Elucidation of the Mechanisms and Molecular Targets of Qishen Yiqi Formula for the Treatment of Pulmonary Arterial Hypertension using a Bioinformatics/Network Topology-based Strategy

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Abstract: Background and Objective: Qishen Yiqi formula (QSYQ) is used to treat cardiovascular disease in the clinical practice of traditional Chinese medicine. However, few studies have explored whether QSYQ affects pulmonary arterial hypertension (PAH), and the mechanisms of action and molecular targets of QSYQ for the treatment of PAH are unclear. A bioinformatics/network topology-based strategy was used to identify the bioactive ingredients, putative targets, and molecular mechanisms of QSYQ in PAH.

Methods: A network pharmacology-based strategy was employed by integrating active component gathering, target prediction, PAH gene collection, network topology, and gene enrichment analysis to systematically explore the multicomponent synergistic mechanisms.

Results: In total, 107 bioactive ingredients of QSYQ and 228 ingredient targets were identified. Moreover, 234 PAH-related differentially expressed genes with a |fold change| >2 and an adjusted P value < 0.005 were identified between the PAH patient and control groups, and 266 therapeutic targets were identified. The pathway enrichment analysis indicated that 85 pathways, including the PI3K-Akt, MAPK, and HIF-1 signaling pathways, were significantly enriched. TP53 was the core target gene, and 7 other top genes (MAPK1, RELA, NFKB1, CDKN1A, AKT1, MYC, and MDM2) were the key genes in the gene-pathway network based on the effects of QSYQ on PAH.

Conclusion: An integrative investigation based on network pharmacology may elucidate the multicomponent synergistic mechanisms of QSYQ in PAH and lay a foundation for further animal experiments, human clinical trials and rational clinical applications of QSYQ.

Keywords: Qishen Yiqi formula, pulmonary arterial hypertension, network pharmacology, bioinformatics, mechanism, gene ontology.

1. INTRODUCTION

Pulmonary arterial hypertension (PAH) is a progressive and fatal disease characterized by pulmonary artery stenosis and an elevated pulmonary arterial pressure that eventually leads to right ventricle pressure overload and results in right-sided heart failure and death [1]. Pulmonary vascular remodeling, which includes the abnormal proliferation and apoptosis of pulmonary arterial smooth muscle cells (PASMCs), myogenesis of distal pulmonary arteries, deposition of extracellular matrix (ECM) proteins, and inflammation of the blood vessels, is the key pathological feature of PAH [2]. In a cohort of Korean PAH patients, the 1-, 3-, and 5-year estimated survival rates were 85%, 54%, and 46%, respectively [3]. Moreover, without appropriate treatment, the median survival time of PAH patients after diagnosis is 2.8 years [4]. Dyspnea, syncope, fatigue, and chest pain are the main symptoms of PAH, and if untreated, PAH could lead to right-sided heart failure. The standard therapy for patients with a PAH diagnosis is to decrease the mean pulmonary artery pressure (PAP) and the cardiac index using pharmaceutical medicines, prolonging survival [5]. Although these treatments can relieve disease symptoms, they are not sufficient to reverse the progression of pulmonary hypertension and improve the survival rate, so there is an urgent need for new treatments to improve the poor prognosis of PAH.
In recent years, great progress has been made in finding treatments for cancers. Ali and colleagues found that increasingly more anti-tumor drugs, including nano anti-cancer drugs [6], curcumin 1-based ligands and their ruthenium(III) complexes [7], thalidomide based dithiocarbamate Cu(II), Ni(II) and Ru(III) complexes [8], imidazoles [9], pyrazoline-based ligand and copper(II), nickel(II) and iron(III) complexes [10], and heterocyclic compounds [11], kill tumor cells through various pathways, and are considered potential anticancer drug candidates. As with anti-tumor drugs, there has been increasing evidence that traditional Chinese medicine (TCM) is a promising strategy for PAH treatment. Several studies of PAH have revealed that Chinese medicine monomers such as sodium tanshinone IIA sulfonate, tetramethylpyrazine, and baicalin possess multiple therapeutic effects in treating PAH [12-14]. However, the mechanisms of action and molecular targets of Qishen Yiqi formula (QSYQ) for the treatment of PAH are not clear.

QSYQ, a Chinese medicine compound, is a mixture of 4 Chinese medicine extracts: Astragalus membranaceus (AM, Huang qi, Fabaceae family), Salvia miltiorrhiza (SM, Danshen, mint family, Lamiaceae), Panax notoginseng (PN, Sanqi, Araliaceae), and Dalbergia odorifera (DO, Jiangxiang, Dalbergia family, Leguminosae). In 2003, QSYQ was approved by the China Food and Drug Administration (CFDA) for the treatment of cardiac dysfunction [15, 16]. Recent studies have shown that QSYQ acts beneficially in the systemic circulation, including the cardiovascular system and arterial vessels. Chen et al. reported that QSYQ acts beneficially in pressure overload-induced cardiac hypertrophy by enhancing energy metabolism and counteracting oxidative stress [17]. As previously reported, QSYQ significantly reduces myocardial ischemia reperfusion injury effects by promoting the expression of superoxide dismutase (SOD) and catalase (CAT) and inhibiting the expression of NADPH oxidase (NOX) [18]. Furthermore, an imbalance of thromboxane A2 (TXA2) and prostaglandin I2 (PGI2) plays a significant role in the pathogenesis of atherosclerosis, and QSYQ regulates the dynamics of TXA2 and PGI2 equilibria [19, 20]. However, whether QSYQ has similar beneficial effects on the pulmonary circulation remains unknown. Thus, the anti-PAH effect of individual components of QSYQ needs further investigation.

Because herbal formulations contain multiple ingredients exhibiting a wide range of pharmacological effects, it is a tremendous challenge to obtain an in-depth understanding of the networks of active components-targets and protein-protein interactions (PPIs) and the corresponding biological functions and pathways of the key molecular targets. Network pharmacology has been proposed to address these problems [21]. A network pharmacology approach can be employed to identify networks of active components-targets, PPIs, and target-disease interactions. This approach not only elucidates the complicated interactions between compounds and potential mechanisms of multiple components and multiple target drugs at a systematic level but also conforms to the systematic and holistic perspective of the TCM theory [22, 23].

In this work, network pharmacology and bioinformatics methods were used to predict the mechanisms of action and molecular targets of QSYQ in the treatment of PAH. The Traditional Chinese Medicine Systems Pharmacology (TCMSP) and DrugBank databases were used to screen and predict the bioactive components and potential targets of QSYQ. Then, the gene expression profiles obtained from PAH patients and healthy individuals were searched and downloaded from the Gene Expression Omnibus (GEO) database. This study aimed to investigate the effects of QSYQ on PAH and explore the mechanism underlying this effect through Gene Ontology (GO) and pathway analyses.

2. MATERIALS AND METHODS

2.1. Active Ingredient Screening

The chemical composition of QSYQ was extracted from the TCMSP database and analysis platform [24] (http://tcmspw.com/tcmsp.php), and the threshold values for the selection of biologically active ingredients were set as follows: oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18 [25]. One hundred and thirty bioactive ingredients were obtained: 20 in AM, 65 in SM, 8 in PN, and 37 in DO. Ultimately, 125 active ingredients were obtained after removing duplicates.

2.2. Identification of Potential Targets

The 125 bioactive ingredients were imported into the DrugBank database [26] (https://www.drugbank.ca/) to validate the corresponding targets of QSYQ. A total of 107 bioactive ingredients were ultimately selected after removing 18 ingredients that were not associated with any related target. The targets of 107 bioactive ingredients were retrieved. A total of 1,973 targets were identified: 825 in SM, 404 in AM, 521 in DO, and 223 in PN. A total of 228 targets were obtained after removing duplicates.

2.3. PAH-related Targets

The gene expression profiles of PAH patients were downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/) under accession number GSE15197. The Linear Models for Microarray Data (limma) package in Bioconductor (R software) was used to investigate the differentially expressed genes (DEGs) [27]. The cut-off criteria were set as follows: |fold change| > 2 and adjusted P value < 0.005. Genes that met these criteria were considered significantly differentially expressed and thus PAH-related targets.

2.4. Construction of the PPI Network

The network of potential targets of QSYQ against PAH was constructed and visualized using Cytoscape software (version 3.7.1, http://www.cytoscape.org/). A PPI network was constructed using the Bisogenet plugin of Cytoscape software version 3.7.1 [28]. This plugin imports data from the Database of Interacting Proteins (DIP), Biological General Repository for Interaction Datasets (BIOGRID), Human Protein Reference Database (HPRD), IntAct Molecular Interaction Database (INTACT), Molecular
INTeraction database (MINT), and biomolecular interaction network database (BIND) [29-34].

2.5. Identification of Candidate QSYQ Targets Responsible for the Treatment Effects on PAH

The PPI networks of QSYQ putative pharmacological targets and PAH-related targets were merged with Cytoscape software. In the graphical networks, nodes represent the components or targets, and edges represent the component-target interactions. Furthermore, six topological parameters, namely degree centrality (DC), betweenness centrality (BC), closeness centrality (CC), eigenvector centrality (EC), network centrality (NC), and local average connectivity (LAC), were calculated to evaluate the component-target network based on data obtained using the Cytoscape plugin CytoNCA [35, 36]. The values of the topological parameters are directly associated with the importance of the node in the network. Following the topological screening, all targets in the core PPI network were considered candidate target genes.

2.6. GO and Pathway Enrichment Analyses

GO analysis was utilized to determine the biological functions of the candidate targets and comprises three independent ontologies: biological processes (BPs), cellular components (CCs), and molecular functions (MFs) [37]. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was used to determine the enriched pathways of the candidate targets [38]. The clusterProfiler package in R software was used to analyze and visualize the GO and KEGG pathway enrichment analyses of the DEGs [39]. A P-adjusted value < 0.05 was used as the cutoff criterion. The set of genes that significantly correlated with the pathways was further analyzed. The gene-pathway network was constructed to identify key targets involved in the effects of QSYQ on PAH.

3. RESULTS

3.1. Ingredient-Target-Pathway Network Analysis

One hundred and seven bioactive ingredients of QSYQ (Table 1) were selected as candidate compounds. The limma package in R software revealed 234 PAH-related DEGs from the GEO database under accession number GSE15197: 57 of these DEGs were significantly upregulated, and the remaining 177 were significantly downregulated. As shown in Fig. (1), a volcano plot was constructed to display the distribution of the DEGs: significant DEGs are indicated with green/red dots, and nonsignificant genes are indicated with black dots.

Table 1. Final QSYQ compounds subjected to analysis.

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| MOL00006 | Luteolin                   | 36.16| 0.25| SM     | MOL0071  | Przewaquinone E             | 42.85| 0.45| SM 
| MOL00569 | Digallate                  | 61.85| 0.26| SM     | MOL0071  | Tanshinone iia              | 49.89| 0.40| SM  
| MOL001601| 1,2,5,6-tetrahydrotanshinone| 38.75| 0.36| SM     | MOL0071  | (6S)-6-(hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-dione | 65.26| 0.45| SM 
| MOL001659| Poriferasterol             | 43.83| 0.76| SM     | MOL0000  | Quercetin                   | 46.43| 0.28| PN  
| MOL001771| Poriferast-5-en-3beta-ol   | 36.91| 0.75| SM     | MOL0000  | Quercetin                   | 46.43| 0.28| PN  
| MOL001942| Isoimperatorin             | 45.46| 0.23| SM     | MOL0003  | Beta-sitosterol             | 36.91| 0.75| PN  
| MOL002222| Sugiol                     | 36.11| 0.28| SM     | MOL0004  | Stigmasterol                | 43.83| 0.76| PN  
| MOL002651| Dehydrotanshinone II A     | 43.76| 0.40| SM     | MOL0014  | Mandenol                    | 42.00| 0.19| PN  
| MOL007036| 5,6-dihydroxy-7-isopropyl-1,1-dimethyl-2,3-dihydrophenanthren-4-one | 33.77| 0.29| SM   | MOL0017  | DFV                        | 32.76| 0.18| PN  
| MOL007041| 2-isopropyl-8-methylphenanthrene-3,4-dione | 40.86| 0.23| SM   | MOL0028  | Diop                       | 43.59| 0.39| PN  
| MOL007045| 3a-hydroxytanshinoneIIa    | 44.93| 0.44| SM     | MOL0053  | Ginsenoside rh2             | 36.32| 0.56| PN  
| MOL007048| (E)-3-[2-(3,4-dihydroxyphenyl)-7-hydroxy-benzofuran-4-y1]acrylic acid | 48.24| 0.31| SM     | MOL0002  | Mairin                     | 64.26| 0.42| DO  

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<td>0.45</td>
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OB, oral bioavailability; DL, drug-likeness; AM, Astragalus membranaceus; SM, Salvia miltiorrhiza; PN, Panax notoginseng; DO, Dalbergia odorifera.
The ingredient-target-pathway interaction network diagram of QSYQ was constructed by Cytoscape software, as shown in Fig. (2). The network contained 335 nodes (107 bioactive ingredients in QSYQ and 228 ingredients-targets) and 1973 edges, which indicated ingredient-target-pathway interactions. The network showed that 107 candidate bioactive ingredients had a median degree of 18.4 (range, 1–281), suggesting that most candidate bioactive ingredients of QSYQ act on multiple targets. Quercetin, formononetin, and kaempferol acted on 281, 66, and 56 targets, respectively. The OBs of quercetin, formononetin, and kaempferol were 46.43, 36.91, and 31.10%, respectively. The DLs of quercetin, formononetin, and kaempferol were 0.28, 0.75, and 0.67, respectively. Therefore, quercetin, formononetin, and kaempferol may be the most crucial bioactive ingredients of QSYQ because of their important positions in the network.
3.2. PPI Network Analysis

PPIs are of importance in large-scale biological processes because they play an important role in understanding cell-to-cell interactions, metabolic control, and developmental control [40]. For a deeper understanding of the relationship between putative QSYQ targets and PAH-related targets, PPI networks were visualized using the Bisogenet plugin of Cytoscape software. The PPI network of putative QSYQ targets contained 7,883 nodes and 176,147 edges, representing 7,883 interacting proteins and 176,147 interactions, respectively. The PPI network of PAH-related targets contained 4,397 nodes and 110,722 edges, representing 4,397 interacting proteins and 110,722 interactions, respectively.

3.3. Identification of Candidate QSYQ Targets Responsible for the Treatment Effects on PAH

There is a paucity of research focused on understanding the mechanisms of action underlying the effects of QSYQ on PAH. The PPI networks of putative QSYQ targets and PAH-related targets were merged to identify candidate therapeutic targets. As shown in Fig. (3A), this network consists of 3,217 nodes and 92,240 edges. Based on previous reports, a node is a significant target if its degree is more than two-fold greater than the median degree of all nodes in a network [41]. In our study, the median degree of all nodes was 36, and nodes with more than 72 degrees were recognized as significant targets. Thus, a network of significant therapeutic targets based on the effects of QSYQ on PAH was constructed, and it contained 781 nodes and 35,457 edges (Fig. 3B). The median DC, BC, CC, EC, LAC, and NC values were 75, 332.95, 0.52, 0.03, 19.44, and 21.41, respectively. Therefore, the candidate therapeutic targets with DC >75, BC>332.95, CC >0.52, EC>0.03, LAC>19.44, and NC>21.41 were further screened. As a result, 266 therapeutic targets were identified based on the effects of QSYQ on PAH (Figure 3C, Supplementary Table S1).

3.4. GO and Signaling Pathway Enrichment Analyses

To achieve a more in-depth understanding of the selected 266 therapeutic target genes, GO functional enrichment analysis and KEGG pathway enrichment analysis were performed using the clusterProfiler package. The GO term analysis included the BP, CC and MF groups. A total of 1,404 GO terms were significantly enriched: 1,087 in BPs, 158 in CCs, and 159 in MFs. The GO analysis data are shown in Supplementary Table S2. As indicated in Fig. (4A) and Supplementary Table S2, for the BPs, the target genes were mainly enriched in the mRNA catabolic process, RNA catabolic process, establishment of protein localization to the membrane, regulation of the apoptotic signaling pathway, regulation of smooth muscle cell proliferation, regulation of gene silencing, and cellular response to oxidative stress. For the CCs (Fig. 4B), the genes were mainly enriched in nuclear chromatin, focal adhesion, cell-substrate adherens junctions, and cell-substrate junctions. The MFs of the target genes were mainly enriched in ubiquitin protein ligase binding, ubiquitin-like protein ligase binding, cadherin binding, and cadherin binding, and cell adhesion molecule binding (Fig. 4C). A range of pathways that were significantly affected by QSYQ in the process of treating PAH were identified by KEGG pathway enrichment analysis. The top 30 significantly enriched KEGG pathways are shown in Fig. (5) and include viral carcinogenesis, the ribosome, the phosphatidylinositol 3-hydroxy kinase/protein kinase B (PI3K)-Akt signaling pathway, the mitogen-activated protein kinase (MAPK) signaling pathway, the hypoxia inducible factor-1 (HIF-1) signaling pathway, the cell cycle, proteoglycans in cancer, and cellular senescence. The KEGG pathway analysis data are shown in Supplementary Table S3.

3.5. Gene-pathway Network Analysis

Based on the top 30 significantly enriched KEGG pathway terms and 184 target genes enriched in transcription pathways, we constructed a gene-pathway network, which is shown in Fig. (6). The network contains target genes (dark yellow) and KEGG pathways (red). More importantly, we demonstrated that TP53 had the highest degree of centrality and was revealed as the core target gene. Additionally, MAPK1, RELA, NFkB1, CDKN1A, AKT1, MYC, and MDM2 also had high degree centrality, indicating that they are also key target genes based on the effects of QSYQ on PAH.

4. DISCUSSION

QSYQ is a TCM used to treat cardiac dysfunction and is comprised of four plant herbs: AM, SM, PN, and DO. The relationship between PAH and the deterioration of cardiac function is well known; however, the relationship between...
Fig. (4). GO terms of candidate targets based on the effects of QSYQ on PAH. Top 20 significantly enriched GO terms of candidate targets based on the effects of QSYQ on PAH: BPs (A), CCs (B) and MFs (C). (A higher resolution/colour version of this figure is available in the electronic copy of the article).
Fig. (5). KEGG pathway enrichment analysis of candidate targets based on the effects of QSYQ on PAH. The top 30 KEGG pathways with an adjusted P value <0.05 were identified. The size of the spot represents the number of genes, and the color represents the adjusted P value. (A higher resolution/colour version of this figure is available in the electronic copy of the article).

Fig. (6). Gene-pathway network based on the effects of QSYQ on PAH. The topological analysis of 30 pathways and 184 target genes was carried out with degree centrality. The dark yellow rectangles represent target genes, and the red V-shapes represent pathways. The larger the rectangle/V-shape, the larger the degree centrality. (A higher resolution/colour version of this figure is available in the electronic copy of the article).

QSYQ as a whole and PAH has not been previously reported and validated. To our knowledge, the therapeutic mechanisms of QSYQ are still obscure. Currently, network pharmacology is a powerful new approach for investigating complex TCM herbal components and exploring the underlying mechanisms of TCMs. In the present work, active component gathering, target prediction, PAH gene collection, network topology, and gene enrichment analysis were performed to provide important clues on QSYQ.

In the present study, an ingredient-target-pathway network of QSYQ was constructed by using 107 bioactive ingredients and 228 ingredient targets. The results of the ingredient-target network suggested that the bioactive
ingredients of QSYQ affect multiple targets; for instance, quercetin, formononetin, and kaempferol act on 281, 66, and 56 targets, respectively. Therefore, quercetin, formononetin, and kaempferol are most likely to be crucial pleiotropically bioactive ingredients of QSYQ. Although the number of putative targets in the compound of every single herb was different, there were a large number of overlapping targets in different herbs; specifically, multiple bioactive ingredients of QSYQ may have the same target, providing synergistic effects. Quercetin is a typical flavonoid compound with multiple pharmacological activities, such as anticancer, anti-allergy, anti-inflammatory, anti-atherosclerotic, and anti-platelet aggregation activities [42-44]. Previous investigations have suggested that quercetin is an active bio-flavonoid that plays critical roles in treating PAH [45-47]. Formononetin is an important natural phytoestrogen that has protective effects on cardiovascular disease due to its pro-apoptotic, anti-inflammatory and antitumor activities [48-50]. Kaempferol is also an active flavonoid and has various biological functions, including antioxidant, anti-inflammatory, and anticancer activities. However, literature on the effects of formononetin and kaempferol on PAH is scarce. Our findings demonstrate a relationship between formononetin or kaempferol and PAH, but further clinical trials and animal experiments are needed to elucidate this mechanism. Moreover, our present study illustrates that quercetin, formononetin, and kaempferol regulate most of the targets associated with PAH, and they all possess anti-inflammatory properties. Although quercetin, formononetin, and kaempferol are ubiquitous and widely known compounds, there is some evidence that these compounds also possess anti-inflammatory effects [51-53]. These compounds also have high OB and are derived from three herbs of QSYQ. Therefore, quercetin, formononetin, and kaempferol might be considered compounds in QSYQ that can be used to treat PAH.

The PPI networks of putative QSYQ pharmacological targets and PAH-related targets were constructed and merged to obtain the core targets. Six parameters, DC, BC, CC, EC, NC, and LAC, were used to screen nodes and construct a new network to obtain more accurate targets. Ultimately, 266 therapeutic targets were recognized, and we used bioinformatics approaches to elucidate the mechanisms involved in the anti-PAH effects of QSYQ. The targets were subjected to GO enrichment analysis, and the related BPs, CCs, and MFs were obtained. As shown in Fig. (4A) and Supplementary Table S2, our findings suggest that QSYQ regulates certain biological processes, such as regulation of the apoptotic signaling pathway, regulation of smooth muscle cell proliferation, regulation of gene silencing, and the cellular response to oxidative stress. It is well reported that excessive proliferation, apoptosis resistance, and oxidative stress play crucial roles in the pathophysiology of PAH [54]. For example, aldehyde dehydrogenase 2 attenuates the development of PAH by regulating the level of 4-hydroxynonenal and inhibits the proliferation of pulmonary smooth muscle cells [55]. It has been found that the apoptotic process plays a crucial role in the maintenance of pulmonary vascular remodeling, and an imbalance in apoptosis contributes to the development and persistence of PAH [56]. Additionally, markers of oxidative stress, such as malondialdehyde, total antioxidant capacity, and catalase activity, have been related to adverse clinical outcomes in patients with PAH [57]. Therefore, our findings further strengthen the notion that QSYQ may help regulate proliferation, apoptosis, and oxidative stress by intervening in these biological processes. It has also been reported that the pathogenesis of PAH is associated with nuclear abnormalities, ubiquitin protein ligase binding, histone deacetylase binding, and cell adhesion molecule binding [58-61], all of which were significantly enriched in the present study. Therefore, QSYQ may exert an important regulatory function in PAH pathogenesis and affect certain cellular components and molecular functions, including nuclear chromatin, the cytosol, the ribosome, DNA binding, mRNA binding, and unfolded protein binding. In accordance with our findings, studies have also demonstrated ultrastructural abnormalities in mitochondrial vacuolization and modifications in the chromatin structure, which play an important role in PAH development [62, 63].

TCM compounds may embody the characteristics of multiple components, targets and channels. QSYQ, as a TCM, also has the same characteristics. Overall, it is known that QSYQ affects PAH through multiple pathways. In the present study, a total of 85 KEGG pathways, including the PI3K-Akt signaling pathway, MAPK signaling pathway, and HIF-1 signaling pathway, were significantly enriched. The MAPK signaling pathway can regulate smooth muscle cell proliferation, apoptosis, autophagy, and the response to oxidative stress, which is one of the mechanisms involved in pulmonary arteriolar remodeling [64-66]. It has also been reported that the PI3K-Akt signaling pathway is an important signaling pathway for PAH and pulmonary vascular remodeling by affecting cell proliferation, apoptosis, migration, protein synthesis, cell cycle activities in the pulmonary vasculature and crosstalk between HIF-1 [67, 68]. Platelet-derived growth factor promotes the Warburg effect by activating the PI3K-Akt and HIF-1 signaling pathways in proliferative PASMcs [69], leading to PAH. HIF-1 is the key cellular survival protein under hypoxia and is associated with the proliferation, oxidative stress, and vascular remodeling of PASMcs [44, 70]. These findings demonstrate a relationship between proliferation, apoptosis, oxidative stress and pulmonary arteriolar remodeling, which plays an important role in the development of PAH and may be a therapeutic target. Taken together, our findings further strengthen the notion that QSYQ regulates proliferation, apoptosis, and oxidative stress through related pathways in the process of PAH treatment. In our study, several pathways related to viruses were also significantly enriched. It is important to recognize that viral infection plays a role in the pathogenesis of PAH. For instance, human immunodeficiency virus, human herpesvirus-8, hepatitis B and hepatitis C may contribute to angioproliferation by reducing inflammation and impairing the immune regulatory system, leading to the development of PAH [71, 72]. The inflammatory mediator response triggered by viral infection may be regulated by QSYQ through specific viral pathways, such as viral carcinogenesis, hepatitis B infection, human papillomavirus infection, hepatitis C infection, human T-cell leukemia virus 1 infection, and Epstein-Barr virus infection. Additionally, QSYQ may function by modulating other pathways, including the cell cycle, proteoglycans in cancer, cellular senescence, and DNA replication. Overall, previous
investigations have suggested that the regulation of cell cycle regulatory molecules by silence information regulator 1 is beneficial for PAH [73]. Cellular senescence has also been increasingly recognized as a crucial contributor to the pathobiology of PAH [74]. In summary, the mechanism of QSYQ in treating PAH is complex and diverse, and there is a direct or indirect relationship with multiple signaling pathways.

The gene-pathway network was constructed to screen the key target genes based on the effects of QSYQ on PAH. The results demonstrated that TP53 had the highest maximum degree centrality and may be the core target gene. The 7 other top genes, namely, MAPK1, RELA, NFKB1, CDKN1A, AKT1, MYC, and MDM2, were selected as the key target genes. TP53, a tumor inhibitor, was reported to play a critical role in regulating cell proliferation, the cell cycle, or apoptosis and contributes to the development of PAH [75, 76]. Mizuno et al. demonstrated that TP53-knockout mice formed more serious pulmonary hypertension in response to chronic hypoxia than wild-type mice [76]. Therefore, TP53 may be a promising target for reversing the pulmonary vascular remodeling process that characterizes PAH. The protein expression and transcriptional activity of TP53 are tightly regulated at multiple levels, including transcription, translation, and postranslation. In particular, posttranscriptional regulation (i.e., ubiquitination and acetylation) is necessary for the function of the TP53 protein. MDM2, a specific TP53 ubiquitin ligase and transcriptional inhibitor, seems to participate in both pathways [75]. MDM2 is a pivotal regulator of the fate and activity of TP53, and the expression level of TP53 is maintained by a negative feedback loop through the regulation of MDM2 [77, 78]. Several recent studies have shown that an MDM2 inhibitor, Nutlin-3a, exerts antitumor activity through TP53 activation in various cancer cells and induces tumor regression [79]. In addition, Mouraret et al. used different models of experimental PAH and observed that Nutlin-3a protected mice from PAH development mainly through a direct alteration in TP53 expression and regulation [75]. These results indicate that TP53/MDM2 could be important for regulating cell proliferation and the cell cycle in the development of PAH. Furthermore, the CDKN1A protein is a transcriptional target of TP53 and can block cell cycle progression from G0/G1 to S phase, which is associated with the upregulation of TP53 and CDKN1A [80]. Huang et al. reported that salidroside inhibits PASC proliferation through the TP53/P21 pathway [81]. MAPK1 (also known as ERK-2) is a member of the MAPK subfamily of Ser/Thr protein kinases and is involved in a wide variety of cellular processes, such as proliferation, differentiation, transcriptional regulation and development [82, 83]. Wei and colleagues found that compared with controls, PAH patients showed higher levels of MAPK1, which is highly related to pulmonary vascular remodeling in PAH [84]. RELA, as an important subunit of NF-kB, plays a critical role in inflammation, cell survival, and cancer development [85, 86]. Feng and colleagues [36] demonstrated that RELA phosphorylation is related to the inflammatory response of pulmonary arterial endothelial cells in PAH [87]. MYC has also been suggested to coordinate proliferation and cell cycle regulation in the development and progression of pulmonary vascular remodeling [88]. A large body of evidence indicates that NFKB1 and AKT1 play distinct roles in regulating cell proliferation, apoptosis, inflammation and protein synthesis in the pathogenesis of PAH [89, 90]. Accordingly, TP53, MAPK1, RELA, NFKB1, CDKN1A, AKT1, MYC, and MDM2 are likely to become potential drug targets for the treatment of PAH.

However, our findings should be interpreted with caution due to some potential limitations. First, the bioactive components and targets screened may be inconsistent with the original ingredients actually absorbed in the blood of patients with PAH. Further studies using liquid chromatography-mass spectrometry, two-dimensional liquid chromatography or quadrupole-time-of-flight mass spectrometry are needed to confirm the bioactive components and targets. Second, animal experiments and human clinical trials are needed to verify our network pharmacology results, and the generalizability of our findings may be limited. Therefore, we plan to validate these findings and further explore the relationship between TCM pattern types and gene polymorphisms using animal experiments and human clinical trials.

**CONCLUSION**

In this study, we proposed a bioinformatics/topology-based strategy for the identification of the molecular mechanisms of QSYQ against PAH. In this work, this hybrid strategy was employed to determine that the pharmacological mechanisms of QSYQ in the treatment of PAH may be associated with its involvement in the PI3K-Akt, MAPK and HIF-1 signaling pathways, providing new evidence for drug development for the prevention and treatment of PAH. Among these crucial biological functions, eight hub genes were identified as key potential drug targets, and quercetin, foronmononetin, and kaempferol have become novel therapeutic agents.

**LIST OF ABBREVIATIONS**

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>QSYQ</td>
<td>Qishen Yiqi formula</td>
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<tr>
<td>PAH</td>
<td>Pulmonary Arterial Hypertension</td>
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<td>PASMCs</td>
<td>Pulmonary Arterial Smooth Muscle Cells</td>
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<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
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<td>TCM</td>
<td>Traditional Chinese Medicine</td>
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<td>AM</td>
<td>Astragalus membranaceus</td>
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<td>SM</td>
<td>Salvia Miltiorrhiza</td>
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<td>PN</td>
<td>Panax Notoginseng</td>
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<td>DO</td>
<td>Dalbergia Odorifera</td>
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<td>SOD</td>
<td>Superoxide Dismutase</td>
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<td>TCMS</td>
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<td>GEO</td>
<td>Gene Expression Omnibus</td>
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OB = Oral Bioavailability
DL = Drug-likeness
DEGs = Differentially Expressed Genes
BIOGRID = Interaction Datasets
HPRD = Human Protein Reference Database
INTACT = IntAct Molecular Interaction Database
MINT = Molecular INTeraction database
BIND = Biomolecular Interaction Network Database
DC = Degree Centrality
BC = Betweenness Centrality
CC = Closeness Centrality
EC = Eigenvector Centrality
NC = Network Centrality
LAC = Local Average Connectivity
BPs = Biological Processes
CCs = Cellular Components
MFs = Molecular Functions
KEGG = Kyoto Encyclopedia of Genes and Genomes
PI3K-Akt = Phosphatidylinositol 3-hydroxy kinase/protein kinase B
MAPK = Mitogen-activated Protein Kinase
HIF-1 = Hypoxia Inducible Factor-1

AUTHORS’ CONTRIBUTIONS
PLW, XNX, MYC, and JWS designed the study, participated in the statistical analyses, and drafted the manuscript. LQC, JOW, and LY collected the data and revised the manuscript. XYH and LXW participated in the study design and coordinated the research groups. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Not applicable.

HUMAN AND ANIMAL RIGHTS
No animals/humans were used in studies that are the basis of this research.

CONSENT FOR PUBLICATION
Not applicable.

AVAILABILITY OF DATA AND MATERIALS
The data and materials generated or analyzed during this study are included in this manuscript and its supplementary information files.

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CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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Declared none.

SUPPLEMENTARY MATERIAL
Supplementary material is available on the publisher’s website along with the published article.

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