myocardial tissue.

Declarations

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

HJ and XW conducted the animal experiments. WL and KL analysed the data and drafted the manuscript. YL and XZ critically revised the manuscript.

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Figures

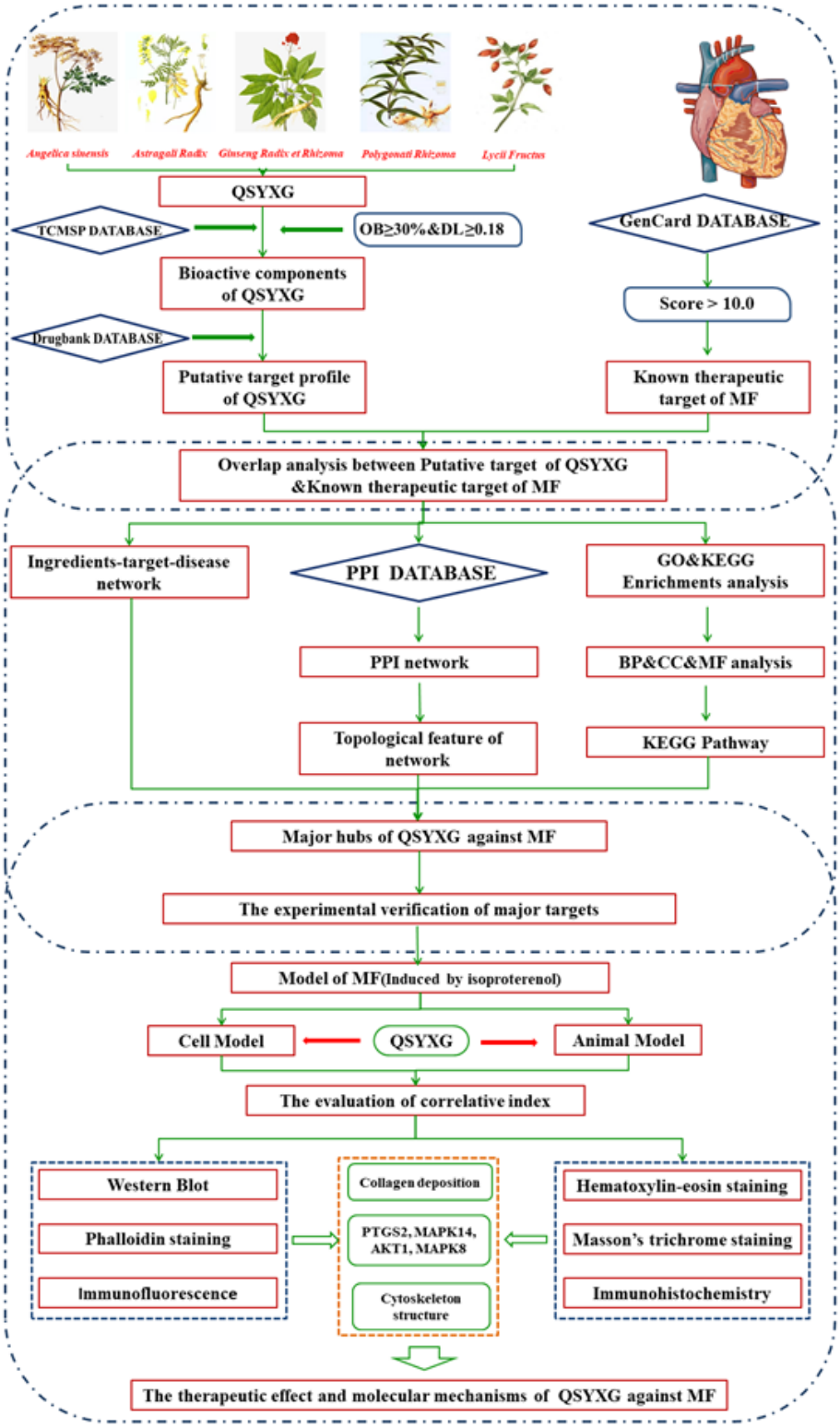


Figure 1

Flowchart of network pharmacology–based strategy for revealing the mechanisms of QSYXG against gastritis. Abbreviations: QSYXG, Qishen Yixin Granules; TCMSP, Traditional Chinese medicine systems pharmacology; PPI, protein-protein interaction; OB: oral bioavailability; DL: drug-likeness.

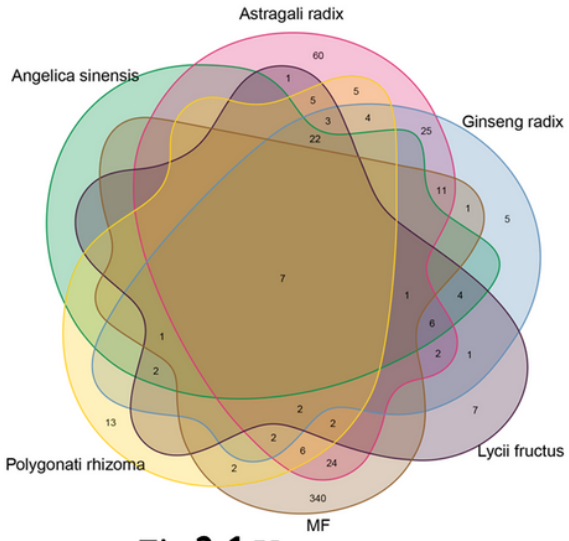


Fig.2-1 Venn

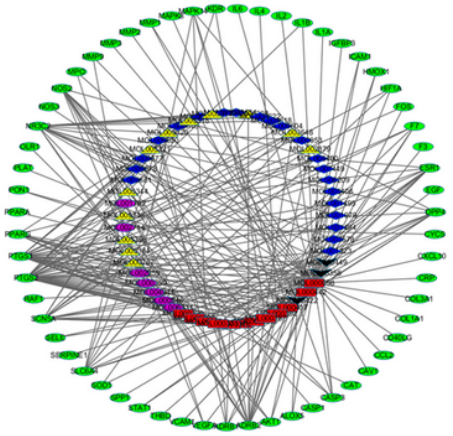
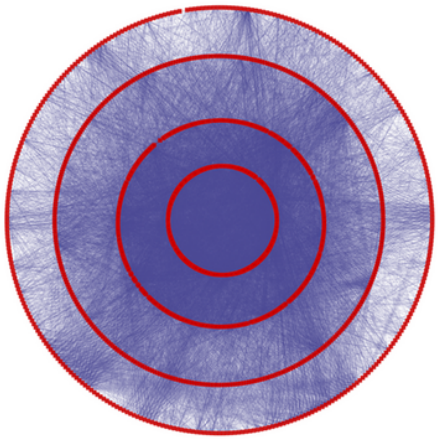
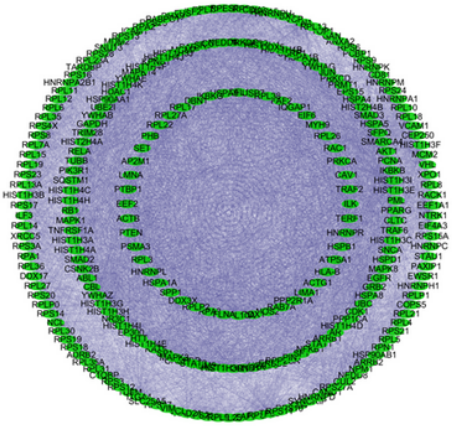


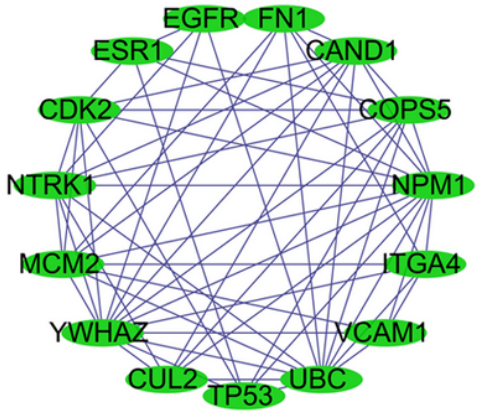
Fig.2-2 Component-target Network



A.Overlap of putative QSYXG targets and known MF-related targets



B.The topological parameter of 'Degree'



C.Calculating the topological parameter of 'Betweenness centrality'.

Fig.2-3 The PPI network

Figure 2

The prominent ingredients, potential targets and interactions of QSYXG for the treatment of MF

2-1 Venn diagram of candidate targets in QSYXG and MF; 2-2 The component-target network. The outside circles refer to 59 putative targets of QSYXG for the treatment of MF. The inside circles represent 55 active components in QSYXG; 2-3 A The PPI network was constructed by the overlap of putative QSYXG targets and known MF-related targets; 2-3 B topological analysis of the PPI by the topological parameter of 'Degree'; 2-3 C Topological analysis of the PPI by calculating the topological parameter of 'Betweenness centrality'.

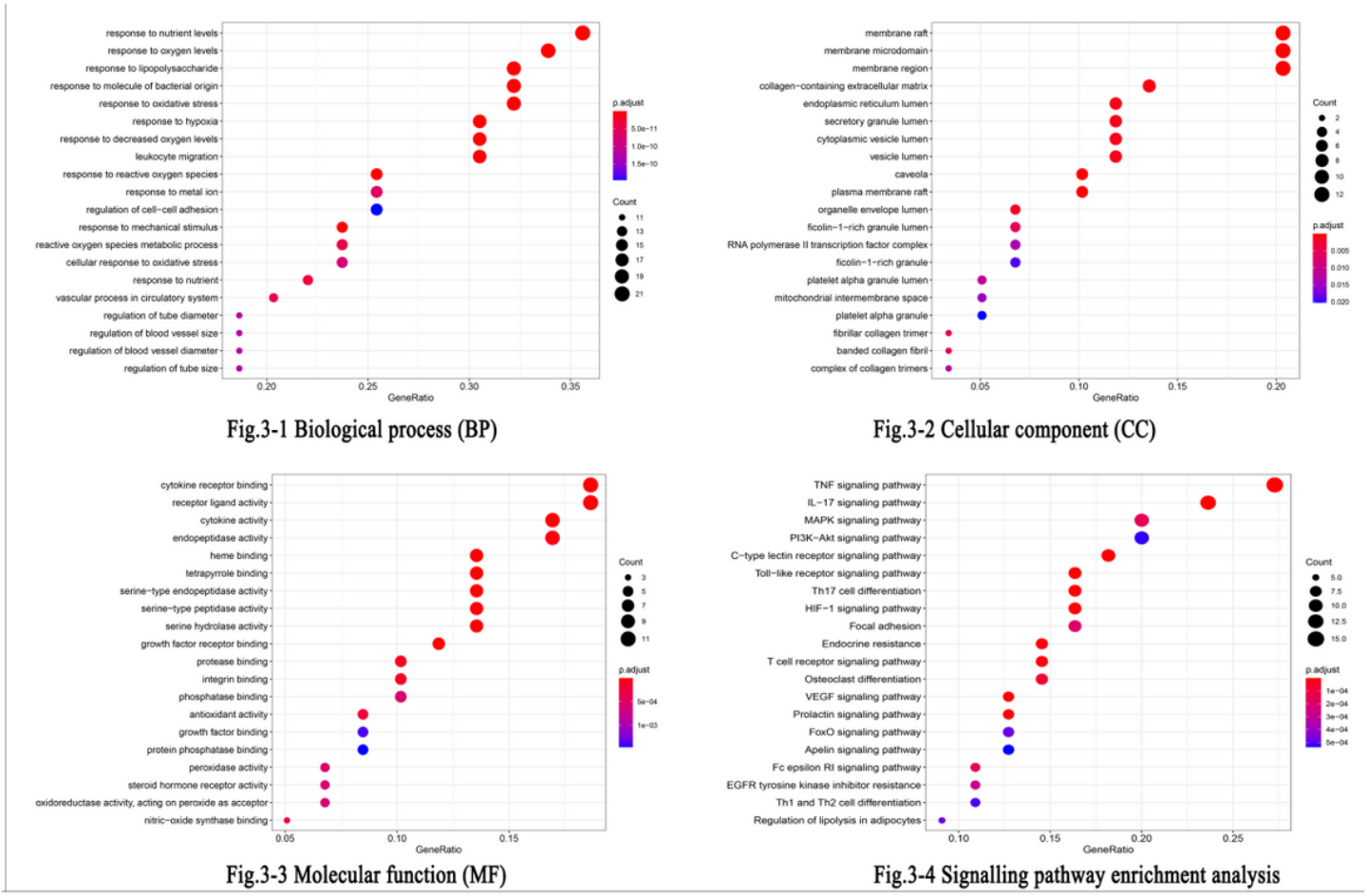


Figure 3

The Gene Ontology and signal pathway enrichment analyses of QSYXG for the treatment of MF

3-1 Representative bubble plots of biological process analysis of the core targets; 3-2 Representative bubble plots of cellular components of identified targets; 3-3 Representative bubble plots of molecular function among candidate targets. Gene ratio = count/set size; 3-4 Representative bubble plots of signalling pathway enrichment analysis of core targets of QSYXG for MF. Gene ratio = count/set size.

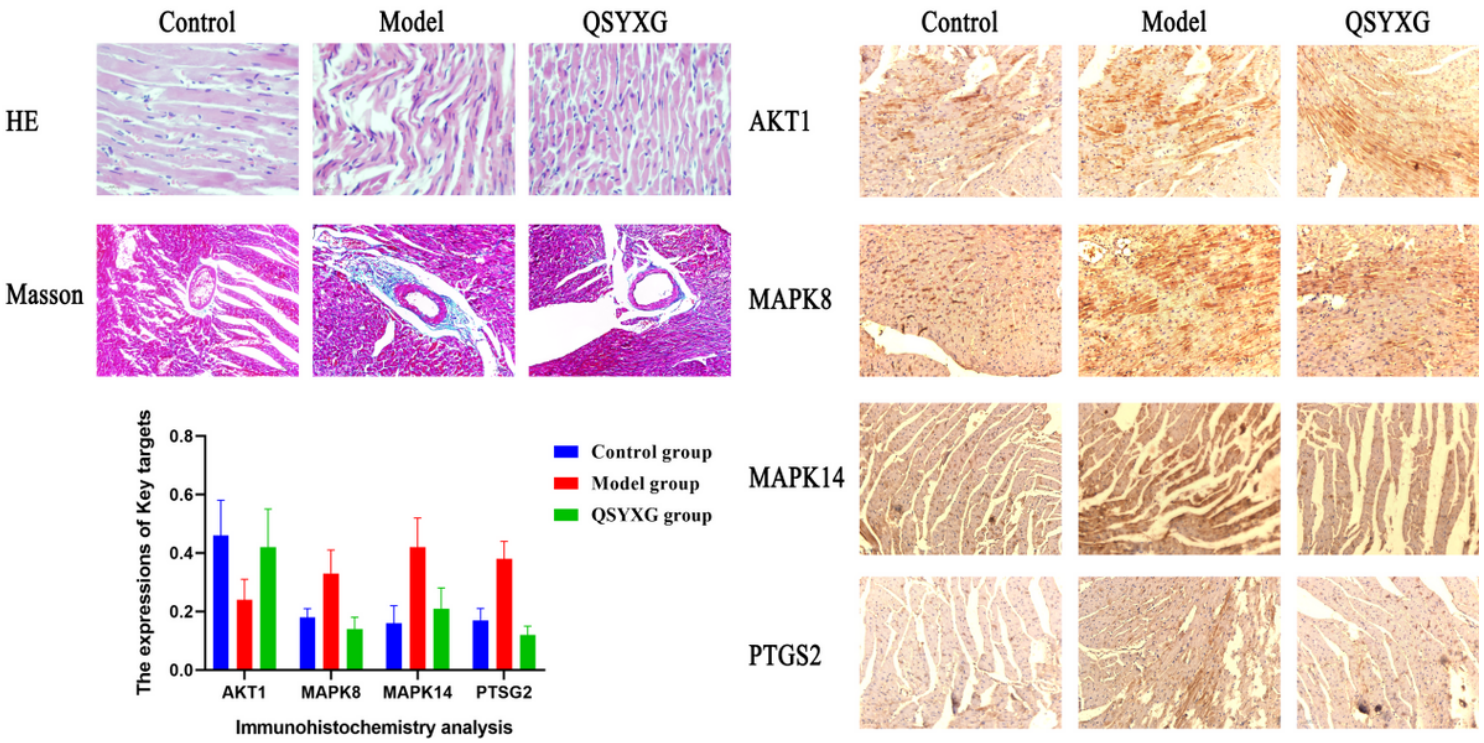


Figure 4

The therapeutic effect of QSYXG on MF assessed by H&E staining; The therapeutic effect of QSYXG in alleviating the deposition of collagen; The effect of QSYXG on the expression of AKT1, MAPK8, MAPK14 and PTGS2.

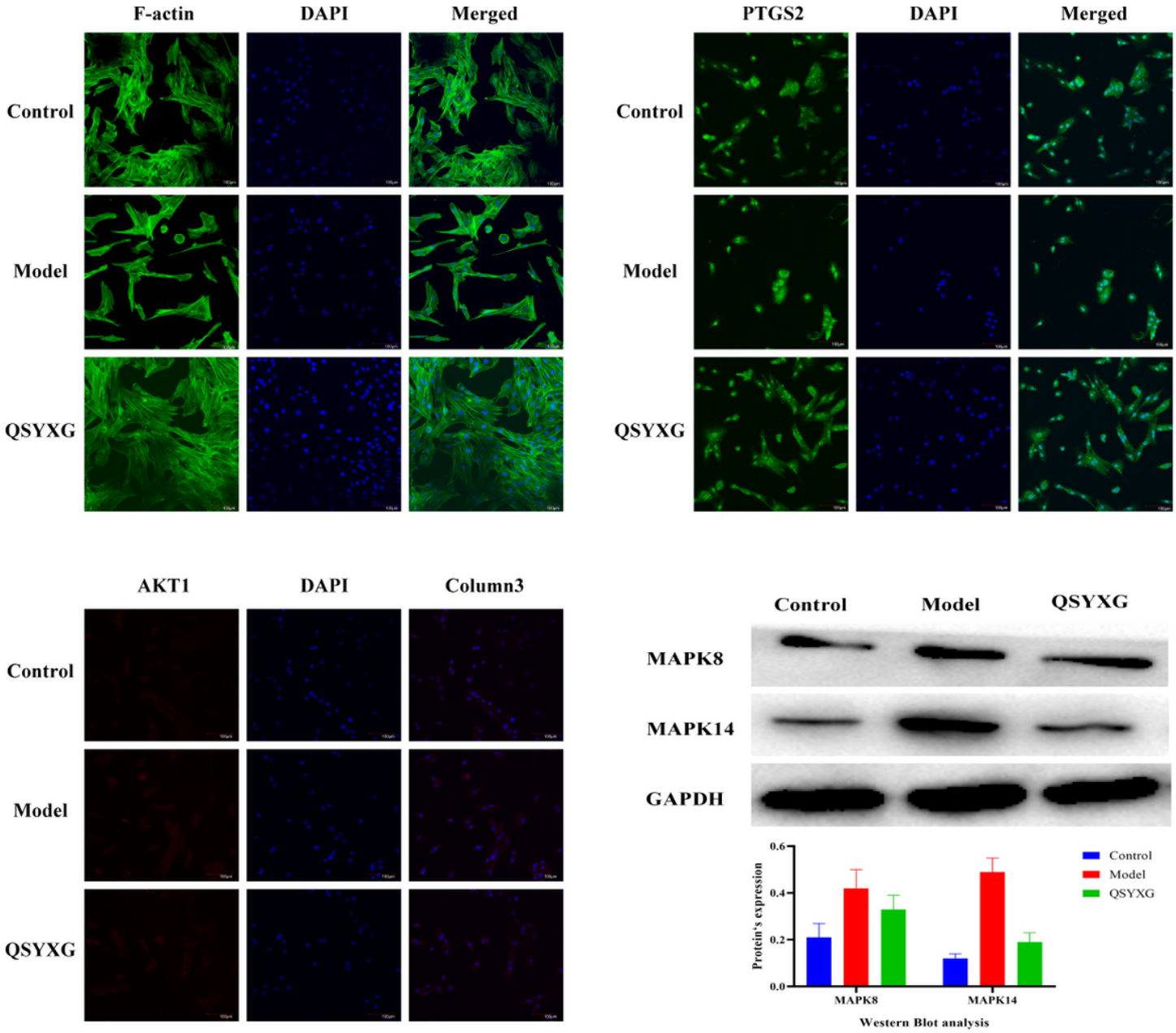


Figure 5

The protein-level expression of MAPK8 and MAPK14 as determined by Western blot; The changes in cytoskeleton structure of isoproterenol-induced H9C2 cells; The effect of QSYXG on the expression of AKT1 and PTGS2.

Supplementary Files

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