Chapter 9
Drug-Disease-Based Network Pharmacology Practice Process

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Abstract The chemical composition of traditional Chinese medicine (TCM) is inordinately complex; therefore, it is a daunting task to reflect on its integrity and methodology by means of adopting the reductionist philosophy of western medicine. It cannot quintessentially manifest the scientific nature of TCM. The augmentation of modern scientific research and the introduction of network pharmacology have gradually transformed the research idea of single component and single target to the overall regulation of a complex system. Network pharmacology studies problems from the perspective of interrelation, which coincides with the core holistic view of Chinese medicine. Therefore, the application of network pharmacology in the study of TCM has unique advantages, and is conducive to a thorough understanding of the value and significance of the overall view of TCM. Several related studies have also emerged. In this chapter, single medicinal components and compound preparations are taken as examples to demonstrate the commonly used drug-disease-based network pharmacology analysis methods from two research examples.

9.1 Study on Network Pharmacology of Exocarpium Citri Grandis in the Treatment of Respiratory Diseases

Exocarpium Citri Grandis is an authentic medicinal materials in Lingnan. It has a significant effect on relieving cough and reducing phlegm and has a history of thousands of years of clinical application. Studies have validated that Exocarpium Citri Grandis not only has antitussive and expectorant effects, but also has an obvious inhibitory effect on acute and chronic respiratory inflammation, and can also promote the regression of inflammation [1–3]. However, due to the complexity of the ingredients and mechanism of action of TCM, it is difficult to carry out in-depth research on the pharmacodynamic mechanism of Exocarpium Citri Grandis, and such research conducted so far has lacked pertinence. Network

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pharmacology provides new research ideas and technical channels to resolve this issue. The overall research idea is first, clarify the chemical component basis of TCM by means of HPLC-MS and construct a library of chemical ingredients. At the same time, use data mining methodology to collect disease-related protein targets and construct a protein target library. Subsequently, calculate the correlation between ingredients and targets by means of molecular docking, and construct the component-target network. Furthermore, employ proteomics, transcriptomics, and disease-related biochemical indicators to verify the prediction results, and establish a regulatory network linking ingredients, targets, pathways, and efficacy of TCM, to explore the complex mechanism of TCM from the holistic perspective.

9.1.1 Prediction of Action Target of Exocarpium Citri Grandis in the Treatment of Respiratory Diseases

9.1.1.1 Analysis of Chemical Ingredients in Exocarpium Citri Grandis Based on UFLC-Triple TOF-MS/MS

We implemented a systematic online separation and identification of chemical components in medicinal components of Exocarpium Citri Grandis by employing UFLC-Triple TOF-MS/MS technology. By comparing with reference substances, accurate molecular weight, and analysis of mass spectrometry cracking behavior, a total of 48 compounds were confirmed and indicated, including 19 flavonoids, 16 coumarins, eight limonoids, and five organic acid compounds (as shown in Fig. 9.1). In addition, the flavonoid components in Exocarpium Citri Grandis mostly exist in the form of glycosides. After oral administration, flavonoid glycosides rapidly remove the glycosylates and turn into corresponding glycosides [4] under the action of intestinal flora β-glucosidase. Therefore, when the molecules are docked, the corresponding aglycones can be used for calculation. The chemical structure of each compound (shown in Table 9.1) was saved in the MOL file format, and used as the ligand for molecular docking calculation.

9.1.1.2 Construction of Target Database for Respiratory Diseases

Protein target information related to respiratory diseases were mined and sorted from multiple public databases such as ChEMBL, DrugBank, ClinicalTrials, BindingDB, Scifinder, SuperTarget, Protein Data Bank, KEGG, and other literature. At present, there are 426 protein targets included in the database, which are divided into the following categories: (1) 153 enzymes, such as 6-phosphate glucose dehydrogenase, histone deacetylase, and arachidonic acid-5-lipoxygenase, phosphodiesterase, etc.; (2) 105 membrane receptors, such as adenosine receptors, adrenergic receptors, chemokine receptors, etc.; (3) 73 unclassified proteins, such as calmodulin protein,

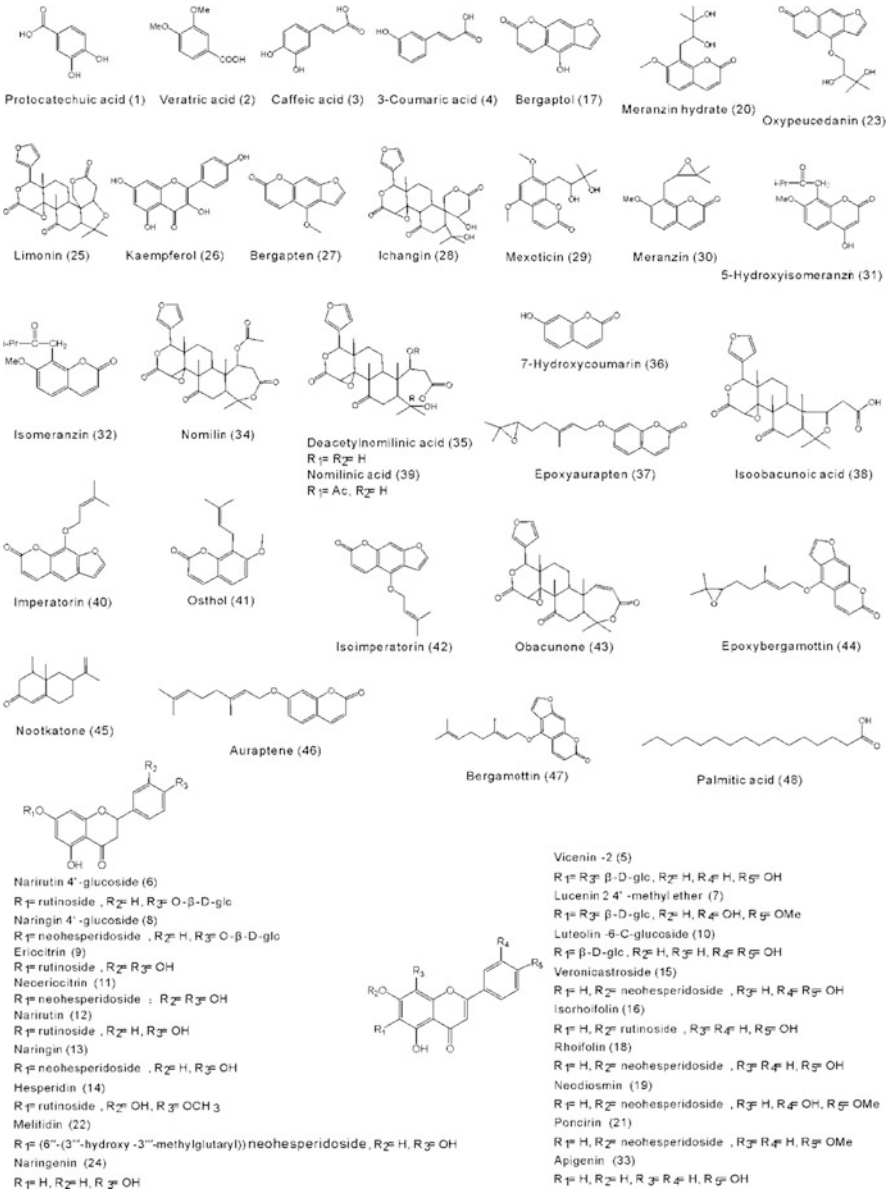

Fig. 9.1 Chemical components of Exocarpium Citri Grandis

integrin protein, heat shock protein, etc.; (4) 37 ion channel proteins, such as voltage-dependent potassium channel, calcium-activated potassium ion channel, sodium channel proteins, etc.; (5) 19 transcription factors, such as peroxisome proliferator activated receptor, glucocorticoid receptor, etc.; (6) 15 secretory

Table 9.1 Compounds used for molecular docking calculation

No.	Compounds	No.	Compounds
F01	Naringenin (Naringin, Narirutin, Melitidin)	C11	Imperatorin
F02	Apigenin (Rhoifolin, Isorhoifolin)	C12	Osthole
F03	Eriodictyol (Eriocitrin, Neoeriocitrin)	C13	Isoimperatorin
F04	Luteolin (Isoorientin, Lonicerin)	C14	Epoxybergamottin
F05	Diosmetin (Neodiosmin)	C15	Auraptene
F06	Hesperetin (Hesperidin)	C16	Bergamottin
F07	Isosakuranetin (Poncirin)	L01	Limonin
F08	Kaempferol	L02	Ichangin
F09	Lucenin-2 4′-methyl ether	L03	Nomilin
F10	Vicenin-2	L04	Deacetylnomilin acid
C01	Bergaptol	L05	Isoobacunoic acid
C02	Meranzin hydrate	L06	Nomilinic acid
C03	Oxypeucedanin	L07	Obacunone
C04	Bergaptene	L08	Nootkatone
C05	Mexoticin	O01	Protocatechuic acid
C06	Meranzin	O02	Veratric acid
C07	Hydroxyisohesperidin	O03	Caffeic acid
C08	Isomeranzin	O04	Coumaric acid
C09	Hydroxycoumarin	O04	Palmitic acid
C10	Epoxyaurapten		

Note: *F* flavone, *C* coumarin, *L* limonin, *O* organic acid. The flavone glycosides represented by aglycones are in brackets

proteins, such as tumor necrosis factor, interleukin, chemokine, etc.; (7) 14 transporter proteins, such as solute transporter, ATP binding cassette, etc.; (8) seven adhesion proteins, such as intercellular adhesion molecules, selectins, etc.; (9) three surface antigen proteins, such as T cell wall glycoprotein, T lymphocyte activation antibody, etc. The three-dimensional structure of each protein was downloaded from the Protein Data Bank as a receptor for the molecular docking calculations.

9.1.1.3 Target Prediction and Network Analysis

AutoDock Vina software was employed in molecular docking calculation. The docking score was evaluated and ranked based on the interaction between the compound molecule and the target protein structure, as in the case of hydrogen bonding, van der Waals force, hydrophobic interaction, etc. The docking score represents the predicted value of $-\log_{10}(K_d)$ between the compound and the target protein. The target with the docking score >6, that is, the protein dissociation constant K_d value $<10^{-6}$, was selected as the potential action target of the target compound. Cytoscape software was employed to visualize the results of molecular docking. Nodes represent chemical components, action targets, and signaling pathways, and the connecting lines represent the connections between components,

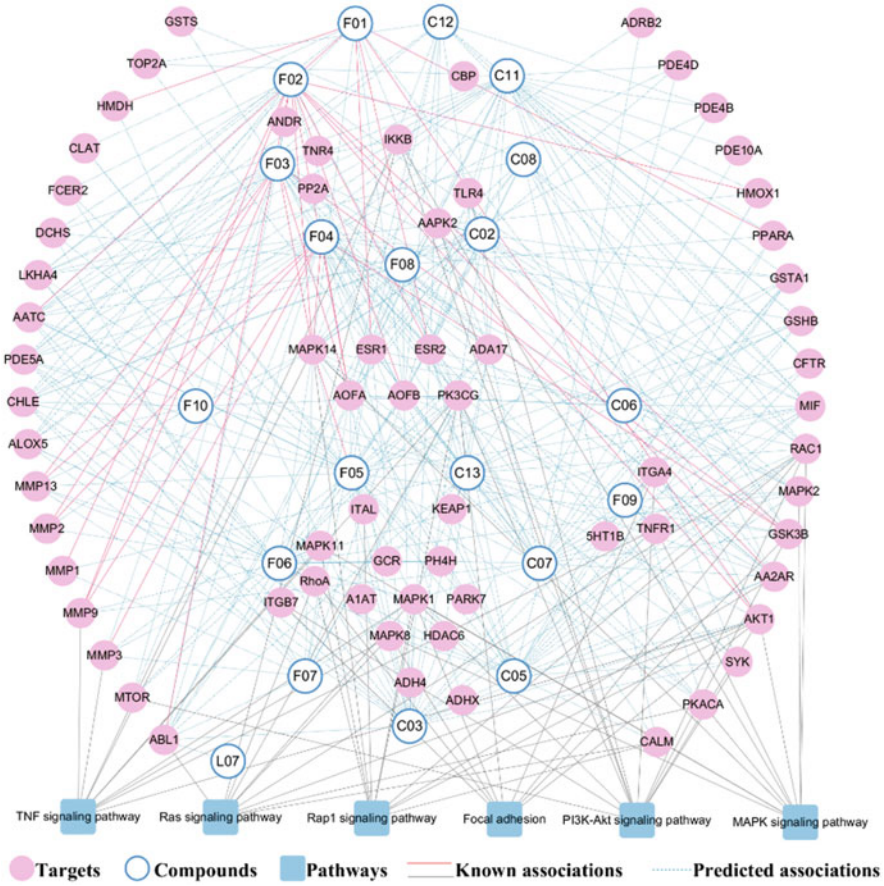

Fig. 9.2 Component-target-pathway network diagram of Exocarpium Citri Grandis

targets, and pathways to construct a network diagram (as shown in Fig. 9.2). The signaling pathway information was obtained using DAVID analysis tools [5, 6], and all the targets in the respiratory disease target library were used as the background; the calculated potential targets of the compounds were subjected to KEGG signaling pathway enrichment analysis.

The results demonstrate that most flavonoids and coumarins have more targets that associate with each other, with all the organic acids, and with most of the limonin and individual coumarin components. A total of 19 compounds were excluded due to fewer related targets (<5). Hence, flavonoids and coumarins are the main active components of Exocarpium Citri Grandis in the treatment of respiratory diseases. The chemical components with the largest number of related targets are diosmetin (F05), imperatorin (C11), luteolin (F04), oxypeucedanin (C03), and eriodictyol (F03). The targets with the largest number of related compounds include MAPK14, AOFB, ESR1, ESR2, AOFA, and PK3CG. The interactions

between a handful of ingredients and targets have been confirmed in literature and are shown in pink lines in Fig. 9.2. The potential targets of Exocarpium Citri Grandis are closely related to many signaling pathways, including key inflammatory signal transduction pathways such as TNF signaling pathway, PI3K-Akt signaling pathway, and MAPK signaling pathway; and cell connection-related pathways such as Ras signaling pathway, Rap1 signaling pathway, and Focal adhesion. The calculation results were combined with the experimental verification data for a comprehensive analysis.

9.1.2 Verification of the Action Targets of Exocarpium Citri Grandis in the Treatment of Respiratory Diseases

Due to the complexity of target prediction results, high-throughput iTRAQ quantitative proteomics technology was employed for experimental verification. Employing a mouse model of acute lung inflammation caused by cigarette smoke, iTRAQ was used to investigate the regulatory effect of Exocarpium Citri Grandis on the overall protein expression level, and to comprehensively analyze the mechanism of action of Exocarpium Citri Grandis in the treatment of respiratory diseases by combining with the target prediction results.

9.1.2.1 Animal Model

The stimulation of cigarette smoke can initiate various pathological changes in the respiratory system, such as increasing parasympathetic excitability, bronchoconstriction and spasms, mucosal hyperemia, edema and increased secretion, inflammation, etc. Therefore, the mouse model of acute pulmonary inflammation caused by cigarette smoke was selected for the experiments. The Balb/c mice were divided into six groups randomly, each with ten mice, namely: normal group, model group, positive drug dexamethasone (DEX) group (5 mg/kg), and Exocarpium Citri Grandis extract (CGE) group (80 mg/kg). Intragastric administration was given 1 h before the first cigarette smoking event every day. The intragastric administration volume was 0.1 mL/10 g body weight. The normal group and the model group were given an identical amount of normal saline.

The animals were first adapted to the new environment and raised for 7 days before initiating the smoking events and modeling. The smoking events were conducted twice a day, with an interval of 4 h. During each smoking event, eight cigarettes were smoked for 1 h, for five consecutive days. The mice were sacrificed by removing the cervical vertebrae 16 h after the last smoking event; the thorax was incised, and the left and right lung tissues were removed, quickly placed on ice, and washed with PBS buffer solution. The right lung was fixed with 4%

paraformaldehyde after cleaning and used to make hematoxylin-eosin-stained tissue sections. The left lung was cut into pieces with small scissors, the residual blood was fully washed, and it was then stored in a sealed bag in at refrigerator at −80 °C, for proteomics analysis.

9.1.2.2 Histopathological Examination

First, the pathological changes in the lung tissue of the mouse caused by the cigarette smoke were observed in the pathological sections. As shown in Fig. 9.3, the results of hematoxylin-eosin-stained lung tissue slices of mice after the acute smoking model demonstrate that compared to the normal group, the alveolar cavity of the model group was reduced, and the alveolar wall and alveolar compartment were thickened. There was proliferation of fibrous tissue and normal alveolar tissue had changed. At the same time, thickening of bronchiole wall, inflammatory cell infiltration, and exudation of inflammatory cells in the lumen were also observed (as shown by the arrow). After administration of positive drug and Exocarpium Citri Grandis, the thickening of alveolar wall and bronchial wall was reduced to various degrees and the degree of pulmonary edema and lesion level alleviated.

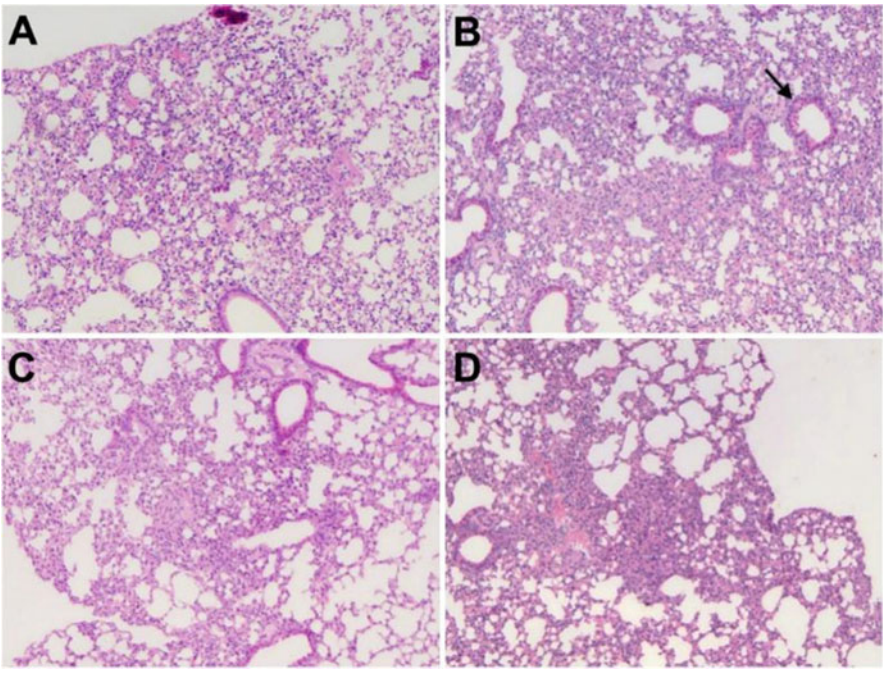

Fig. 9.3 Hematoxylin-eosin staining results of mouse lung tissue. (**a**) Normal group; (**b**) Model group; (**c**) Dexamethasone group; (**d**) Exocarpium Citri Grandis group

9.1.2.3 Proteomic Analysis of iTRAQ

Protein Identification and Differential Protein Expression Analysis

The iTRAQ kit (AB Sciex) was employed for proteomics detection. There are four groups of samples, namely the normal group (CON), the model group (MOD), the dexamethasone group (DEX), and the Exocarpium Citri Grandis extract group (CGE), and each set has one technical replicate. A total of 12,232 peptides and 3528 proteins were identified. IQuant (BGI) software was used for quantitative analysis. The results of each pair of neighboring samples of the four groups of samples were compared. According to the volcanogram of protein expression differential distribution (as shown in Fig. 9.4), proteins with differential expression Q value <0.05 were selected as differential proteins (as shown in Table 9.2).

Bioinformatics Analysis of Differentially Expressed Proteins

Cluster analysis: Cluster analysis was performed on differential proteins, and maps were employed for visualization processing (as shown in Fig. 9.5) to investigate the

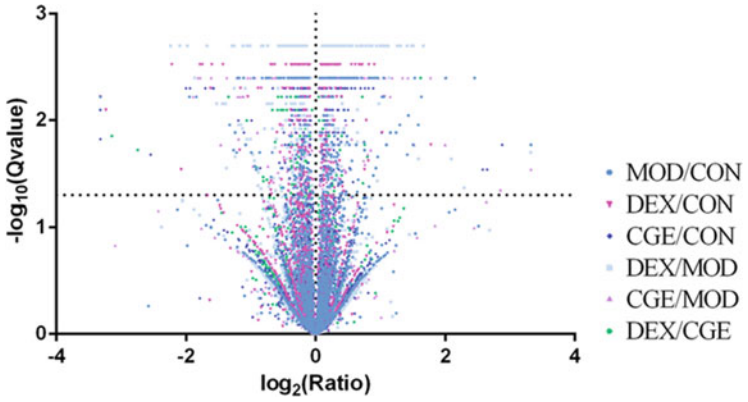

Fig. 9.4 Volcanogram of differential distribution of protein expression between groups

Table 9.2 Statistics of differentially expressed proteins between groups

Comparison group	Up-regulated protein number	Down-regulated protein number	Total number of differential proteins
MOD/CON	57	60	117
DEX/MOD	119	113	232
CGE/MOD	63	58	121
DEX/CON	94	86	180
CGE/CON	46	41	87
DEX/CGE	79	57	136

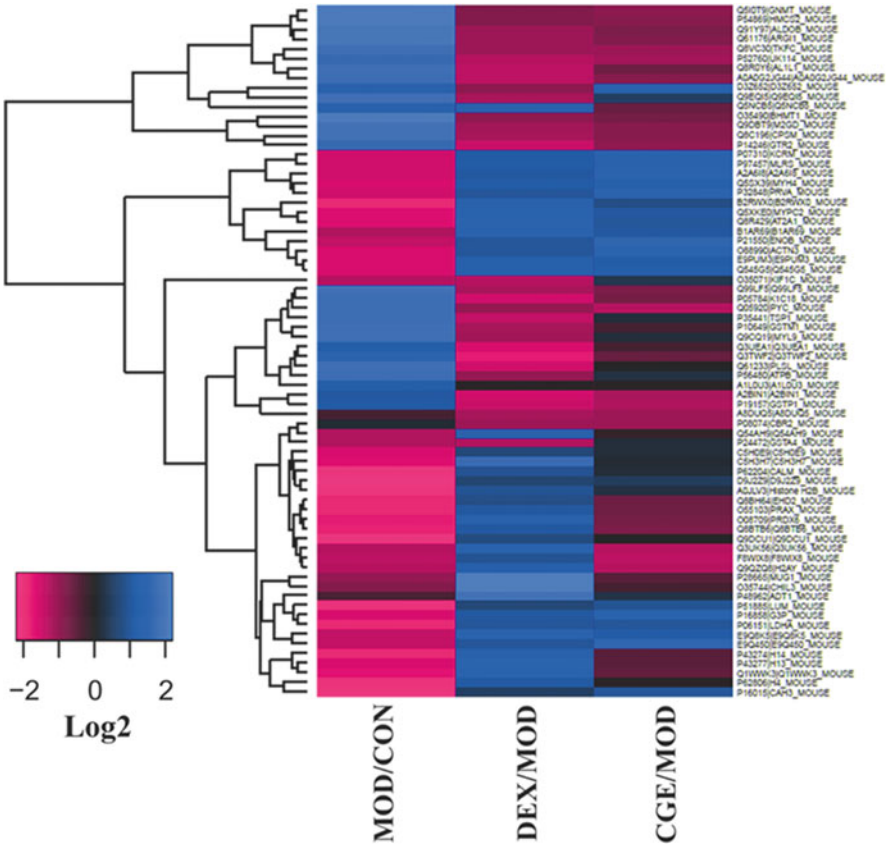

Fig. 9.5 Cluster analysis of differentially expressed proteins

correlation and difference of differential proteins between different groups. The results demonstrate that the positive drug dexamethasone (DEX) has distinct degrees of callback to the differential expression protein (MOD/CON) caused by modeling, which indicates that dexamethasone had a wide, rapid, and powerful therapeutic effect on acute inflammation. Exocarpium Citri Grandis (CGE) has similar regulation effect to dexamethasone on differential proteins related to cell junction and metabolism but has no effect on mitochondrial function and histone-related differential proteins.

Gene Ontology (GO) enrichment analysis: The enrichment results are demonstrated in Table 9.3. The enrichment results of different proteins between the model group and the normal group in terms of biological processes show the presence of stimulation of the mouse lung tissue by the cigarette smoke and activation of the metabolism process of exogenous small molecules. At the same time, the smoke stimulation also affects the process of actin, which may be related to the morphological changes of pulmonary vascular smooth muscle cells. In terms of molecular

Table 9.3 Enrichment analysis results of differentially expressed protein GO

Group	Cellular component	Molecular function	Biological process
MOD/CON	Actin cytoskeleton Myosin complex Filamentous actin	Calcium ion binding	Generation of precursor metabolites and energy Small molecule metabolic process Response to chemical stimulus Muscle system process
DEX/MOD	Extracellular region part Myosin complex Extracellular space	Oxygen binding	Response to lipid Response to extracellular stimulus Response to purine-containing compound Response to nutrient levels Response to glucocorticoid stimulus Response to endogenous stimulus
CGE/MOD	Contractile fiber Actin cytoskeleton Myosin complex	Modified amino acid binding Structural constituent of cytoskeleton	Muscle system process Cellular amino acid metabolic process

functions, differential proteins are mainly enriched in calcium ion binding activities. On the one hand, calcium ions directly act on actin, and are also important second messengers, participating in various signal regulation processes including the release of inflammatory factors. In terms of cellular components, the differentially expressed proteins are mainly enriched in the actin cytoskeleton and myosin structure, which indicates that the smoking modeling may also affect the proliferation and migration of pulmonary vascular smooth muscle cells and the integrity of epithelial cells. The differentially expressed proteins affected by dexamethasone are involved in the response to glucocorticoids in biological processes, including regulating the synthesis and metabolism of nutrients such as lipids and purine compounds, and regulating the immune response induced by endogenous and extracellular response. In terms of cellular components, there is enrichment of extracellular components and compartments and the myosin complex, which may be related to the pharmacological effects in regulating vascular permeability and inhibiting the directional migration of inflammatory cells. The enrichment results of Exocarpium Citri Grandis show that its effect is mainly reflected in the regulation of cytoskeleton and amino acid modification binding activity.

Enrichment analysis of KEGG signaling pathway: Proteins usually perform certain biological functions through mutual connection. Based on the KEGG database, differentially expressed proteins were analyzed for signaling pathway enrichment. The enrichment results are shown in Table 9.4. The signaling pathways closely related to the differential proteins between the model group and the normal group include: Metabolism of xenobiotics by cytochrome P450 related to the metabolism of exogenous substances, tight junction pathways associated with cellular connectivity and barrier function, focal adhesion pathways, leukocyte

Table 9.4 Enrichment analysis results of KEGG signaling pathway of differentially expressed proteins

Group	Pathway	DEPs with pathway annotation	*P* value
MOD/CON	Carbon metabolism	10	1.41E-04
	Biosynthesis of amino acids	9	1.90E-04
	Focal adhesion	9	1.29E-03
	Tight junction	8	1.45E-02
	Metabolism of xenobiotics by cytochrome P450	7	1.48E-03
	Glycolysis/gluconeogenesis	5	4.74E-03
	Leukocyte transendothelial migration	5	3.65E-02
DEX/MOD	Carbon metabolism	14	6.86E-05
	Focal adhesion	14	7.54E-03
	Biosynthesis of amino acids	11	2.36E-04
	Glycolysis/gluconeogenesis	8	9.04E-03
	ECM-receptor interaction	8	2.79E-02
	Leukocyte transendothelial migration	8	9.39E-03
	Metabolism of xenobiotics by cytochrome P450	7	3.54E-02
	Small cell lung cancer	6	2.40E-02
CGE/MOD	Carbon metabolism	14	1.20E-10
	Biosynthesis of amino acids	11	5.00E-09
	Glycolysis/gluconeogenesis	10	1.80E-08
	Tight junction	8	5.20E-04
	Focal adhesion	8	5.10E-03
	Adrenergic signaling in cardiomyocytes	6	1.80E-02
	Metabolism of xenobiotics by cytochrome P450	6	1.20E-02
	Glutathione metabolism	5	2.30E-03
	Leukocyte transendothelial migration	5	3.50E-02
	Dilated cardiomyopathy	5	1.00E-02
	Cardiac muscle contraction	4	4.40E-02

transendothelial migration, material and energy metabolism related pathways, carbon metabolism, biosynthesis of amino acids, and glycolysis/gluconeogenesis. The enrichment results of dexamethasone show that its effect is related to maintaining the stability of actin filament, which is the specific protective effect of the glucocorticoid [7]. At the same time, it may also play a role in reducing vascular permeability, reducing tissue congestion and leucocyte exudation, and recruitment of white blood cells through adhesion plaques, extracellular matrix receptors, and leukocyte migration signaling pathways across the endothelium, in order to reduce tissue inflammation reaction, which is consistent with its known mechanism of action. There are many signaling pathways enriched by the differentially expressed proteins caused by

Exocarpium Citri Grandis, which reflects the complexity of the regulation methods of multi-component drugs. The role of Exocarpium Citri Grandis is related to pathways such as tight junctions, focal adhesion, and actin cytoskeleton regulation. In addition, it is also involved in the process of muscle excitation and contraction.

9.1.3 Analysis of the Regulatory Network of Exocarpium Citri Grandis in the Treatment of Respiratory Diseases

The overall regulatory network of Exocarpium Citri Grandis in the treatment of respiratory diseases is discussed combining the results of molecular docking, iTRAQ proteomics testing, and reported pharmacological experiment results (as shown in Fig. 9.6). In terms of antitussive effect, the mechanism may be related to airway smooth muscle relaxation related to cGMP level, wherein the PDE5 is the key target. At the same time, it may also act on PP2A and CALM in the tight junction pathway to maintain the stability of the airway barrier function, thereby reducing the exposure of peripheral RARs receptors, inhibiting the release of substance P, and reducing cough. In addition, the predicted major potential targets of Exocarpium Citri Grandis are the upstream regulatory molecules of PI3K-Akt and Mapk14 pathway. There have been several studies that demonstrate that Exocarpium Citri Grandis has an agreeable anti-inflammatory effect on respiratory inflammation [1–3], and can affect the gene expression of inflammatory cytokines such as TNF-α. Therefore, based on comprehensive analysis it is speculated that the regulatory network is associated with anti-respiratory inflammation in Exocarpium Citri Grandis. Adhesion spot pathway and other cell connection-related pathways can mediate cell adhesion and migration and are closely related to promoting the release of inflammatory factors and the development of tissue inflammation. They may also be a key link in the anti-inflammatory mechanism of Exocarpium Citri Grandis. Inflammation also has a vast impact on the secretion of mucin in sputum. Our previous research demonstrated that naringin, the main active ingredient of Exocarpium Citri Grandis, can inhibit the increase of MUC5AC content and goblet cell proliferation by inhibiting MAPKs-AP-1 and IKKs-IkB-NF-κB B pathways [1–3], which is consistent with the speculated regulatory network of Exocarpium Citri Grandis, and can also explain the mechanism of Exocarpium Citri Grandis in relieving of cough and phlegm.

The association between the binding ingredients and the target was analyzed. The key proteins ITGB, RhoA, and PP2A in the adhesion junction regulation pathway have flavonoids acting on their own, suggesting that flavonoids may play a major role in maintaining the airway barrier function. At the same time, CALM, the target of coumarins acting on their own may affect actin synthesis through calcium ion signals, and on the other hand, affect barrier function. Most of the targets in inflammation-related signaling pathways are common targets of flavonoids and coumarins. This also reflects several possible mechanisms of multi-component

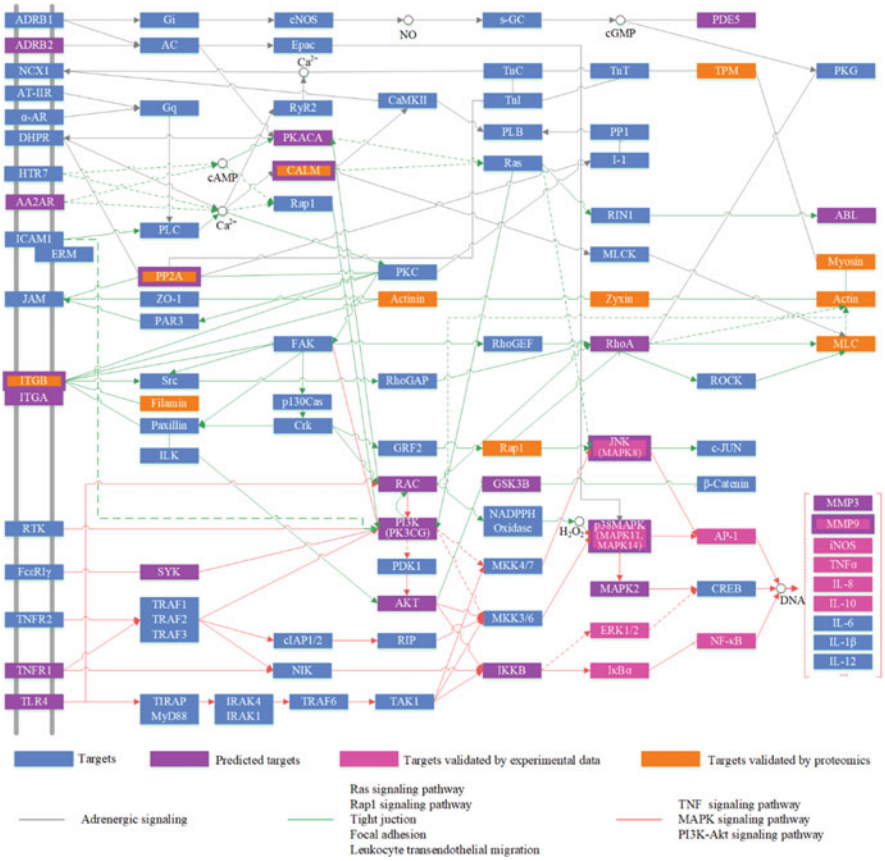

Fig. 9.6 Regulatory network of Exocarpium Citri Grandis in the treatment of respiratory diseases

synergistic action of TCM, including different components acting on the same target, acting on different targets of the same signaling pathway, or interrelated different signaling pathway targets. The results of this study provide an important basis for further exploration of the mechanism of action of Exocarpium Citri Grandis and its active ingredients and guide the clinical application of Exocarpium Citri Grandis.

9.2 Network Pharmacological Study on the Treatment of Cardiovascular Diseases with Danhong Injection

Danhong injection is a TCM preparation composed of Salvia miltiorrhiza and safflower. It is widely used clinically in the treatment of coronary heart disease, angina pectoris, myocardial infarction, and other cardiovascular diseases. The

pathogenesis of cardiovascular disease is complex, involving coagulation, inflammation, oxidative stress, and other systems; at the same time, Danhong injection is a complex mixture. Network pharmacology provides a new method to resolve such complex problems. This section employs network pharmacology technology to study the overall regulatory mechanism of Danhong injection in the treatment of cardiovascular disease and the scientific compatibility of its prescription.

9.2.1 Prediction of Action Targets of Danhong Injection in the Treatment of Cardiovascular Diseases

9.2.1.1 Chemical Composition Analysis of Danhong Injection Based on UFLC-Triple ToF-MS/MS

The chemical components of Danhong injection were detected online and sorted using UFLC-DAD-Q-TOF-MS/MS technology. Through comparison of reference substances, precise molecular weight search, and secondary lysis pattern analysis, a total of 82 chemical components were confirmed and identified in Danhong injection, including two alkaloids, three nucleosides, six amino acids, five organic acids, four iridoid glycosides, seven flavonoids, five quinone chalcones, 39 phenolic acids, eight tanshinone, and three other compounds. At the same time, the attribution of each component was determined by comparing with a single medicinal component. There are 17 components in both salvia and Carthamus tinctorius, mainly amino acids and nucleosides; there are 32 components only attributable to Salvia, mainly including phenolic acids and tanshinones; and there are 22 components only attributable to Carthamus tinctorius, mainly flavonoids, quinone chalcones, and iridoid glycosides. In addition, 11 kinds of phenolic acids were not detected in the two medicinal components, and were newly generated during the production process. The molecular structure of each compound is stored in MOL format as a ligand for molecular docking.

9.2.1.2 Action Target Prediction and Network Construction Analysis of Danhong Injection

The existing cardiovascular and cerebrovascular disease target database was employed for molecular docking calculation. The database includes 984 candidate protein targets and involves multiple pathophysiological processes such as thrombosis, endothelial function, energy metabolism, inflammation reaction, oxidative stress, etc. [8] The 82 chemical components of Danhong injection were molecularly docked with 984 protein targets, and a total of 64 active chemical components and 470 potential action targets were screened. The higher the number of components associated with the target, the stronger the influence of the components of Danhong, therefore it may be the main target of Danhong. The targets ranked high in the

Table 9.5 Potential action targets of chemical components of Danhong Injection (number of associated compounds ≥34)

No.	Target	Target name	Number of associated compounds
1	RENI	Renin	45
2	PDE5A	cGMP-specific 3′,5′-cyclic phosphodiesterase	45
3	HMDH	3-hydroxy-3-methylglutaryl-coenzyme A reductase	43
4	GDN	Glia-derived nexin	43
5	PDE4D	cAMP-specific 3′,5′-cyclic phosphodiesterase 4D	41
6	FOLH1	Glutamate carboxypeptidase 2	41
7	ACE	Angiotensin-converting enzyme	37
8	DPP4	Dipeptidyl peptidase 4	35
9	ANT3	Antithrombin-III	35
10	MK14	Mitogen-activated protein kinase 14	34

number of associated compounds are shown in Table 9.5, and mainly include: PDE5A, PDE4D, and MK14 that are associated with inflammatory response; ACE related to renin angiotensin system (angiotensin-converting enzyme); Reni (renin), FA10 (coagulation factor X), ANT3 (SERPINC1, antithrombin), PAFA, and PROC (protein C—coagulation factor VA and VIIIA inhibitors) associated with fibrinolysis system; HMDH associated with lipid metabolism; NOS3 (endothelial nitric oxide synthase) associated with endothelial function; and HMOX1 (heme oxygenase gene 1) associated with blood oxygen metabolism. Similarly, the more targets the compound is associated with, the stronger the regulatory activity of the component, thus it may be the main active component of Danhong. As shown in Table 9.6, the components with a large number of related targets are mainly salvianolic acid H, salvianolic acid I, and salvianolic acid A, followed by flavonoids such as Kaempferol-O-rutinoside, Kaempferol-di-O-glucoside, etc., as well as quinone chalcone such as Cartormin and Isocarthamin.

The correlation between the calculated components and targets was visualized by employing Cytoscape software. At the same time, the ClueGO plug-in was used to analyze the KEGG signal pathway of the targets in the network, to interpret the biological significance of the calculation results and the correlation between the targets, and to construct a component-target-signal pathway network. The results demonstrate that the potential action targets of Danhong injection are mainly enriched in antigen processing and presentation, B cell receptor signaling pathway and other pathways related to inflammation and immunity, complement and coagulation cascades signaling pathway related to the coagulation process, fluid shear stress and atherosclerosis pathway associated with cardiovascular disease, focal adhesion and other pathways related to endothelial function, and the key signal transduction pathways, namely PI3K Akt signaling pathway, MAPK signaling pathway, TNF signaling pathway, and Rap1 signaling pathway.

Table 9.6 Potential active ingredients of Danhong Injection (number of associated targets ≥100)

Compound serial no.	Compound name	Number of associated targets
SR-52	Salvianolic acid H	203
SR-53	Salvianolic acid I	199
SR-70	Salvianolic acid A	196
SR-65	Salvianolic acid E	174
SR-66	Salvianolic acid B	160
SR-56	Salvianolic acid D	157
SR-50	Salvianolic acid K	152
SR-59	Monomethyl lithospermate	152
SR-58	Salvianolic acid G	145
SR-62	Lithospermic acid	142
SR-61	Rosmarinic acid	109
SR-76	Salvianolic acid C	105
SR-67	Ethyl lithospermate	101

Furthermore, the associations between Danhong injection components and signaling pathways were explored by analyzing the number of targets associated with components in each signaling pathway. The results are shown in Fig. 9.7. The key signal transduction pathways, namely PI3K Akt signaling pathway, MAPK signaling pathway, TNF signaling pathway, Rap1 signaling pathway, and HIF-1 signaling pathway are strongly related to phenolic acids, flavonoids, and other ingredients. In addition, phenolic acids are also closely related to inflammation and immune-related pathways, such as antigen processing and presentation, B cell receptor signaling pathway, etc.; flavonoids are closely related to complement and coagulation cascades. The amino acids, nucleosides, organic acids, tanshinones, and other components in Danhong injection are associated with fewer targets, suggesting that the drug efficacy is also weak.

In summary, through molecular docking and network pharmacology analysis, the main active ingredients of Danhong injection in the treatment of cardiovascular diseases may be salvianolic acid H, salvianolic acid I, salvianolic acid A, and other salvianolic acids, as well as flavonoids such as Kaempferol-O-rutinoside and Kaempferol-di-O-glucoside, and quinoid chalcones such as Cartormin and Isocarthamin. The targets of these ingredients are enriched in the key signal transduction pathways PI3K-Akt and the MAPK signal pathway. In addition, the action target of Danhong injection is firmly correlated with inflammation, immunity, coagulation, endothelial function, and other related pathways. The phenolic acids derived from salvia focus more on inflammation and immune-related pathways, while the flavonoids derived from Carthamus tinctorius focus more on blood coagulation-related pathways, suggesting a mutual collaboration between the two herbs.

Class	No	PI3K-Akt signaling pathway	MAPK signaling pathway	Fluid shear stress and atherosclerosis	Cytokine-cytokine receptor interaction	TNF signaling pathway	HIF-1 signaling pathway	Complement and coagulation cascades	Focal adhesion	Sphingolipid signaling pathway	Hematopoietic cell lineage	Rap1 signaling pathway	Ras signaling pathway	p53 signaling pathway	Natural killer cell mediated cytotoxicity	B cell receptor signaling pathway	T cell receptor signaling pathway	Antigen processing and presentation	Identification	Source
Alkaloids	SR-02	2	2	2	1	1	3	2	1	2	0	2	1	1	0	0	1	0	Adenine	SM/CT
Alkaloids	SR-05	1	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	0	Uracil	SM/CT
Amino acids	SR-03	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	L-Pyroglutamic acid	SM/CT
Amino acids	SR-08	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	Leucine	SM/CT
Amino acids	SR-12	0	1	1	0	1	0	0	0	1	0	1	0	0	0	0	1	0	Phenylalanine	SM/CT
Amino acids	SR-20	3	2	2	1	1	3	4	1	2	0	1	0	3	1	0	1	1	Tryptophane	SM/CT
Flavonoids	SR-31	4	3	3	1	1	4	4	2	2	1	1	2	0	0	1	1	0	6-Hydroxykaempferol-tri-O-glucoside	CT
Flavonoids	SR-36	5	3	3	1	1	6	5	3	1	2	3	3	1	1	1	1	0	Quercetin-di-O-glucoside	CT
Flavonoids	SR-46	3	5	5	2	2	5	6	1	3	0	3	2	2	0	1	2	0	6-Hydroxykaempferol-di-O-glucoside	CT
Flavonoids	SR-51	8	5	7	4	4	5	5	4	4	3	7	4	0	1	1	2	0	Kaempferol-di-O-glucoside	CT
Flavonoids	SR-57	7	5	12	2	3	3	3	3	4	3	6	4	0	4	4	6	2	Kaempferol-O-rutinoside	CT
Iridoid glycosides	SR-34	7	3	3	5	2	4	1	3	2	5	3	3	2	2	2	2	2	Ixoroside	CT
Iridoid glycosides	SR-40	1	0	1	1	0	0	0	2	1	0	1	0	0	0	0	0	0	Roseoside	SM/CT
Iridoid glycosides	SR-41	10	7	9	4	2	3	1	3	4	2	6	5	1	3	3	3	2	Morroniside	CT
Nucleosides	SR-04	1	2	0	0	0	1	0	0	1	0	1	1	1	0	0	0	2	Uridine	SM/CT
Nucleosides	SR-09	1	2	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	Adenosine	SM/CT
Organic acids	SR-06	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	Succinic acid	SM/CT
Organic acids	SR-23	0	2	2	0	2	1	1	0	1	0	1	0	1	0	1	2	0	Neochlorogenic acid	CT
Organic acids	SR-27	0	2	2	0	1	2	4	1	1	0	1	0	1	0	0	1	0	Coumaric acid-O-hexoside	CT
Organic acids	SR-30	2	2	4	2	2	1	1	1	2	0	3	1	0	1	1	2	1	Chlorogenic acid	CT
Other	SR-32	1	3	2	2	0	2	0	2	0	0	1	0	0	0	0	0	1	Syringin	SM/CT
Phenolic acids	SR-11	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Gallic acid	NF
Phenolic acids	SR-16	2	1	2	0	1	1	1	0	0	0	0	1	0	0	1	1	0	Danshensu	SM
Phenolic acids	SR-18	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	Vanillic acid	NF
Phenolic acids	SR-19	0	1	1	0	1	0	0	0	1	0	1	0	0	0	0	1	0	Danshensu methyl ester	NF
Phenolic acids	SR-21	1	2	3	0	2	2	4	0	2	0	1	0	1	0	1	2	0	3,4-Dihydroxybenzenepropionic acid	SM/CT
Phenolic acids	SR-25	2	5	6	2	2	5	5	2	3	1	2	2	2	3	2	4	2	Prolithospermic acid	NF
Phenolic acids	SR-26	1	4	2	0	2	1	4	1	1	0	2	1	1	0	1	2	0	Caffic acid-O-hexoside	SM/CT
Phenolic acids	SR-33	1	1	3	1	1	1	0	0	2	0	1	0	0	0	0	1	0	Caffeic acid	SM
Phenolic acids	SR-50	17	13	16	13	7	11	8	7	9	3	14	14	5	6	7	6	5	Salvianolic acid K	SM
Phenolic acids	SR-52	23	15	17	14	6	11	11	8	8	10	14	12	7	5	4	8	4	Salvianolic acid H	SM
Phenolic acids	SR-53	23	17	20	14	11	14	8	14	11	5	16	18	7	9	9	9	4	Salvianolic acid I	NF
Phenolic acids	SR-54	6	4	8	4	3	3	3	2	2	2	3	2	2	1	1	3	1	Salviaflaside	SM
Phenolic acids	SR-56	18	16	14	7	8	8	7	13	11	3	12	12	6	6	7	8	4	Salvianolic acid D	SM
Phenolic acids	SR-58	14	12	14	9	6	6	5	6	9	4	8	9	4	7	6	9	4	Salvianolic acid G	SM
Phenolic acids	SR-59	17	19	16	12	6	13	9	13	12	6	14	16	4	8	9	8	6	Monomethyl lithospermate	NF
Phenolic acids	SR-61	13	12	13	7	6	6	8	7	10	1	9	8	4	5	6	7	4	Rosmarinic acid	SM
Phenolic acids	SR-62	21	14	18	15	5	13	6	9	7	7	13	13	7	6	6	5	2	Lithospermic acid	SM
Phenolic acids	SR-64	0	1	2	1	2	0	2	0	1	0	1	0	0	0	0	1	0	9''-Methyl lithospermate B	NF
Phenolic acids	SR-65	18	9	12	10	6	14	9	9	8	9	10	10	6	4	3	4	3	Salvianolic acid E	SM/CT
Phenolic acids	SR-66	18	8	8	10	2	12	12	8	8	8	12	15	4	8	6	6	4	Salvianolic acid B	SM
Phenolic acids	SR-67	8	10	12	6	8	8	8	4	6	2	6	7	3	3	3	6	3	Ethyl lithospermate	NF
Phenolic acids	SR-68	13	8	6	8	5	9	6	3	3	7	6	7	4	0	2	3	0	Salvianolic acid L	SM
Phenolic acids	SR-69	4	3	4	2	1	6	7	2	2	0	3	2	2	0	0	1	0	Romarinic acid methyl ester	SM
Phenolic acids	SR-70	23	19	19	15	9	14	11	11	11	8	15	15	6	10	9	10	4	Salvianolic acid A	SM
Phenolic acids	SR-73	2	4	4	1	2	5	7	2	2	0	2	1	2	0	1	2	0	Isosalvianolic acid A	NF
Phenolic acids	SR-74	2	3	3	1	1	4	3	2	2	0	2	1	0	0	0	1	0	Salvianolic acid C isomer	SM
Phenolic acids	SR-76	11	12	13	4	4	8	8	6	8	1	6	9	6	3	5	6	2	Salvianolic acid C	SM
Quinochalcones	SR-28	1	1	3	2	0	2	2	2	0	1	0	1	1	2	1	0	0	Hydroxysafflor yellow A	CT
Quinochalcones	SR-55	6	7	7	3	3	4	4	5	1	0	5	4	3	0	1	2	1	Isocarthamin	CT
Quinochalcones	SR-60	6	2	5	3	2	3	6	4	3	4	3	3	1	2	2	2	2	Cartormin	CT
Tanshinones	SR-63	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	Tanshindiol A	SM
Tanshinones	SR-71	0	1	2	0	1	0	0	0	1	0	1	1	0	0	0	1	0	Tanshindiol B	SM
Tanshinones	SR-77	2	4	3	0	2	1	2	1	2	0	2	1	0	0	1	2	0	Phenanthro[1,2-b]furan-10,11-dione, 1	SM
Tanshinones	SR-78	1	2	2	1	1	2	1	1	0	0	1	1	0	0	1	1	0	17-Hydroxycryptotanshinone	SM
Tanshinones	SR-81	1	1	0	1	0	1	1	1	0	0	1	1	0	0	0	0	0	3-Hydroxycryptotanshinone	SM

Fig. 9.7 Distribution of target points of active ingredients of Danhong injection in signal pathways of cardiovascular diseases (the numbers in the table refer to the number of targets)

9.2.2 Verification of Action Targets of Danhong Injection in the Treatment of Cardiovascular Diseases

9.2.2.1 Investigation of the Effect of Danhong Injection on Gene Expression in Rats with Acute Blood Stasis Based on Transcriptome

Animal Model

The SD rats were randomly divided into three groups: blank group (control), acute blood stasis model group (model), and Danhong injection group (3 mL/kg/d) (DHI), respectively, with three rats in each group. The Danhong injection group was administered intramuscular injection; and the blank control group and model group were injected with the same volume of normal saline, once a day for a period of ten consecutive days.

Thirty minutes after the last administration, except in the blank control group, rats in the other groups were injected subcutaneously with adrenaline hydrochloride 0.8 mg/kg, and rats in the blank group were injected subcutaneously with the same amount of normal saline. After 2 h, the rats in each group except the blank control group were immersed in ice water at 0–4 °C for 5 min. After 2 h, they were injected with adrenaline hydrochloride 0.8 mg/kg again [9]. After treatment, rats in each group were kept on fasting for 12 h and then administered with DHI or normal saline, respectively. And 15 min later, the rats were anesthetized by intraperitoneal injection of 10% chloral hydrate 0.35 mL/100 g, and blood was taken from the heart and they were sacrificed. One milliliter of whole blood was taken from each rat; three times the amount of trizol was added to the blood and it stored in a refrigerator at −80 °C for transcriptome detection.

Analysis of Amount of Gene Expression and Detection of Differentially Expressed Genes

Employing the BGISEQ-500RS sequencing platform, quantitative gene analysis and differential gene screening were conducted on the samples. The genes with Fold Change $\geq$4.00 and Adjusted P value $\leq$0.001 were employed as differentially expressed genes, and bioinformatics analysis was performed on them. The test results demonstrate that the average output of each sample is 23.70 M of data, the average comparison rate of the sample comparison genome is 96.24%, and the average comparison rate of the comparison gene set is 94.08%; a total of 13,416 genes were detected. The percentage of bases after filtering low-quality data was >90%, indicating that the sequencing quality is good. Compared to the model group, a total of 176 up-regulated genes and 145 down-regulated genes were detected in the blank group; compared to the Danhong group, a total of 1671 up-regulated genes and 14 down-regulated genes were detected in the model group.

Accuracy Verification of Transcriptome Sequencing

RT-qPCR method was employed to detect and calculate the relative expression amount of 15 genes, namely FN1, TLR4, PIK3cb, iNOS, IL-1R1, ITGB3, AP-1, JNK, NFκB, MHCII, IL-6R, Hif-1α, Ctsb, Xop7, and Retn, and the difference multiple of relative expression amount between the Danhong group and model group (as shown in Fig. 9.8), where Log_2 Fold Change (DHI/model) = Log_2 (Relative expression amount of Danhong group/relative expression amount of model group). The results demonstrate that the relative expression trend of each gene in qPCR results is consistent with that of transcriptome, signifying the accuracy and reliability of the transcriptome results.

Bioinformatics Analysis of Differentially Expressed Genes

Gene Ontology (GO) enrichment analysis: Go function enrichment analysis was conducted for the differentially expressed genes with respect to three aspects—molecular function, cell composition, and biological process. When comparing the model group and Danhong group (Model-VS-DHI) (as shown in Fig. 9.9), in terms of molecular functions, the differentially expressed genes were significantly enriched in adenyl ribonucleotide binding, kinase binding, transcription factor binding, and cation binding; in terms of cell composition, the differentially expressed genes were significantly enriched in MHC protein complex, cell surface, and vacuole; in terms of biological processes, the differentially expressed genes were significantly enriched in response to host immune response, response to cytokine, toll-like receptor signaling pathway, immune response-activating cell surface receptor signaling pathway, macrophage activation, antigen processing and presentation, regulation of MAPK cascade, and cellular protein modification process. It is suggested that upon administration of Danhong injection, the processes of RNA processing in cells, intracellular antigen presentation, MAPK cascade reaction, etc., are affected.

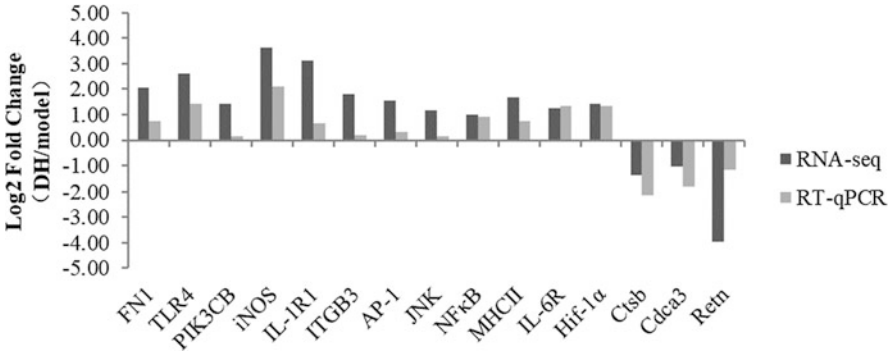

Fig. 9.8 qPCR-verified transcriptome sequencing results

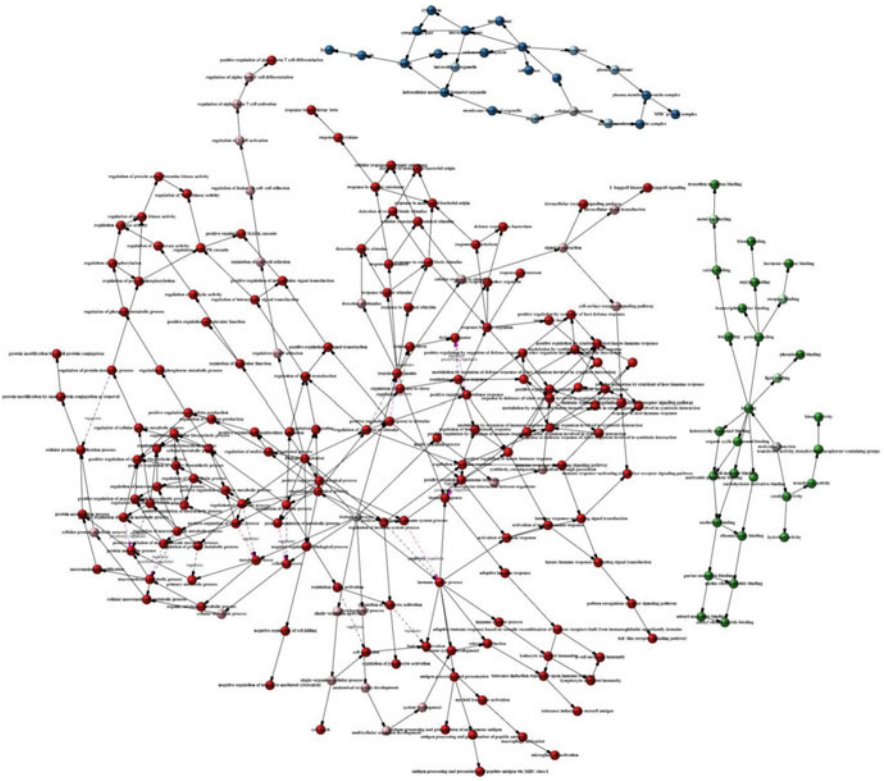

Fig. 9.9 GO Term network relationship diagram of Model group vs. Danhong group (Model-VS-DHI)

Enrichment analysis of KEGG signaling pathway: KEGG signal pathway enrichment analysis was conducted for differentially expressed genes, and the results of signal pathways with significant enrichment of differential genes among groups are as shown in Tables 9.7 and 9.8. The results demonstrate that the key pathways involved in the pharmacodynamic effect of Danhong injection are immune inflammation-related pathways such as antigen processing and presentation, platelet activation, T cell receptor signaling pathway, Fc epsilon RI signaling pathway, leukocyte transendothelial migration, chemokine signaling pathway, cell community pathway such as focal adhesion and regulation of actin cytoskeleton, and signal transduction pathways such as TNF signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway, and Jak-STAT signaling.

Association Analysis Between Differentially Expressed Genes and Prediction Targets

By comparing and analyzing the obtained target predicted by network pharmacology with the differentially expressed genes in transcriptomics, it was found that in the

Table 9.7 Enrichment results of differentially expressed gene signaling pathways in blank group vs. model group (Control-VS-Model)

Pathway	Control-VS-Model (297)	All-gene (15026)	*P* value	*Q* value	Level 1	Level 2
Antigen processing and presentation	16	134	9.06E-09	2.39E-07	Organismal systems	Immune system
Fluid shear stress and atherosclerosis	13	214	0.000341	3.56E-03	Human diseases	Cardiovascular diseases
TNF signaling pathway	9	138	0.00169	1.35E-02	Environmental information processing	Signal transduction
Chemokine signaling pathway	11	212	0.003351	2.37E-02	Organismal systems	Immune system
Focal adhesion	12	265	0.006594	3.96E-02	Cellular processes	Cellular community
PI3K-Akt signaling pathway	15	386	0.010052	5.88E-02	Environmental information processing	Signal transduction

component-target-pathway network of the under-construction Danhong injection, 25.1% (118/470) of the targets were verified by transcriptomics (as shown in Fig. 9.10). The signaling pathways enriched by verified targets (as shown in Table 9.9) mainly involve biological processes such as cell signal transduction, inflammation and immunity, cytoskeleton morphological adhesion, and cell apoptosis. The components that have interaction with the verified targets are mentioned below according to the number of associated targets (numbers in brackets), from high to low: Salvianolic acid A (19), Salvianolic acid I (18), Salvianolic acid B (17), Salvianolic acid H (16), Lithospermic acid (16), Salvianolic acid K (16), Monomethyl lithospermate (15), Salvianolic acid E (15), Salvianolic acid G (14), Salvianolic acid D (11), Rosmarinic acid (10), Ethyl lithospermate (9), Salvianolic acid C (9), and Kaempferol-O-rutinoside (8).

9.2.2.2 Effect of Danhong Injection on Biochemical Indexes in Rats with Acute Blood Stasis

Animal Model

The SD rats were randomly divided into eight groups: blank control group, acute blood stasis model group, positive drug low molecular weight heparin calcium group (50μL/kg/d), positive drug aspirin group (10 mg/kg/d), Danhong injection low-dose

Table 9.8 Enrichment results of differentially expressed gene signaling pathways in model group vs. Danhong group (Model-VS-DHI)

Pathway	Model-VS-DHI_high (1541)	All-gene (15026)	*P* value	*Q* value	Level 1	Level 2
Antigen processing and presentation	47	134	8.26E-15	1.28E-12	Organismal systems	Immune system
TNF signaling pathway	42	138	4.47E-11	9.93E-10	Environmental information processing	Signal transduction
NF-kappa B signaling pathway	30	100	3.75E-08	5.08E-07	Environmental information processing	Signal transduction
Toll-like receptor signaling pathway	33	117	4.24E-08	5.49E-07	Organismal systems	Immune system
B cell receptor signaling pathway	27	87	8.18E-08	1.02E-06	Organismal systems	Immune system
Chemokine signaling pathway	46	212	6.46E-07	7.17E-06	Organismal systems	Immune system
Apoptosis	44	207	2.02E-06	2.09E-05	Cellular processes	Cell growth and death
MAPK signaling pathway	52	284	2.39E-05	2.07E-04	Environmental information processing	Signal transduction
Platelet activation	33	168	0.000184	1.33E-03	Organismal systems	Immune system
Jak-STAT signaling pathway	35	184	0.00023	1.62E-03	Environmental information processing	Signal transduction
Regulation of actin cytoskeleton	45	277	0.00124	7.71E-03	Cellular processes	Cell motility
Focal adhesion	43	265	0.001608	9.75E-03	Cellular processes	Cellular community
T cell receptor signaling pathway	24	124	0.001629	9.75E-03	Organismal systems	Immune system
Fc epsilon RI signaling pathway	17	81	0.003098	1.69E-02	Organismal systems	Immune system

Leukocyte transendothelial migration	29	167	0.003221	1.73E-02	Organismal systems	Immune system
Fluid shear stress and atherosclerosis	34	214	0.006504	3.16E-02	Human diseases	Cardiovascular diseases
HIF-1 signaling pathway	22	126	0.008884	3.84E-02	Environmental information processing	Signal transduction
PI3K-Akt signaling pathway	53	386	0.016814	6.41E-02	Environmental information processing	Signal transduction

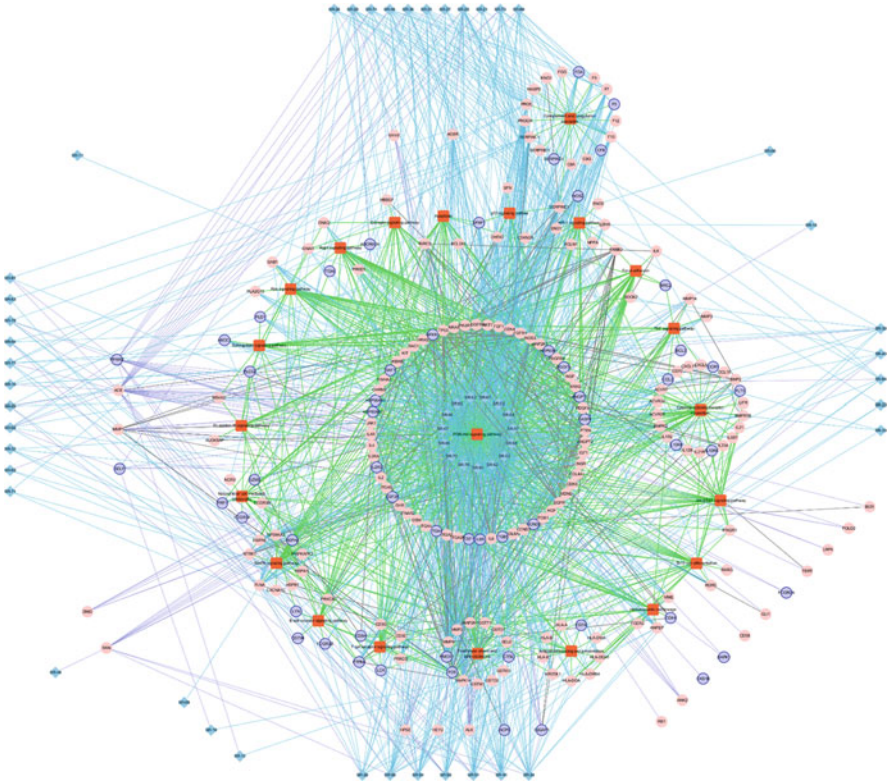

Fig. 9.10 Target verified by transcriptome in component-target-pathway network of Danhong injection (marked in purple)

group (0.75 mL/kg/d) (Clinical dose), medium dose group (1.5 mL/kg/d), and high-dose group (3 mL/kg/d), with ten rats in each group. Aspirin was given by gavage and Danhong injection was given intramuscularly. The blank control group and model group were intramuscularly injected with the same volume of normal saline once a day for ten consecutive days. After the last administration for 30 min, except for the blank control group, acute blood stasis modeling was performed on all rats in all the other groups, using the same method as mentioned in Sect. 6.2.2. After treatment, animals were put on fasting for 12 h and then administered once with DHI or normal saline, respectively; 15 min later, they were anesthetized with an intraperitoneal injection of 10% chloral hydrate 0.35 mL/100 g, and blood was taken from the heart, and they were sacrificed.

The collected rat whole blood was tested with a coagulation analyzer, blood rheometer, dynamic erythrocyte sedimentation rate tester, and platelet aggregation meter, to detect related indexes of hemorheology and coagulation function. Automatic biochemical analyzer, colorimetric kit, and ELISA determination kit were used to detect glutamic-pyruvic transaminase (ALT), glutamic oxaloacetic transaminase

Table 9.9 Signaling pathways enriched by verified targets

Enrichment pathway	Number of enriched targets	Biological process
PI3K-Akt signaling pathway	15	Cell signal transduction
Jak-STAT signaling pathway	7	
Ras signaling pathway	7	
HIF-1 signaling pathway	6	
TNF signaling pathway	6	
Cytokine-cytokine receptor interaction	8	Signal molecular interaction
B cell receptor signaling pathway	8	Inflammation and immunity
Natural killer cell mediated cytotoxicity	8	
Fc gamma R-mediated phagocytosis	7	
Antigen processing and presentation	6	
Focal adhesion	7	Cell community
Regulation of actin cytoskeleton	8	Cytoskeleton morphology
Apoptosis	4	Cell apoptosis

(AST), alkaline phosphatase (ALP), total protein (TP), creatinine (Cr), uric acid (UA), lactate dehydrogenase (LDH), creatine kinase isoenzymes (CK-MB), α-hydroxybutyrate dehydrogenase (α-HBDH), superoxide dismutase (SOD), malondialdehyde (MDA), myeloperoxidase (MPO), nitric oxide (NO), platelet activating factor (PAF), hypersensitive C-reactive protein (hs-CRP), interleukin1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM). The measurement data obtained are all expressed as mean ± standard deviation. SPSS version 18.0 was used to analyze the data using One-Way ANOVA and T testing methods. P values <0.05 and P values <0.01 are considered statistically different.

Detection Results of Multiple Biochemical Indexes

In acute blood stasis rats, obvious abnormalities were found in inflammation and immune response, vascular endothelial function, oxidative stress, platelet aggregation, myocardial energy metabolism, and liver and kidney function. Danhong injection was significantly effective for 19 indexes of IgM, IgA, IgG, IL-1β, TNF-α, IL-6, IL-8, LDH, CK-MB, PAF, SOD, MDA, MPO, hs-CRP, NO, TP, ALP, Cr, and UA, indicating that Danhong injection can inhibit the inflammatory response in rats with acute blood stasis, improve immune function, protect cardiomyocytes, reduce body peroxidation damage, protect liver and kidney function, inhibit platelet aggregation, and improve vascular function (as shown in Table 9.10).

Most of the pharmacodynamic indicators investigated are representative indicators in the pathological process. The signal pathways where these indicators are

Table 9.10 Effects of Danhong injection on several biochemical indexes in rats

Efficacy	Index	Effect of Danhong injection
Hemorheology	Whole blood viscosity	↓
	Erythrocyte aggregation, electrophoresis, rigidity index	↓
	Maximum platelet aggregation rate	↓
	PAF	↓
Immune response	IgG	↓
	IgM	↓
	IgA	↓
Inflammatory response	IL-1β	↓
	TNF-α	↓
	IL-6	↓
	IL-8	↓
Myocardial enzyme spectrum	LDH	↓
	CK-MB	↓
Oxidative stress	MDA	↓
Endothelial function	NO	↑
Liver function	TP	↑
	ALP	↓
Renal function	Cr	↓
	UA	↑

located were fairly consistent with the predicted targets and the signal pathways enriched in transcriptomics, differentially expressed the genes. For example, Danhong injection can regulate the expression of pro-inflammatory factors by activating the TNF signaling pathway; The fluid shear stress and atherosclerosis pathway regulate the expression of eNOS, thereby affecting the production of NO and endothelial function.

9.2.3 Analysis of Regulatory Network of Danhong Injection in the Treatment of Cardiovascular Diseases

The regulatory network of Danhong injection in the treatment of cardiovascular diseases was analyzed after integrating target prediction, transcriptome detection, and measurement results of multiple biochemical indexes in rats with acute blood stasis (as shown in Fig. 9.11). The mechanism mainly involves the following aspects:

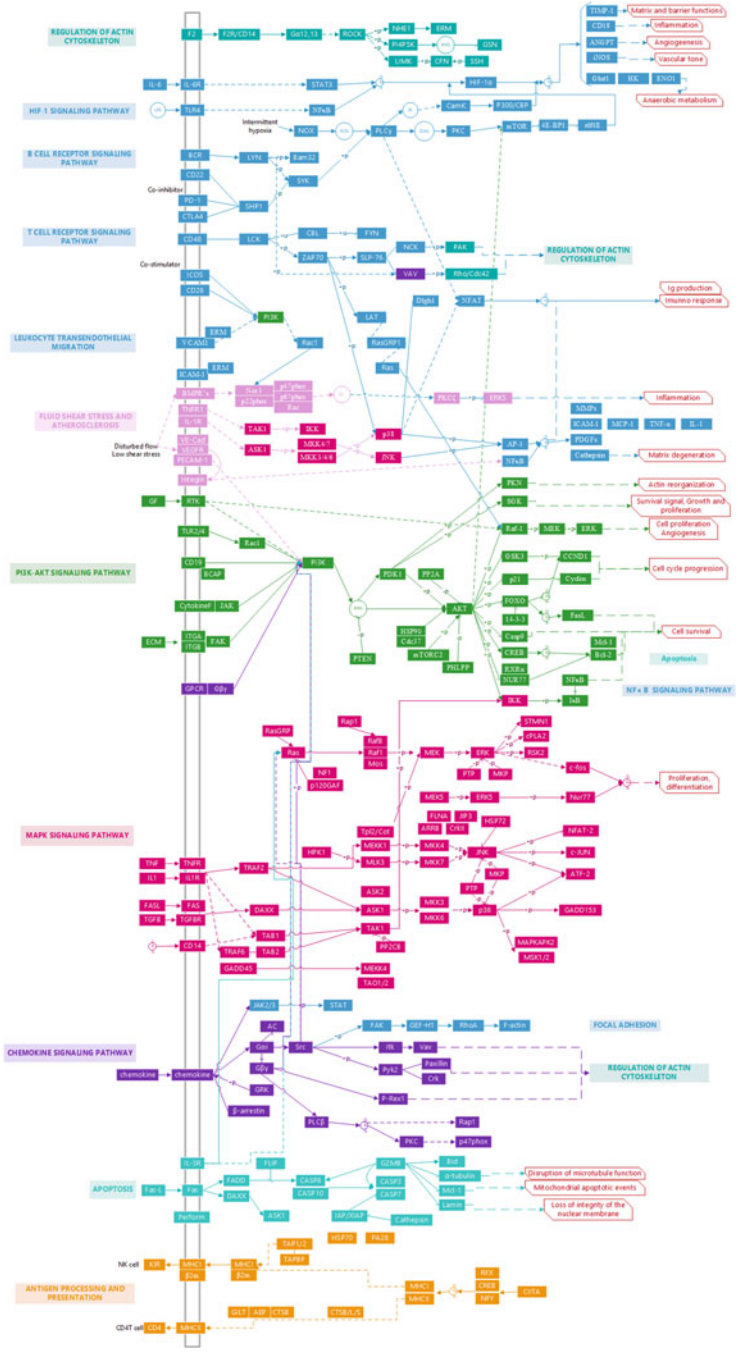

Fig. 9.11 Molecular mechanism of Danhong injection based on network pharmacology and transcriptomics

9.2.3.1 Improving Hemorheology

Danhong injection can bind to F2R receptors, chemokine receptors, and integrins through F2 (coagulation factor II), chemokines, and extracellular matrix, respectively, to activate the regulation of actin cytoskeleton, adhesion plaques, and PI3K-Akt signaling pathway, which plays a role in reducing red blood cell aggregation, electrophoresis, rigidity index, whole blood viscosity, and platelet aggregation. At the same time, chemokine receptors and integrins can also activate the PI3K-Akt signaling pathway and mediate inflammation response. Inflammation response is closely related to platelet activation and atherosclerotic disease progression. Therefore, Danhong injection inhibits platelet activation and reduces whole blood viscosity, which may be related to the regulation of PI3K-Akt pathway-related gene expression.

9.2.3.2 Modulating Immune Response

Danhong injection can inhibit the abnormal increase of immunoglobulin. On the one hand, its mechanism may regulate the expression of MHCI and MHCII on the cell surface by regulating the related genes in the antigen processing and presentation pathway, thus affecting the B cell and T cell receptor signaling pathways and regulating the immune response. On the other hand, it may also affect the activation of MAPK cascade reaction and the transcriptional regulation of immunoglobulin by NFAT through the TNFR1 receptor in the TNF signaling pathway, in order to regulate the immune response.

9.2.3.3 Inhibiting Inflammatory Response

The action target of Danhong injection is closely related to the TNF signaling pathway, PI3K-Akt signaling pathway, and MAPK signaling pathway, and can regulate the expression of inflammatory cytokines IL-1β, TNF-α, IL-6, and IL-8 by affecting the transcriptional regulation of AP-1 and NFκB nuclear transcription factors.

9.2.3.4 Reducing Oxidative Stress

Danhong injection can regulate the expression of related genes in the HIF-1 signaling pathway and fluid shear stress and atherosclerosis pathway, especially the key gene NOX. NOX is a key source of reactive oxygen species in the body, thus, regulating its expression can regulate the degree of oxidative stress in the body, affecting the expression of SOD, MDA, and other efficacy indicators. In addition,

inflammatory factors such as IL-1β and TNF-α can activate NOX, therefore, a decrease in inflammatory levels also reduces oxidative stress levels.

9.2.3.5 Protecting Endothelial Function

Similar to the effect of oxidative stress, the mechanism of Danhong injection in protecting and improving endothelial function may be related to the regulation of HIF-1 signaling pathway and fluid shear stress and the expression of related genes in the atherosclerotic pathway. Among them, NOX can reduce the degradation of eNOS and reduce the level of NO in cells, while NO can in turn inhibit the activity of NOX, alleviating oxidative stress and vascular endothelial damage from the source [10]. NO has been proven to protect endothelial cells, improve endothelial cell function, and at the same time promote angiogenesis, and plays an important protective role in ischemic injury. In addition, Danhong injection may induce the production of HIF-1α by activating the PI3K or MAPK pathway. HIF-1α can activate the transcription of eNOS in endothelial cells, thereby promoting the production of NO. The biochemical index test results also show that the level of NO increases correspondingly after the administration of Danhong injection.

9.2.3.6 Protecting Myocardial, Liver, and Kidney Function

Danhong injection is closely related to PI3K-Akt signaling pathway and cell apoptosis pathway. These pathways not only regulate cell apoptosis, proliferation, and differentiation, but also participate in the regulation of oxidative stress and inflammatory response. Therefore, the protective effect of Danhong injection on myocardium, liver, kidney, and other organs may be related to the reduction of cell apoptosis, the alleviation of inflammation, and tissue damage caused by oxidative stress.

To sum up, this section adopts the network pharmacology method to establish the composition-target-pathway network of Danhong injection, and further experimentally verifies the prediction results through transcriptomics analysis and multiple biochemical indicator tests, and comprehensively analyzes and clarifies the molecular mechanism and regulatory network of Danhong injection.

Due to the complexity of components and functions of TCM, the limitations of technology in the past added to the lack of overall understanding of the mechanism of its action led to an unclear direction for in-depth research as well as lack of pertinence. Network pharmacology, which has developed strongly in recent years, aims to study complex issues from the holistic perspective and internal relevance. It coincides with the core ideas of Chinese medicine and has become a new strategy [11] for scientifically explaining the effectiveness of Chinese medicine. This chapter introduces the common methods of network pharmacology in the TCM research by taking Exocarpium Citri Grandis and Danhong injection as examples. It mainly includes the construction of component and target data, the prediction and analysis

of active components and potential action targets, as well as the comprehensive analysis of the relationship between components, targets, pathways, and efficacy, in order to explore the overall complex mechanism of action of TCM. The development of network pharmacology research will provide an important basis for guiding the in-depth research and development and clinical application of Chinese medicine. With the development of network pharmacology and its integration with new technologies, network analysis methods will also have broader application prospects in Chinese medicine research.

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