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Is acupuncture effective in the treatment of COVID-19 related symptoms? Based on bioinformatics/network topology strategy

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Abstract

Acupuncture is an important part of Chinese medicine that has been widely used in the treatment of inflammatory diseases. During the coronavirus disease 2019 (COVID-19) epidemic, acupuncture has been used as a complementary treatment for COVID-19 in China. However, the underlying mechanism of acupuncture treatment of COVID-19 remains unclear. Based on bioinformatics/topology, this paper systematically revealed the multi-target mechanisms of acupuncture therapy for COVID-19 through text mining, bioinformatics, network topology, etc. Two active compounds produced after acupuncture and 180 protein targets were identified. A total of 522 Gene Ontology terms related to acupuncture for COVID-19 were identified, and 61 pathways were screened based on the Kyoto Encyclopedia of Genes and Genomes. Our findings suggested that acupuncture treatment of COVID-19 was associated with suppression of inflammatory stress, improving immunity and regulating nervous system function, including activation of neuroactive ligand–receptor interaction, calcium signaling pathway, cancer pathway, viral carcinogenesis, *Staphylococcus aureus* infection, etc. The study also found that acupuncture may have additional benefits for COVID-19 patients with cancer, cardiovascular disease and obesity. Our study revealed for the first time the multiple synergistic mechanisms of acupuncture on COVID-19. Acupuncture may play an active role in the treatment of COVID-19 and deserves further promotion and application. These results may help to solve this pressing problem currently facing the world.

Key words: acupuncture; interventions; COVID-19; bioinformatics; network topology

Introduction

As of 16 February 2021, the global coronavirus disease 2019 (COVID-19) pandemic has caused more than 100 million infections and 2.4 million deaths [32]. The clinical manifestations of COVID-19 vary greatly, from asymptomatic or mild symptoms to the common cold, and even to severe acute respiratory distress syndrome, which may eventually lead to severe respiratory impairment [16]. The high contagiousness of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cause person-to-person transmission directly through respiratory droplets during sneezing or coughing or indirectly through contaminated surfaces [16]. Due to currently lack of effective drugs, a series of basic and clinical studies involving COVID-19 have been performed, aiming to explore and evaluate effective therapies against SARS-CoV-2 [16]. Notably, a large number of COVID-19 patients in China were integrative treated by Chinese and Western medicine [81]. In addition, during COVID-19 epidemic, traditional Chinese medicine (TCM) experts participated in the entire therapeutic process, and TCM was also included in the diagnostic and therapeutic guidelines of COVID-19 [61, 81]. Acupuncture, Chinese patent medicine, decoction and other characteristic therapy of TCM are also widely used to treat and prevent COVID-19 in China.

SARS-CoV-2 may cause fatal inflammatory responses and acute lung injury [23]. Hyperinflammatory symptoms are manifested in part of COVID-19 patients, which is similar to cytokine storm [62]. The dramatically increased content of pro-inflammatory cytokines (e.g. IL-1, IL-6, IL-8, IL-12) exacerbates disease progression [10, 20, 62]. Moreover, suppression of pro-inflammatory family members has been revealed to be involved in treating multiple types of inflammatory disorders, including viral infections [7, 10]. Acupuncture has been historically and widely used in all aspects of health, aiming to promote the physiological recovery of diseases, which has also been proven to be safe [59]. Acupuncture intervention has been demonstrated to positively relieve inflammatory responses by suppressing inflammatory cytokines (such as IL-6, IL-1β.TNF-α, etc.), activating cholinergic anti-inflammatory pathway and regulating immunity [42, 47, 64, 69, 86, 87]. In addition, electroacupuncture can notably decrease the levels of TNF-α and IL-1β in bronchoalveolar lavage fluid in animal models [87]. Similarly, another study showed that acupuncture may relieve chronic obstructive pulmonary disease (COPD)-triggered inflammatory response and subsequently protect lung function by attenuating cell inflammation and the production of TNF-α and IL-8 [42]. In addition, electroacupuncture can enhance the ratio of CD4 + T cells/CD8 + T cells in experimental autoimmune encephalitis rats and restore the balance of Th1/Th2/Th17/Treg Th cell subset responses [47]. Systematic reviews and/or meta-analysis have shown that acupuncture is effective for acute pancreatitis [82], COPD [70], osteoarthritis [66], etc. Based on the properties of acupuncture therapy, it is reasonable to conceive that acupuncture may be one of the complementary therapies for the effective management of COVID-19. Notably, the guideline for acupuncture and moxibustion intervention on COVID-19 (Second edition) has been recently proposed and issued by Chinese association of acupuncture and moxibustion [45]. A recent observational clinical study found the effectiveness of acupuncture combined with routine regimens in treating COVID-19, all 33 COVID-19 patients (including five patients with severe illness) were cured and discharged, with significantly relieved symptoms of chest distress, fatigue, anxiety, loss of appetite and insomnia [24]. No adverse reactions or complications were found, and no acupuncturists were infected. A number of randomized controlled trials (RCTs) are currently being conducted to treat COVID-19 patients with TCM, including acupuncture [80, 85]. Meanwhile, some systematic reviews and meta-analyses concerning efficacy and safety of acupuncture in treating COVID-19 are under way [30, 80, 85]. Functionally, acupuncture therapy may ease the negative emotions in patients, relieve symptoms such as cough, breathlessness, abdominal pain, nausea, vomiting and fatigue [30, 70, 80, 82, 85], playing a role in the prevention, treatment and rehabilitation of COVID-19. In particular, the meta-analyses of RCTs have shown that acupuncture is an effective adjunctive non-pharmacological treatment to improve symptoms in patients under medical treatment for respiratory disease, such as asthma and COPD [31, 70]. This also provides a basis for further study on the feasibility of acupuncture in the treatment of COVID-19 related symptoms. However, it remains largely unclarified of the therapeutic mechanism of acupuncture treatment against COVID-19.

The traditional drug discovery model of 'one-compound-one-target' has failed, and recently switched to the 'multi-target approach' for developing and designing agents to target various intracellular constituents and signaling pathways [67]. Bioinformatics approaches are playing essential roles in drug translational research in academia. The amount of data generated during all stages of drug discovery is increasing, and the application of these data for calculations can solve key challenges in this process [75]. Network topology has been widely adopted in multiple systems, such as physics, chemistry, biology etc., with great progress [58]. And biological networks are dynamic networks based on the signal interactions between biochemical reactions and active proteins [38]. These networks can hopefully provide deep insights into the mechanisms of understanding the organisms at the systematic level. With the development of system biology, pharmacology, network biology and formation of network pharmacology concept, it is based on the application of systemic biological and multi-omics technology [78]. Network pharmacology is widely used in drug discovery, mechanism research and target prediction, especially in TCM [5, 78]. Relevant studies have recently proved that acupuncture involves multiple systems of the human body and has multi-target therapeutic effects, therefore, it is urgent to introduce novel research ideas into the research of the potential mechanisms of acupuncture [41]. In this study, we aimed to reveal the molecular mechanisms of acupuncture against COVID-19 through bioinformatics, network topology and network pharmacology approach, in order to further deepen the current understanding of the therapeutic nature of acupuncture on COVID-19.

Methods

Collection of active ingredients produced by the human body after acupuncture treatment which may be effective for COVID-19

In this analysis, we systematically searched the following six databases: Web of Science, Embase, PubMed, CNKI, VIP and Wanfang Databases (the latest up-date was 31 December 2020) by searching the following terms: COVID-19, SARS-CoV-2, fever, cough, pneumonia, respiratory disease, inflammation, coronavirus, pain, acupuncture, body acupuncture, electroacupuncture, etc.

Eligible studies: (1) Acupuncture was considered the stimulation for acupuncture points that used the needle to pierce

the skin, with or without electric stimulation; studies using other forms of stimulation, including acupressure without needles, moxibustion, transcutaneous electrical nerve stimulation and laser acupuncture were excluded. (2) We included randomized controlled humans or animal trials, as well as non-randomized comparative trials (prospective and retrospective) and removed single-group observational studies that assessed outcomes prior to and following interventions. (3) Control interventions might take the form of placebo acupuncture, sham acupuncture, no treatment, another active treatment or medication; studies that only compared different forms of acupuncture were excluded; (4) The outcomes of interest were reported.

Two researchers independently searched the databases using prespecified inclusion and exclusion criteria, and chose proper literature in full-text edition. Researchers' controversies were addressed by negotiation. In case that no consensus was obtained, the third researcher solved the dispute.

By searching the above databases, we found that dopamine [69] and β-endorphin [8, 88] are the active compounds after acupuncture treatment, which may be effective for COVID-19. To be specific, COVID-19 can cause potent immune response and inflammatory storm [76]. Dopamine inhibits cytokine production through type 1 dopamine receptor, thereby suppressing systemic inflammation [69]. Another study has found that dopamine controls systemic inflammation by inhibiting NLRP3 inflammasome [77]. Animal studies have shown that electroacupuncture can activate the aromatic l-amino acid decarboxylase by inducing the vagus nerve, thereby producing dopamine in the adrenal medulla and controlling systemic inflammation [69]. Moreover, dopamine can functionally suppress the generation of cytokine through dopamine type 1 receptors, whose activation could inhibit systemic inflammation [69]. Another animal study has demonstrated that acupuncture can raise plasma level of dopamine in COPD rats, and the effect of acupuncture on lung function may be associated with decreased levels of inflammatory factors and increased dopamine level [25]. β-endorphin, one of the most prominent endogenous peptides, is irreplaceably involved in peripheral and central analgesia in the human body via three main mechanisms including three parts (peripheries, the supraspinal cord and the spinal cord) [49], which widely exists in the hypophysis cerebri and hypothalamus. According to the present epidemiological data, fever, cough and shortness of breath are considered as the most common symptoms of COVID-19 [74], along with relatively rare neurological complications. In terms of neurological complication, headache and myalgia are relatively common [4]. To manage pain, acupuncture represents an intriguing approach. The analgesic effect of acupuncture may help relieve neurological symptoms of COVID-19, such as headache and myalgia. Over five decades ago, the assumption that nerve stimulation and endorphin secretion are the biological mechanism of acupuncture was supported by the gating control model for pain signals [13]. The current study has validated that the secretion of β-endorphin after acupuncture treatment is an important biological mechanism. The analgesic effects of acupuncture are likely to be correlated with the capacity of acupuncture in modulating pituitary and hypothalamic β-endorphin levels [8, 44, 76, 88]. On the other hand, the analgesic and anti-inflammatory effects of β-endorphin on inflammatory pain have also been confirmed [27]. Taken together, acupuncture may play a positive role in COVID-19 associated pain.

Screening of protein targets of active ingredients produced by body after acupuncture

Targets with high binding affinity with the dopamine and β-endorphin were acquired from the STITCH [68] and SwissTargetPrediction [15] database. STITCH is an aggregated database of 430 000 chemicals linked to an easy-to-use resource, aiming to facilitate the study into interactions between proteins and chemicals [68]. SwissTargetPrediction is a web server on the basis of combined 2D and 3D similarity measures and known ligands which can accurately predict biologically active molecular targets [15].

Screening of potential therapeutic targets of COVID-19

In order to facilitate the translational significance of our research, human genes were selected for research. GeneCards, a compendium of human genes, is a comprehensive database containing all the information of annotated and predicted genes, which enables researchers to effectively navigate and correlate the broad fields of human genes, variants, diseases, cells, proteins and biological pathways [65]. To determine the key targets of COVID-19, we searched for GeneCards database that may be associated with COVID-19, using keywords such as heat, cough, viral pneumonia and coronavirus.

Screening of the correlative targets of acupuncture against COVID-19

The obtained protein targets were analyzed using Venn tool (http://bioinfogp.cnb.csic.es/tools/venny) [55]. Afterwards, the amount of overlap between targets associated with the acupuncture related active ingredients and COVID-19 associated with targets was visualized by a Venn diagram.

Construction of protein–protein interaction networks

STRING11.0 (http://string-db.org/cgi/input.pl) was used to construct the protein–protein interaction (PPI) network [50]. To be specific, the possible therapeutic targets of acupuncture against COVID-19 were up-loaded into the search tool (STRING11.0) to retrieve interacting genes/proteins by selecting humans as the species. The PPI results were sorted by the degree value, and the key targets with the top 20 connectivity were identified as the key targets.

Gene Ontology and the Kyoto Encyclopedia of Genes and Genomes enrichment analysis

We analyzed the correlative targets of acupuncture against COVID-19 and possible signaling pathways for further functional investigation. To this end, Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed, followed by plotting and visualization of effector targets via DAVID database (https://david.ncifcrf.gov/home.jsp) [18].

Establishment of the compound-target and compound-disease-target network

Cytoscape 3.7.2 was utilized for visualizing compound-target and compound-disease-target network of acupuncture on COVID-19.

Therapy
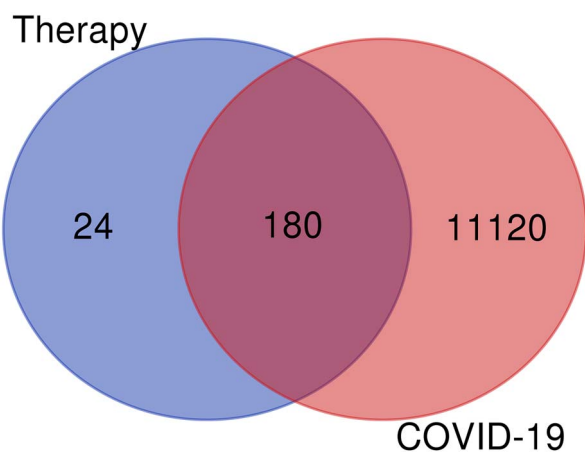
Figure 1. The intersection generated between acupuncture-associated target and COVID-19-associated genes. Using online databases, we identified 180 shared biotargets of acupuncture against COVID-19.

Table 1. Top 20 core targets

Target name	Degree	Target name	Degree
BDKRB1	41.0	OPRK1	25.0
ADCY5	34.0	OPRL1	25.0
CXCR4	31.0	MTNR1B	25.0
FPR1	31.0	DRD3	25.0
C3AR1	28.0	DRD4	25.0
OPRM1	27.0	C5AR1	25.0
OPRD1	27.0	ADORA3	25.0
HTR1A	26.0	ADRA2A	25.0
DRD2	25.0	HTR1B	25.0
PDYN	25.0	HRH3	25.0

Results

Target screening of acupuncture in treating COVID-19

The application of STITCH and SwissTargetPrediction database gave rise to genes correlated with the effects of active ingredients after acupuncture. COVID-19- associated genes were collected from GeneCard database. In total, human targets were matched following normalization of gene names in the original files. Subsequently, 204 ingredient acupuncture-associated target (Supplementary Table S1 and S2), and 11 300 COVID-19 targets (Supplementary Table S2 and S3) were utilized for plotting a Venn diagram, which yielded to coincident targets (Figure 1). Collectively, our analysis showed that acupuncture generated 180 potential therapeutic targets of COVID-19.

Establishment and analysis of PPI network

PPI network was constructed based on STRING database and visualized by Cytoscape after topological analysis, showing 180 nodes. 'Degree' is defined as the number of connections of a node in the network graph, reflecting the interaction information between nodes. The larger value indicates the greater importance of the node [2]. The degree of targets was shown in Supplementary Table S4. BDKRB1 had the greatest degree (41), followed by ADCY5 (34), CXCR4 (31), FPR1 (31) and C3AR1 (28). Top 20 core targets sorted by the value of degree were shown in Table 1, which were identified as the key targets of acupuncture in COVID-19.

GO and KEGG enrichment analysis

GO analysis can be used to reflect target functions from three aspects, including cellular components (CC), molecular functions (MF) and biological processes (BP) [1]. In our study, 522 GO entries were enriched ($P < 0.05$) (Supplementary Table S5). The top 10 pathways with the most enriched GO analysis were shown in Figure 2. Top 10 BP of acupuncture against COVID-19 was shown in Table 2. The phospholipase C-activating G-protein coupled receptor signaling pathway, positive regulation of cytosolic calcium ion concentration and response to drug were the top three GO terms in BP with low P-adjust value. Plasma membrane, integral component of plasma membrane and integrin complex were the top three GO terms in CC with low P-adjust value. And drug binding, enzyme binding and virus receptor activity were the top three GO terms in MF with low P-adjust value.

In terms of KEGG analysis, 61 most enriched signal pathways were acquired ($P < 0.05$) (Supplementary Table S6). The top 20 pathways with high significance were selected and presented in Figure 3, including neuroactive ligand–receptor interaction, serotonergic synapse, calcium signaling pathway, focal adhesion, alcoholism, pathways in cancer, cocaine addiction, cGMP–PKG signaling pathway, viral carcinogenesis, etc. Top 10 KEGG pathways of acupuncture against COVID-19 were displayed in Table 3.

The construction of compound-target and compound-disease-target network

The compound-target and compound-disease-target network of acupuncture on COVID-19 was shown in Figures 4 and 5, respectively. The degree of dopamine and β-Endorphin were 93 and 96, respectively. Among the keywords of COVID-19, the degree of heat was the highest (161), followed by cough (140), viral pneumonia (109) and coronavirus (18).

Discussion

Due to the global spread of COVID-19 pandemic, effective therapeutic strategies for COVID-19 are urgently needed. Acupuncture might be an additional therapeutic selection for COVID-19 and provide new ideas for the global fight against the epidemic.

Although acupuncture may be effective against COVID-19 [24, 30, 80, 85], no study has systematically investigated its potential mechanisms against COVID-19. Here, by using a potent strategy of bioinformatics and network topology, we aimed to uncover the integrative mechanism of acupuncture against COVID-19. The bioinformatics/network topology analysis demonstrated that acupuncture exerts an overall regulatory role through multi-target. Our findings suggest that multiple nervous system-, immune and inflammation-associated gene functions and signaling pathways are simultaneously involved, which is the basis of acupuncture treatment in COVID-19. Acupuncture can change the levels of dopamine and β-endorphin in the body. As a member of monoamine neurotransmitter family, dopamine along with its D1 and D2 receptors participates in electroacupuncture-induced analgesia [79], while analgesic effects of electroacupuncture can be reserved by dopamine receptor antagonists [79]. In addition, electroacupuncture analgesia can be enforced by hypothalamic expression of the dopamine beta-hydroxylase gene [34]. Lung immunity might be shaped by dopamine receptors, which were expressed by lung terminal nerves, lung macrophages and alveolar epithelial cells according to relevant animal studies [6, 73]. A murine model of endotoxin-induced acute lung injury revealed that dopamine
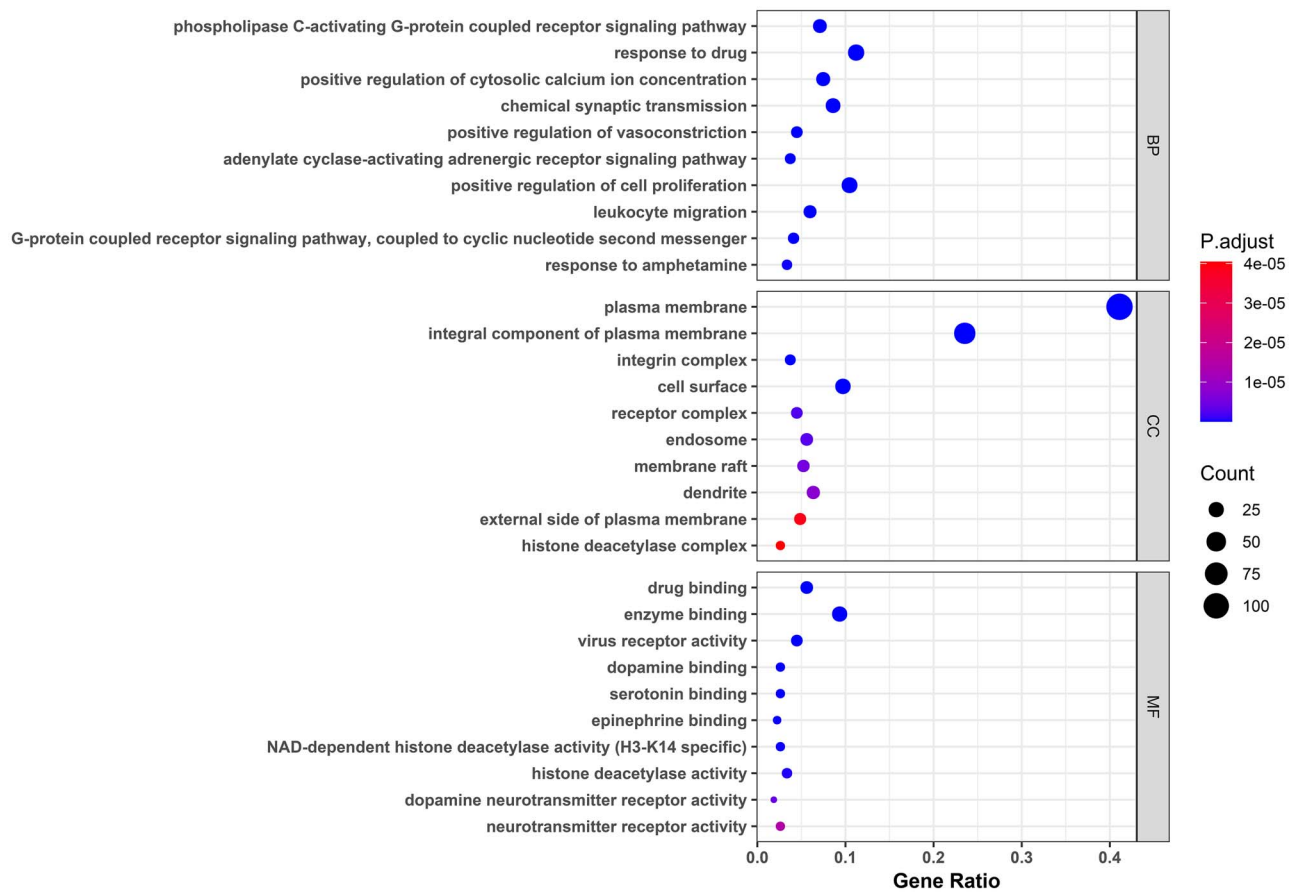
Figure 2. GO enrichment analysis (P-adjust value < 0.05). GO: Gene Ontology; BP: biological processes; CC: cellular components; MF: molecular functions.

Table 2. Top 10 biological processes of acupuncture against COVID-19

ID	Description	P-value	P-adjust	Number of gene
GO:0007200	Phospholipase C-activating G-protein coupled receptor signaling pathway	5.12E-21	9.38E-18	19
GO:0042493	Response to drug	1.20E-19	1.10E-16	30
GO:0007204	Positive regulation of cytosolic calcium ion concentration	2.11E-16	1.36E-13	20
GO:0007268	Chemical synaptic transmission	1.02E-14	4.68E-12	23
GO:0045907	Positive regulation of vasoconstriction	1.48E-14	5.41E-12	12
GO:0071880	Adenylate cyclase-activating adrenergic receptor signaling pathway	1.16E-13	3.55E-11	10
GO:0008284	Positive regulation of cell proliferation	6.11E-13	1.60E-10	28
GO:0050900	Leukocyte migration	3.04E-12	6.96E-10	16
GO:0007187	G-protein coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger	4.07E-11	8.30E-09	11
GO:0001975	Response to amphetamine	8.72E-10	1.60E-07	9

D1 receptor agonist fenoldopam could suppress inflammation, lung permeability as well as pulmonary edema [6]. Acupuncture is globally prevalent to treat both chronic and acute pain. Endogenous opioid peptides in the central nervous system have been demonstrated to be vitally involved in mediating the analgesic effect of acupuncture [8, 44, 88]. A recent RCT of laser acupuncture to treat knee osteoarthritis has demonstrated that patients treated by laser had significantly relieved pain and increased serum beta-endorphin compared to those treated by sham laser [51]. Accessible evidence implicates pain as a common symptom of COVID-19. COVID-19 patients also show neurological symptoms, such as myalgia and/or arthralgia) and headache [22]. Moreover, symptoms associated with COVID-19, including gastrointestinal disorders and nausea could be aggravated by the administration of opioids and other medicines to relieve neuropathic pain [22]. Therefore, acupuncture may be a safe and effective method for pain management in COVID-19 patients.
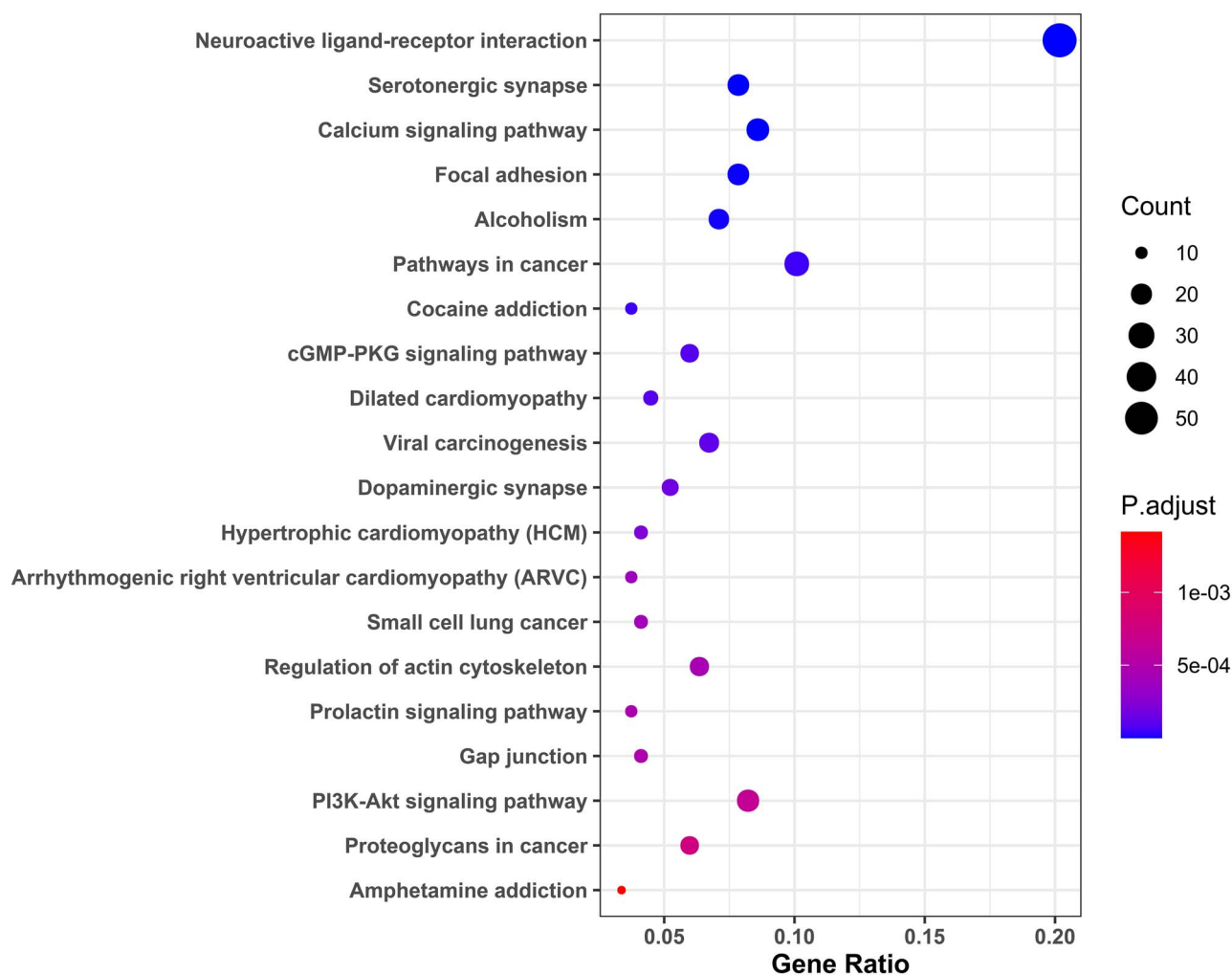
Figure 3. KEGG pathways enrichment analysis (P-adjust value < 0.05). KEGG: Kyoto Encyclopedia of Genes and Genomes.

Table 3. Top 10 KEGG pathway of acupuncture against COVID-19

ID	Description	P-value	P-adjust	Number of gene
hsa04080	Neuroactive ligand–receptor interaction	5.03E-35	1.00E-32	54
hsa04726	Serotonergic synapse	8.14E-13	8.10E-11	21
hsa04020	Calcium signaling pathway	1.50E-10	9.93E-09	23
hsa04510	Focal adhesion	6.63E-08	3.30E-06	21
hsa05034	Alcoholism	1.56E-07	6.23E-06	19
hsa05200	Pathways in cancer	1.47E-06	4.87E-05	27
hsa05030	Cocaine addiction	1.72E-06	4.89E-05	10
hsa04022	cGMP-PKG signaling pathway	4.28E-06	9.46E-05	16
hsa05414	Dilated cardiomyopathy	3.93E-06	9.78E-05	12
hsa05203	Viral carcinogenesis	6.13E-06	1.22E-04	18

The PPI network of candidate therapeutic targets for acupuncture of COVID-19 was established based on the active ingredients and COVID-19 target networks with 180 overlapping genes. By using the median values for the degree (top 20 of degree), 20 targets were considered as significant, including BDKRB1, ADCY5, CXCR4, FPR1, C3AR1, etc. The expression of BDKRB1 was increased after tissue injury and inflammation (hyperalgesia, exudation, hyperemia, etc.) [40]. BDKRB1 plays an important role in regulating immune system both *in vitro*

and *in vivo*, which goes beyond pro-inflammatory effects [63]. Additionally, BDKRB1 belongs to calcium signaling pathway and neuroactive ligand–receptor interaction pathway. KEGG pathways enrichment analysis showed that the above two signaling pathways were highly enriched. Therefore, BDKRB1 may be an important target for acupuncture therapy of COVID-19. Obesity is a global problem. Studies have shown the greater hospitalization risk of obese patients, and when being affected by the influenza, obese patients face greater risk of hospital
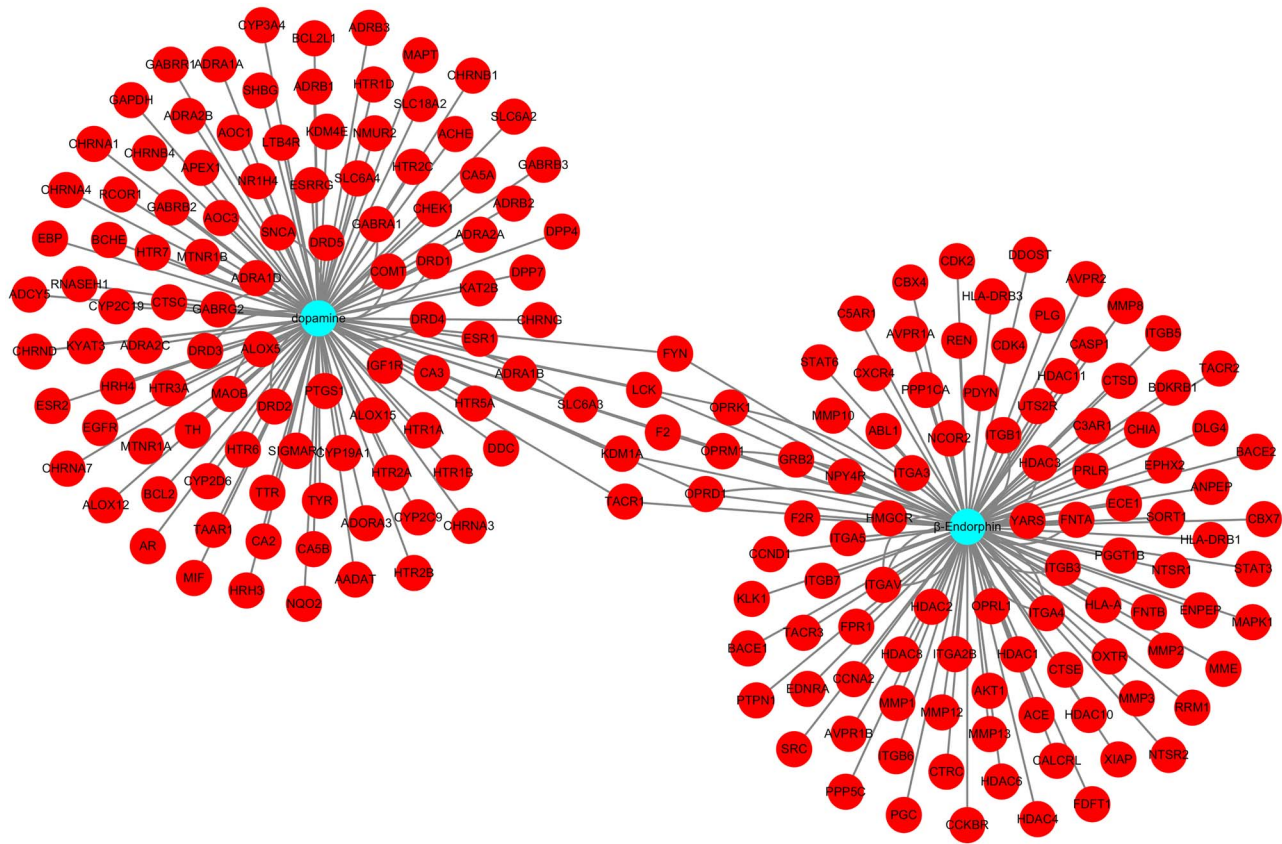
Figure 4. Component-target network of acupuncture for COVID-19. In the network, there are two active components have interactions with 180 protein targets. Red nodes represent protein targets, light blue nodes represent active components.

admission than normal-weight patients [52]. Increasingly, more data have revealed the strong correlation between obesity and COVID-19 infection severity, even with no presence of other comorbidities [36]. A recent meta-analysis proved obese patients' greater mortality risk from COVID-19 infection [28]. On the other hand, most high-risk patients are suffering relevant consequences of obesity, like hypertension, heart conditions and diabetes [89]. Among populations suffering growing abdominal obesity, the pulmonary function in the supine position is further compromised due to reduced diaphragmatic excursion, causing greater difficulty in ventilation [21]. These studies suggest that obese patients may have a more serious course of disease. These researches further stress the urgency of greater vigilance, and the prior detection and aggressive therapy for obese patients infected with the COVID-19. Studies have shown that ADCY5 gene expression in adipose tissue is related to obesity in human [35]. The positive efficacy of acupuncture in managing simple obesity has been readily shown. Clinical researches have shown that acupuncture is capable of attenuating oxidative stress, modulating endocrine system, regulating metabolic molecules and promoting digestion among patients with simple obesity [72]. Therefore, ADCY5 may be an important target for acupuncture treatment of COVID-19 patients with obesity. And acupuncture may be more conducive to the recovery of obese patients. On the other hand, ADCY5 is highly expressed in striatum, a region involved in modulating movement [33]. Therefore, acupuncture may be effective in relieving myalgias symptoms in patients with COVID-19. A variety of processes are modulated by chemokines and chemokine receptors, including

immune responses, morphogenesis as well as angiogenesis. In terms of chemokine receptors, CXCR4 plays a dominant role due to its multiple roles and its involvement in various pathological situations (including malignancies, immune disorder and viral infection) [60]. Therefore, CXCR4 may be a key target in acupuncture treatment. As a plague receptor on murine and human immune cells, the mutation and absence of FPR1 could protect from *Yersinia pestis*. Additionally, FPR1 alleles selection in plague is likely to shape human immune responses towards other infectious disorders [57]. Originally, C3AR1 was thought to be restricted to the innate immune response and play a role in the complement cascade, however, its scope of involvement has been extended to cancer [56]. C3AR plays a vital role in up-regulating caspase-4 and -5 as well as caspase-11 orthologues in primary human macrophages when inflammation occurs. In addition, there is high expression of C3AR1 and caspase-5 transcripts in patients with severe sepsis. These outcomes indicate the importance of these pathways in human sepsis [53]. COVID-19 is greatly associated with sepsis, implicating most death cases are directly caused by of SARS-CoV-2 infection-induced sepsis in intensive care units (ICU) [3]. A recent systematic review has revealed that acupuncture might be a promising complementary strategy for controlling sepsis inflammation [39]. Therefore, C3AR may be a key target of acupuncture against sepsis.

KEGG analysis revealed that most enriched pathways were associated with inflammatory response, immune, pain and cancer. These pathways were mainly enriched in calcium signal-ing pathway, serotonergic synapse, neuroactive ligand–receptor
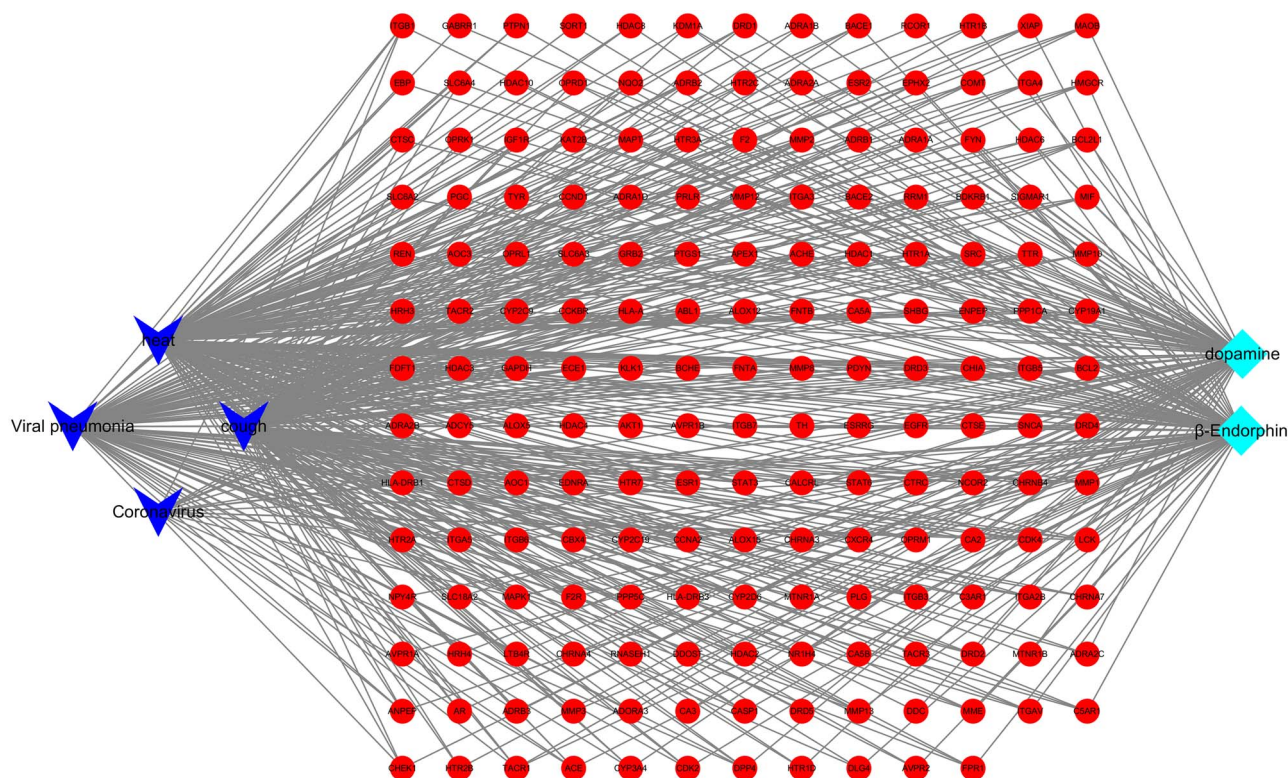
Figure 5. Compound-disease-target network of acupuncture for COVID-19. Red nodes represent targets, light blue nodes represent active components, dark blue nodes represent the key words of COVID-19.

interaction, etc. Notably, myalgias, headache and abdominal pain are all symptoms of COVID-19 [16], which can be regulated by calcium signaling pathway and neuroactive ligand–receptor interaction [17]. A study using RNA-sequencing found that experimental chronic inflammatory pain could be relieved by electroacupuncture via the suppression of calcium voltage-gated channel-mediated inflammation [90]. Although most calcium signaling pathway studies focused on pain and cardiovascular disease research, it has also been shown to be associated with inflammatory responses [29]. Neutrophils are recruited to inflammatory location, where they fight off invading microorganisms by phagocytosis and release antimicrobials. The altered levels of intracellular calcium are caused by the influx of extracellular Ca^{2+} as well as cytosolic Ca^{2+} store depletion. Moreover, calcium signaling pathway is vitally involved in synchronizing neutrophil activation and functions [29]. Tumor pathways, viral carcinogenesis, proteoglycans in cancer, small cell lung cancer and PI3K–Akt signaling pathways are all cancer-related and immune-related pathways. It is worth considering that active cancer can increase the susceptibility to the COVID-19, probably because of the systemic immunosuppressive status provoked directly by tumor growth and indirectly by anticancer treatment effect [26]. With the spread of the pandemic, the incidence rate of cancer patients has exhibited a larger number of severe disease cases. Liang *et al*. [43] discovered that patients with cancer are likely to have increased COVID-19 risks than normal individuals. Moreover, they also found poor prognosis of COVID-19 in cancer patients [43]. The COVID-19 and cancer consortium cohort research data gathered from 1018 patients, also proved the remarkably greater mortality and severe illness risk of COVID-19 cancer patients compared with ordinary people [37]. One retrospective study proved that cancer

patients with COVID-19 more easily suffer fatigue and dyspnea symptoms [14, 84]. Particularly, for COVID-19 patients with lung cancer or lung metastasis, dyspnea occurred much earlier upon COVID-19 diagnosis, than non-cancer patients and patients with other cancers [14, 84]. Therefore, the active treatment of cancer patients is particularly important. Clinical evidence suggests that acupuncture can be used to relieve cancer-related pain and related symptoms, such as postoperative nausea and vomiting, postsurgical gastroparesis syndrome and aromatase inhibitor-associated joint pain [48]. Acupuncture may provide additional benefits for these patients. Meta-analysis shows that cardiovascular disease is one of the major risk factors among COVID-19 patients [71]. Both pulmonary and systemic inflammations are caused by SARS-CoV-2 infection, causing multiple organ dysfunction. Cardiac complications of the COVID-19 cover acute myocardial injury, arrhythmias, cardiogenic shock and even sudden death [19]. Moreover, the interplay between drugs and COVID-19 therapies possibly increases the risk of arrhythmias, cardiomyopathy and sudden death for patients [19]. Concerning the severe complication of severe COVID-19, heart failure, sepsis and acute cardiac injury are most frequent [9]. Calcium signaling pathway, cGMP–PKG signaling pathway and hypertrophic cardiomyopathy pathway are associated with cardiovascular disease [11]. An updated meta-analysis shows that acupuncture therapy might relieve anginal symptoms in patients with stable angina pectoris, which could be used as an adjunctive treatment [46]. Acupuncture therapy may assist to treat COVID-19 patients with cardiovascular disease. *Staphylococcus aureus* infection (containing six targets, including C3AR1, C5AR1, HLA-DRB1, HLA-DRB3, FPR1, PLG), which is closely associated with infectious diseases [54]. It is closely associated with anti-inflammatory effects and might be an

important pathway underlying the anti-inflammatory role of acupuncture.

The COVID-19 pandemic has quickly developed into the global healthcare emergency. Clinical trials should provide high-quality data for an objective assessment about the potential therapies for COVID-19 treatment and prevention [12]. Based on our findings, clinical trials of acupuncture can be further studied to alleviate clinical symptoms of the COVID-19, prevent and treat complications, and improve patients' quality of life and prognosis, especially for patients with underlying diseases (including cancer, cardiovascular disease and obesity).

Limitations

In this study, we only analyzed two representative active substances produced by the human body after acupuncture treatment, while failed to represent all the active substances produced after acupuncture treatment. Further data mining and experimental verification are warranted. Of note, different acupuncture prescriptions/acupoints, treatment length and frequency are important parameters in acupuncture treatment [83]. Nevertheless, due to a lack of literature, these factors were not analyzed in our study.

Conclusions

In our present study, a bioinformatics/network topology strategy was utilized to explore the targets and molecular mechanisms of acupuncture for COVID-19. Our study uncovered that anti-inflammation, immunity activation and nervous system modulation were primary therapeutic pathways of acupuncture against COVID-19. Acupuncture may be effective against COVID-19-related symptoms. Moreover, acupuncture may provide additional benefits, especially for patients with cancer, obesity and cardiovascular disease. Hopefully, acupuncture can be applied in the effective management of COVID-19 based on the identified mechanisms. Despite the encouraging results, more rigorously designed clinical and basic research are warranted to further confirm the current findings.

Key Points

- For the first time, candidate targets and underlying mechanisms of the acupuncture treatment against COVID-19 were identified.
- This research proposed a method to study the therapeutic mechanism of acupuncture.
- The comprehensive research based on bioinformatics/network topology methods may clarify the multifunctional synergy mechanisms of acupuncture in the treatment of COVID-19.
- This research provided ideas for formulating relevant intervention measures for acupuncture treatment of COVID-19.

Supplementary data

Supplementary data are available online at *Briefings in Bioinformatics*.

Data Availability

The data underlying this article are available in the article and in its online supplementary material.

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