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Original Article

Network pharmacology-based study of the molecular mechanisms of Qixuekang in treating COVID-19 during the recovery period

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Abstract: Objective: In this research, the analytical method of network pharmacology was used to explore Qixuekang molecular mechanism in treating Coronavirus 2019 (COVID-19) during the recovery period. Methods: Traditional Chinese Medicine Systems Pharmacology (TCMSP) database was used to collect the active components and corresponding targets of Qixuekang. Disease targets, related to COVID-19 during the recovery period, were collected from the GeneCards database. Protein-Protein interaction (PPI) network was built by using the String database, and analyzing and using Cytoscape 3.7.0 software to screen out hub genes. GO enrichment and KEGG pathway enrichment analysis were analyzed by R 3.6.1 software. Results: 34 active components of Qixuekang were screened out, and 161 common targets of drug and disease were identified. GO enrichment suggested 141 biologic processes, mainly involving nuclear receptor activity, transcription factor activity, and direct ligand regulated sequence-specific DNA binding. KEGG pathway enrichment suggests 96 signaling pathways, mainly including TNF signaling pathway, IL-17 signal pathway, and C-type lectin receptor signal pathway. The hub genes, screened in the PPI network, were mainly inclusive of CXCL8, CXCL2, CXCL10, ADRA2A, and ADRA2C. Conclusion: Qixuekang has numerous components and targets in treating COVID-19 during the recovery period. It is mainly applied in anti-inflammatory action and regulating immune defense, which may guide clinical trials in the later stage.

Keywords: Network pharmacology, COVID-19, Qixuekang, mechanism

Introduction

Coronavirus Disease 2019 (COVID-19) has a rapid onset and infection rate [1], which has a significant impact on the global economy and politics. As of 24:00 on April 3, 2020, 1562 confirmed cases (including 331 severe cases), 76,751 discharged cases, 3326 deaths, 81,639 confirmed cases, and 114 suspected cases had been reported in China [2]. As of 10:00 p.m. on April 2, Central European time, there were 896,450 confirmed cases and 45,526 deaths worldwide, affecting 205 countries and regions [3].

Although some COVID-19 patients met discharge standards, symptoms of fatigue, shortness of breath, palpitations, cough, and sputum were observed in the clinical stage of COVID-19 recovery [4, 5]. Chinese medicine is a critical treatment schemes in the diagnosis and treatment scheme of COVID-19 [6]. It is written in the book compendium of materia medica that "Renshen (Ginseng Radix Et Rhizoma), the first to nourish one's vital qi and Sanqi (Notoginseng Radix Et Rhizoma), the first to enrich the blood, are the most precious treatments of Traditional Chinese medicine (TCM)." Qixuekang oral liquid, containing four Chinese herbs: Sanqi (Notoginseng Radix Et Rhizoma, fresh), Huangqi (Astragali Radix), Renshen (Ginseng Radix Et Rhizoma) and Gegen (Puerariae Lobatae Radix), is applied in the symptoms of weakness, shortness of breath in operation, palpitations, and cough, which are consistent with the symptoms of COVID-19 dur-

ing the recovery period. Since ancient times, TCM has been used because "It is important to prevent disease recurrence after the rehabilitation stage." Proper rehabilitation treatment can promote recovery from disease and prevent recurrence [4]. In this research, we applied network pharmacology methods to screen the active components in Qixuekang and analyze the relevant mechanism of their interactions. We constructed a multi-level interaction network of "component-target-pathway", which provides references for further research and rational clinical-drug-use. As far as we know, this is the first research exploring the mechanism of Qixuekang in the treatment of COVID-19 during the recovery period by the methods of network pharmacology.

Methods

Qixuekang active compounds and potential target collection

We used the TCMSP database (http://ibts.hkbu.edu.hk/lsp/tcmsp.php) to search all chemical compositions and targets of Sanqi, Huangqi, Renshen, and Gegen. We screened the active compounds for oral bioavailability (OB) value ≥30% and drug similarity (DL) value ≥0.18. We used the Uniprot database (https://www.uniprot.org/) for standardization of collected target nomenclatures of Sanqi, Huangqi, Renshen, and Gegen.

Collection targets of COVID-19 during the recovery period

We obtained the related targets of COVID-19 during the recovery period through the GeneCards database (https://www.genecards.org/) by using "palpitations", "short of breath", and "cough" as search words. The mutual target genes of active compounds of Qixuekang and COVID-19 during the recovery period were obtained through VENNY 2.1 online tool (http://bioinfo.cnb.csic.es/tools/venny/index.html), known as COVID-19 during the recovery period disease-associated target genes of Qixuekang.

Qixuekang: active components-disease target network

The Qixuekang-active components-disease target network was made by Cytoscape 3.7.0 software (http://www.cytoscape.org/).

Protein-protein interaction network construction

Using the String (http://string-db.org) database to screen human targets with a confidence score > 0.9, we assessed the Qixuekang PPI network in treating COVID-19 during the recovery period. The key subnetwork of MCC topology analysis and the hub genes were screened by using cytohubba, a plug-in of Cytoscape 3.7.0 software.

Gene ontology and KEGG pathway enrichment analyses

Applying R 3.6.1 software (https://www.r-project.org/) loaded into the cluster profiler software package (http://www.bioconductor.org/packages/release/bioc/html/clusterprofiler.html), we carried out the GO enrichment analysis and KEGG pathway enrichment analysis with the results of $P < 0.05$, drawing relevant graphs.

Results

Active composition and target prediction of Qixuekang

Altogether 119 compounds of Sanqi, 87 compounds of Huangqi, 190 compounds of Renshen, and 18 compounds of Gegen were retrieved. Among the above compounds, eight active compounds in Sanqi, 20 active compounds in Huangqi, 22 active compounds in Renshen and four active compounds in Gegen, were found according to the screening conditions. There were 34 active compounds of Qixuekang after deleting duplicates (**Table 1**). The active components of Sanqi involve 116 targets. The active components of Huangqi include 355 targets. The active components of Renshen include 193 targets. The active components of Gegen include 68 targets. 192 targets were obtained after deleting duplicates.

Identification of COVID-19 during the recovery period disease-associated targets

After collecting 1450 targets associated to palpitations, 827 targets related to short of breath, 4148 targets related to cough, we finally obtained 4917 targets after deleting duplicates. The Venn diagram (**Figure 1**) was obtained by Venny 2.1 online tool.

Table 1. Active compounds and ADME findings of Qixuekang

herb	Mol ID	Molecule Name	OB (%)	DL
Sanqi	MOL001494	Mandenol	42	0.19
Sanqi	MOL001792	DFV	32.76	0.18
Sanqi	MOL002879	Diop	43.59	0.39
Sanqi	MOL000358	beta-sitosterol	36.91	0.75
Sanqi	MOL000449	Stigmasterol	43.83	0.76
Sanqi	MOL005344	ginsenoside rh2	36.32	0.56
Sanqi	MOL007475	ginsenoside f2	36.43	0.25
Sanqi	MOL000098	quercetin	46.43	0.28
Huangqi	MOL000211	Mairin	55.38	0.78
Huangqi	MOL000239	Jaranol	50.83	0.29
Huangqi	MOL000296	hederagenin	36.91	0.75
Huangqi	MOL000033	(3S,8S,9S,10*R*,13*R*,14S,17*R*)-10,13-dimethyl-17-[(2*R*,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78
Huangqi	MOL000354	isorhamnetin	49.6	0.31
Huangqi	MOL000371	3,9-di-O-methylnissolin	53.74	0.48
Huangqi	MOL000374	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	41.72	0.69
Huangqi	MOL000378	7-O-methylisomucronulatol	74.69	0.3
Huangqi	MOL000379	9,10-dimethoxypterocarpan-3-O-β-D-glucoside	36.74	0.92
Huangqi	MOL000380	(6a*R*,11a*R*)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.42
Huangqi	MOL000387	Bifendate	31.1	0.67
Huangqi	MOL000392	formononetin	69.67	0.21
Huangqi	MOL000398	isoflavanone	109.99	0.3
Huangqi	MOL000417	Calycosin	47.75	0.24
Huangqi	MOL000422	kaempferol	41.88	0.24
Huangqi	MOL000433	FA	68.96	0.71
Huangqi	MOL000438	(3*R*)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.67	0.26
Huangqi	MOL000439	isomucronulatol-7,2'-di-O-glucosiole	49.28	0.62
Huangqi	MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48
Huangqi	MOL000098	quercetin	46.43	0.28
Renshen	MOL002879	Diop	43.59	0.39
Renshen	MOL000449	Stigmasterol	43.83	0.76
Renshen	MOL000358	beta-sitosterol	36.91	0.75
Renshen	MOL003648	Inermin	65.83	0.54
Renshen	MOL000422	kaempferol	41.88	0.24
Renshen	MOL004492	Chrysanthemaxanthin	38.72	0.58
Renshen	MOL005308	Aposiopolamine	66.65	0.22
Renshen	MOL005314	Celabenzine	101.88	0.49
Renshen	MOL005317	Deoxyharringtonine	39.27	0.81
Renshen	MOL005318	Dianthramine	40.45	0.2
Renshen	MOL005320	arachidonate	45.57	0.2
Renshen	MOL005321	Frutinone A	65.9	0.34
Renshen	MOL005344	ginsenoside rh2	36.32	0.56
Renshen	MOL005348	Ginsenoside-Rh4_qt	31.11	0.78
Renshen	MOL005356	Girinimbin	61.22	0.31
Renshen	MOL005357	Gomisin B	31.99	0.83
Renshen	MOL005360	malkangunin	57.71	0.63

Renshen	MOL005376	Panaxadiol	33.09	0.79
Renshen	MOL005384	suchilactone	57.52	0.56
Renshen	MOL005399	alexandrin_qt	36.91	0.75
Renshen	MOL005401	ginsenoside Rg5_qt	39.56	0.79
Renshen	MOL000787	Fumarine	59.26	0.83
Gegen	MOL000392	formononetin	69.67	0.21
Gegen	MOL000358	beta-sitosterol	36.91	0.75
Gegen	MOL002959	3'-Methoxydaidzein	48.57	0.24
Gegen	MOL003629	Daidzein-4,7-diglucoside	47.27	0.67

Abbreviations: OB: oral bioavailability value; DL: drug similarity.

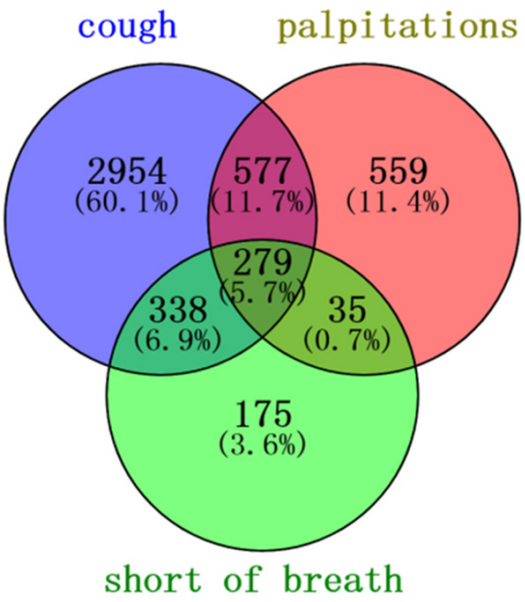

Figure 1. Venn diagram of COVID-19 during the recovery period disease-associated targets.

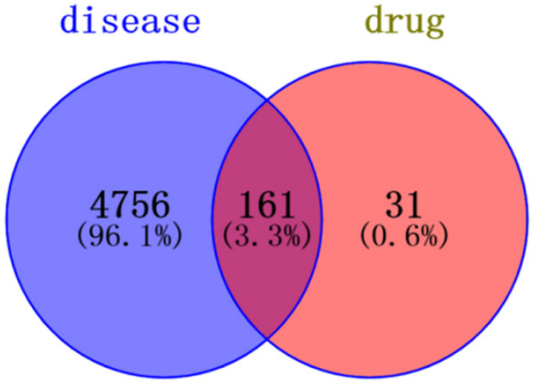

Figure 2. Venn diagram of Qixuekang and COVID-19 during the recovery period disease-associated targets.

COVID-19 during the recovery period disease-associated targets of Qixuekang

The online tool VENNY 2.1 was used to input the targets of Qixuekang and related targets of COVID-19 during the recovery period. Drawing the Venn diagram (**Figure 2**), we obtained a total of 161 common targets.

The network building of Qixuekang-active components-targets-disease

The 34 active components and 161 COVID-19 during the recovery period disease-associated targets of Qixuekang were input into Cytoscape 3.7.0 software to build the network (**Figure 3**). The network consists of 200 nodes and 710 interactions. The blue nodes represent COVID-19 during the recovery period disease-associated targets of Qixuekang. The purple nodes represent the active components. The yellow node represents COVID-19 during the recovery period, and the red nodes represent the herbs of Qixuekang.

PPI network of Qixuekang in treating COVID-19 during the recovery period

With the use of the String database, we constructed the PPI network of active components of Qixuekang in treating COVID-19 during the recovery period. The network (**Figure 4**) consists of 161 nodes and 569 interactions. MCC topology analysis was performed on cytoHubba to obtain the key subnetwork and the hub genes (**Figure 5**), a plug-in of Cytoscape 3.7.0 software. The top ten hub genes include CXCL8, CXCL2, CXCL10, ADRA2A, ADRA2C, CHRM2, PTGER3, OPRM1, OPRD1, and JUN.

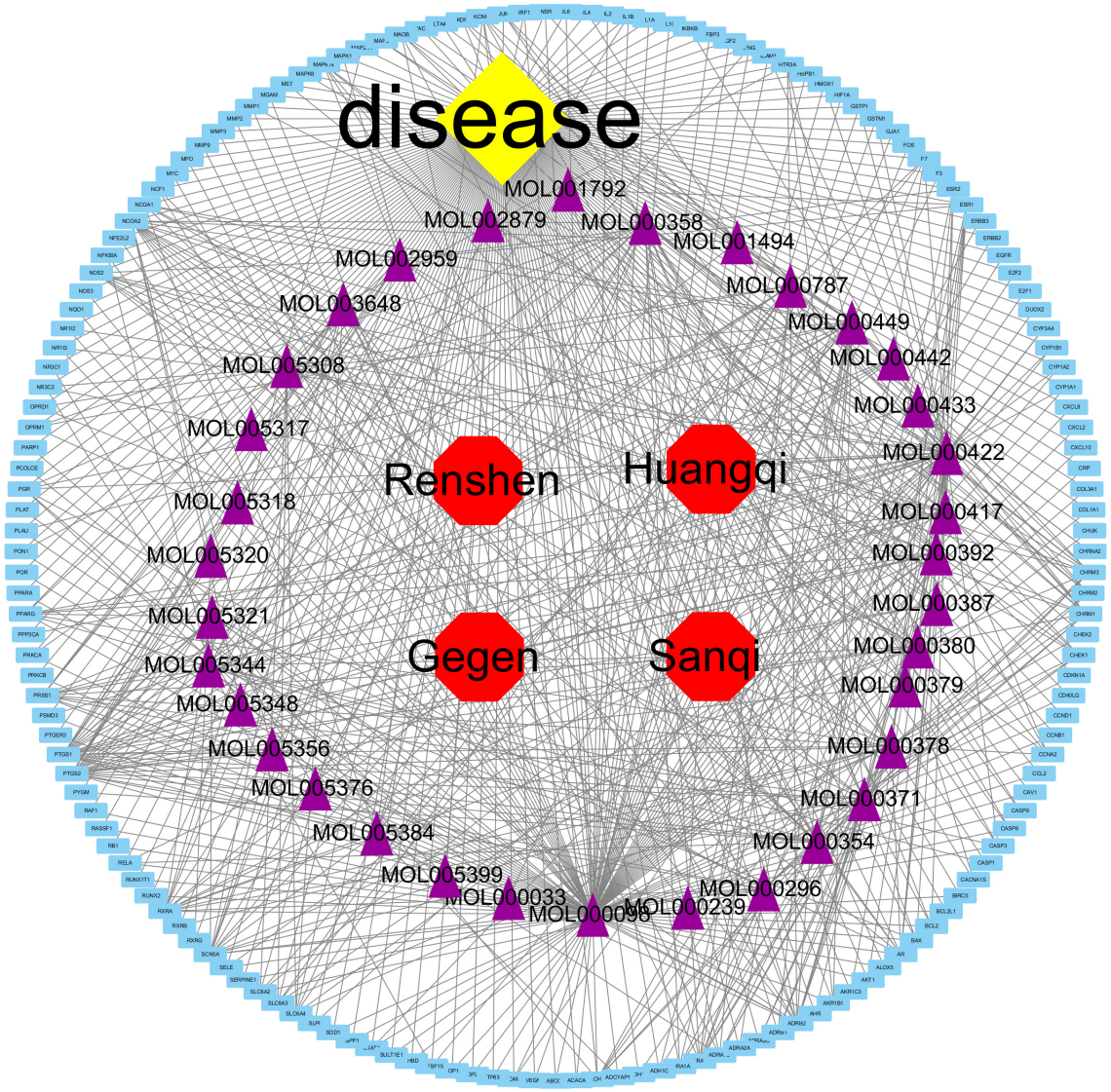

Figure 3. Network of active components of Qixuekang - targets - COVID-19 during the recovery period.

Qixuekang GO functional enrichment analysis in the treatment of COVID-19 during the recovery period

Using GO functional enrichment analysis, we obtained 141 related items. Here list the top 20 entries (**Figure 6**). The top biologic functions included: nuclear receptor activity, transcription factor activity, direct ligand regulated sequence-specific DNA binding, steroid hormone receptor activity, protein heterodimerization activity, G protein-coupled amine receptor activity, cytokine receptor binding, proximal promoter sequence-specific DNA binding, heme binding, DNA-binding transcription activator activity, RNA polymerase II-specific, and tetrapyrrole binding. This indicates that Qixuekang may treat COVID-19 during the recovery period through the above biologic regulation.

KEGG pathway enrichment analysis of Qixuekang in the treatment of COVID-19 during the recovery period

Through KEGG pathway enrichment analysis, we obtained 96 associated items. Here list the top 20 entries (**Figure 7**). The top topics (**Figures 8-10**) of KEGG pathways include TNF signal pathway, IL-17 signal pathway, Th17 cell differentiation, endocrine resistance, C-type lectin receptor signal pathway, Relaxin signal

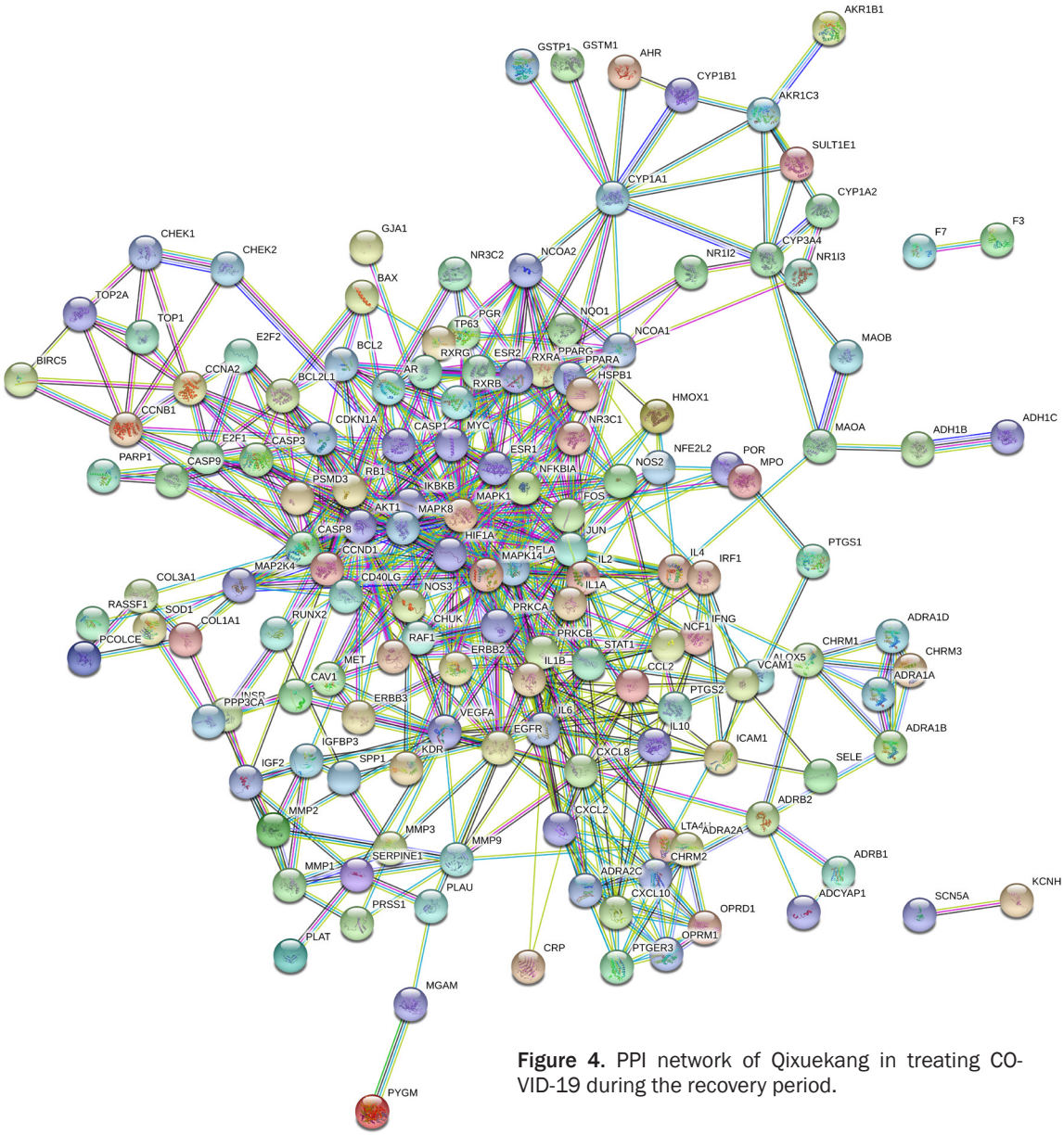

Figure 4. PPI network of Qixuekang in treating COVID-19 during the recovery period.

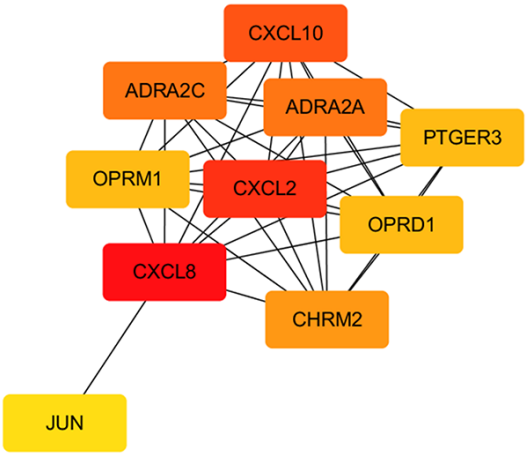

Figure 5. PPI Network of the top ten hub genes.

pathway, Toll-like receptor signal pathway, HIF-1 signal pathway, and MAPK signal pathway, which indicates that Qixuekang may treat COVID-19 during the recovery period through the above signaling pathways.

Discussion

Rehabilitation from COVID-19 should enhance the patient's physical fitness and immunity, improve the prognosis, and ultimately improve patients' life quality, to integrate patients into society as soon as possible. With the development of the epidemic and the increase of discharged patients, it is necessary to focus on the treatment of COVID-19 during the recovery

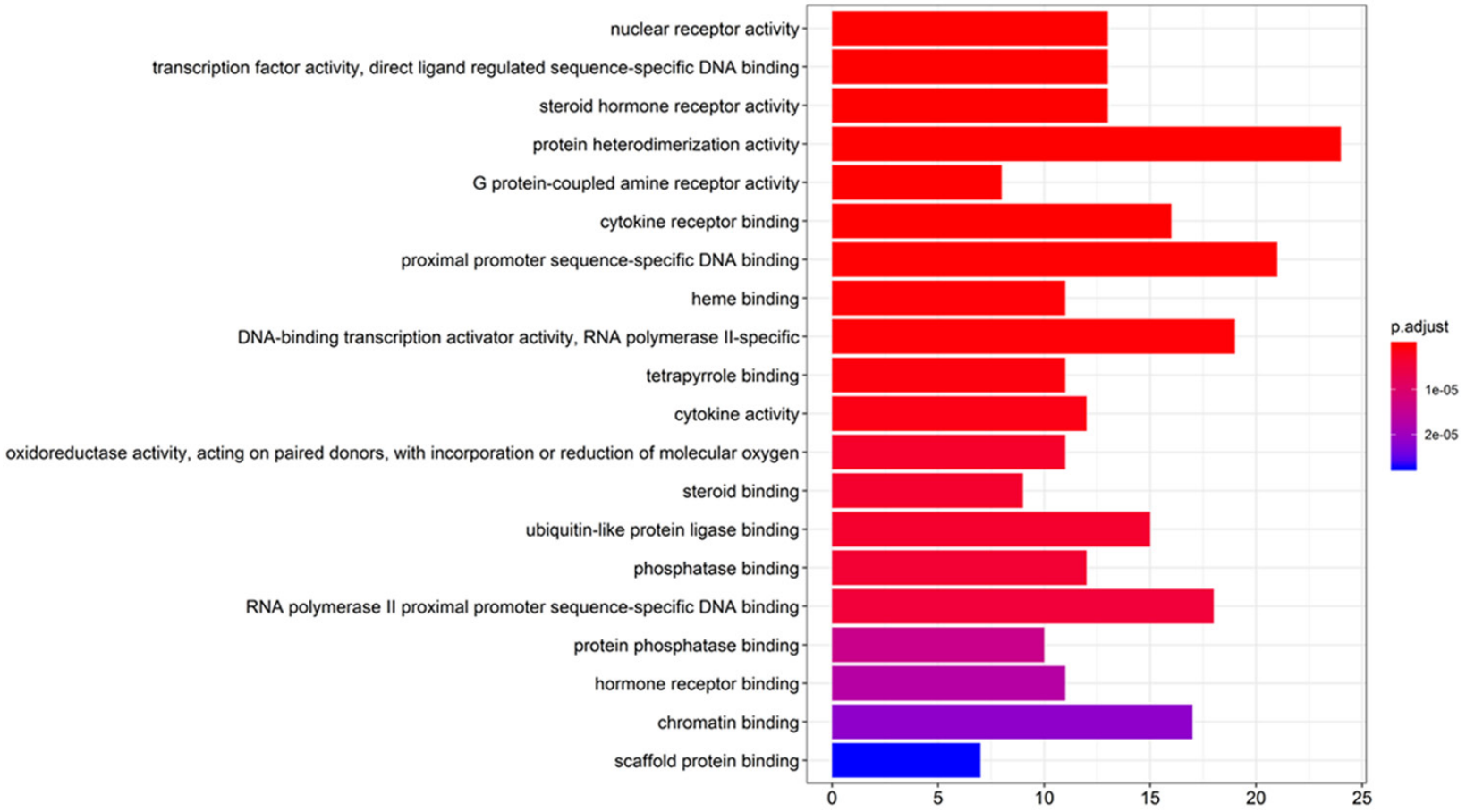

Figure 6. GO functional enrichment analysis histogram of Qixuekang in treating COVID-19 during the recovery period.

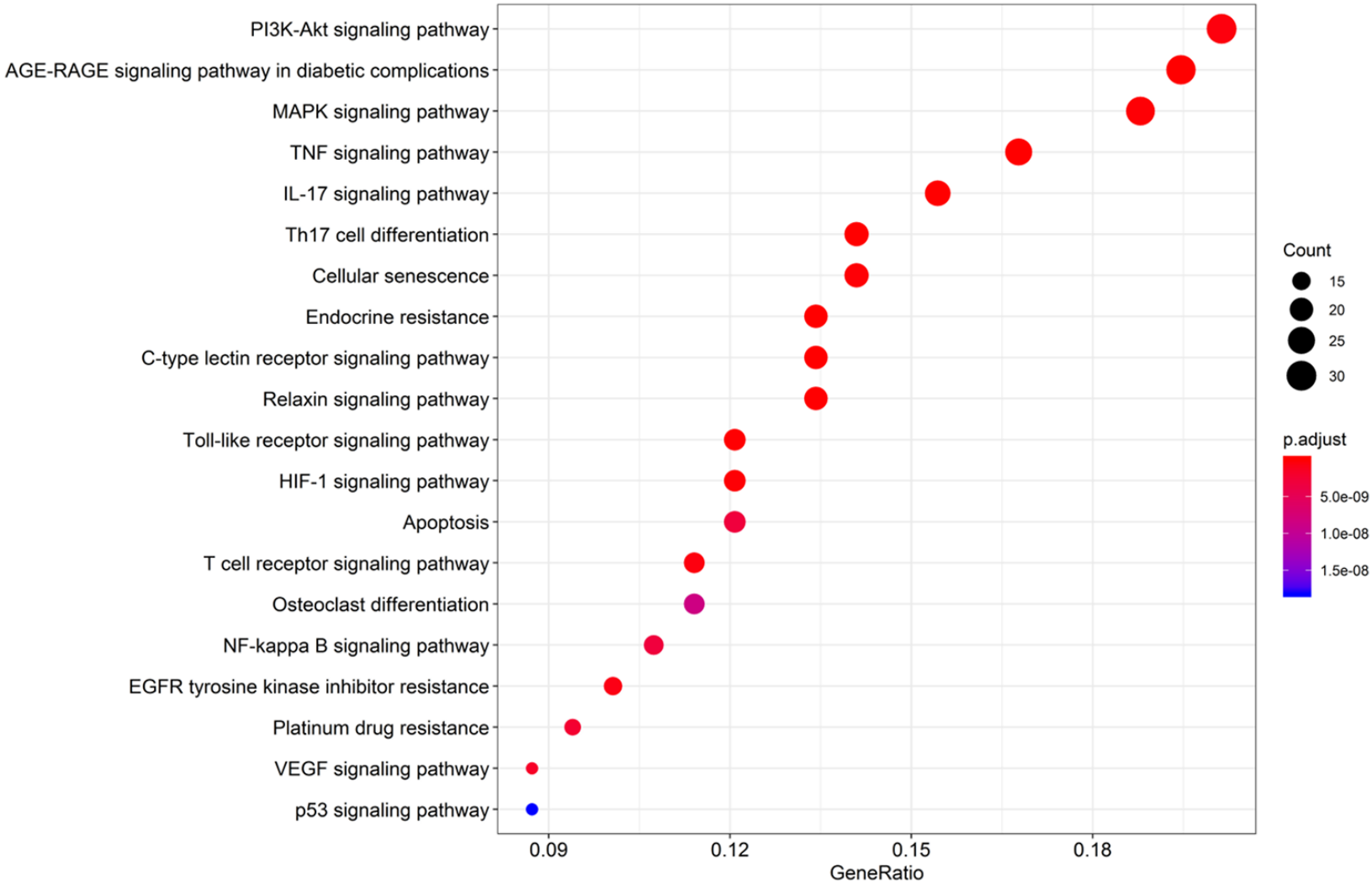

Figure 7. KEGG pathway point diagram of Qixuekang in treating COVID-19 during the recovery period.

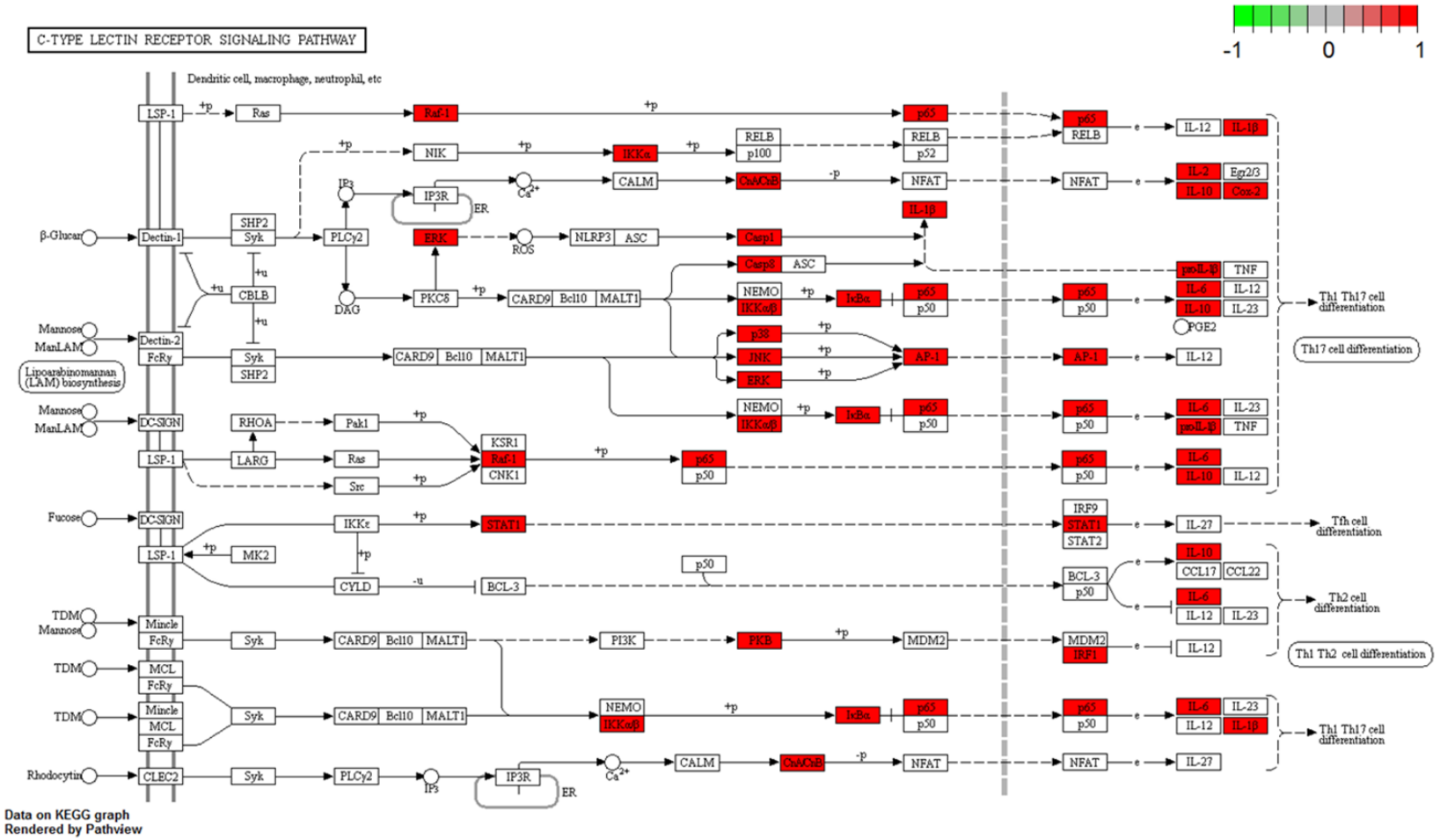

Figure 8. C-type lectin receptor signaling pathway. The red nodes represent COVID-19 during the recovery period-related targets of Qixuekang.

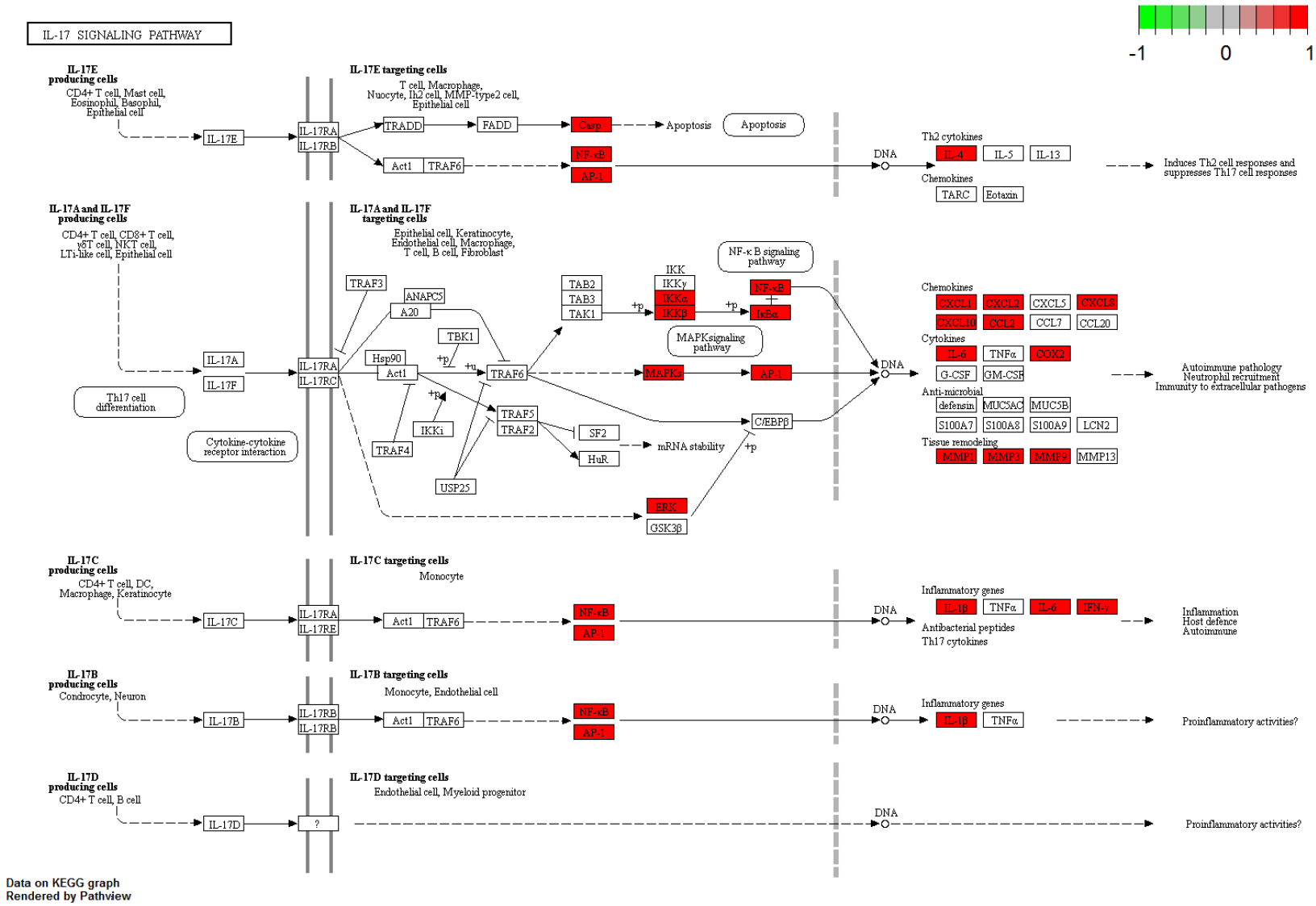

Figure 9. IL-17 signaling pathway. The red nodes represent COVID-19 during the recovery period-related targets of Qixuekang.

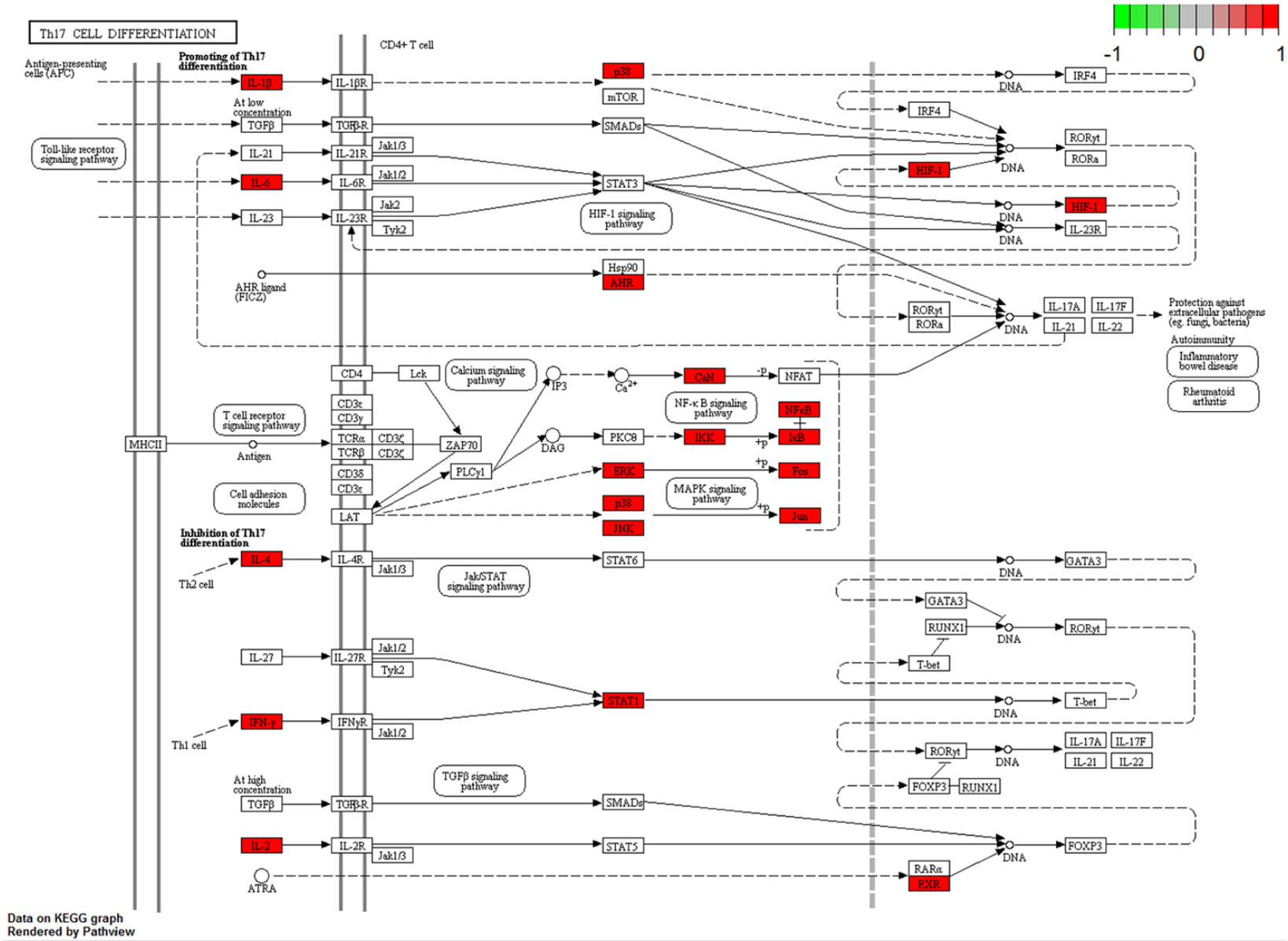

Figure 10. Th17 cell differentiation. The red nodes represent COVID-19 during the recovery period-related targets of Qixuekang.

period [7]. TCM has an essential effect on the prevention and treatment of various emergent infectious diseases. The clinical efficacy of TCM and integrated traditional Chinese and western medicine in treating SARS has been confirmed by the World Health Organization [8, 9]. According to the publication of COVID-19 diagnosis and treatment plan (6th edition) by the national health commission for trial implementation [5], the types of TCM clinical staging are initial stage, intermediate stage, critical stage, and recovery stage. The main symptoms in the recovery period are deficiency of vital qi and blood, and strengthening vital qi to eliminate pathogenic factors is necessary [10]. The vital qi of TCM is closely related to the immune function of modern medicine. Li et al [11]. studied the pharmacodynamics of Qixuekang oral liquid given to three pathological model mice, rats, and normal mice. The results showed that Qixuekang has the effect of strengthening the body in various aspects such as blood supplementation, Qi supplementation, and anti-immunosuppression.

The results showed 34 active components of Qixuekang, such as mandenol, DFV, Diop, beta-sitosterol, stigmasterol, mairin, jaranol, and hederagenin. They play essential roles in the treatment of COVID-19 during the recovery period. For instance, mandenol, known as ethyl linoleate, is an unsaturated fatty acid that is applied in many cosmetics for its various characteristics, such as antibacterial and anti-inflammatory properties [12]. Some studies have proved that ethyl linoleate can reduce the release of inflammatory factors that are induced by bacterial lipopolysaccharide [13]. Stigmasterol has anti-inflammatory effects. Choi et al [14]. found that β-sitosterol can inhibit IL-6 activity of macrophages and reduce the secretion of inflammatory factors such as IL-1 by reducing NO synthesis. Ginsenoside rh2 acts on a variety of immune cells to cause immune enhancement by increasing the number of innate immune cells, promoting the proliferation and maturation of innate immune cells, reducing the expression of immunosuppressive molecules, and promoting the proliferation of immune effector cells [15]. Isorhamnetin has the functions of inhibiting the activation of NF-κB, alleviating inflammation and anti-apoptosis [16-18], as well as significant antioxidant and free radical scavenging functions [19], and has a protective effect on the injury of endothelial cells in vitro caused by oxidative modification of LDL [20]. Formononetin can significantly enhance the immune function of immunosuppressed mice by promoting the proliferation and maturation of immunoreactive cells (T, B lymphocytes of thymus and spleen, and KCs cells of liver) in related immune organs [21]. Calycosin has an antiviral effect. It can not only treat myocarditis which is caused by Coxsackievirus B3 (CVB3), but also significantly improve the survival rate and left ventricular function of animals that are infected with myocarditis, to inhibit virus replication. It can improve heart function and regulate cellular immune function [22, 23].

PPI network analysis showed that the hub genes of Qixuekang for the treatment of COVID-19 during the recovery period are CXCL8, CXCL2, CXCL10, ADRA2A, ADRA2C, CHRM2, PTGER3, OPRM1, OPRD1, and JUN. When inflammation occurs in the respiratory tract, lung tissue cells, such as lung epithelial cells and fibroblasts, can secrete or regulate cell factors and inflammatory mediators, regulating the body's innate immune and adaptive immune response [24]. Chemokines CXCL8 and CXCL10 trigger the chemotaxis of neutrophils [25, 26]. The role of neutrophils is crucial in acute respiratory distress syndrome (ARDS), pneumonia and asthma [27]. Many patients with Chronic Obstructive Pulmonary Disease (COPD) have response to bronchodilator agents. The studies show that polymorphisms in the β2-adrenergic (ADRb2), muscarinic M2 and M3 receptors (CHRM) may take part in the regulation of the receptor responses [28]. Studies have shown that Jun participates in airway inflammation, airway remodeling, and airway hyperresponsiveness in asthma [29].

The GO biological processes, which are for the targets of Qixuekang in treating COVID-19 during the recovery period, mainly include nuclear receptor activity, transcription factor activity, direct ligand regulated sequence-specific DNA binding, steroid hormone receptor activity, protein heterodimerization activity, G protein-coupled amine receptor activity, cytokine receptor binding, proximal promoter sequence-specific DNA binding, heme binding, DNA-binding transcription activator activity, and RNA polymerase II-specific, and tetrapyrrole binding. The KEGG

pathways for the targets of Qixuekang in treating COVID-19 during the recovery period mainly include: TNF signal pathway, IL-17 signal pathway, Th17 cell differentiation, endocrine resistance, C-type lectin receptor signal pathway, Relaxin signal pathway, Toll-like receptor signal pathway, HIF-1 signal pathway, and MAPK signal pathway. For instance, TNF signal pathway, IL-17 signaling, Th17 cell differentiation, and MAPK signal pathway participate in the inflammatory response. C-type lectin receptor (CLRs) can mediate and regulate the production of cytokines and trigger the protective immune response of Th17 cells [30]. Activation of the toll-like receptor signal pathway is of great importance to the body's immune defense process. The signal transduction pathway mediated by it can induce the activation of many rapid reactions, producing such effector molecules as co-stimulant molecules and chemokines to participate in the body's defense response [31]. The HIF-1 signaling pathway is related to lung function [32].

In conclusion, this research investigated the active constituents, related targets, and Qixuekang mechanisms in treating COVID-19 during the recovery period through network pharmacology. Theoretically, it has been verified that Qixuekang is has multiple components and targets for treatment of COVID-19. It is mainly used to treat COVID-19 during the recovery period by anti-inflammatory action and regulating immune defense, which can guide clinical trials in the later stage.

Acknowledgements

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Disclosure of conflict of interest

None.

Abbreviations

COVID-19, Coronavirus 2019; PPI, Protien-Protien interaction; OB, oral bioavailability; DL, drug similarity; CVB3, Coxsackie virus B3; ARDS, acute respiratory distress syndrome; COPD, Chronic Obstructive Pulmonary Disease; ADRb2, b2-adrenergic; CLRs, C-type lectin receptor.

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