Study On the Mechanism of Action of Qili Qiangxin Capsule in the Treatment of Heart Failure: Based on Network Pharmacology and Molecular Docking Method

Hao Sun[1]*, Yongjian Ji[2], Hongwei Dong[1], Yi Sun[1], Peng Wang[1]

[1]Department of Cardiovascular Medicine, Qilu Hospital of Shandong University Dezhou Hospital, Dezhou 253000, Shandong Province, China
[2]Department of Geriatric Medicine, Qilu Hospital of Shandong University Dezhou Hospital, Dezhou 253000, Shandong Province, China

Corresponding author: Hao Sun, msn_222@163.com

Abstract: *Objective:* To investigate the pharmacodynamic substances and mechanism of action of Qili Qiangxin Capsule in the treatment of heart failure based on network pharmacology and molecular docking technology. *Methods:* The active ingredients and drug targets of Qili Qiangxin Capsule were obtained from databases such as TCMSP, GeneCards, OMIM, PharmGkb, TTD, and DrugBank databases were searched for heart failure disease targets. The drug targets and disease targets were corrected by the UniProt database, then the Venn diagram was drawn, and the intersecting genes were screened. Cytoscape 3.8.0 software was used to draw the active ingredient-disease target network for topological analysis. PPI network was constructed in the STRING database platform to predict the core targets. Bioconductor package was used to perform KEGG pathway analysis. autoDock Vina software was used to molecularly dock the core targets with the main active ingredients, and Pymol software visualised the results. *Results:* Screening of 209 active ingredients of Qili Qiangxin Capsule, including quercetin, luteolin, cryptotanshinone, etc. 11,432 heart failure disease targets, 249 genes intersecting with drugs, including the core targets containing *TP53*, *STAT3*, *JUN*, *MAPK1*, etc. These genes are mainly involved in the AGE-RAGE, fluid shear stress, and atherosclerosis, PI3K-Akt signaling pathways. *Conclusion:* This study initially revealed the multi-component, multi-target, multi-pathway mechanism of action of Qili Qiangxin Capsule in the treatment of heart failure, which provides a theoretical basis for further research.

Keywords: Qili Qiangxin Capsule; Heart failure; Network pharmacology; Molecular docking; Mechanism of action

1. Introduction

Currently, about 26 million people worldwide suffer from heart failure, which is the end stage of all types of heart diseases, with high morbidity and high economic burden, and is regarded as the "cancer of the

cardiovascular field" [1]. Of the 13,687 heart failure patients enrolled in the China-HF study from January 2012 to September 2015 in 132 hospitals in China, the in-hospital mortality rate was as high as $4.1 \pm 0.3\%$ [2]. The goals of current heart failure treatment are to improve symptoms, slow disease progression and prolong survival. Various guidelines recommend strategies for the treatment of heart failure, including improvement of ventricular remodelling, vasodilatation, cardiotonic diuresis, etc. Commonly used drugs such as β-blockers, aldosterone receptor antagonists, angiotensin receptor enkephalinase inhibitors, nitrate esters, calcium sensitizers, digitalis, and collaterals are used as diuretics. These drugs can significantly relieve patients' symptoms, such as dyspnea and oedema and improve the quality of life, but some patients are accompanied by a series of adverse reactions, which bring greater physical and mental suffering [3]. In this context, the concept of combining Chinese and Western medicine has created a new situation for the treatment of heart failure in China. Qili Qiangxin Capsule has been recommended by several domestic guidelines to be applied to patients with heart failure, and has achieved good clinical effects [4,5].

Traditional Chinese medicine classifies heart failure under the categories of "asthma", "cardiac paralysis", "cardiac water" and "oedema." Modern Chinese medicine classifies heart failure into three syndromes: "qi" deficiency and blood stasis, "yang qi" deficiency and blood stasis, and "qi yin" deficiency and blood stasis [6]. Academician Wu Yiling believes that heart "qi" deficiency and blood stasis are the central link and fundamental pathogenesis of heart failure, and based on the idea of "by the collaterals to pass, the meeting of the biochemistry", he put forward the "qi, blood, and water treated together but eliminate separately", and then developed an innovative traditional Chinese medicine, Qili Qiangxin Capsule, to treat the heart failure [7]. This paper intends to apply the technical methods of network pharmacology and molecular docking to construct the component-target network of Qili Qiangxin Capsule for the treatment of heart failure, to explore the intrinsic mechanism of action, and to provide theoretical support for subsequent experimental validation and clinical application.

2. Materials and methods

2.1. Active ingredients and predicted targets of Qili Qiangxin Capsule
The TCM Systematic Pharmacology Database and Analysis Platform (TCMSP) was searched for the active ingredients related to 11 Chinese medicines in Qili Qiangxin Capsule, and those with oral bioavailability (OB) ≥ 30% and drug-like properties (DL) ≥ 0.18 were screened out. In the Uniprot database, the search condition was set to Organism: *Homo sapiens* (Human), and the active ingredients and genes were converted to standardize the names of the target genes of the active ingredients that met the criteria.

2.2. Prediction of heart failure-related targets
Using "Heart Failure" as the keyword, we searched related genes in GeneCards, PharmGkb, Drugbank and TTD databases, and combined the valid information retrieved from the five databases to obtain all the disease targets of heart failure.

2.3. Construction of active ingredient-disease target network
The active ingredient targets of Qili Qiangxin Capsule were compared with the disease target genes of heart failure, and the R software was used to find out the targets of the two, and then a Venn diagram was drawn. Cytoscape 3.8.0 software "network analyser" mode was used to construct the "active ingredient-disease target" network diagram.

2.4. Constructing protein-protein interaction (PPI) networks

The protein-protein interaction (PPI) network was constructed by importing the co-interacting targets into the STRING database, setting the species as "*Homo sapiens*", setting the minimum interaction threshold ≥ 0.95, and hiding the free sites. Then, import to Cytoscape 3.8.0 software and use CytoNCA plug-in to carry out protein network topology analysis, and calculate the core protein based on the six centrality topology parameters BC, CC, DC, EC, IC and LAC.

2.5. KEGG pathway enrichment analysis

The data were processed by Bioconductor package in R software, and the top 30 pathways were selected for KEGG analysis according to the number of enriched target genes in order of $P < 0.05$ standard.

2.6. Molecular docking validation of active ingredients with key targets

Screening of drug candidates in Cytoscape 3.8.0 network, finding the 2D structure of candidate components using Pubchem database and force field conformation optimisation by ChemBio3D software. Candidate compound format was saved as pdbqt format using AutoDock Tools as an alternative. Download the pdb format file of the core protein from the PDB database. Pymol software deleted the irrelevant small molecules in the protein, then imported them into AutoDock Tools software to remove water, ligand, and hydrogenation and finally saved them as a pdbqt file. Create the centre grid point of the receptor protein and set the box size. Finally, molecular docking of the drug candidate components with the core target protein receptor was performed by Autodock vina software, and the binding mode with the lowest energy was selected for the graph.

3. Results

3.1. Prediction of active ingredients and targets of Qili Qiangxin Capsule

209 active ingredients of Qili Qiangxin Capsule were finally obtained from TCMSP database, including 5 Chenpi, 65 Danshen, 21 *Rhizoma Pinelliae*, 7 Gui Zhi, 22 Safflower, 20 Astragalus, 22 Panax Ginseng, 12 *Draba nemerosa*, 17 *Cortex periplocae*, 8 Yuzu, and 10 *Alisma plantago-aquatica.* Searching for the targets corresponding to the active ingredients, the Uniprot database was used to find the gene SYMBOL, and a total of 2349 genes were obtained for the predicted targets, of which 82 were Chenpi, 801 were Danshen, 26 were *Rhizoma Pinelliae* , 59 were Gui Zhi, 391 were Safflower, 395 were Astragalus, 212 were Panax Ginseng, 272 were *Draba nemerosa*, 65 were *Cortex periplocae*, 37 were Yuzu indica, and 9 were *Alisma plantago-aquatica*.

3.2. Potential targets of Qili Qiangxin Capsule for the treatment of heart failure

By searching GeneCards (Relevance score 1), OMIM, Drugbank, PharmGkb, and TTD databases, 11,408, 239, 138, 115, and 80 heart failure disease-related target genes were collected, respectively, and a total of 11,432 disease-targeted genes were obtained after de-emphasis (**Figure 1**). Venn analysis (automatic weight removal) was performed on the 2349 predicted target genes corresponding to the active ingredients of Qili Qiangxin Capsule and the 11432 heart failure disease target genes, and the intersection was obtained, which is the intersection of Qili Qiangxin Capsule for the treatment of heart failure with 249 potential effector target genes (**Figure 2**).
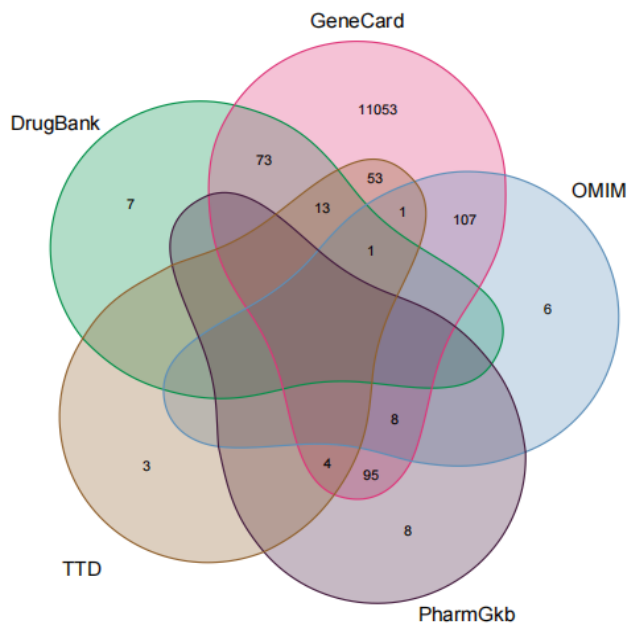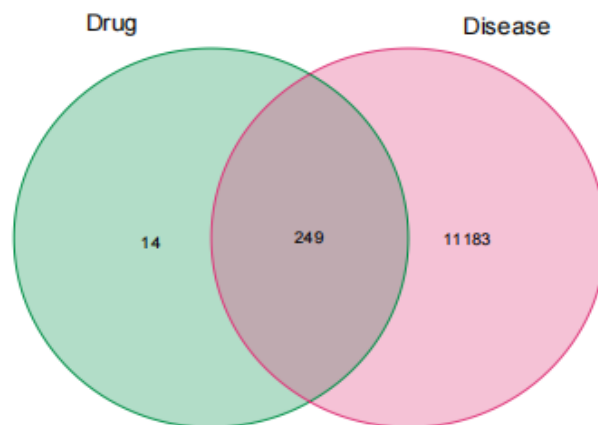
Figure 1. Heart failure disease targets.

Figure 2. Qili Qiangxin Capsule and heart failure target Venn diagrams.

3.3. "Active Ingredient-Disease Target" interaction networks

Cytoscape 3.8.0 software was used to construct the "Active Ingredient-Disease Target" network, in which the left circle represents the active ingredient of Qili Qiangxin Capsule, and the different colours represent the different ingredients of the traditional Chinese medicine, and the right grid represents the target genes of the active ingredient, which are represented by blue rectangles (**Figure 3**).
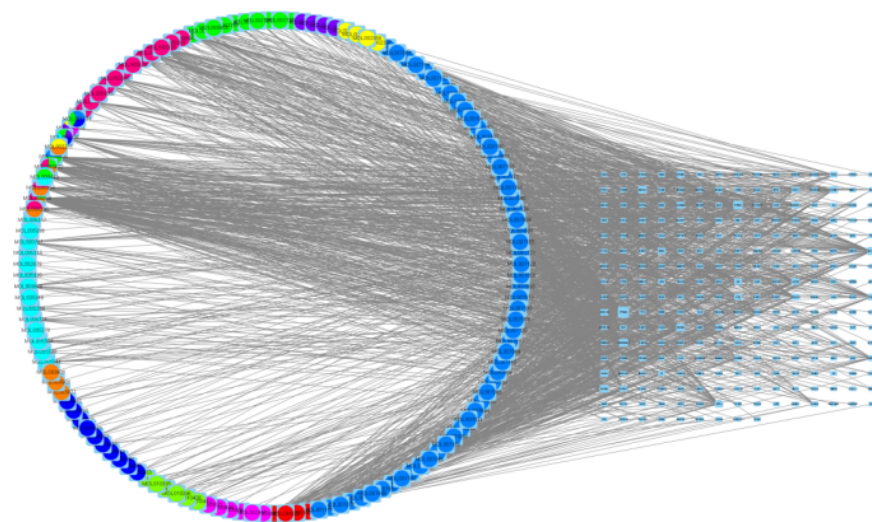

Figure 3. Network diagram of "Active Ingredient-Disease Target."

3.4. PPI network topology analysis and core target screening

The 181 protein interactions obtained from STRING 11.0 platform were imported into Cytoscape 3.8.0 software, and 16 core targets were screened out by using CytoNCA plug-in, which were: *AKT1*, transcription factor JUN, transcription factor *FOS*, *MYC*, *EGFR*, *VEGFA*, *BCL2L1*, *CCND1*, *MAPK14*, *TP53*, *RB1*, *MAPK3*, *CDKN1A*, *MAPK1*, *STAT3*, *RELA* (**Figure 4**).
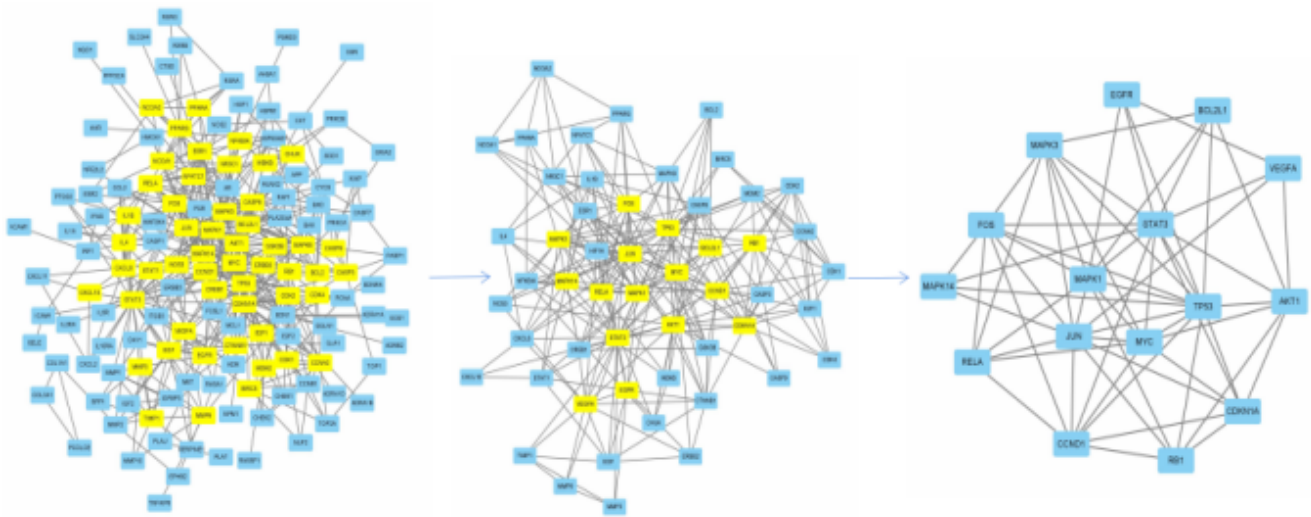
Figure 4. Screening diagram of PPI network.

3.5. Enrichment analysis of core target genes

In order to understand the role of intersecting genes in the treatment of heart failure, KEGG enrichment analysis was performed on 16 core target genes of Qili Qiangxin Capsule, which were mainly enriched in 183 pathways, and the top 30 signalling pathways were extracted to draw a barplot histogram. The vertical coordinate represents the pathway name, the horizontal coordinate represents the number of genes, and the colour represents the significance of the enrichment. The top 30 KEGG-enriched pathways, according to the ascending order of *P*-value, are shown in **Figure 5**. It can be seen that Qili Qiangxin Capsule can act on the effects of lipid and atherosclerosis, AGE-RAGE signalling pathway in diabetic complications, chemical carcinogenesis-receptor activation, fluid shear stress and atherosclerosis, PI3K-Akt signalling pathway, etc. This suggests that the treatment of heart failure with Qili Qiangxin Capsule involves the modulation of different signalling pathways which are interrelated and synergistic.
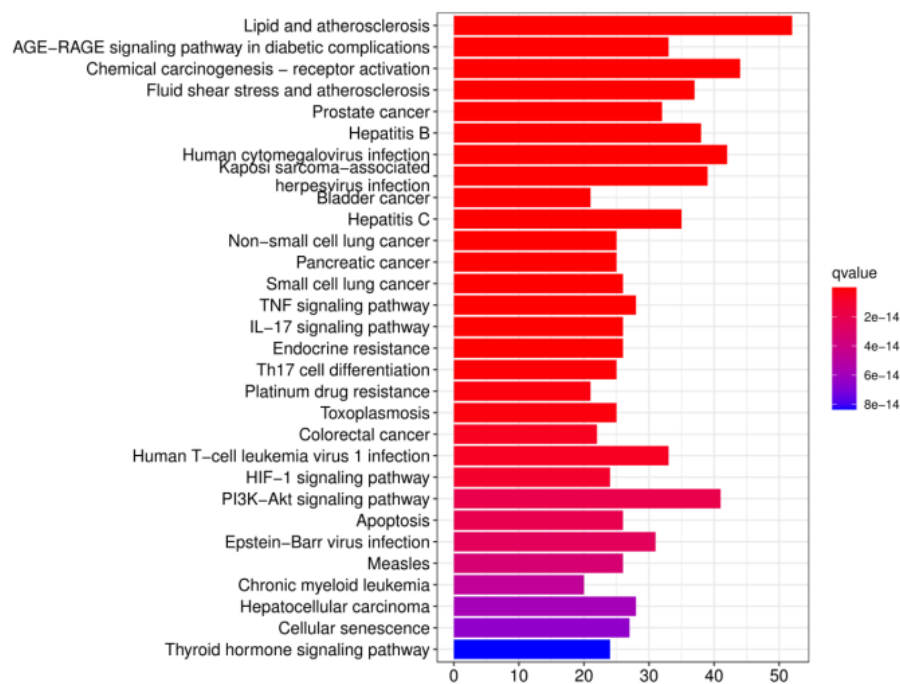

Figure 5. KEGG pathway enrichment results.

3.6. Analysis of molecular docking results

The three relevant active ingredients of Qili Qiangxin Capsule (quercetin, luteolin, cryptotanshinone) and the four core proteins (MAPK-3, STAT3, JUN, TP53) with the largest degree values in the PPI network were subjected to molecular docking by AutoDock Vina software. The minimum binding energy represents the energy required for the compound to bind to the target protein. The less energy, the more stable the binding is, the result is less than -5 kal/mol, which indicates that the binding is important, see **Table 1**. **Figure 6** represents the best combination of the four core proteins docked with the corresponding active ingredients, which includes MAPK1, JUN and TP53 with quercetin and luteolin, respectively, and STAT3 with cryptotanshinone. The docking results illustrated the good binding ability between the active ingredients and target proteins in Qili Qiangxin Capsule.

Table 1 Docking results of the top 4 core proteins with the main active ingredients

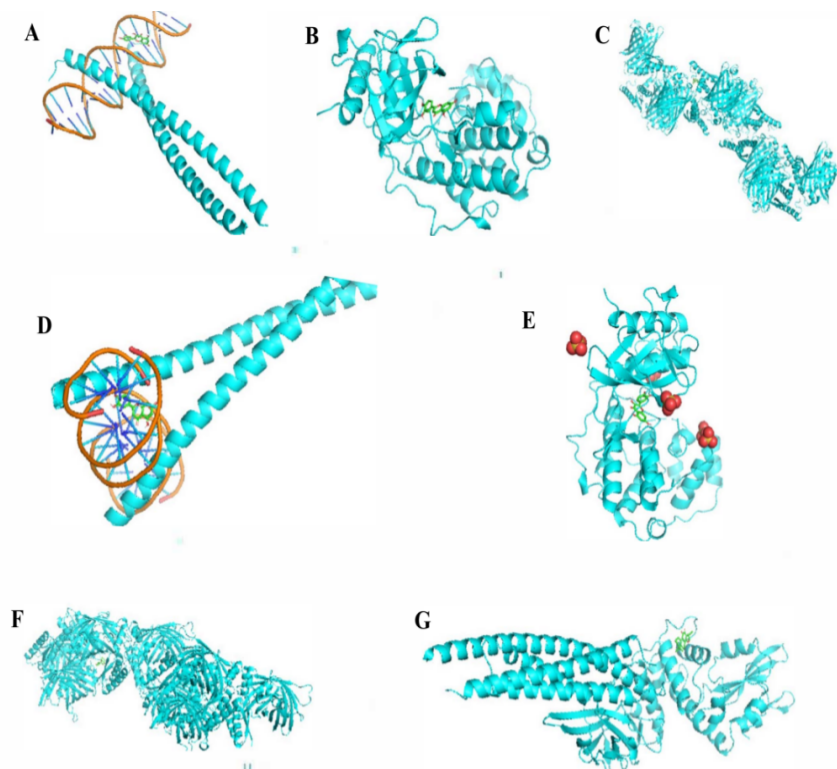| Target gene	PDB ID	Compound	Minimum binding energy (kal/mol)
MAPK1	4FUY	quercetin	-8.9
MAPK1	4FUY	luteolin	-9.0
JUN	5T01	quercetin	-8.6
JUN	5T01	luteolin	-8.8
TP53	7BWN	quercetin	-8.9
TP53	7BWN	luteolin	-8.6
STAT3	6NUQ	cryptotanshinone	-7.7

Figure 6. Molecular docking of JUN with luteolin (A), MAPK1 with luteolin (B), TP53 with luteolin (C), JUN with quercetin (D), MAPK1 with quercetin (E), PT53 with quercetin (F), and STAT3 with cryptotanshinone (G).

4. Discussion

Ancient medical practitioners proposed that the key to treating heart failure lies in diarrhoea and tonifying heart "yang", which is the method of warming yang and water-liquefying, which is the method that later medical practitioners focus on [8]. Modern Chinese medicine practitioners believe that the pathogenesis of heart failure is caused by different reasons that lead to the deficiency of heart "qi" and blood, "yin and yang", and the generation of pathological products such as stasis of blood, water stagnation, and cold congealment, with the mixture of emptiness and solidity, and the underlying deficiency and solidity of this deficiency [9]. Li *et al.* (2013) found that Qili Qiangxin Capsule could significantly reduce plasma NT-proBNP levels and increase left ventricular ejection fraction (LVEF) of patients with heart failure, improve 6-minute walking distance (6MWD), and improve the quality of life of patients with heart failure [10]. Modern pharmacological studies have confirmed that Qili Qiangxin Capsule can play a role in the treatment of heart failure by inhibiting the activation of the RAAS system, protecting the cardiac microvessels, inhibiting lipid metabolism disorders, and diuretic effect, but its active ingredients, targets, and molecular mechanisms are not yet completely clear.

In this study, we found that the active ingredients of Qili Qiangxin Capsule for the treatment of heart failure mainly include: quercetin, luteolin, and cryptotanshinone. Quercetin induces muscle sarcoplasmic reticulum Ca2+-ATPase activity, maintains cellular calcium homeostasis [11], inhibits angiotensin II-induced cardiac fibrosis [12], and delays the development of heart failure [13]. The mechanisms by which luteolin improves cardiac function focus on regulating myocardial contractility, promoting cardiomyocyte autophagy and inhibiting ventricular remodelling [14]. Cryptotanshinone can regulate NO, Ca^{2+}, ROS levels and ATP content in cardiomyocytes under hypoxic conditions, preventing hypoxia-induced cardiomyocyte damage and mitochondrial dysfunction [15].

The PPI network includes 16 core targets, mainly tumour suppressor p53 (TP53), transcriptional activator protein-3 (STAT3), transcription factor JUN, mitogen-activated protein kinase 1 (MAPK1), and so on. In recent years, it has been found that TP53 is maintained at very low levels in the normal heart, and severe or persistent hypoxia induces TP53 expression, resulting in disturbances in energy metabolism of cardiomyocytes, apoptosis, and ultimately heart failure [16]. STAT3 enhances the expression of a variety of encoded genes, such as anti-apoptotic genes (*Bcl-xl*, *MCL-1*), antioxidant genes (*MnSOD*, metallothionein), and vascular endothelial growth factor (VEGF). Studies have shown that STAT3 expression in mitochondria can affect ATP synthesis, the opening of mPTP, and reactive oxygen species production. Thus, STAT3 plays an important role in promoting myocardial differentiation, participating in neovascularisation, regulating β-adrenergic function, and maintaining extracellular matrix homeostasis [17]. Mitogen-activated protein kinases (MAPKs) have been shown to be key signalling factors involved in cardiac remodelling, with family members mainly including p38 kinase, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase 1/2 (ERK1/2), as well as BMK1, which, once activated by phosphorylation, are involved in the regulation of a number of biological processes such as growth, differentiation, proliferation, inflammation, stress, and others. MAPK1 is also known as the *ERK2* gene, and Jochmann S *et al.* (2019) found that the ERK1/2 signalling pair inhibited cardiac fibrosis and cardiomyocyte apoptosis in a stress-loaded mouse model [18]. In addition, targets such as MYC, FOS, and MAPK3 are also present. A total of 183 signalling pathways related to heart failure therapy were obtained by signal pathway enrichment analysis. Among them, the significantly enriched and star pathways in recent years included AGE-RAGE signalling pathway, fluid shear stress and atherosclerosis, PI3K-Akt signalling pathway, etc. AGEs (advanced glycosylation end products) are produced and accumulated in plasma and vascular tissues for a long period of time, which induces monocyte chemotaxis, oxidative stress response in vascular smooth muscle cells, and release of cytokines (IL6, TNF-α), growth factors (TGF-β1), etc. thereby causing an

inflammatory response, leading to vasodilatory and constrictive dysfunction; although the specific molecular mechanism of the AGE-RAGE axis involved in heart failure is still unclear, AGE and esRAGE have become an important predictor of the occurrence and development of heart failure [19]. Fluid shear stress can affect the morphology and function of endothelial cells and plays an important role in cardiovascular system diseases. PI3K/Akt signalling pathway is widely present in human cardiomyocytes as an important pathway for the transduction of membrane receptor signals to the intracellular level. AKt is a key downstream target of PI3K, whose Akt1 isoforms have a close relationship with the progression of heart failure, and PI3K/Akt signalling pathway can be mediated through the the downstream target BAD by mediating the effects of a variety of apoptotic factors (*Bcl-2*, *Bcl-x1*, etc.) [20], and it can also attenuate cellular inflammatory response and improve myocardial energy metabolism.

Through molecular docking, it is known that effective compounds such as quercetin, luteolin and cryptotanshinone can bind well with receptor proteins and play a role in intervening in the development of heart failure by regulating cell autophagy, improving oxidative stress, inhibiting cell fibrosis, and other biological processes, and on the other hand, the activation of receptor proteins modulates the relevant signalling pathways to regulate cardiovascular tissues. The target sites are enriched with various signals.

In conclusion, the treatment of heart failure with Qili Qiangxin Capsule is a multi-component, multi-target, multi-pathway process, and it can be used through multiple core targets, such as TP53, STAT3, JUN, MAPK1, and multiple pathways such as AGE-RAGE, fluid shear stress, and signalling pathways such as atherosclerosis and PI3K/Akt. This study was conducted on an existing public database, and the completeness of the data has an important impact on the predicted results. Since this study focuses on theory, more basic experiments are needed to validate the predicted targets and related pathways, which will provide a strong theoretical basis for the clinical use of Qili Qiangxin Capsule in the treatment of heart failure.

Funding

Disclosure statement

The author declares no conflict of interest.

References

[1] Ponikowski P, Anker SD, AlHabib KF, et al., 2014, Heart Failure: Preventing Disease and Death Worldwide. ESC Heart Failure, 1(1): 4–25.

[2] Zhang Y, Zhang J, Butler J, et al., 2017, Contemporary Epidemiology, Management, and Outcomes of Patients Hospitalized for Heart Failure in China: Results From the China Heart Failure (China-HF) Registry. Journal of Cardiac Failure, 23(12): 868–875.

[3] Nakou ES, Vardas PE, 2013, New Therapeutic Options in Heart Failure What's on the Horizon?: An overview. International Journal of Cardiology,170(2): 95–106.

[4] Mao J, Wu Y, Shi D, 2021, Guidelines for Clinical Application of Proprietary Chinese Medicines in the Treatment of Coronary Heart Disease (2020). Journal of Integrative Cardiovascular and Cerebrovascular Diseases, 2021(9): 1409–1435.

[5] Wang H, Liang Y, 2018, Chinese Guidelines for the Diagnosis and Treatment of Heart Failure. Chinese Journal of Cardiovascular Disease, 2018(10): 760–789.

[6] Chen K, Wu Z, Zhu M, et al., 2016, Expert Consensus on Combined Chinese and Western Medicine Diagnosis and Treatment of Chronic Heart Failure. Journal of Integrated Cardiovascular and Cerebrovascular Diseases of Chinese and Western Medicine, 2016(3): 225–232.

[7] Zhao Q, Li H, Yin Y, et al., 2019, Progress of Research on the Mechanism of Action of Qili Qiangxin Capsule in the Treatment of Chronic Heart Failure. Tianjin Traditional Chinese Medicine, 2019(4): 313–318.

[8] Zhou J, Ma Zandong, Li Zhongxuan, 2018, Research on the Collation of Ancient Chinese Medical Literature on Heart Failure. China Medical Innovation, 2018(31): 54–57.

[9] Zhao Xue, Dai Guohua, 2021, Multi-targeted Intervention of Chinese Medicine in Chronic Heart Failure. Journal of Chinese Medicine, 2021(6): 1217–1221.

[10] Li X, Zhang J, Huang J, et al., 2013, A Multicenter, Randomized, Double-blind, Parallel-group, Placebo-controlled Study of the Effects of Qili Qiangxin Capsules in Patients with Chronic Heart Failure. Journal of the American College of Cardiology, 62(12): 1065–1072.

[11] Blaškovič D, Zizžková P, Držík F, et al., 2013, Modulation of Rabbit Muscle Sarcoplasmic Reticulum Ca2+-ATPase Activity by Novel Quercetin Derivatives. Interdisciplinary Toxicology, 6(1): 3–8.

[12] Wang L, Tan A, An X, et al., 2020, Quercetin Dihydrate Inhibition of Cardiac Fibrosis Induced by Angiotensin II in vivo and in vitro. Biomedicine & Pharmacotherapy, 2020(127): 110205.

[13] Yamagata K, 2019, Polyphenols Regulate Endothelial Functions and Reduce the Risk of Cardiovascular Disease. Current Pharmaceutical Design, 25(22): 2443–2458.

[14] Luo Y, Shang P, Li D, 2017, Luteolin: A Flavonoid that Has Multiple Cardio-Protective Effects and Its Molecular Mechanisms. Frontiers in Pharmacology, 2017(8): 692.

[15] Jin HJ, Li CG, 2013, Tanshinone IIA and Cryptotanshinone Prevent Mitochondrial Dysfunction in Hypoxia-Induced H9c2 Cells: Association to Mitochondrial ROS, Intracellular Nitric Oxide, and Calcium Levels. Evidence-Based Complementary and Alternative Medicine, 2013(1): 610694.

[16] Kimata M, Matoba S, Iwai-Kanai E, et al., 2010, p53 and TIGAR Regulate Cardiac Myocyte Energy Homeostasis Under Hypoxic Stress. American Journal of Physiology: Heart and Circulatory Physiology, 299(6): 1908–1916.

[17] Pipicz M, Demján V, Sárközy M, et al., 2018, Effects of Cardiovascular Risk Factors on Cardiac STAT3. International Journal of Molecular Sciences, 19(11): 3572.

[18] Jochmann S, Elkenani M, Mohamed BA, et al., 2019, Assessing the Role of Extracellular Signal-Regulated Kinases 1 and 2 in Volume Overload-Induced Cardiac Remodelling. ESC Heart Failure, 6(5): 1015–1026.

[19] Paradela-Dobarro B, Agra RM, Álvarez L, et al., 2019, The Different Roles for the Advanced Glycation End Products Axis in Heart Failure and Acute Coronary Syndrome Settings. Nutrition, Metabolism and Cardiovascular Diseases, 29(10): 1050–1060.

[20] Li S, Li P, Kang N, et al., 2021, Research Progress on the Relationship Between PI3K/Akt Signalling Pathway and Cardiovascular Diseases. Beijing Medical, 2021(1): 66–69.