Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Biomedicine & Pharmacotherapy 128 (2020) 110316

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Analysis of the molecular mechanism of Pudilan (PDL) treatment for COVID-19 by network pharmacology tools

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ARTICLE INFO

Keywords:
SARS-CoV-2 infection
COVID-19
Traditional Chinese herbs
Targeted therapy
Network pharmacology

ABSTRACT

Background: Pudilan (PDL), a four-herb prescription with the traditional function of heat-clearing and detoxifying, has been clinically used as an anti-SARS-CoV-2 infectory agent in China. PDL might also have therapeutic potentials for COVID-19 while the underlying mechanisms remain to be clarified.

Methods: We used network pharmacology analysis and selected 68 co-targeted genes/proteins as targets of both PDL and COVID-19. These co-targeted genes/proteins were predicted by SwissDock Server for their high-precision docking simulation, and analyzed by STRING for proteins to protein interaction (PPI), pathway and GO (gene ontology) enrichment. The therapeutic effect for PDL treatment on COVID-19 was validated by the TCMATCOV (TCM Anti COVID-19) platform.

Results: PDL might prevent the entrance of SARS-CoV-2 entry into cells by blocking the angiotensin-converting enzyme 2 (ACE2). It might inhibit the cytokine storm by affecting C-reactive protein (CRP), interferon-γ (IFN-γ), interleukin- 6 (IL-6), interleukin- 10 (IL-10), tumor necrosis factor (TNF), epidermal growth factor receptor (EGFR), C-C motif chemokine ligand 5 (CCL5), transforming growth factor-β1 (TGFβ1), and other proteins. PDL might moderate the immune system to shorten the course of the disease, delay disease progression, and reduce the mortality rate.

Conclusion: PDL might have a therapeutic effect on COVID-19 through three aspects, including the moderate immune system, anti-inflammation, and anti-virus entry into cells.

1. Introduction

Since the outbreak of the 2019 novel coronavirus disease (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread to the whole world with nearly 4.9 million diagnosed patients and caused more than 320 thousand deaths (updated 20 May 2020). Unfortunately, few effective drugs were available for treating COVID-19 patients.

After the four-months of combating COVID-19, China has accumulated a lot of experience and lessons in preventive and therapeutic aspects. The Chinese government and medical scientists recommended some drugs that are potentially useful for COVID-19 treatment. Among them, several traditional Chinese medicine (TCM) prescriptions are included [1]. More than 85 % of SARS-CoV-2 infected patients had received TCM treatment in China [2].

TCM, a traditional medical system, has more than two thousand years of clinical practice. Compared with modern medicine, the herb-based TCM shows several advantages, including significant curative effects, few side-effects, and low cost. Clinical practice showed that early intervention by TCM is a practical medical way to improve the cure rate, shorten the disease course, delay the disease progression, and reduce the mortality rate [3,4]. However, the underlying mechanisms remain unclear mainly due to the complicated ingredients of TCM. The proposed mechanisms include blocking the SARS-CoV-2 infection, balance the physiological activity, regulation of the immune response, inhibition of the inflammatory storm, and promoting patient recovery [3].

Pudilan (PDL) is a four-herb prescription that includes Pu Gong Ying

Abbreviations: ACE2, angiotensin I converting enzyme 2; ARDS, acute respiratory distress syndrome; BXTM, banxia tianma baizhu tang (TCM herbs); COVID-19, 2019 novel coronavirus disease; LHQW, lian hua qing wen (TCM herbs); HSZF, hanshi zufei fang (TCM herbs); PDL, Pudilan (TCM herbs); SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFJD, shufeng jiedu fang (TCM herbs); TCM, traditional Chinese medicine

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https://doi.org/10.1016/j.biopha.2020.110316
Received 1 May 2020; Received in revised form 21 May 2020; Accepted 22 May 2020

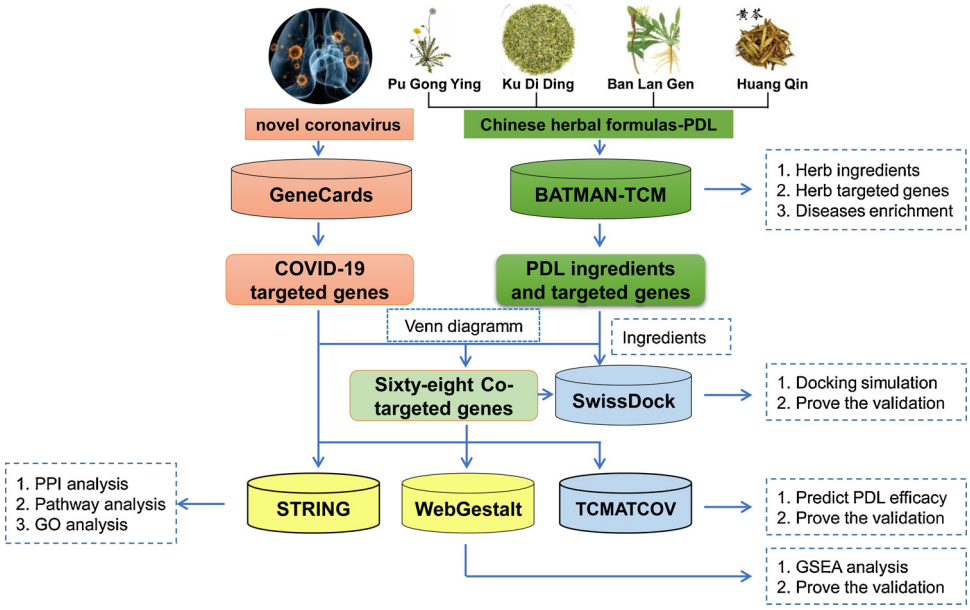

Fig. 1. The flow chart of this whole analysis for this study.

(*Taraxacum mongolicum* Hand.-Mazz, Mongolian dandelion), Ku Di Ding (*Corydalis bungeana* Turcz., Bunge corydalis), Ban Lan Gen (*Isatis indigotica* Fort., Indigowoad root), and Huang Qin (*Scutellaria baicalensis* Georgi., Baikal skullcap). PDL has three pharmaceutical forms in China, that are Pudilan Xiaoyan tablet, Pudilan Xiaoyan capsule, and Pudilan Xiaoyan oral liquid. The traditional functions of PDL are Qingre Jiedu (heat-clearing and detoxifying) and Kangyan Xiaozhong (anti-inflammatory and reduce swelling).

PDL was recorded in the Chinese Pharmacopoeia (2015 Edition) and has been recommended as a preferred drug for the prevention and treatment of H1N1 and hand, foot, and mouth disease (HFMD). PDL is also useful in the treatment of COVID-19 and is recommended for SARS-CoV-2 infection in children [5]. Our experimental studies using hACE2 mice and Vero E6 cells revealed that PDL oral liquid has a therapeutic effect against SARS-CoV-2 by anti-virus, anti-inflammatory, and moderate immunity [6].

To explore the molecular mechanism for PDL against COVID-19, we tried to integrate the bioinformatics and network pharmacology tools to predict the target genes and proteins and to analyze the interactions between PDL ingredients with the targeted genes.

2. Methods

2.1. Ingredients targeted genes and functional analysis

The query four herbs of PDL were first transferred into a list of compositive ingredients/ingredients based on the formula-herb-ingredient association data collected and integrated by the TCMID (Traditional Chinese Medicine Integrated Database) database (http://www.megabionet.org/tcmid) [7].

For each ingredient, candidate targets were predicted based on the target prediction method of BATMAN-TCM (Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine, http://bionet.ncpsb.org/batman-tcm), which is a bioinformatics tool used for analyzing the molecular mechanism of TCMs by predicting the potential targets of the ingredients of TCMs, and then performing functional analyses on these targets including known ingredient-target interactions, protein interaction networks, and KEGG pathway data [8].

2.2. Disease-associated gene mining

GeneCards (https://www.genecards.org) provides gene-centric information that is automatically mined and integrated from myriad data sources, resulting in the web-based card for COVID-19 disease targeted genes by searching the “novel coronavirus” in GeneCards and obtained a list of COVID-19-targeted genes [9].

2.3. PPI and GSEA enrichment analysis

With STRING (https://string-db.org), we analyzed the co-targeted proteins that are encoded by COVID-19-associated genes that interact with PDL ingredient-targeted genes to explore their relationship within a PPI network, GO, and Reactome pathway analysis [10]. WebGestalt (http://www.webgestalt.org) was used as the enrichment method for COVID-19 and PDL co-targeted GSEA [11]. The Reactome Knowledgebase (https://reactome.org) provides molecular details of pathways and reactions in human biology. We used Reactome to draw two pathways that COVID-19 and PDL co-targeted gene set enriched [12]. With pathway builder tool 2.0, we simulated the possible ways for PDL treatment on COVID-19.

2.4. Classic anti−COVID-19 prescription validation

TCM Anti COVID-19 (http://tcmatcov.bbtcml.com, TCMATCOV) was a platform to predict the efficacy of the anti-coronavirus pneumonia effect of TCM. TCMATCOV is based on the interaction network imitating the disease network of COVID-19 [13]. TCMATCOV utilizes a quantitative evaluation algorithm to analyze disease network disturbance after multitarget drug attacks to predict potential drug effects. Based on the TCMATCOV platform, PDL was calculated and predicted to have a high disturbance score and to account for a high proportion of the classic anti−COVID-19 prescriptions used by clinicians.

2.5. Study design

The steps used in the entire analysis performed in this study are shown in Fig. 1. COVID-19 disease targeted genes/proteins were mined by GeneCards. The PDL ingredients were identified targeted by TCMID and their targeted genes/proteins and pathways were identified by BATMAN-TCM. These co-targeted genes/proteins were enriched by

STRING, WebGestalt, and predicted by SwissDock, and TCMATCOV.

2.6. Statistical methods

All analyses were performed with the default values for each of the tools used. Continuous variables were commonly described as the median and range. The cutoff of the FDR value was set as 0.01. Only the predicted candidate target proteins with scores > = 20 are presented in the query results of BATMAN-TCM. All reported P values are two-tailed, and $P < 0.01$ was considered statistically significant.

3. Results

3.1. PDL ingredients targeted genes and functional analysis

The PDL ingredients were identified targeted by TCMID and their targeted genes/proteins and pathways were identified by BATMAN-TCM. PDL includes four kinds of herbs, which contain 181 ingredients. Among them, 67 ingredients have no structural information, and thus their targets could not be predicted. Finally, 114 ingredients were predicted to interact with 1281 targeted genes, and 64 ingredients had potential targets with scores larger than 20 (Supplementary Table S1). The results of the PDL ingredients targeted gene-disease enrichment analysis in TTD (Therapeutic Target Database) indicate that PDL might treat some respiratory system disease including asthma, chronic obstructive pulmonary disease (COPD), obstructive airway disease, and cough, which are closely related to COVID-19 (Table 1, $P < 0.01$, Enrich ratio < 1.5).

3.2. COVID-19 disease-associated gene targeted by PDL

COVID-19 disease targeted genes/proteins were mined by GeneCards. We searched for “Novel Coronavirus” in GeneCards and obtained 350 COVID-19 related genes with targeted scores (Supplementary Table S2). Several TCM herb prescriptions, including Lianhuaqingwen (LHQW), and Shufengjiedu (SFJD) were reported to be useful for the treatment of COVID-19, similar to PDL. We compared their targeted genes and the data are shown in Fig. 2A. The 68 co-targeted genes that were among both the PDL targeted genes and the COVID-19 disease-associated genes are shown in the Venn diagram of Fig. 2A and Fig. 2B. Sixty-eight genes were identified as the COVID-19, PDL, LHQW, SFJD co-targeted genes. These genes may be the hub genes involved in the therapeutic effects of PDL, LHQW, and SFJD on COVID-19.

Table 2 showed the top 10 target prediction results for COVID-19 disease-associated genes interaction with PDL ingredients with predicted scores. Among which, ACE2 is the receptor for SARS-CoV-2 entry into cells. TNF, SPIDR, IFN-γ, IL-6, TP53, CRP, EGFR, and CCL5 proteins play important roles in the pathogenic process of COVID-19. The result may explain the efficacy of PDL oral liquid therapy in COVID-19 patients.

Table 1
PDL ingredients targeted genes enrichment analysis in TTD related to COVID-19.

Term description	p-value	Enrich ratio
Asthma	$2.41e^{-03}$	1.8
Chronic Obstructive Pulmonary Disease (COPD)	$2.45e^{-03}$	3.8
Diabetes Mellitus Type 2	$7.02e^{-03}$	3.3
Inflammatory Bowel Disease	$7.44e^{-03}$	2.4
Dyspnea	$1.05e^{-02}$	4.6
Malignant Hyperthermia	$1.05e^{-02}$	4.6
Pulmonary Hypertension	$3.51e^{-02}$	3.4
Chronic Rhinitis	$4.80e^{-02}$	4.6
Obstructive Airway Disease	$4.80e^{-02}$	4.6
Cough	$4.80e^{-02}$	4.6

3.3. The association networks of PDL targeted functional proteins

Using STRING, we analyzed the interactions of 68 proteins that are COVID-19-associated genes interaction with PDL ingredient-targeted genes, and the multiple proteins to protein interaction (PPI) enrichment were obvious ($P < 1.0e^{-16}$) (Fig. 2B). Separate interaction scores are available as well as part of the underlying evidence. The interaction scores from STRING represent the expression of approximate confidence that the association is true given all the available evidence.

With PDL ingredient-targeted genes, we performed GO enrichment analysis. The GO enrichment analysis identified the cellular response to chemical stimulus (GO:0070887), regulation of biological quality (GO:0065008), regulation of cell death (GO:0010941), response to organic substances (GO:0010033), cellular response to organic substances (GO:0071310), and regulation of apoptotic process (GO:0042981), etc (Table 3). The major pathology of COVID-19 is viral pneumonia with pulmonary edema and patchy inflammatory cellular infiltration. The above biological processes or activities may infer in the pathogenic of COVID-19 and these pathological changes may be treated by PDL.

3.4. Prediction of PDL − COVID-19 disease treatment by TCMATCOV

With TCMATCOV, Fig. 2C showed the network of PDL ingredient-drug target-DEGs consists of ingredient-target relations (from BATMAN-TCM, confidence score ≥ 20), and drug target-disease protein relations (protein-protein interaction from the string, confidence score = 0.4). Fig. 2D is the enlarged part of the TCMATCOV network from Fig. 2C.

The influence of drug target on the topological characteristics of the disease network is used to evaluate the intervention effect of drugs on disease network constructed using COVID-19 based SARS transcriptome data. The cutoff of the protein-protein interaction confidence score was 0.4. The data showed that the PDL therapeutic effect on COVID-19 was very close to the positive control (HSZF), which had been reported to be useful in clinical (Table 4, $P = 0.0007$). We also validated the four herbs in PDL prescription by TCMATCOV platform, and the data showed that Ban Lan Gen, Ku Di Ding and Huang Qin are the more therapeutic herbs for the COVID-19 treatment than Pu Gong Ying (Table 4, $P = 0.0001$). The results were consistent with that in Table 2.

3.5. Reactome pathways enrichment and simulation diagrams

Using STRING, we also analyzed the PDL ingredient-targeted Reactome pathways enrichment. The results indicated that the pathways were enriched in cytokine signaling in the immune system, signaling by interleukins, the immune system, interleukin-4, and interleukin-13 signaling, signal transduction, and interleukin-10 signaling among other pathways (Table 5). These pathways are important in cytokine storms caused by COVID-19. With the Reactome knowledgebase, we draw the simulation diagrams for PDL treatment during SARS-CoV-2 infection in cytokine signaling in the immune system (HSA-1280215, Fig. 3A) and signaling by interleukins (HSA-449147, Fig. 3B), which showed the possible targets for PDL and SARS-CoV-2 with hit gene numbers and false discovery rate (FDR) scores. These simulation diagrams have vividly illustrated the mechanism of PDL treatment for COVID-19.

3.6. The GSEA enrichment of PDL − COVID-19 co-targeted genes

To make a GSEA pathway enrichment, we used WebGestalt as the enrichment tool with COVID-19 and PDL co-targeted genes with scores for GSEA enrichment. The GSEA enrichment results are shown in Fig. 4A–B and the gene set enrichment plots with P values and enrichment scores were listed in Fig. 4C. As the results showed, the 68 PDL − COVID-19 co-targeted genes were enriched. Ten positively related categories were identified, including tuberculosis, human

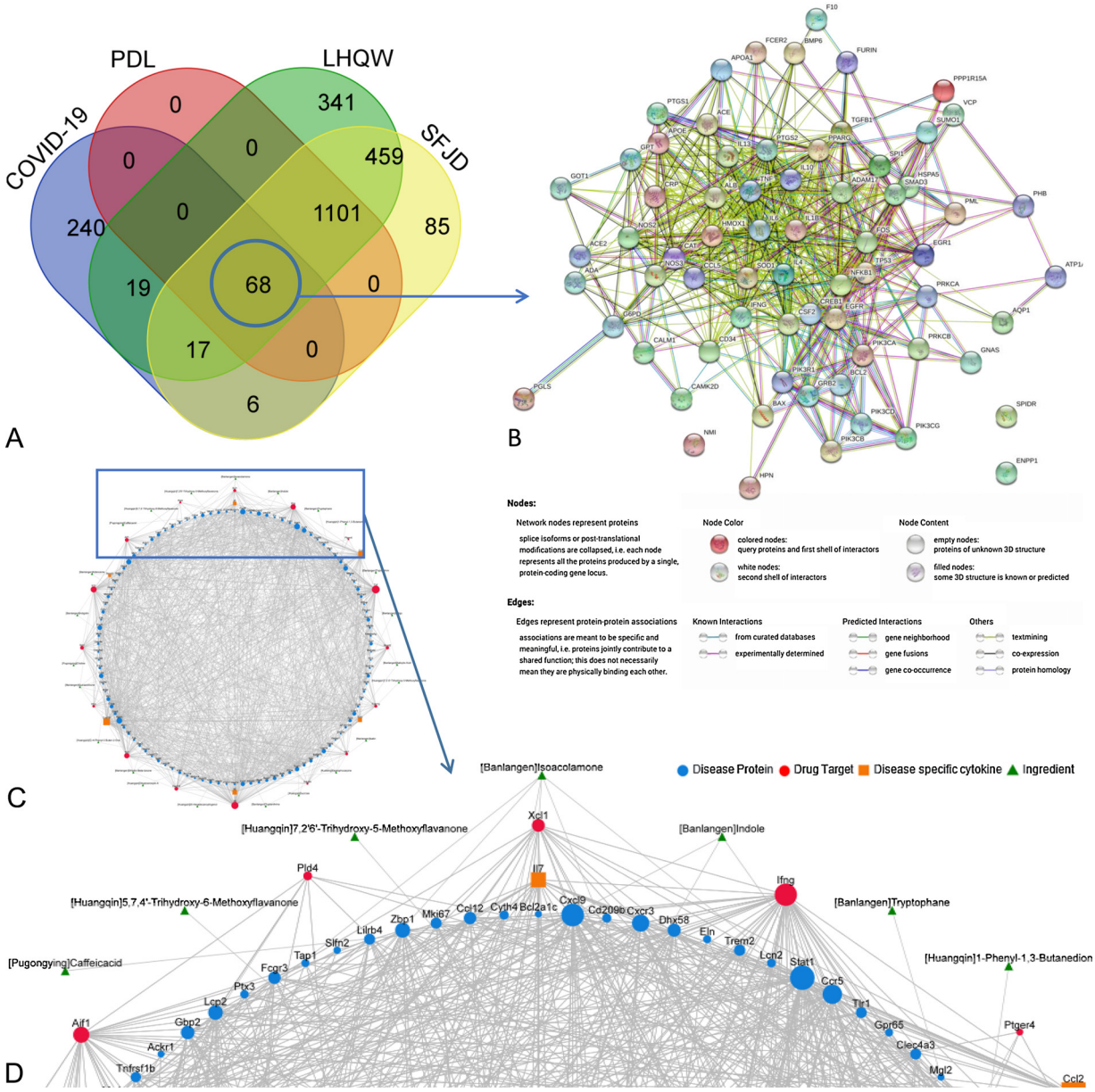

Fig. 2. An association network of PDL targeted proteins associated with COVID-19. (A) Venn diagram of COVID-19, and TCM herbs of PDL, LHQW, SFJD targeted genes; (B) The network for 68 co-targeted genes/proteins had been selected as input for PPI analysis in STRING. Their size is proportional to the enrichment measure (PPI enrichment p-value < 1.0e-16) provided by STRING; (C) TCMATCOV network of ingredient-drug target-DEGs, that consists of ingredient-target relations (from BATMAN-TCM, confidence score 20), and drug target-disease protein relations (protein-protein interaction from the string, confidence score 0.4); (D) Enlarged part of TCMATCOV network from Fig. 2C.

cytomegalovirus infection, C-type lectin receptor signaling pathway, and Influenza A. Four negatively related categories were also identified, including cholinergic synapse, inflammatory mediator regulation of TRP channels, cAMP signaling pathway, and metabolic pathways.

3.7. Molecular docking

CRP, IL-6, IL-10, and TNF-α were remarkably higher in severe cases than in moderate cases of COVID-19 [14]. We selected 6 more potential PDL and COVID-19 co-targeted proteins with ingredients for molecular docking using the SwissDock server. The data show these PDL ingredients are well docking with PDL and COVID-19 co-targeted proteins (Fig. 5A–F). Among them, IL-6 is an important factor elevated during the pathology of COVID-19 with a cytokine storm [15]. The percentage of IFN-γ producing $CD4^+$ T cells and $CD8^+$ T cells was increased in severe patients of COVID-19 [16]. Among the PDL ingredients, quinazolinone, and oxysophocarpine may be useful in the treatment of COVID-19. These results can prove that PDL ingredients work with COVID-19 targeted proteins in molecular docking simulation. The results may serve as the validation of the activity of the single substance components of the herb mixture.

4. Discussion

Our previous study analyzed the importance of ACE2 and TMPRSS2 in the susceptibility of SARS-CoV-2 infection [17].Other reports also supposed that integrins [18] and CD147 [19] might be the potential receptors of SARS-CoV-2, and integrins were targeted as the COVID-19 targeted genes, but they were not predicted in PDL−COVID-19 co-targeted genes. Therefore, PDL might have not effect on integrins and

Table 2
Target prediction result for COVID-19 disease-associated genes interaction with PDL ingredients with predicted scores (top10).

Co-targeted genes	Gene description	Disease relevance score	Predicted ingredients (score)	TCM Herbs
ACE2	Angiotensin I Converting Enzyme 2	28.74	(E)-4-Phenyl-3-Buten-2-One(22.373)	Huang qin
			Indigotin(22.373);Indigo(22.373); Tryptanthrine(22.373)	Ban lan gen
TNF	Tumor Necrosis Factor	17.68	Isoacolamone(22.373);Adenosine(22.373);Quinazolinone(80.882);Salicylic Acid (22.373);Dihydro-Beta-Ionone(22.373)	Ban lan gen
			Oxysophocarpine(22.373)	Ku di ding
			Sucrose (48.000)	Huang qin
SPIDR	Scaffold Protein Involved In DNA Repair	17.5	Indole(22.373)	Ban lan gen
IFN-γ	Interferon Gamma	14.91	Quinazolinone(22.373);Salicylic Acid(23.000)	Ban lan gen
			Sucrose (48.000)	Huang qin
			Caffeicacid (23.000)	Pu gong ying
IL-6	Interleukin 6	14.2	Quinazolinone(22.373)	Ban lan gen
TP53	Tumor Protein P53	11.8	Isoacolamone(22.373);Salicylic Acid(48.000); Dihydro-Beta-Ionone(22.373)	Ban lan gen
			Oxysophocarpine(22.373)	Ku di ding
CRP	C-Reactive Protein	9.83	Isoacolamone(22.373);Gamma-Aminobutyric Acid(22.373);Adenosine (22.373);Quinazolinone(22.373);Dihydro-Beta-Ionone(22.373)	Ban lan gen
			Choline(22.373)	Pu gong ying
			Oxysophocarpine(22.373)	Ku di ding
EGFR	Epidermal Growth Factor Receptor	9.24	Indole(22.373);Indigotin(22.373);Indigo(22.373);Tryptanthrine (22.373);Tryptanthrin(22.373)	Ban lan gen
			(E)-4-Phenyl-3-Buten-2-One(22.373)	Huang qin
CCL5	C-C Motif Chemokine Ligand 5	8.43	Indigotin(22.373);Indigo(22.373); Tryptanthrine(22.373)	Ban lan gen
			(E)-4-Phenyl-3-Buten-2-One (22.373)	Huang qin
IL-1β	Interleukin 1β	5.41	Salicylic Acid(55.444); Isaindigodione(22.373); Quinazolinone(22.373)	Ban lan gen
			Stigmasterol(22.373);Nothosmyrnol(22.373)	Huang qin

Table 3
PDL and COVID-19 co-targeted genes ontology (GO) enrichment analysis of the biological process (top10).

GO-term	Description	PDL and COVID-19 co- targeted 68 proteins (FDR)	COVID-19 350 proteins (FDR)
GO:0070887	cellular response to chemical stimulus	$1.12e^{-30}$	$1.24e^{-81}$
GO:0065008	regulation of biological quality	$1.12e^{-30}$	$2.30e^{-37}$
GO:0010941	regulation of cell death	$9.13e^{-29}$	$1.06e^{-43}$
GO:0010033	response to organic substance	$1.70e^{-28}$	$1.01e^{-74}$
GO:0071310	cellular response to organic substance	$2.27e^{-28}$	$4.38e^{-75}$
GO:0042981	regulation of apoptotic process	$5.12e^{-28}$	$4.65e^{-42}$
GO:0006950	response to stress	$5.12e^{-28}$	$5.61e^{-81}$
GO:0042221	response to chemical	$7.51e^{-28}$	$2.58e^{-71}$
GO:0048583	regulation of response to stimulus	$7.55e^{-27}$	$8.78e^{-63}$
GO:0009893	positive regulation of metabolic process	$7.55e^{-27}$	$2.23e^{-37}$

FDR: false discovery rate.

CD147.

PDL, a famous TCM formula recorded in Chinese Pharmacopeia, is widely prescribed for the treatment of acute and chronic inflammation. The reported side effects of PDL include gastrointestinal symptoms and allergic reactions. PDL oral liquid alleviates LPS-induced respiratory injury by decreasing nitroxidative stress and blocking toll-like receptor 4 (TLR4) activation along with nuclear factor kappa B (NF-κB) phosphorylation in mice [20,21], and reduces the levels of pro-inflammatory mediators including IL-10, TNF-α, and NF-κB in serum [22].

Pudilan (PDL) is a four-herb prescription, among which Pu Gong Ying could alleviate inflammatory injury by inhibiting phosphorylation of NF-κB and TLR4/NF-κB signal pathway [23]. Ku Di Ding could inhibit the protein expression of iNOS, TNF-α, IL-6 and IL-1β in vitro and in vivo [24]. Ban Lan Gen could dose-dependently inhibited cleavage activity of the 3C-like protease (3CLpro) of SARS-coronavirus [25]. Baicalin is a bioactive flavone extracted from the Huang Qin was predicted to inhibit the activity of SARS-CoV-2 [26]. The study of the

Table 4
PDL (herbs) and related TCM prescriptions validation results by TCMATCOV platform.

TCM herbs	Sum score	Average Degree	Average shortest path	Degree centrality	Closeness centrality
Negative Control (BXTM)	12.59	−1.84	3.53	−0.76	−6.46
Positive Control (HSZF)	20.85	−4.09	9.01	−1.12	−6.63
LHQW	24.13	−4.63	11.73	−1.32	−6.45
SFJD	23.35	−4.76	10.85	−1.30	−6.44
PDL	18.67	−4.83	6.37	−1.15	−6.32
Ban Lan Gen (herb)	18.97	−5.4	5.87	−1.28	−6.43
Ku Di Ding (herb)	17.61	−3.97	3.87	−3.68	−6.1
Huang Qin (herb)	16.79	−4.38	2.19	−4.28	−5.94
Pu Gong Ying (herb)	3.99	−0.31	−1.64	0.57	−5.89

Note: BXTM: Ban Xia Tian Ma Bai Zhu Tang; HSZF: Han Shi Zu Fei Fang.

Table 5
PDL and COVID-19 co-targeted genes Reactome pathways enrichment analysis(top10).

Pathway	Description	PDL and COVID-19 co- targeted 68 proteins (FDR)	COVID-19 350 proteins (FDR)
HSA-1280215	Cytokine signaling in immune system	$1.45e^{-24}$	$1.18e^{-74}$
HSA-449147	Signalling by interleukins	$1.55e^{-22}$	$1.54e^{-51}$
HSA-168256	Immune system	$1.36e^{-18}$	$1.35e^{-74}$
HSA-6785807	Interleukin-4 and interleukin-13 signalling	$1.58e^{-17}$	$7.62e^{-25}$
HSA-162582	Signal transduction	$1.39e^{-12}$	$1.14e^{-19}$
HSA-6783783	Interleukin-10 signalling	$1.15e^{-09}$	$4.70e_{-18}$
HSA-109582	Hemostasis	$2.21e^{-09}$	$2.64e^{-24}$
HSA-76002	Platelet activation, signalling and aggregation	$9.85e^{-09}$	$4.83e^{-22}$
HSA-9006925	Intracellular signaling by second messengers	$1.85e^{-08}$	$1.30e^{-10}$
HSA-9027276	Erythropoietin activates Phosphoinositide-3-kinase (PI3K)	$1.68e^{-07}$	$5.34e^{-05}$

FDR: false discovery rate.

molecular mechanism for PDL and COVID-19 interactions has contributed extensively to the understanding of PDL therapeutic effect on COVID-19 including inflammatory cytokines.

Acute respiratory distress syndrome (ARDS) with cytokine storms might be the main cause of death due to COVID-19. Many inflammatory cytokines (IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-α, and TGFβ) and chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, and CXCL10) were detected in COVID-19 patients [27]. Human coronaviruses (HCoVs) may modulate various cellular processes, such as apoptosis, innate immunity, mitogen-activated protein kinase (MAPK) pathway, and nuclear factor kappa B (NF-κB) pathway [28]. When the host immune system is exposed to viral pathogens, it reacts straightaway by triggering a diverse array of defense mechanisms to establish a more efficacious shield, as characterized by the increased production of type I interferons (IFN-α and IFN-β) and other inflammatory cytokines. The cytokine family of interferons is dedicated

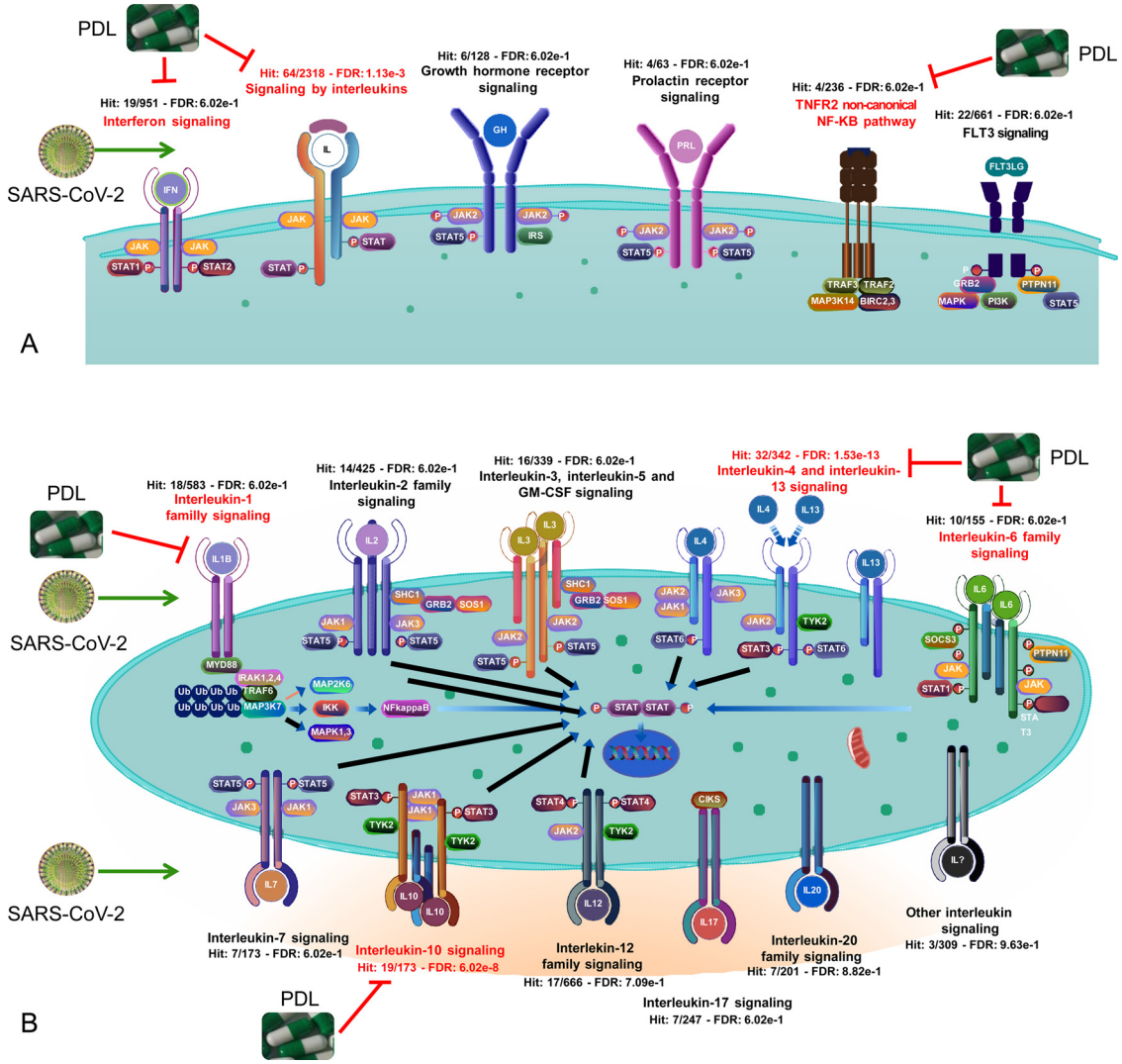

Fig. 3. Simulation diagram for PDL treatment during SARS-CoV-2 infection. (A) PDL treatment in cytokine signaling in the immune system (HSA-1280215); (B) PDL treatment in signaling by interleukins (HSA-449147).

Acknowledgment: These pictures were drawn based on the database of Reactome.

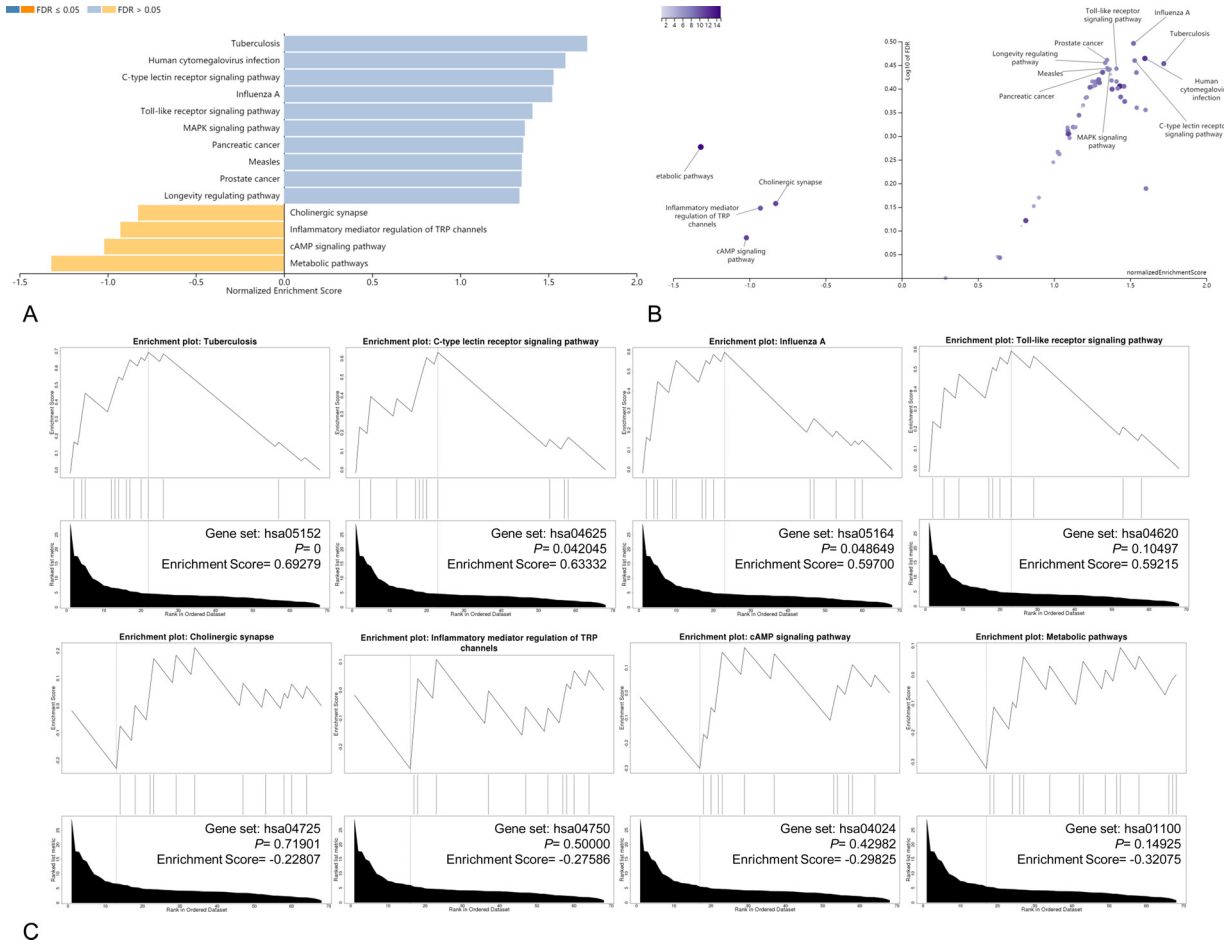

Fig. 4. The GESA enrichment results for 68 co-targeted genes/proteins with scores had been selected as input for WebGestalt analysis. (A) The GSEA enrichment results in bar chart; (B) The GSEA enrichment results in volcano plot; (C) The GESA enrichment plots of eight enriched gene sets in Fig. 4A-B.

to the conveyance of the presence of infection [29].

As reported, anti-inflammatory drugs (such as hormones and other molecules), and TCM (such as LHQW capsules and SFJD capsule), are the drug treatment options for COVID-19 [3,30]. Based on the beneficial effects of clinical practices in treating COVID-19 patients, some TCM prescriptions are on clinical trials against COVID-19 in China (www.chictr.org.cn/), including LHQW, Re Du Ning injection, Shen Fu injection, etc.. The reported clinical evidence has shown the beneficial effect of TCM on the treatment of COVID-19 patients in China [4].

LHQW significantly inhibited SARS-CoV-2 replication in Vero E6 cells and remarkably reduced pro-inflammatory cytokine (TNF-α, IL-6, CCL-2/MCP-1, and CXCL-10/IP-10) expression at the mRNA level [30]. A recent report indicated that these herbal products could markedly relieve major symptoms such as fever and cough and could promote the recovery. For example, Shen Fu injection inhibited the lung inflammation and decrease the levels of IL-1β, IL-6, and other cytokines [4]. Re Du Ning injection markedly reduced the levels of IL-1β, TNF-α, IL-8, and IL-10 in acute lung injury in a rat model [2].

PDL was recommended in the treatment for COVID-19, due to its anti-inflammation effects, its capability to reduce fever and to clear the infection, especially in children [5,31]. PDL also exhibited potential treatment for COVID-19 and produced good outcomes in the hACE2 mouse model and Vero cells with SARS-CoV-2 infection [6].

In the network pharmacology analysis, 68 co-targeted genes/proteins were selected as targets of both PDL and COVID-19. PDL works efficiently to block SARS-CoV-2 entry into cells by blocking the ACE2 protein.

Sixty-eight genes were identified as COVID-19, PDL, LHQW, and SFJD co-targeted genes, including ACE2, TNF, IFN-γ, IL-6, TP53, CRP, EGFR, CCL5, IL-10, TGFβ1, BCL2, HSPA5, BAX, IL-1β, PIK3CA, and other genes. Many of these genes were inferred to be involved in the ARDS and cytokine storms. PDL may attenuate cytokine storms by affecting TNF, IFN-γ, IL-6, CRP, EGFR, CCL5, IL-10, TGFβ1, and other genes. These genes may be the hub genes involved in the effects of PDL, LHQW, and SFJD on COVID-19.

5. Conclusions

In conclusion, our study showed that PDL, a TCM formula, might be useful in the treatment of COVID-19 through regulating and targeting many cytokines and chemokines. PDL could balance the physiological activity, regulate the immune response, inhibit the inflammatory storm in animal and cell experiments. However, these potential targets predicted by bioinformatic and network pharmacology tools need further investigation to confirm.

Authors contribution

Qi Kong designed the project and drafted the manuscript. Xiuping Chen attended to design the project, reviewed the manuscript, and provided comments and suggestions. Other authors were involved in data analysis and interpretation.

Funding information

This work was supported by the CAMS Initiative for Innovative

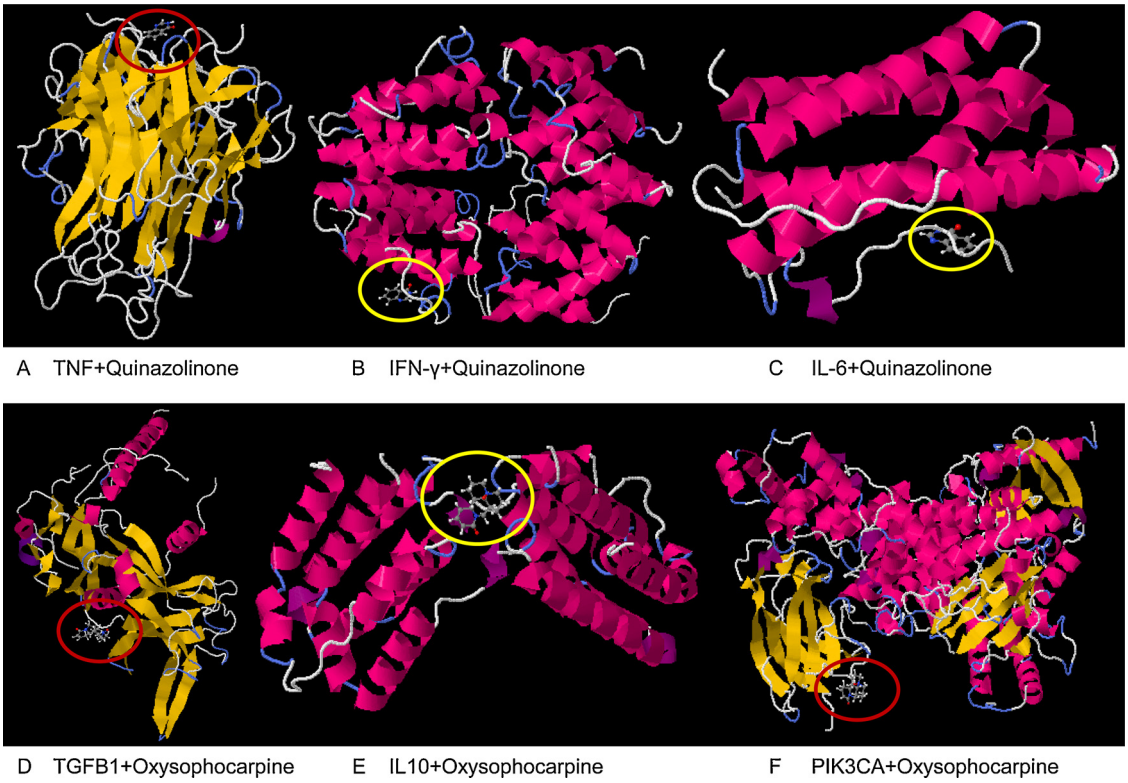

Fig. 5. PDL ingredients and COVID-19 co-targeted proteins molecular docking by the SwissDock server and the docking positions were circled. (A) Molecular docking simulation for TNF protein with quinazolinone (in red circle); (B) Molecular docking simulation for IFN-γ protein with quinazolinone (in yellow circle); (C) Molecular docking simulation for IL-6 protein with quinazolinone (in yellow circle); (D) Molecular docking simulation for TGFB1 protein with oxysophocarpine (in red circle); (E) Molecular docking simulation for IL10 protein with oxysophocarpine (in yellow circle); (F) Molecular docking simulation for PIK3CA protein with oxysophocarpine (in red circle). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Medicine of China (2016-I2M-2-006), and National Mega Projects of China for Major Infectious Diseases (2017ZX10304402).

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgment

We thank American Journal Experts (www.aje.com) for language standard editing.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2020.110316.

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