

Study on the potential mechanism of Pien Tze Huang Capsules against coronavirus disease 2019 based on network pharmacology

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Abstract

Objective: To discuss the possible underlying mechanism of the effect of Pien Tze Huang capsules (PTHCs) against coronavirus disease 2019 (COVID-19) using network pharmacology research methods.

Methods: Through the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), in this study, we searched for the active ingredients of PTHC, including Sanqi (*Panax notoginseng*), Shedan (snake's bile), Niu-huang (*Calculus Bovis* or ox bezoars), and musk, as well as information on the corresponding targets. The GeneCards database was used to obtain relevant information on COVID-19 targets. The search tool for the retrieval of interacting genes/proteins (STRING) database was used to draw the protein–protein interaction network, and the R Programming Language was used to carry out gene ontology functional enrichment analysis and the Kyoto encyclopedia of genes and genomes pathway enrichment analysis.

Results: In PTHC, there were 12 compounds that met the conditions and 232 gene targets. Along with the 394 gene targets associated with COVID-19, there were 41 potential targets related to the PTHC ingredients against COVID-19. The key targets included RELA, interleukin (IL)-6, IL-1 β , mitogen-activated protein kinase (MAPK) 1, and C-X-C motif chemokine ligand 8, whereas the potential signaling pathways were nuclear factor- κ B, cytokine-cytokine receptor interaction, the hypoxia-inducible factor 1 signaling pathway, the chemokine signaling pathway, and the MAPK signaling pathway.

Conclusion: PTHCs have the potential to combat COVID-19 by modulating key targets and potential signaling pathways that regulate immune function, providing theoretical support for more TCM treatments against COVID-19.

Keywords: Pien Tze Huang Capsule, COVID-19, Network pharmacology

Background

Since the diagnosis of the first coronavirus disease 2019 (COVID-19) patient in December 2019, the increasing number of patients has demonstrated that the disease is highly contagious. Here, the number of people infected far exceeds that of the severe acute respiratory syndrome (SARS) in 2003. This time, the epidemic is very serious, and the efficacy of syndrome differentiation in Traditional Chinese Medicine (TCM) has been well confirmed. With its unique advantages, TCM syndrome differentiation has been widely recognized in all versions of the *Diagnosis and Treatment Protocol for COVID-19* [1].

Pien Tze Huang Capsule (PTHC) is a national confidential prescription of TCM that contains four

Chinese medicines, including Sanqi (*Panax notoginseng*), Shedan (snake's bile), Niu-huang (*Calculus Bovis* or ox bezoars), and musk; the active ingredients include notoginsenoside R, ginsenoside Rg1, and ginsenoside Rb. The capsule has the effects of clearing heat and detoxifying, cooling the blood and removing stasis, and eliminating phlegm and diminishing swelling [2]. PTHC is widely prescribed in clinical practice, especially in the treatment of infectious diseases, and has been included in the *Expert Guidance on the Treatment of Ebola Hemorrhagic Fever by Traditional Chinese Medicine* (1st Edition) and the *Diagnostic Guideline for Dengue Fever* (2nd Edition, 2014) as well as in other relevant guidelines. Similar to Ebola hemorrhagic fever and

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dengue fever, COVID-19 is also an “epidemic disease.” To combat epidemic diseases, TCM often adopts methods such as “avoiding filth and resolving turbidity” and “heat clearing and detoxifying,” which are consistent with the effects of PTHC. However, whether PTHC can be used for the prevention and control of COVID-19 (as well as the pathological mechanism) is not clear. Network pharmacology provides strong theoretical support regarding the mechanism of multi-target, multi-component, and holistic treatment via Chinese medicine compound prescriptions. This study adopted the network pharmacology method to study the potential therapeutic mechanism of PTHC, with its known components against COVID-19, which provides a reference for the theoretical basis of using PTHC more extensively in the treatment of COVID-19 and other diseases.

Methods

Screening of the active ingredients of PTHC and prediction of potential targets

This study used the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP; <http://tcmispw.com/tcmisp.php>) to identify the targets of the active ingredients of the four main Chinese medicines in PTHCs. The screening criteria were set as: Oral Bioavailability (OB) $\geq 30\%$ and Drug Likeness (DL) ≥ 0.18 .

Predicting COVID-19 targets

This study searched for genes associated with COVID-19 and collected target genes through the GeneCards (<https://www.genecards.org/>) and the Online Mendelian Inheritance in Man (OMIM) (<https://omim.org/>) databases using “coronavirus disease 2019” as the keyword.

Building the TCM-active ingredient-target network and the protein–protein interaction (PPI) network

The intersections between the relevant targets of the Chinese medicine active ingredients and the relevant targets of the disease were used to build the Chinese medicine–active ingredient–target network using Cytoscape. The intersecting target information was loaded into the search tool for the retrieval of interacting genes/proteins (STRING) database, with the species selected as “human” and the screening condition set to Confidence > 0.9 . We then downloaded the PPI information of the intersected targets and visualized it using Cytoscape.

Analyzing the targeted genes’ biological information enrichment

We loaded the intersected targets of PTHC and COVID-19 into the Database for Annotation,

Visualization and Integrated Discovery (DAVID), with the species limited to human and then performed gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis. After screening and obtaining the biological process and signaling pathways when $P < 0.05$, we used the R language (ClusterProfiler package) for GO and KEGG analysis and visualized it by drawing.

Results

Collecting the active ingredients of the PTHC and COVID-19 targets

Through retrievals from the TCMSP, the number of active ingredients of PTHC that met the standard was 12. The predicted targets of the obtained active ingredients were loaded into the Uniprot database for gene normalization. After duplicate values were removed, 232 target genes were obtained. In total, 394 potential target genes associated with COVID-19 were collected using the GeneCard and OMIM databases. After mapping using Venny 2.1.0, 41 intersected targets were finally obtained. Therefore, PTHC can act on COVID-19 through 41 target genes.

Network analysis of disease–active pharmaceutical ingredient–target

The 41 common targets corresponded to six active ingredients of TCM, including quercetin, Mandenol, diflucortolone valerate, ginsenoside rh2, stigmasterol, and β -sitosterol, which can be considered the key active ingredients of PTHC that act on COVID-19. Cytoscape was used to construct the TCM–active ingredient–target network (Figure 1). There were 49 nodes (consisting of 6 active targets for TCM chemical compositions and 41 common targets) and 109 edges representing inter-target interactions. In Figure 1, the diamond nodes are the active ingredients of the drug, the ellipses are the common targets, the red rectangle is the disease, and the green rectangle is the drug. This figure reflects the complex features of PTHC with multiple ingredients and multiple targets.

Building and analyzing the PPI network

The PPI network of PTHC potential targets was analyzed using the online STRING database (Figure 2). The core PPI network contained 37 nodes and 119 edges. In Figure 2, the nodes represent the proteins, and each edge represents protein interactions. The thinner the edge, the lower the degree of that protein’s interactions; the higher the node degree, the larger the circle becomes. The top five node degrees correspond to target proteins, which are RELA (degree = 16), interleukin (IL)-6 (degree = 16), IL-1 β (degree = 12), mitogen-activated protein kinase (MAPK) 1 (degree = 12), and C-X-C motif chemokine ligand 8 (CXCL8) (degree = 11), indicating that these five core targets

play a key role in the network.

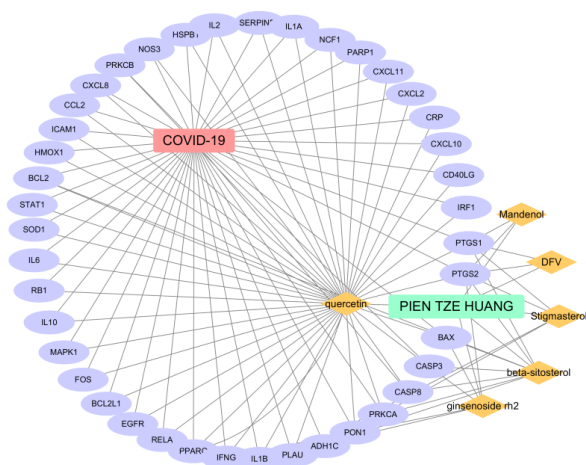


Figure 1 The Network of Disease–Active Pharmaceutical Ingredient–Target

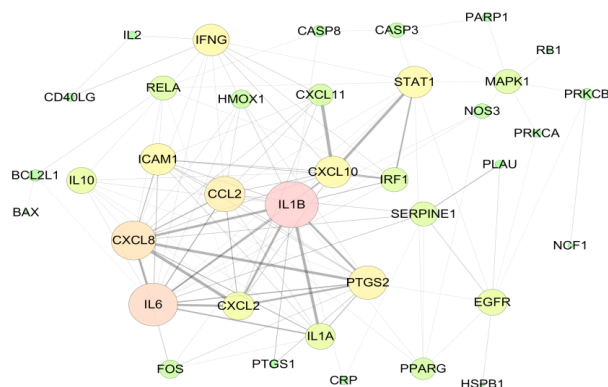


Figure 2 The Core Protein-Protein Interaction Network of the Pien Tze Huang Capsule

Bioinformatics analysis

STRING was used to perform functional annotation analysis (GO analysis) on the relevant target proteins, including cellular components, molecular function, and biological process. The results were illustrated by selecting the top 10 GO terms with the lowest *P* values (Figure 3). It was found that these proteins were mainly involved in the following biological processes: response to stimulus, biological regulation, and cellular process; the following molecular functions: binding, protein binding, and signaling receptor binding; and the following cellular components: cytoplasmic part, intracellular organelle part, and extracellular region. It was inferred that the efficacy of

PTHC, including clearing heat and detoxifying, cooling the blood and removing stasis, and eliminating phlegm and diminishing swelling were possibly related to the functional processes listed above.

Pathway analysis

KEGG signaling pathway enrichment analysis ($P \leq 0.05$) was performed on the 41 screened targets using the R language (ClusterProfiler package). The screening yielded 14 signaling pathways, involving: the nuclear factor-kappa B (NF- κ B) signaling pathway, cytokine-cytokine receptor interactions, the hypoxia-inducible factor (HIF)-1 signaling pathway, the chemokine signaling pathway, and the MAPK signaling pathway.

Discussion

From the perspective of TCM, COVID-19 is a disease that develops as a result of a mix of unseasonal qi and evil toxic dampness. The virus along with dampness enters the body through the mouth and nose, disturbing the functional activities of the upper and middle jiao (two types of Triple Burner) and leading to exhaustion and stasis [3]. Patients may have symptoms such as weakness, soreness, coughing, phlegm and chest tightness, shortness of breath, poor appetite, nausea, vomiting, scalloped tongue or tongue with light redness, and a thick-white-greasy or a white-greasy tongue coating. Meanwhile, the feedback mechanism of COVID-19 patients promotes the release of a large number of inflammatory factors, such as tumor necrosis factor (TNF), interleukins, and interferon (IFN), resulting in “cytokine storm syndrome” [4]. In clinical practice, PTH is used for the treatment of viral hepatitis, Yong Ju (carbuncle), Ding Chuang (deep-seated sore), and various inflammatory diseases [5]. Among the various components of PTH, niu-huang is cool in nature and sweet in flavor, with the effects of reducing phlegm for resuscitation and orifice opening, detoxifying, and clearing liver heat. Musk, warm and pungent, has swelling-reducing, pain-relieving, brain-activating, and orifice-opening effects. It can promote blood circulation and has antibacterial and anti-inflammatory effects [6]. Tianqi, or Sanqi, is sweet and slightly bitter in flavor and warm in nature. Modern studies have confirmed that *Panax notoginseng* polysaccharides have immunity boosting effects. Sanqi flavonoids have significant effects such as coronary blood flow increasing, cell proliferation suppressing, anti-inflammation, and anti-viral activities [7]. Shedan, cold and bitter in nature, can clear heat and detoxify, remove phlegm and suppress spasms; it is often used in treating pertussis, bronchitis, cough,

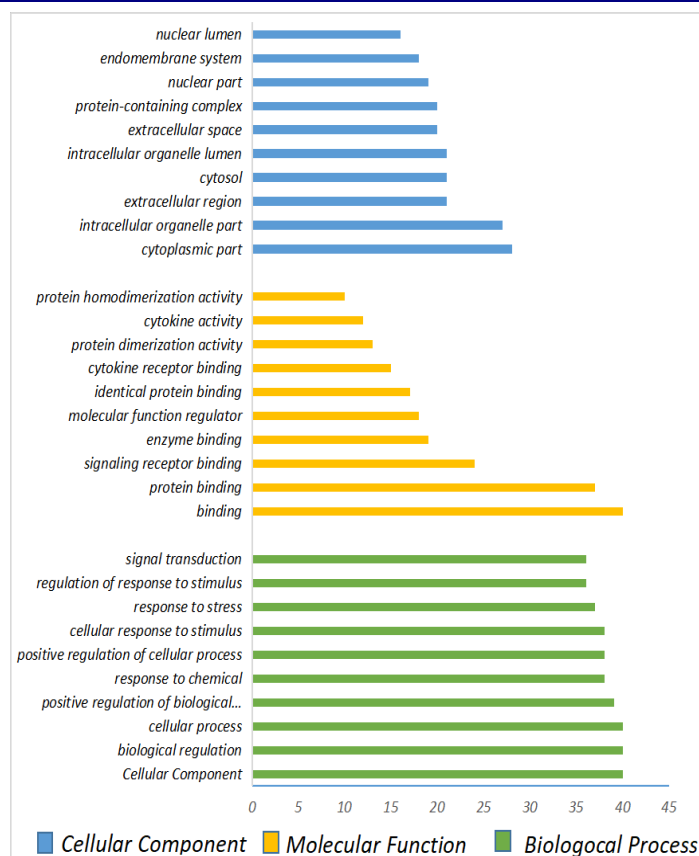


Figure 3 Gene Ontology Enrichment Analysis of the Relevant Protein Targets of Pien Tze Huang Capsules

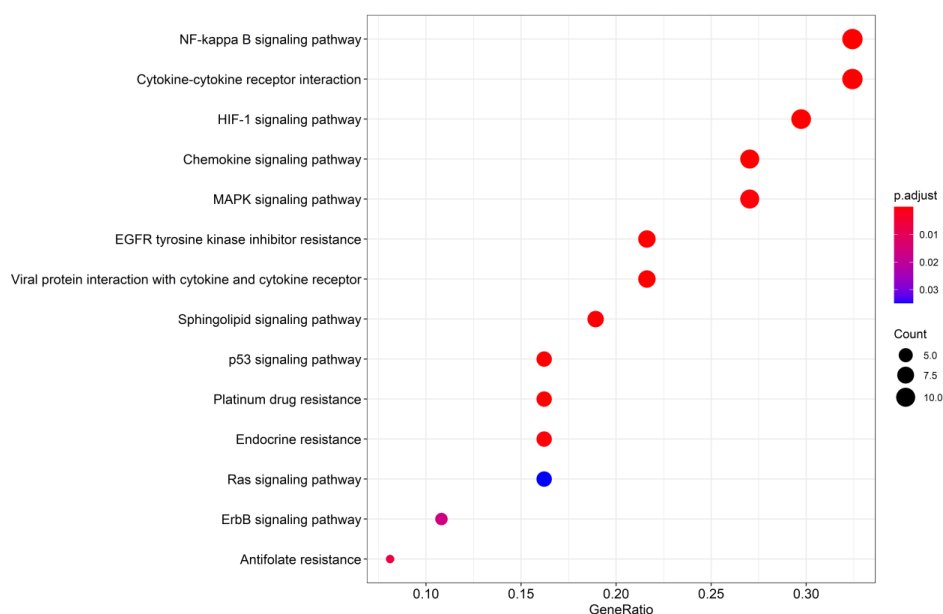


Figure 4 The Bubble Chart of the Kyoto Encyclopedia of Genes and Genomes Enrichment of the Effective Ingredients of Pien Tze Huang Capsules

and asthma with phlegm. Prescribed with other drugs, it has the effects of clearing heat and removing toxins, cooling blood and removing stasis, and reducing swelling and eliminating phlegm. According to the analysis of its known drug ingredients, PTHC might have the function of regulating inflammatory factors,

thus alleviating the occurrence of cytokine storm syndrome.

This study obtained the following results through GO and KEGG enrichment analyses. Through targets including IL-1 β , RELA, and CXCL8, the NF- κ B pathway is involved in the gene regulation of various

cytokines and chemokines, such as IL-8, IL-6, intercellular adhesion molecule-1 (ICAM-1), and TNF- α . Chemokines induce chemotaxis of the target cells, activating leukocytes to sites of inflammation or injury. Both play an important role in the non-specific immune regulation of the patient's body [8]. AMP-activated protein kinase (AMPK) indirectly regulates NF- κ B activity by regulating downstream proteins, such as sirtuin 1, p53, IL-1 β , and IL-6. Thereby, it further suppresses inflammatory factor expression and achieves anti-inflammatory and anti-fibrotic effects as well as improves the inflammatory response and pulmonary fibrosis in patients [9]. Activation of the AMPK signaling pathway protects the liver and improves liver function by improving mitochondrial function and reducing intrahepatic lipogenesis [10].

In addition, the expression of HIF-1 is detected in inflammatory diseases, such as immune inflammation and viral infections [11]. Studies have shown that HIF-1 engages in the regulation of cell growth, proliferation, migration, and apoptosis through MAPK1, IL-6, and RELA. NF- κ B promotes the function of HIF-1 α in inflammation [12] by positively regulating HIF-1 α downstream, to modulate the survival of immune cells. Studies have also shown that depression is positively correlated with the serum cytokines C-C motif chemokine ligand 11 (CCL11) and CXCL10. Therefore, NF- κ B may also help with mood disorders in COVID-19 patient [13].

In summary, through the abovementioned targets and the NF- κ B, AMPK, and HIF-1 signaling pathways, and with cytokines such as IL-6 and IL-10 as intermediates, PTHCs suppress the expression of inflammatory factors and provide adjuvant treatment for COVID-19 by regulating immune function, improving liver function, and regulating mood. This study elaborated the underlying mechanism of PTHC at the molecular level, corresponding to the efficacy of PTHCs in clearing heat, removing toxins, activating blood circulation and diminishing swelling, which provides a theoretical reference for the clinical use of Chinese medicine to treat COVID-19.

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