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Network analysis for elucidating the mechanisms of Shenfu injection in preventing and treating COVID-19 combined with heart failure

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ABSTRACT

Background: The emergence of the novel coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to millions of infections and is exerting an unprecedented impact on society and economies worldwide. The evidence showed that heart failure (HF) is a clinical syndrome that could be encountered at different stages during the progression of COVID-19. Shenfu injection (SFI), a traditional Chinese medicine (TCM) formula has been widely used for heart failure therapy in China and was suggested to treat critical COVID-19 cases based on the guideline for diagnosis and treatment of COVID-19 (the 7th version) issued by National Health Commission of the People’s Republic of China. However, the active components, potential targets, related pathways, and underlying pharmacology mechanism of SFI against COVID-19 combined with HF remain vague.

Objective: To investigate the effectiveness and possible pharmacological mechanism of SFI for the prevention and treatment of COVID-19 combined with HF.

Methods: In the current study, a network analysis approach integrating active compound screening (drug-like-ness, lipophilicity, and aqueous solubility models), target fishing (Traditional Chinese Medicine Systems Pharmacology, fingerprint-based Similarity Ensemble Approach, and PharmMapper databases), compound-target-disease network construction (Cytoscape software), protein-protein interaction network construction (STRING and Cytoscape software), biological process analysis (STRING and Cytoscape plug-in Clue GO) and pathway analysis (Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis) was developed to decipher the active ingredients, potential targets, relevant pathways, and the therapeutic mechanisms of SFI for preventing and treating COVID-19 combined with HF.

Results: Finally, 20 active compounds (DL ≥ 0.18, 1 < Alog P ≤ 5, and −5 ≤ LogS ≤ −1) and 164 relevant targets of SFI were identified related to the development of COVID-19 combined with HF, which were mainly involved in three biological processes including metabolic, hemostasis, and cytokine signaling in immune system. The C-T-D network and reactome pathway analysis indicated that SFI probably regulated the pathological processes of heart failure, respiratory failure, lung injury, and inflammatory response in patients with COVID-19 combined with HF through acting on several targets and pathways. Moreover, the venn diagram was used to identify 54 overlapped targets of SFI, COVID-19, and HF. KEGG pathway enrichment analysis showed that 54 overlapped targets were highly enriched to several COVID-19 and HF related pathways, such as IL-17 signaling pathway, Th17 cell differentiation, and NF-kappa B signaling pathway.

Conclusions: A comprehensive network analysis approach framework was developed to systematically elucidate the potential pharmacological mechanism of SFI for the prevention and treatment of SFI against COVID-19 combined with HF. The current study may not only provide in-depth understanding of the pharmacological mechanisms of SFI, but also a scientific basis for the application of SFI against COVID-19 combined with HF.
1. Introduction

COVID-19 is a highly contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection that has led to the unprecedented global pandemics, as well as various scientific and societal challenges. Recently, different variants of SARS-CoV-2 emerged since the first COVID-19 case reported in December 2019, including Alpha, Beta, Gamma, Delta, and newly discovered Omicron. These variants have brought new challenges for diagnosis and treatment, especially for the vaccine efficacy, disabusing the notion of infection control by global vaccination [1,2].

According to the “Guideline on Diagnosis and Treatment of COVID-19” (the 7th version) issued by the National Health Commission of China (National Health Commission & State Administration of Traditional Chinese Medicine, 2020), patients with COVID-19 disease were divided into four groups: mild, moderate, serious, and critical illness. COVID-19 cases manifest with a wide range of illness from asymptomatic infection or mild respiratory ailments to acute respiratory distress and death. The clinical manifestations of COVID-19 are mainly characterized by fever, fatigue, and dry cough. Some patients present with nasal congestion, sore throat, runny nose, diarrhea, and myalgia. In severe cases, the infection may cause acute respiratory distress syndrome, respiratory failure, heart failure (HF), shock, multiple organ dysfunctions and even death [3]. The membrane invagination of SARS-CoV-2 depends on the trimer spike protein binding to the Angiotensin-converting enzyme 2 (ACE2) of the host cells which is coordinated by transmembrane serine protease 2 (TMPRSS2) [4]. Of note, ACE2 is involved in the cardiomyopathy which is proved by disrupted cardiac function in ACE2 knockout mice and elevated gene expression in spontaneously hypertensive rats [5].

HF is a common and life-limiting disorder that occurred in the pathological stages of COVID-19 infection [6]. HF is considered a secondary event from COVID-19 infection. As the mostly affected organ, COVID-19 infection disrupts the lung function and decreases the blood oxygen saturation which may result in the cardiac ischemia [7]. Moreover, the inflammatory process caused by COVID-19 infection can damage the heart. SARS-CoV-2 also enters the endothelial cells lining the inner surfaces of veins and arteries, and results in the inflammation and clots in blood vessel, which compromise blood flows to the heart [8]. A recent evidence in Switzerland suggested that the HF is present in around 20% COVID-19 patients with cardiovascular disease and 13% total COVID-19 patients, which could increase the risk of severe symptoms and high in-hospital mortality [9]. The existing HF poses a unique set of challenges in the treatment of COVID-19 patients, since the complexity of presentation, management, and prognosis of COVID-19. COVID-19 combined with HF raises the risk of morbidity and mortality due to the reduced immunity, general frailty, and hemodynamic ability of HF and the widespread activation of the systemically inflammatory response related to severe COVID-19 infection. A recent study revealed that close to 9000 COVID-19 patients in 169 hospitals from three continents were encountered coronary artery disease and HF with the mortality of 15.3% compared to those without HF (5.6%) [10]. Monocytes in HF patients producing higher tumor necrosis factor alpha (TNF-α) and lower IL-10 than healthy subjects [6] exacerbated the systemic inflammatory storm in severe COVID-19 individuals which needs supports from cardiac performance (lacking in HF patients) [11]. Therefore, it is imperative to develop multi-mechanism medications and understand their pharmacological mechanisms for the prevention of COVID-19 combined with HF.

Traditional Chinese medicine (TCM) has been exploited in the fight against epidemic diseases over thousands of years and exhibits a significant therapeutic effectiveness for various infectious diseases, due to the comprehensive view and multi-component, multi-target properties. Shenfu injection (SFI) is a modified medication from Shenfu decoction, that is firstly recorded in Yan’s Prescriptions for Rescuing Lives in the 1250s [12]. SFI composed of Red Ginseng (RG) and Radix Aconitii Lateralis Preparata (RA) can invigorate qi to engender blood and restore yang (Supplementary 1). It has been used in treating HF and has exhibited good efficacy in improving the symptoms for a long time [13]. Moreover, SFI has become the recommended TCM formula for the severe cases of COVID-19 therapy in the trial 7th edition of the “Guideline on Diagnosis and Treatment of COVID-19” issued by the National Health Commission of China (National Health Commission & National Administration of Traditional Chinese Medicine, 2020) [14]. Some studies have conducted the pharmacodynamic evaluation of SFI in both clinic and animals (rats with ischemic heart failure) suggesting anti-hypoxia effects and normal hemodynamic characteristic of SFI [15]. Although SFI has been shown to be effective in combating HF and COVID-19. However, the active compounds, related targets and the synergistic pharmacological mechanisms of SFI in preventing COVID-19 combined with HF are poorly understood.

Fortunately, network analysis approach has emerged as a promising tool to reveal the underlying mechanisms of multi-component and multi-target agents, as well as the therapeutic effects of TCM for various diseases [16,17]. It provides a more comprehensive understanding of the complex relationships between active compounds, targets, pathways, and certain diseases. Therefore, in the current study, a network analysis approach methodology was proposed to explore the active ingredients, potential targets, related pathways, and the synergistic therapeutic mechanisms of SFI against COVID-19 combined with HF. The present study provides a promising strategy to dissect effectiveness and possible pharmacological mechanism of SFI for the prevention and treatment of COVID-19 combined with HF, which will facilitate the development and application of TCM for various complicated diseases.

2. Materials and methods

2.1. Chemical database for SFI

SFI consists of two herbs including RG and RA. All chemical constituents of SFI were obtained from Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (http://tcmspw.com/tcmsp.php) [18], which consists of 499 Chinese herbs registered in the Chinese pharmacopoeia with 29,384 ingredients. TCMSP is a powerful tool of TCM for searching a mass of information on herbal entries and ingredients. Finally, a total of 119 ingredients from the two herbs in SFI including 67 in RG and 52 in RA were collected. The molecular structures were extracted from TCMSP and PubChem databases [18], which were saved as mol2 format for further analysis [19].

2.2. Generation of ADME filters

ADME is an important indicator to evaluate the drug activity at the early stage of drug development. The drug-likeness (DL) provides valuable guidelines for the clinical trials at early phrase of a potential drug with considerable bioavailability [20]. Since TCM injections including SFI are dissolved in water or saline, lipophilicity (AlogP) and aqueous solubility (LogS) are critical characteristics. Lipophilicity is a special feature in the early drug screening and affects many parameters of ADME. Lipophilicity is closely correlated to ADME properties and negatively related to aqueous solubility [21]. The aqueous solubility plays an important role in the drug design for the administration by injection [22]. Therefore, to obtain the active compounds from SFI with satisfactory pharmacokinetics properties, the three in silico models including DL, AlogP, and LogS were applied in the current study.

Drug-likeness (DL) is a qualitative parameter employed the early stages of drug discovery and development to evaluate the drug-ability of a specific candidate molecule [23]. The DL index of each compound was predicted based on Tanimoto coefficient, which is defined as $T(X, Y) = (X \cdot Y)/(|X|^2 + |Y|^2 - A \cdot B)$. In this equation, X indicates the molecular descriptor of the ingredients in SFI, and Y displays the average DL index of all the compounds in Drugbank database [24]. The DL $\geq 0.18$ (average value for DrugBank) was considered as the threshold to screen
the candidate active compounds in SFI for further research [25].

Lipophilicity is an important feature of the ADME properties which defined as the partition coefficient P. AlogP is a widely-used parameter to measure the ability of chemicals absorption and excretion in biological system [26]. In the current study, the values of AlogP were calculated by the ALOGPS 2.1 program using an in silico model. Tetko et al. developed a model with good predictability integrating 12,908 log P data and 75 input parameters of the chemicals using 64 neural networks (root mean square = 0.49 and standard mean error = 0.26) [27]. Finally, we set the Alog P range from 1 to 5 base on the Lipinski’s rule of five [28].

Aqueous solubility (LogS) is another critical property of ADME in the early phases of drug discovery, which significantly affects the bioavailability of a compound [29]. LogS is also calculated using a well-known online software ALOGPS 2.1 [30] and the threshold value is ranged from −5 to −1 [31].

2.3. Identification of potential targets for SFI, COVID-19 and HF

The potential targets of the active compounds for SFI were identified based on TCMS (http://tcmspw.com/tcmsp.php) [18], fingerprint-based Similarity Ensemble Approach (SEA, http://sea.bslab.org/) [32], and PharmMapper (http://www.lilab-ecust.cn/pharmmapper/) [33] databases. TCMS predicts targets by a systematic drug-target interactions model (SysDT) that integrates the chemical, genomic and pharmacological information. SEA was used to identify related targets depending on chemical structure similarity among the small molecule ligands, while PharmMapper was performed to predict candidate drug targets through the chemical similarities and pharmacophore models of bioactive components (Supplemental 2).

In addition, GeneCards database is a comprehensive database that integrates concise genome, proteome, transcriptome, disease and functional data information from large public resources [34]. The keywords “COVID-19”, “SARS-CoV-2”, “novel coronavirus pneumonia” and “heart failure” were put into GeneCards to obtain COVID-19 and HF relevant target genes.

Finally, all the targets were combined, duplicated and further mapped to UniProt Database to confirm and convert into corresponding gene names, restricted the species to Homo sapiens [35]. The Venn diagram was involved to visualize the intersected genes between the SFI, COVID-19 and HF.

2.4. Construction of the protein-protein interaction network and pathway analysis

To further elucidate the interactions of intersected genes between the SFI, COVID-19 and HF, we introduced the STRING platform (http://string-db.org/) including a large number of protein interaction relationships acquired from experiments or bioinformatics methods (Supplementary 2) [37]. The overlapping targets of SFI, COVID-19 and HF were put into the STRING to gain the network of the protein-protein interactions. The obtained results were imported into Cytoscape v3.2.1 (https://cytoscape.org/) to visualize the protein-protein interaction network and the network analyzer plug-in was used to analyze the network topological properties (Supplementary 2) [38]. Then the pathway enrichment analysis of above common targets was perform based on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using DAVID to explore the underlying mechanisms of SFI against COVID-19 combined with HF [39,40].

2.5. Network construction and analysis

To elucidate the potential pharmacological effects of SFI for the prevention and treatment of COVID-19, compound-target-disease (C-T-D) and target-pathway (T-P) networks were developed to systematically clarify the complicated relationships among compounds, targets, diseases and pathways at the system level. The C-T-D network was built based on active compounds, their corresponding targets and related diseases, while T-P network was generated by linking potential targets and their enriched pathways. All the networks were visualized by Cytoscape v3.2.1, an available software for biomolecular interaction network visualization, data integration and analysis.

3. Results and discussion

3.1. Identification of active compounds in SFI

Despite a single herb or TCM formula usually contains multiple components, only a few active compounds have desirable pharmacodynamic and pharmacokinetic properties. ADME filtering is an attractive alternative to experimental methods for high throughput screening the promising compounds, significantly reducing time and costs of the drug development process. In the current study, three ADME parameters (DL, AlogP and LogS) were employed to filter out the potential active compounds of SFI. The results showed that a total of 20 active compounds passed through the filtering criteria of DL ≥ 0.18, 1 ≤ AlogP ≤ 5, and −5 ≤ LogS ≤ −1, which account for 16.8% of all the 119 ingredients. The detailed information of these active compounds in SFI was shown in Table 1.

In RG, 10 active components satisfied out the filtering criteria, such as protopanaxatriol (DL = 0.78, AlogP = 4.62 and LogS = −4.48), notoginsenoside R2 (DL = 0.28, AlogP = 1.64 and LogS = −3.53), ginsenoside Rb1 (DL = 0.57, AlogP = 2.87 and LogS = −3.93) and ginsenoside Rs1 (DL = 0.46, AlogP = 4.42 and LogS = −4.95) and so on. Interestingly, most of them exhibited significant biological activity related to COVID-19 and heart failure. For instance, Ginsenoside Rg3 (DL = 0.22, AlogP = 2.3 and LogS = −3.9) was found to exert significant regulatory effect on glucose metabolism and insulin resistance through activation of the AMPK pathway in chronic heart failure induced by transverse aortic coarctation [41]. Ginsenoside Rf (DL = 0.24, AlogP = 1.13 and LogS = −3.28) has been reported to exhibit anti-inflammatory activities on inhibiting hypoxia-induced cyclooxygenase 2 expression through binding to peroxisome proliferator-activated receptor γ [42].

Among the 52 ingredients in RA, 10 of them have reasonable ADME parameters, such as karajin (DL = 0.34, AlogP = 2.94 and LogS = −4.02), deudatine (DL = 0.67, AlogP = 2.25 and LogS = −2.98), deltoin (DL = 0.37, AlogP = 2.48 and LogS = −4.84), Deoxyandrographolide (DL = 0.31, AlogP = 3.02 and LogS = −3.51) and so on. Surprisingly, some of these active compounds have been reported to possess profound pharmacological activities. For example, the evidence showed that karajin suppressed inflammatory mediators production released by immune cells via its antioxidant activity and immune modulatory effects [43]. Deoxyandrographolide (DL = 0.31, AlogP = 3.02 and LogS = −3.51) was found to have anti-inflammatory activity by inhibiting the production of lipopolysaccharide-induced proinflammatory mediators, such as TNF-α and interleukin 6 (IL-6), and decreasing mRNA level of inducible nitric oxide synthase [44].

3.2. Potential target collection of SFI, COVID-19 and HF

Generally, the efficacy of TCM formulas in the prevention and treatment of complex diseases depends on the synergistic effects of multiple compounds and targets [45]. To further decipher the therapeutic mechanism of SFI against COVID-19 combined with HF, the targets of the active compounds were identified using three databases, including TCMS [18], SEA [32], and PharmMapper [33].

In the current study, 164 candidate targets for the 20 active compounds with 334 interactions between them were obtained (Table S1). The results showed that most active ingredients of SFI act against more than one biological target, revealing various potentially beneficial pharmacological effects of the bioactive compounds. For instance, compound ginsenoside Rf from RG can interact with 53 proteins, while
### Table 1
Chemical information of 20 active ingredients.

| Herb | Molecule Name                  | DL  | AlogP | LogS  | Structure |
|------|--------------------------------|-----|-------|-------|-----------|
| RG   | Protopanaxatriol               | 0.78| 4.62  | −4.48 |           |
| RG   | Notoginsenoside R2             | 0.28| 1.64  | −3.53 |           |
| RG   | Ginsenoside Rh1                | 0.57| 2.87  | −3.93 |           |
| RG   | Ginsenoside Rs1                | 0.46| 4.42  | −4.95 |           |
| RG   | Ginsenoside Rh4                | 0.6 | 3.84  | −4.93 |           |
| RG   | Ginsenoside Rh2                | 0.56| 4.04  | −4.71 |           |
| RG   | Ginsenoside Rg3                | 0.22| 2.3   | −3.9  |           |
| RG   | Ginsenoside Rg1                | 0.28| 1.13  | −3.42 |           |
| RG   | Ginsenoside Rf                 | 0.24| 1.13  | −3.28 |           |
| RG   | Ginsenoside Rg2                | 0.26| 2.02  | −3.66 |           |

*(continued on next page)*
(R)-norcoclaurine from RA targets on 61 targets. In addition, 1741 COVID-19 putative targets and 6783 HF disease genes were obtained from GeneCards.

3.3. Biological processes regulated by SFI targets

To further explore the potential pharmacological mechanisms of the SFI targets, the biological processes were performed. In this section, three sub-networks of different biological processes for the targets of SFI were constructed (Fig. 1). The results showed that SFI acted on the targets specific for three biological processes, including metabolism, hemostasis, and cytokine signaling in immune system.

The metabolism sub-network (Fig. 1A) showed that SFI targeted on some core receptor proteins, such as nuclear receptor coactivator 1 (NCOA1), nuclear receptor coactivator 2 (NCOA2), glucocorticoid receptor (NR3C1), lysine acetyltransferase 2B (KAT2B), retinoid X receptor (RXRA), retinoic acid receptor α (RARA), retinoic acid receptor β (RARB), retinoic acid receptor γ (RARG), estrogen receptor alpha (ESR1), and androgen receptor (AR), indicating the pharmacological effect of SFI for the regulation of metabolism. In addition, some protein kinases and cytochrome P450 enzymes were involved in several sub-networks, including mitogen-activated protein kinase 8 (MAPK8),

Table 1 (continued)

| Herb | Molecule Name     | DL  | AlogP | LogS | Structure |
|------|-------------------|-----|-------|------|-----------|
| RA   | Karanjin          | 0.34| 2.94  | -4.02|           |
| RA   | Denudatine        | 0.67| 2.25  | -2.98|           |
| RA   | (R)-Norcoclaurine | 0.21| 2.57  | -3.06|           |
| RA   | Deltoin           | 0.37| 2.48  | -4.84|           |
| RA   | Delavaconitine    | 0.37| 1.38  | -3.49|           |
| RA   | 1-Benzoylnapelline| 0.53| 3.12  | -3.73|           |
| RA   | Deoxyandrographolide | 0.31| 3.02  | -3.51|           |
| RA   | Delphin           | 0.28| 1.67  | -3.71|           |
| RA   | 14-Deoxy-11,12-didehydroandrographolide | 0.32| 2.1   | -3.67|           |
| RA   | Ignavine          | 0.25| 1.22  | -2.46|           |
mitogen-activated protein kinase 14 (MAPK14), cytochrome P450 1A1 (CYP1A1), cytochrome P450 2C9 (CYP2C9), and cytochrome P450 1B1 (CYP1B1), which were associated with the metabolism process. Of note, the angiostatin (1–7) produced by the ACE2 plays a crucial role in inhibiting MAPK cascades that are found in our targets pool, indicating the therapeutic potential of SFI in correcting ACE2 dysfunction caused by SARS-CoV-2 infection [46].

Some hub targets hit by SFI belonged to protein kinases involved in hemostasis of sub-network (Fig. 1B), including protein kinase CAMP-activated catalytic subunit alpha (PRKACA), phosphoinositide-dependent protein kinase 1 (PDK1), proto-oncogene tyrosine-protein kinase Src (SRC), tyrosine-protein kinase ABL1 (ABL1), cyclin E/cyclin-dependent kinase 2 (CDK2), and tyrosine-protein kinase Lck (LCK). These protein kinases were related to the processes of coagulation, which indicated that SFI might play a role in hemostasis by regulating coagulation. In the subnetwork of cytokine signaling in immune system (Fig. 1C), SFI acted on several cytokines, such as tumor necrosis factor (TNF), interleukin 1 beta (IL-1B), vascular endothelial growth factor A (VEGFA), insulin-like growth factor 1 (IGF1), interleukin-2 (IL-2), and interleukin-4 (IL-4). These cytokines were involved in cytokine storm disorders, producing an excessive inflammatory and immune response, which were responsible for the pathophysiology of COVID-19. Moreover, IL-6 plays a pivotal role in the pathophysiology of COVID-19, which can be suppressed by the SFI treatment [47,48]. Previous studies have shown that TNF-α mediates the expression of IL-6 through phosphorylation of MAPK [49,50]. Intriguingly, the sub-network uncovered that the above mentioned factors were targeted by SFI, indicating the potential effects on IL-6 by SFI. These results suggested that SFI might prevent or relieve cytokine storm and multi-organ failure through modulating the biological process of cytokine signaling in immune system.

3.4. C-T-D network and reactome pathway analysis of SFI

To decipher pharmacological mechanism of SFI in treating COVID-19 combined with HF from a global perspective, the C-T-D network linking compounds, targets and their related diseases was developed. In the current study, 20 active compounds (10 of RG and 10 of RA), a total of 164 target proteins (94 of RG and 110 of RA) as their targets, and 5 diseases were gathered to construct the C-T-D network to explore the mechanism of SFI treating COVID-19 combined with HF (Fig. 2, Table S1).

The result showed that the targets hit by active compounds in SFI were connected with the pathological process of heart failure, respiratory failure, lung injury, inflammation, and immune response in COVID-19 combined with HF. For instance, galectin-3 (LGALS3) hit by 4 active compounds of SFI has been shown to be a useful biomarker and biotarget in prognosis and risk stratification in HF. Inhibition of its activation might be a promising therapeutic strategy in the prevention and treatment of HF [51]. IGF1 hit by 5 active compounds was involved in the pathogenesis of acute respiratory failure in regulating vascular permeability and regeneration through inactivation of epithelial sodium channel (ENaC) and promotion of vascular endothelial growth factor (VEGF), respectively [52]. Moreover, IGF-1 has been indicated to phosphorylate endothelial nitric oxide synthase (eNOS) [53], which was diminished in COVID-19 and HF, respectively [54,55]. 4 compounds were against the matrix metalloproteinase-2 (MMP2) which was a proteolytic enzyme responsible for the pathogenesis of lung injury via degrading the extracellular matrix (ECM) of blood-air barrier [56]. Hypoxia-inducible factor 1-alpha (HIF1A) hit by 4 compounds was suggested to directly interfere with mRNA indication and promoter activation of brain natriuretic peptide (BNP) [57], which was closely related to HF and other cardiac diseases [58]. Interestingly, BNP/NT-proBNP concentration was significantly correlated with the disease severity of patients with COVID-19 [59]. Tumor necrosis factor (TNF, hit by 2 active compounds), as a well-known pro-inflammatory cytokine played a key role in pathogenesis of inflammation induced by certain immunological reactions, infection, or tissue damage [60]. While 6 active compounds were found to target IL-2, which has been reported to be a powerful immune growth factor induced by antigen stimulation and played a critical role in regulating the adaptive immune system through modulating the survival and proliferation of regulatory T-cells (Treg) [61]. These results suggested that SFI could exert a significant pharmacological effect on the prevention and treatment of COVID-19 combined with HF from a system level.

The pathway enrichment analysis for the 164 potential targets of SFI based on Reactome was conducted to clarify the potential pharmacological effects of SFI on COVID-19 combined with HF from the pathway level. The top 20 Reactome pathways or super pathways were significantly enriched with these targets (Fig. 3, Table S2). Interestingly, these pathways were closely related to the pathological processes in COVID-19 combined with HF. For instance, signal transduction pathway (81 targets involved) showed the highest degrees and were highly related to the pathological processes of COVID-19. Block of the mentioned signal transduction pathway would be a promising method for the treatment of severe COVID-19 patients [62]. 80 targets were involved in immune system pathway suggested the key pathways referred to the regulation of COVID-19 and HF since immune system responsible for the defense of...
the body against pathogens and production specific antibodies to kill bacteria and viruses [63,64]. In addition, other pathways closely related to COVID-19 and HF such as metabolism pathway, cytokine signaling in immune system pathway, and infectious disease pathway. These results showed that SFI might exert therapeutic effects via regulating various cellular pathways targeting on multiple proteins.

3.5. Protein-protein interaction network and pathway analysis of the intersecting target

To interpret the potential pharmacological effects of SFI against COVID-19 combined with HF, 54 overlapped targets of SFI, COVID-19, and HF were analysed by the venn diagram (Fig. 4A). The protein-protein interaction network with 41 nodes and 167 edges was generated using STRING to further explore the regulatory roles of the overlapped targets (Fig. 4B). The protein-protein interaction network showed 10 hub genes with highest coreness and betweenness, including TNF, IL-1B, VEGFA, MAPK14, IL-2, IL-4, PTGS2, caspase 3 (CASP3), albumin (ALB), and heat shock protein 90 alpha family class a member 1 (HSP90AA1). Interestingly, these hub genes have been indicated to closely correlate with HF, respiratory failure, lung injury, inflammation, and immune response, which may be regarded as pivotal targets of SFI in the prevention and treatment of COVID-19 combined with HF. For instance, among these hub genes, several cytokines were involved in the processes of inflammation and immune response contributing to the cytokine storm in COVID-19, such as TNF, IL-1B, IL-2, and IL-4. These results revealed that the hub genes played important roles in the therapeutic mechanism of SFI for COVID-19 and HF.

In addition, KEGG pathways were enriched with the 54 overlapped targets to explore the therapeutic mechanism of SFI against COVID-19 combined with HF from the pathway level. As a result, the top 20 significantly pathways that related to the pathological process of COVID-19 combined with HF were identified, such as IL-17 signaling pathway, Th17 cell differentiation, TNF signaling pathway, T cell receptor signaling pathway, fluid shear stress and atherosclerosis pathway, NF-kappa B signaling pathway, apoptosis and so on (Fig. 5A). The T-P network that linked the overlapped targets and related pathways was shown in Fig. 5B (Table S3).
In T-P network, 15 targets were involved in IL-17 signaling pathway, which has the highest degree and plays an important role in the immunopathology of COVID-19 and HF [65,66]. As a proinflammatory T-cell subset, Th17 cell differentiation is related to 11 targets involved in pathogenesis of multiple immune disorders [67], including COVID-19 and HF. TNF signaling pathway (9 targets involved) and NF-kappa B signaling pathway (7 targets involved) have been suggested as the potential targets of COVID-19 [68,69], while 6 targets were marked in Toll-like receptor signaling pathway that has been recognized as a component of innate immunity contributing to the pathological processes of COVID-19 infection [70]. These results suggested that SFI might involve in the prevention and treatment of COVID-19 and HF by regulating various pathways.

4. Discussions

The outbreak of COVID-19 remains largely uncontained with infection numbers continuing to grow around the world. The clinical
The application of TCM has greatly increased in response to the COVID-19 pandemic and has been proven remarkably effective for treating patients. However, the potential therapeutic mechanism of SFI for the prevention and treatment of COVID-19 and HF remains unclear. Therefore, in the current study, a comprehensive network analysis framework that integrated active compound screening, target fishing, C-T-D network construction, protein-protein interaction network construction, biological process analysis and pathway analysis was developed to elucidate the therapeutic mechanisms of SFI against COVID-19 combined with HF.

The main findings were presented as follows. Firstly, 20 active compounds and 164 targets were found to play important roles in prevention and treatment of SFI on COVID-19 combined with HF, which mainly involved three biological processes including metabolic, hemostasis, and cytokine signaling in immune system. Secondly, The C-T-D network and reactome pathway analysis revealed that SFI was probably involved in the pathological processes of heart failure, respiratory failure, lung injury, inflammation, and immune response related to COVID-19 combined with HF by acting on significant targets and pathways. Thirdly, the venn diagram and protein-protein interaction network uncovered 54 overlapped targets of SFI, COVID-19, and HF as hub genes related to COVID-19 combined with HF. Finally, 54 overlapped targets were marked by KEGG pathway enrichment analysis to identify several pathways, such as IL-17 signaling pathway, Th17 cell differentiation, TNF signaling pathway, and NF-kappa B signaling pathway, which explored the pharmacological effect of SFI for the prevention and treatment of COVID-19 combined with HF at the pathway level. The present work will propose a deeper understanding of the therapeutic mechanism of SFI against COVID-19 combined with HF.

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Declaration of competing interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.compbiomed.2022.105845.

References

[1] T. Fiolet, Y. Kherabi, C.-J. MacDonald, J. Ghosn, N. Peiffer-Smadja, Comparing COVID-19 vaccines for their characteristics, efficacy and effectiveness against SARS-CoV-2 and variants of concern: a narrative review, Clin. Microbiol. Infect. (2021).
[2] S.S.A. Karim, Q.A. Karim, Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic, Lancet 398 (2021) 2126–2129.
C. Zhang, Z. Wu, J.-W. Li, H. Zhao, G.-Q. Wang, Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality, Int. J. Antimicrob. Agents 55 (2020), 105954.

M.A. Chowdhury, N. Hossain, M.A. Kashem, M.A. Shahid, A. Alam, Immune response in COVID-19: a review, J. Infect. Publ. Health 13 (2020) 1619–1629.

D. Mari, F. Di Berardino, M. Cugno, Chronic heart failure and the immune system, Clin. Rev. Allergy Immunol. 23 (2002) 325–340.

T. Shibabaw, Inflammatory cytokine: IL-17A signaling pathway in patients present with COVID-19 and current treatment strategy, J. Inflamm. Res. 13 (2020) 673.

S.-L. Chang, Y.-W. Hsiao, Y.-N. Tsai, S.-F. Lin, S.-H. Liu, Y.-J. Lin, L.-W. Lo, F.-P. Chung, T.-F. Chao, Y.-F. Hu, Interleukin-17 enhances cardiac ventricular remodeling via activating MAPK pathway in ischemic heart failure, J. Mol. Cell. Cardiol. 122 (2018) 69–79.

I.I. Ivanov, L. Zhou, D.R. Littman, Transcriptional Regulation of Th17 Cell Differentiation, Seminars in Immunology, Elsevier, 2007, pp. 409–417.

S. Choudhary, K. Sharma, H. Singh, O. Silakari, The interplay between inflammatory pathways and COVID-19: a critical review on pathogenesis and therapeutic options, Microb. Pathog. (2020), 104673.

M. Kandasamy, NF-kB signalling as a pharmacological target in COVID-19: potential roles for IKK inhibitors, N. Schmied. Arch. Pharmacol. 394 (2021) 561–567.

S. Khanmohammadi, N. Rezaei, Role of Toll-like receptors in the pathogenesis of COVID-19, J. Med. Virol. 93 (2021) 2735–2739.