Genes & Genomics (2021) 43:55–67
https://doi.org/10.1007/s13258-020-01021-8

Online ISSN 2092-9293
Print ISSN 1976-9571

RESEARCH ARTICLE

# Screening druggable targets and predicting therapeutic drugs for COVID-19 via integrated bioinformatics analysis

**Siyou Tan[1] · Wenyan Chen[1] · Hongxian Xiang[2] · Gaoyin Kong[1,5] · Lianhong Zou[3,4] · Lai Wei[1,5]**

Received: 26 June 2020 / Accepted: 25 November 2020 / Published online: 11 January 2021


## Abstract

**Background** Since the outbreak of coronavirus disease 2019 (COVID-19) in China, numerous research institutions have invested in the development of anti-COVID-19 vaccines and screening for efficacious drugs to manage the virus.

**Objective** To explore the potential targets and therapeutic drugs for the prevention and treatment of COVID-19 through data mining and bioinformatics.

**Methods** We integrated and profoundly analyzed 10 drugs previously assessed to have promising therapeutic potential in COVID-19 management, and have been recommended for clinical trials. To explore the mechanisms by which these drugs may be involved in the treatment of COVID-19, gene-drug interactions were identified using the DGIdb database after which functional enrichment analysis, protein–protein interaction (PPI) network, and miRNA-gene network construction were performed. We adopted the DGIdb database to explore the candidate drugs for COVID-19.

**Results** A total of 43 genes associated with the 10 potential COVID-19 drugs were identified. Function enrichment analysis revealed that these genes were mainly enriched in response to other invasions, toll-like receptor pathways, and they play positive roles in the production of cytokines such as IL-6, IL-8, and INF-β. TNF, TLR3, TLR7, TLR9, and CXCL10 were identified as crucial genes in COVID-19. Through the DGIdb database, we predicted 87 molecules as promising druggable molecules for managing COVID-19.

**Conclusions** Findings from this work may provide new insights into COVID-19 mechanisms and treatments. Further, the already identified candidate drugs may improve the efficiency of pharmaceutical treatment in this rapidly evolving global situation.

**Keywords** SARS-CoV-2 · Drug · Toll-like receptors · Bioinformatics analysis

Siyou Tan and Wenyan Chen contributed equally to this study.

✉ Lai Wei
448027999@qq.com

[1] Department of Anesthesiology, Hunan Provincial People's Hospital, The First Affiliated Hospital of Hunan Normal University, No.61 Jiefang West Road, Furong District, Changsha 410002, Hunan, China

[2] Department of Cardiothoracic Surgery, Hunan Provincial People's Hospital, The First Affiliated Hospital of Hunan Normal University, Changsha 410002, Hunan, China

[3] Hunan Provincial Institute of Emergency Medicine, Hunan Provincial People's Hospital, The First Affiliated Hospital of Hunan Normal University, Changsha 410002, Hunan, China

[4] Hunan Provincial Key Laboratory of Emergency and Critical Care Metabonomics, Changsha 410002, Hunan, China

[5] Clinical Research Center for Anesthesiology of ERAS in Hunan Province, Changsha 410002, Hunan, China

**Abbreviations**

| | |
|---|---|
| COVID-19 | Coronavirus disease 2019 |
| SARS-CoV-2 | Severe acute respiratory syndrome coronavirus 2 |
| GO | Gene ontology |
| KEGG | The Kyoto Encyclopedia of Genes and Genomes |
| BP | Biological process |
| CC | Cellular component |
| MF | Molecular function |
| PPI | Protein–protein interaction |
| STRING | The search tool for the retrieval of interacting genes |

**Gene list**

| | |
|---|---|
| *ACE2* | Angiotensin-converting enzyme 2 |
| *TMPRSS2* | Transmembrane protease serine 2 |
| *BSG* | Basigin |
| *TNF* | Tumor necrosis factor |

| | |
|---|---|
| *TLR3* | Toll-like receptor 3 |
| *TLR7* | Toll-like receptor 7 |
| *TLR9* | Toll-like receptor 9 |
| *CXCL10* | C-X-C chemokine 10 |

## Introduction

As of August 16, 2020, COVID-19 infection has spread to more than 200 countries, with nearly 20 million confirmed cases and 765, 000 deaths globally. Under the severe situation of the COVID-19 epidemic, institutions and enterprises in various countries have accelerated the research on the development of effective drugs to curb the spread of the virus. However, no effective specific antiviral drug against the global pandemic has been uncovered. Since the outbreak of severe acute respiratory syndrome (SARS) in 2003 and Middle East respiratory syndrome (MERS) in 2018, to the recent outbreak of COVID-19, clinicians and scientists have actively been involved in the exploration and research to combat the epidemic. Several potentially effective antiviral drugs, including Remdesivir, lopinavir/ritonavir, convalesce plasma, and monoclonal antibodies, have been recommended to date, but their efficacy and safety in COVID-19 patients are yet to be confirmed through further clinical trials (Costanzo et al. 2020). In addition, the mechanisms of these drugs in the prevention and treatment of COVID-19 require in-depth assessment.

In this study, 10 drugs (lopinavir, ritonavir, chloroquine diphosphate, arbidol, darunavir, hydroxychloroquine, favipiravir, remdesivir, ribavirin, and interferon-α) were identified, and using DGIdb database, we revealed 43 genes that interact with these drugs. Then, gene ontology (GO) analysis and biological pathway enrichment analysis were performed to evaluate the underlying molecular mechanisms by which these genes are linked to COVID-19. The 43 genes were subsequently used to construct a PPI network that incorporated angiotensin-converting enzyme 2 (ACE2), transmembrane protease serine 2 (TMPRSS2), and basigin (BSG). These genes have been reported to exert a functional role in promoting the invasion of host cells by SARS-CoV (Chen et al. 2005; Sanders et al. 2020; Yurchenko et al. 2006). Based on the functional enrichment analysis results, *TNF*, *TLR3*, *TLR7*, *TLR9*, and *CXCL10* were identified as key genes that potentially play crucial roles in the progression of COVID-19. Among the 5 genes, *TLR7* and *TLR9* were particularly involved in the entire process of the pattern recognition receptors (PRRs) activation mediated toll-like receptor pathway, leading to virus-induced cytokine storm (Ye et al. 2020). On the other hand, *TLR7*, *TLR9*, *TNF*, and *TLR3* were associated with IL-6 regulation, which critically drives the excessive immune response (Sanders et al. 2020). Loss-of-function variation of *TLR7* has been revealed to potentially result in high mortality among young male patients with severe COVID-19 (van der Made et al. 2020). Besides, *CXCL10* may be associated with olfactory dysfunction in COVID-19 patients (Oliviero et al. 2020). In addition, using DGIdb database, we explored the candidate drugs that target the 5 key genes (*TNF*, *TLR3*, *TLR7*, *TLR9*, and *CXCL10*), *ACE2*, *TMPRSS2*, and *BSG* and their first neighbor genes (*APOE* and *MMP1*) after which the miRNA–gene network was constructed for more information on the upstream regulation. An overview of the workflow is highlighted in Fig. 1.

## Materials and methods

### Identifying target genes

After collecting the drugs recommended for COVID-19 treatment, we used the DGIdb database (http://www.dgidb.org/; version: 3.0.2-sha1 ec916b2) to identify the interactive genes with these drugs. Currently, the DGIdb database contains more than 40,000 genes and 10,000 drugs involved in over 15,000 drug-gene interactions. The user can enter into the database, a list of genes to retrieve all known or potentially druggable genes. Also, if an exact match is found to a DGIdb drug, DGIdb searches for drug-gene interactions associated with the drug in question (Cotto et al. 2018). With

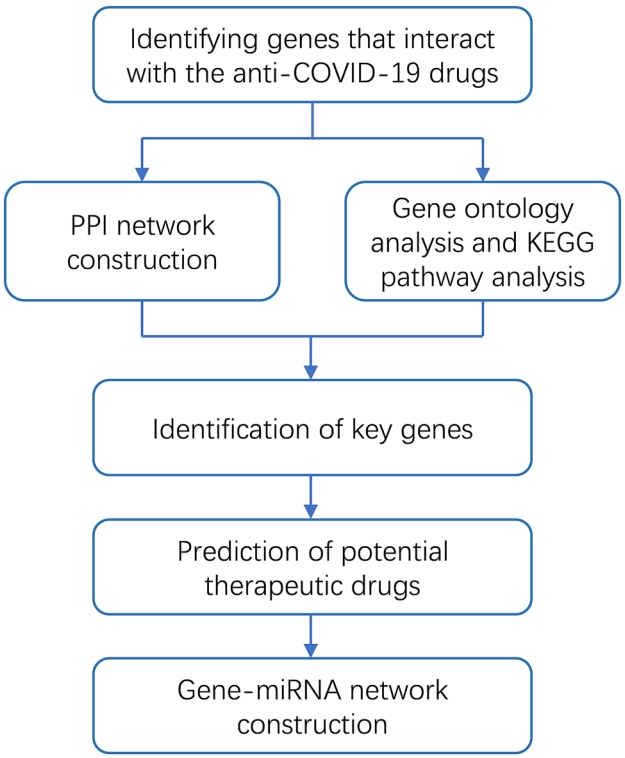


**Fig. 1** Workflow of bioinformatics analysis process. KEGG, Kyoto Encyclopedia of Genes and Genomes

this approach, researchers have been able to identify target genes/proteins from known drugs or compounds through network pharmacology-based studies (Wang et al. 2020; Zhang et al. 2019). Here, we used Cytoscape software (version: 3.6.1) to construct the drug-gene interaction network. In this network, the red diamond node denoted the drug and the green circular node denoted the identified gene. Their type of interactions was denoted by arrows of different colors, and the size of the green node was adjusted based on the number of supporting evidence for the interaction between the drug and genes.

## GO and KEGG pathway analysis

GO analysis map genes to known functional information sources, including biological process (BP), cellular component (CC), and molecular function (MF), and detect statistically significantly enriched terms. Here, we performed GO analysis using g: Profiler database (https://biit.cs.ut.ee/gprofiler/gost), which is a web server that analyzes functional enrichment and conversions of gene lists for users to grasp biological characteristics (Raudvere et al. 2019). We submitted a total of 43 genes to g: Profiler with $P < 0.05$ as a cut-off criterion. GO results of important terms for BP, CC, and MF were ranked by *p*-value and visualized in a bar chart. Each bar chart contained up to 10 analysis terms. $P < 0.05$ was considered statistically significant. Kyoto Encyclopedia of Gene and Genome (KEGG) pathway analysis was performed using ClueGo plug-in in Cytoscape software (Bindea et al. 2009), $P < 0.05$ was set as the cut-off criterion.

## PPI network construction and analysis

We visualized the protein–protein interaction (PPI) information on 43 identified genes, ACE2, TMPRSS2, and BSG using STRING (Search Tool for the Retrieval of Interacting Genes) (Franceschini et al. 2013). Then, Cytoscape software was adopted to construct PPI networks. An interaction with a combined score > 0.4 and the maximum number of interactors = 0 were set as the cut-off criterion. In this network, the size of the edge was adjusted according to the comprehensive score, whereas the size of the nodes was determined by the number of connections. To visualize the gene modules in the PPI network, we used the MCODE app in Cytoscape (Bandettini et al. 2012; Treister and Pico 2018). The module containing 16 genes was identified as the most significant by MCODE with degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and Max depth = 100. The first and the second neighbor genes of ACE2, TMPRSS2, and BSG were mapped into PPI using the STRING tool. Moreover, the g: Profiler database was used for KEGG pathway analysis of module 1, neighbor genes, and the three genes (ACE2, TMPRSS2, and BSG).

## Identifying the key genes in COVID-19 pharmaceutical therapy

To find the genes that are critically important in pharmaceutical COVID-19 treatment, we selected the following 10 enrichment terms, according to the GO analysis results: 'response to other organisms', 'response to virus', 'PRRs activity', 'regulation of NF-κB signaling', 'regulation of IL-6 production', 'positive regulation of IL-8 production', 'positive regulation of interferon-β (IFN-β) biosynthetic process', 'influenza A', and 'defense response'. Besides, we selected the genes in module 1 and the first and the second neighbor genes to ACE2, TMPRSS2, and BSG based on the PPI network. Key genes were identified by the intersections of the gene combinations. The overlapping genes among these combinations were identified using the FunRich tool (version: 3.0) (Benito-Martin and Peinado. 2015).

## Predicting potential drugs that target key genes

The 5 key genes, along with *ACE2*, *TMPRSS2*, *BSG*, and their first neighbor genes *APOE* and *MMP1*, were considered as the promising targets for drug search through the DGIdb database (http://www.dgidb.org/; version: 3.0.2-sha1 ec916b2). Drugs supported by at least one database or PubMed literature were selected as ideal candidates. For the 5 key genes, screened drugs were comprised of only those that have been approved by the Food and Drug Administration (FDA), whereas for *ACE2*, *TMPRSS2*, and *BSG*, their potential interactive drugs were selected. In this network, the green circular node or orange triangle node denoted the gene, while the purple or pink circular node denoted the potential drugs. For the size of the node that denoted drugs, adjustments were made based on the number of supporting evidence for the interactions.

## miRNA–gene network construction

miRNA targeting the 5 key genes and the 3 coronavirus-related genes (ACE2, TMPRSS2, and BSG) was predicted via the established miRNA target prediction databases, including PITA, mirmap, microT, miRanda, PicTar, and TargetScan. Then, miRNA predicted by at least one database was selected as the target miRNA of the gene. Cytoscape software was used to construct a co-expression network based on the correlation analysis of genes and miRNA.

## Results

### Identifying genes that interact with the anti-COVID-19 drugs

Through a literature search, we identified 10 drugs with therapeutic potential for COVID-19 treatment, including lopinavir, ritonavir, chloroquine diphosphate, arbidol, ribavirin, interferon-α, darunavir, hydroxychloroquine, favipiravir, and remdesivir (Costanzo et al. 2020; Du and Chen 2020; Grein et al. 2020). Using the DGIdb database, a total of 43 genes that interacted with these drugs were revealed (Fig. 2a). In the network, the size of the green circular node was revealed by the number of supporting evidence for each interaction.

### Gene ontology and KEGG pathway analysis

All 43 genes were analyzed via g: Profiler database, where GO analysis demonstrated that (1) for biological processes (BP), these genes were particularly enriched in response to other organisms or external biotic stimulus, virus, defense response, and positive regulation of IL-6, IL-8, and IFN-β; (2) for molecular function (MF), these genes were highly associated with signaling receptor binding, among which 3 genes (*TLR4*, *TLR7*, and *TLR9*) had the potential to regulate the PRRs activity; (3) for cellular component (CC), identified genes were significantly enriched in the extracellular region, endoplasmic reticulum, and endosome (Fig. 3a). KEGG pathway analysis demonstrated that the 43 genes were particularly enriched in the toll-like receptor signaling pathway, drug metabolism, cholesterol metabolism, and amyotrophic lateral sclerosis (Fig. 3b).

### Construction of the PPI network and module analysis

Using the STRING database and Cytoscape software, the protein interaction relationships between the 43 genes, *ACE2*, *TMPRSS2*, and *BSG* were constructed into a network, including 45 nodes and 161 edges (Fig. 2b). Results based on the STRING database showed that Chromosome 12 open reading frame 43 (*C12orf43*), which have been identified to participate in lipid metabolism, blood coagulation, and atherosclerosis (Shahzadi et al. 2016), did not interact with the other 45 proteins, thus was not incorporated in the network. A total of 20 genes were identified as the first and the second neighbor to ACE2, TMPRSS2, and BSG (Fig. 4a), and the module (MCODE score = 7.333) containing 16 nodes and 55 edges was identified as the most significant cluster using MCODE plug-in in Cytoscape software (Fig. 4c). We also analyzed the biological pathways of the neighbor genes (Fig. 4b), gene module (Fig. 4d), and gene combination consisting of *ACE2*, *TMPRSS2*, and *BSG* (Fig. 4f) via the g: Profiler database. Pathway enrichment analysis indicated that the neighbor gene was mainly associated with influenza A, toll-like receptor signaling pathway, and cholesterol metabolism. Module 1 was particularly enriched

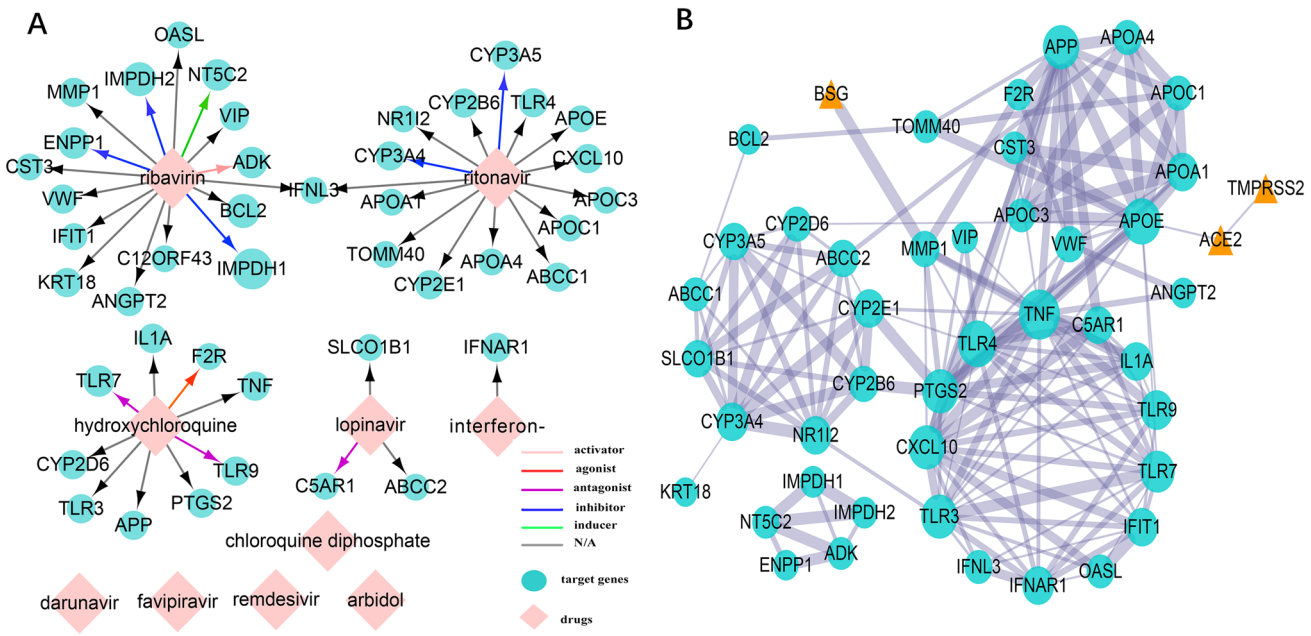


**Fig. 2** The drug-gene interaction network and the PPI network. **a** A drug-gene interaction network. **b** A PPI network constructed using STRING online database. The red diamond node denoted the drug. The green circular node denotes the gene. The orange triangle node denotes ACE2, TMPRSS2, and BSG. The arrow represents interactions (colour figure online)

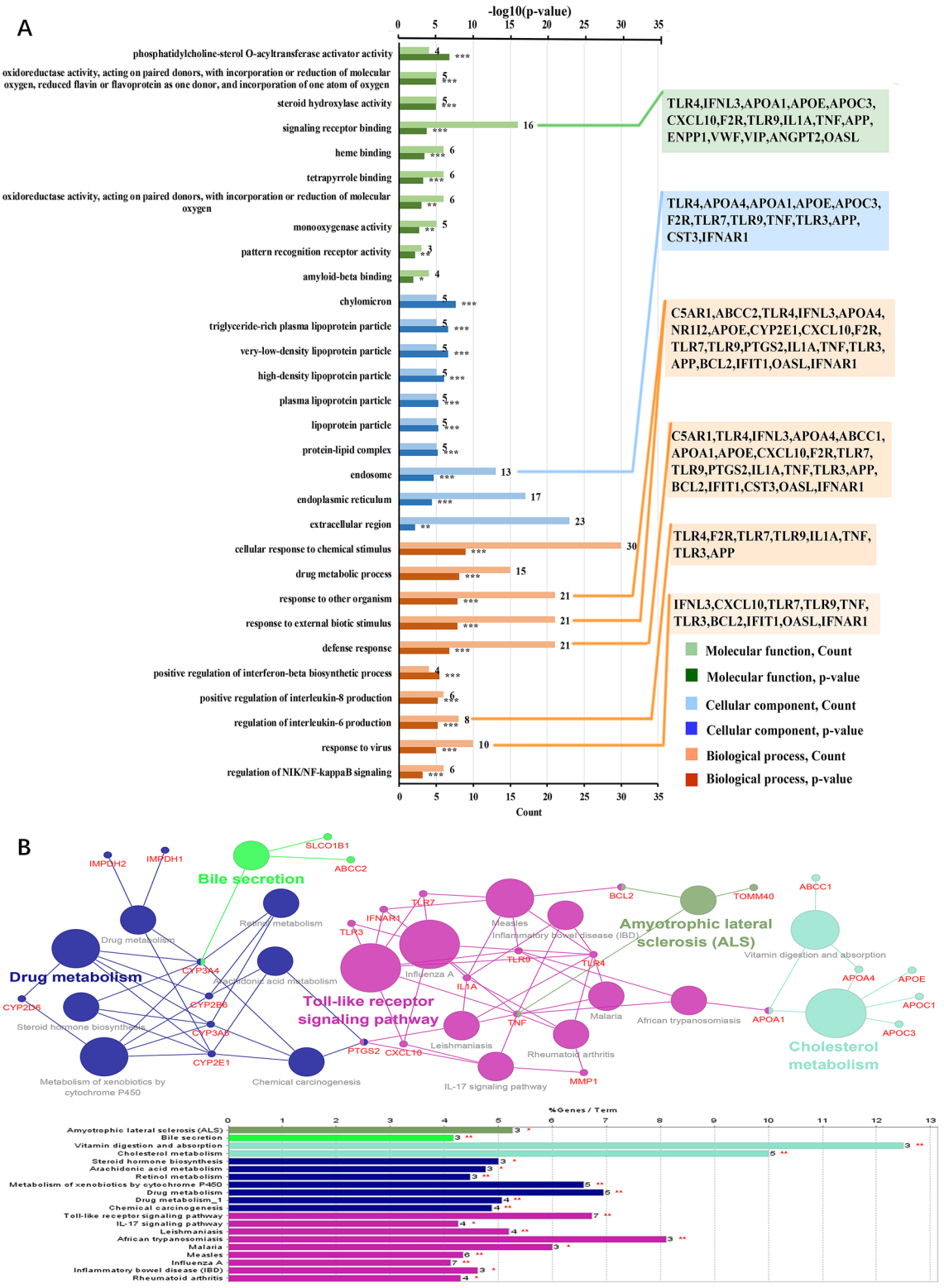


**Fig. 3** GO analysis and KEGG pathway analysis for the 43 identified genes. **a** GO analysis was performed using the g: Profiler database (biological process, cellular component, and molecular function). **b** The KEGG pathway analysis was constructed using ClueGO plug-in in Cytoscape software. Green represents the molecular function. Blue represents the cellular component. Orange represents the biological process. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$ (colour figure online)

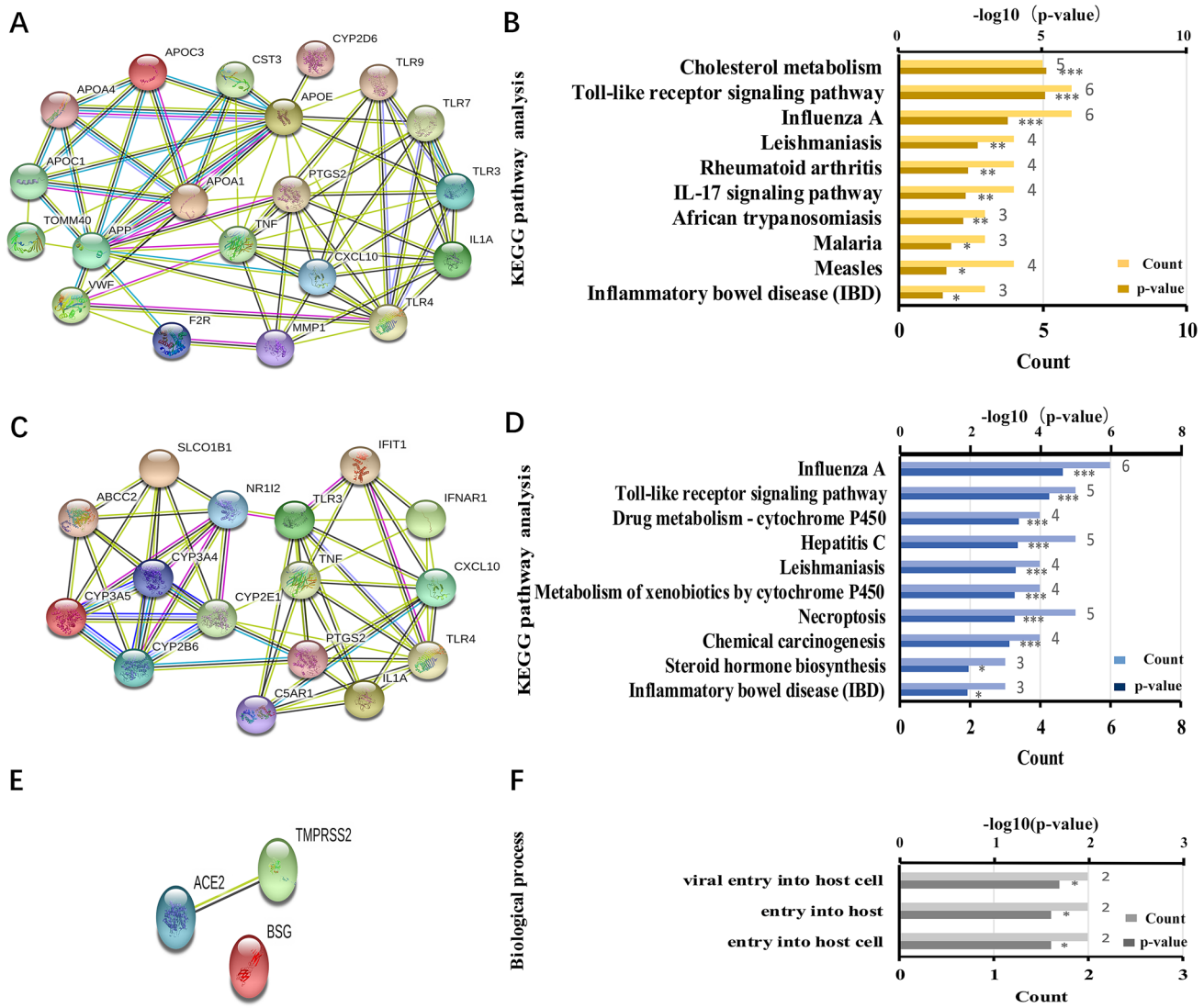


**Fig. 4** PPI network and KEGG pathway analysis for neighbor genes, module 1, and genes combination. **a** A PPI network of neighbor genes. **b** KEGG pathway analysis for neighbor genes. **c** A PPI network for module 1. **d** KEGG pathway analysis for module 1. **e** A PPI network of ACE2, TMPRSS2, and BSG. **f** KEGG pathway analysis of ACE2, TMPRSS2, and BSG

in drug metabolism, toll-like receptor signaling pathway, and influenza A. These findings revealed that both neighbor genes and module genes were highly involved in the biological process of influenza A and the toll-like receptor pathway. Of note, *ACE2* and *TMPRSS2* were highly associated with viral entry into host cells, suggesting that clinical interventions that target the two genes might be crucial for COVID-19 treatment.

## Identification of key genes

Based on the GO and KEGG pathway analysis results, we found that the 43 genes were mainly and highly involved in the body's response to pathogens ($n=21$, $P<0.001$) and inflammatory reactions ($n=21$, $P<0.001$). KEGG analysis also demonstrated that these genes are highly correlated with toll-like receptor pathways ($n=7$, $P<0.01$), and biological processes such as influenza A ($n=7$, $P<0.01$). This tendency led us to attach importance to and focus on the selection of these items. To reveal genes that play an important role in this process, we selected 9 enrichment items with significant *P* values, among which, 'response to other organisms', 'response to virus', 'PRRs activity', 'regulation of NF-κB signaling', 'regulation of IL-6 production', 'positive regulation of IL-8 production', and 'positive regulation of IFN-β biosynthetic process' were used to simulate a series of inflammatory responses triggered by virus invasion. By overlapping genes in these selected analysis terms, we found that *TLR7* and *TLR9* were involved in virus invasion, activation of PRRs, and production of related inflammatory factors

(Fig. 5). Besides, the 10 genes involved in the response of virus invasion were used to perform intersections with the 16 module genes and 20 neighbor genes, as well as genes in GO analysis terms influenza A and defense response, respectively. *CXCL10*, *TNF*, and *TLR3* were identified as the other 3 key genes (Fig. 5). Eventually, 5 genes (*TLR3*, *TLR7*, *TLR9*, *CXCL10*, and *TNF*) were recognized to be critical in pharmaceutical therapy of COVID-19. Considering that COVID-19 patients usually develop multiple organ function impairment, we hypothesized that PRRs activation mediated toll-like receptor pathway might play an important role in COVID-19 progression.

## Candidate drugs that target key genes

We supposed that 10 genes, including 5 key genes, *ACE2*, *TMPRSS2*, *BSG*, and their first neighbor genes *APOE* and *MMP1*, may serve as potential targets for COVID-19 treatment. Using the DGIdb database, 87 drugs were screened as candidate drugs, among which CHEMBL260273, CHEMBL429844, gavilimomab have not been approved by FDA (Fig. 6). Of the 4 drugs targeted at ACE2, 3 namely CHEMBL260273, CHEMBL429844, and lisinopril were inhibitors (Chen et al. 2002). However, it is unclear whether ACE2 inhibitors should be used in preventing SARS-CoV-2 infection since there is not enough evidence to support or refute the application of ACE inhibitors for the benefit or harm on COVID-19 patients (Danser et al. 2020). Notably, clinical observation of COVID-19 patients in China showed that inpatient use of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) was associated with a lower risk of all-cause mortality and better outcomes compared with ACEI/ARB non-users (Meng et al. 2020; Zhang et al. 2020). For now, the use of ACE2 inhibitors or anti-hypertension agents should be continued in people at risk of infection and COVID-19 patients until when definitive data shall be availed (Vaduganathan et al. 2020).

## miRNA–gene network

To explore the regulatory relationship between the 5 key genes, 3 coronavirus-related genes (*ACE2*, *TMPRSS2*, *BSG*), and their target miRNA, we used 6 miRNA target prediction databases to predict the target miRNA of these genes. A total of 271 miRNA-gene interactions were screened. A co-expression network based on the relationships among these genes and 220 miRNAs was shown in Fig. 7.

# Discussion

SARS-CoV-2 is a single-strand RNA enveloped virus that belongs to the family of β-coronavirus, together with SARS-CoV and MERS-CoV. SARS-CoV-2 invades into host cells by binding to the ACE2 receptor on the host cell via the

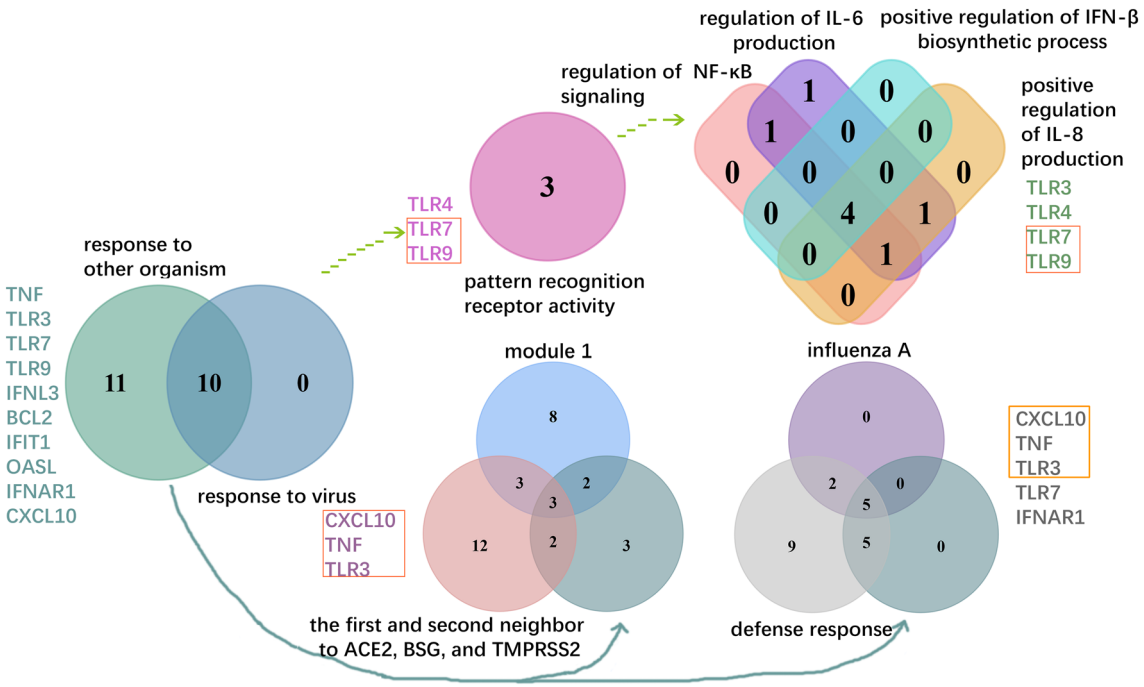


**Fig. 5** Venn diagrams. Identification of key genes from 9 selected GO terms, module 1, and neighbor genes using FunRich software. Different colors represent different gene combinations. Cross areas represents overlapping genes

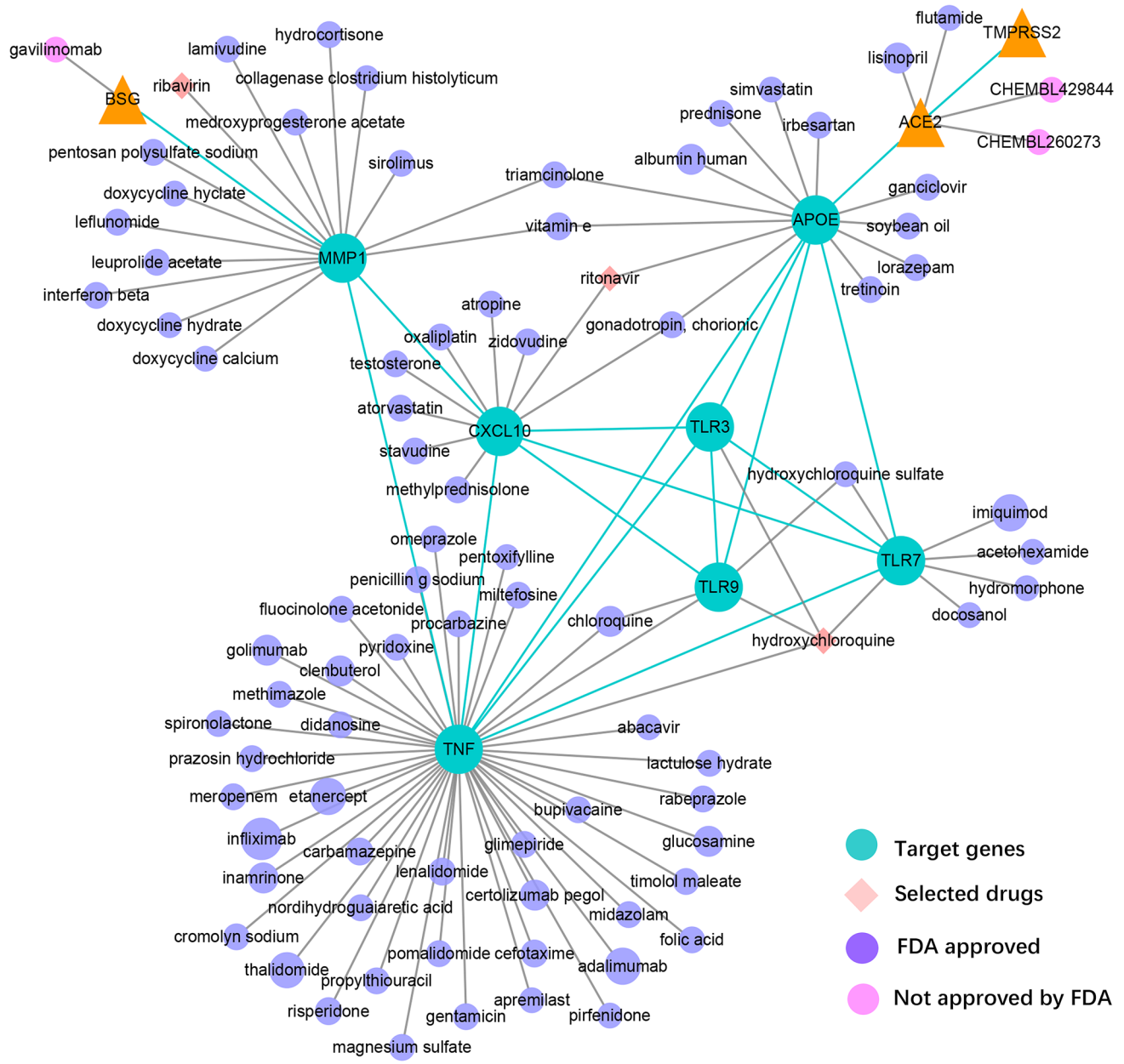


**Fig. 6** Gene-drug interaction network. The green circle represents target genes. The red diamond node represents the selected drug. The purple circular node represents a drug that has been approved by the FDA. The pink circular node represents drugs that have not been approved by the FDA. The green edge represents the interaction between target genes. The grey edge represents interactions between drugs and genes (colour figure online)

spike (S) protein. In this process, the TMPRSS2 protease and BSG (also called EMMPRIN and CD147) mediate the SARS-CoV invasion of host cells (Biswas et al. 1995; Hoffmann et al. 2020; Yurchenko et al. 2006). Also involved in the progression of the disease are TLR family members, which regulate the body's anti-viral response to invaders and the upregulation of pro-inflammatory mediators. When a series of events including viral entry into cells and the innate immune response are uncontrolled, infected patients occasionally experience a harmful systemic response that leads to a "cytokine storm". This is followed by the down-regulation of T cells, dendritic cells, and macrophages, causing multi-organ failure (Coperchini et al. 2020).

Controlling viral infection requires an appropriate and innate coordination of host antiviral immunity. This response is activated by various sensors, including PRRs that recognize pathogen-associated molecular patterns (PAMPs). For many viruses, viral RNA acts as PAMP and is recognized

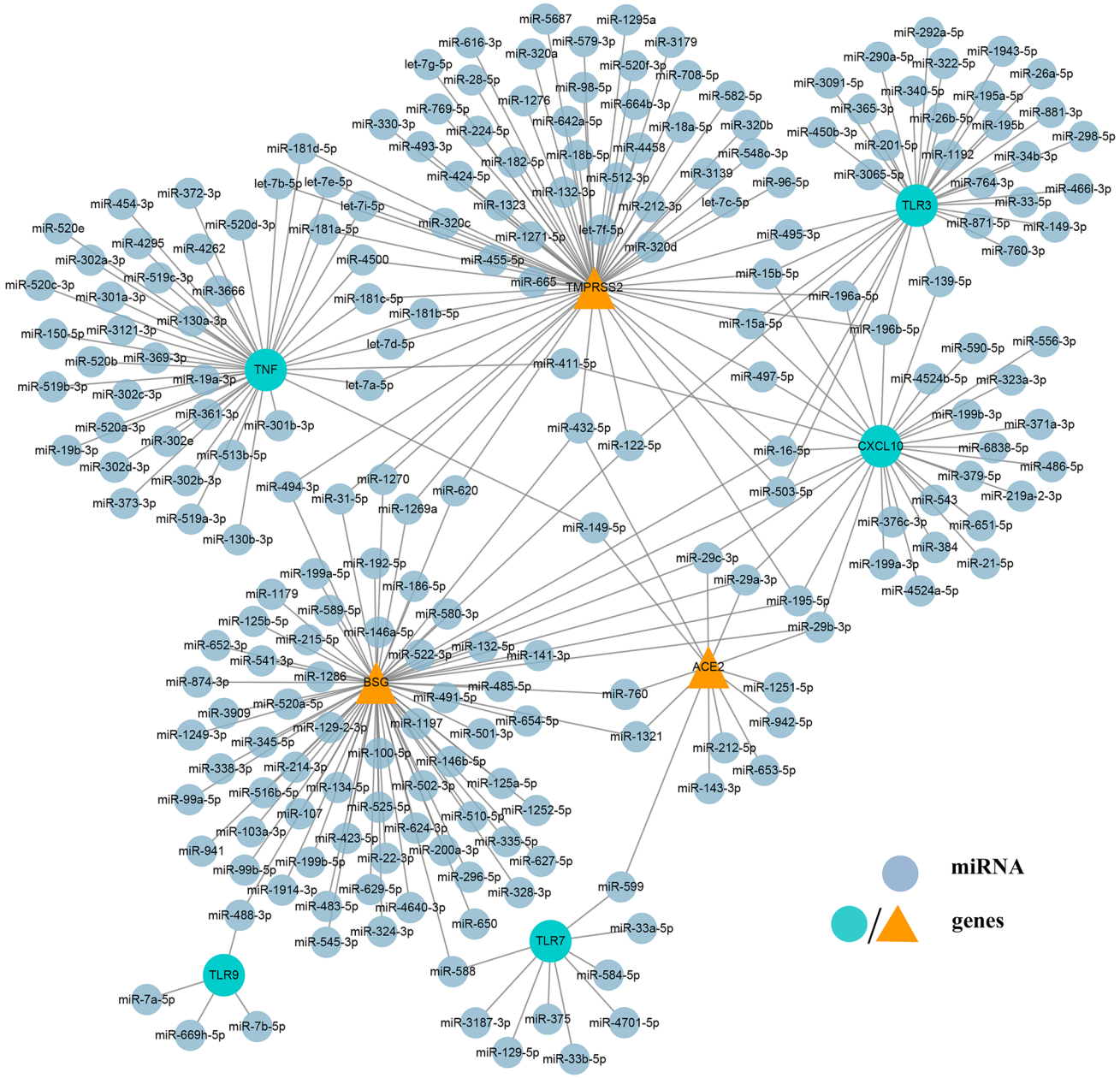


**Fig. 7** miRNA-gene interaction network. The blue circular node represents miRNAs. The orange triangle and green circular node represent genes (colour figure online)

by many different receptors, such as TLR3 (recognition of double-stranded RNA [DS]), TLR7 and TLR8 (recognition of single-stranded RNA [SS]), and melanoma differentiation association gene 5 (MDA-5), which recognize long dsRNA (Chow et al. 2018). However, the specific process and mechanism by which these receptors recognize the coronavirus genome remain elusive.

Information on the associations between inflammatory reaction and anti-virus defense response may provide new insight into the treatment of COVID-19. Usually, some specific metabolic activities may be directly involved in disease progression. For instance, COVID-19 patients tended to develop functional impairment of more than one vital organ such as acute lung injury and hepatic insufficiency (Nabil et al. 2020). Some researchers believed that the fundamental cause of such impairment is associated with cytokine storm, and IL-6 seemed to be a key driver of this inflammatory disorder (Sanders et al. 2020). When the body is invaded by pathogens or tissue cells are damaged, the PRRs, including PAMPs and damage-associated molecular

patterns (DAMPs), will be activated, consequently activating the toll-like receptor pathway. This is followed by the activation of the downstream NF-κB signaling pathway, and ultimately causes systemic inflammatory responses and multi-organ damage (Erard and Ryffel 2008; Ibrahim et al. 2013; Tsan and Gao 2007). In this study, *TLR3*, *-7*, *-9*, *TNF*, and *CXCL10* were identified as hub genes in COVID-19. We found that these genes were particularly enriched in PRRs activation mediated TLR pathways, suggesting that TLR pathways may be remarkably involved in the progression of the disease. Among the 5 genes, *TLR7* and *TLR9* were involved in the entire recognition process of viral response, PRRs response, activation of toll-like receptor pathway, and production of downstream inflammatory mediators, suggesting that *TLR7* and *TLR9* may play a crucial role in the process. Recently, data from several sources have implicated *TLR7* as the most biologically credible candidate gene for young male patients with severe COVID-19, which may be associated with impaired type I and type II interferon responses resulting from functional impairment of the *TLR7* gene variant on the X chromosome (van der Made et al. 2020). In mice, TLR7 is considered to be an important PRR for the identification of MERS-CoV and SARS-CoV ssRNA. A relevant study have shown that the TLR7- IFN regulatory factor 7 (IRF7) pathway contributes to the production of IFN in airway epithelial cells after MERS-CoV infection in mice (van der Made et al. 2020). In addition, the whole genome sequencing of SARS-CoV, MERS-CoV, and SARS-CoV-2 showed that, compared with the SARS-CoV, the SARS-COV-2 genome contained more ssRNA motifs that could interact with TLR7, suggesting that the TLR7 pathway may be more relevant in the COVID-19 pathogenesis (Moreno-Eutimio et al. 2020). Similar to TLR7, the TLR3 receptor can induce IRF3 activation relatively parallel to the TLR7-IRF7 pathway, which induces IFN gene transcription via migration into the nucleus. However, TLR7 was also documented to accelerate the acute lung injury by enhancing the NOX2 oxidase-dependent oxidative burst in macrophages (To et al. 2014). Elsewhere, a study by Shi et al. reported that TLR3 and TLR7 could enhance the inflammatory damage caused by the overproduction of IL-1 and IL-6 in virus infection (Shi et al. 2020). Even though TLR3, -7 and − 9 usually accelerates the inflammatory reaction, in some circumstances, they recognize viral nucleic acids and rapidly trigger different signaling cascades that contribute to the production of IFNs to antiviral defense (Zhu et al. 2019). C-X-C chemokine 10 (CXCL10) and tumor necrosis factor (TNF) are pro-inflammatory chemokines, both of which play crucial roles in severe lung damage caused by cytokine storms (Coperchini et al. 2020). The chemokine CXCL10, previously known as the IFN-γ inducible 10-kDa protein or IP-10, has been found to induce olfactory nerve demyelination via CXCL10-dependent chemotaxis of T lymphocytes in central nerves system, causing olfactory dysfunction in COVID-19 patients (Oliviero et al. 2020). All these studies suggest that the 5 genes may be critical in extensively understanding the COVID-19 pathogenesis and to uncover highly effective treatments for the disease.

Moreover, we found that hydroxychloroquine could target *TLR3*, *-7*, and − *9*, which might render a potential immunomodulatory role in COVID-19 (Gies et al. 2020). In the recent past, many drugs for the management of COVID-19 are currently under evaluation or development, of which, hydroxychloroquine remains the most controversial drug. Hydroxychloroquine has a long history in the treatment of malaria as well as systemic lupus erythematosus and rheumatoid arthritis. A few assessments have shown that hydroxychloroquine may benefit patients by inhibiting the virus entry into host cells and its potential immune regulatory effect (Ferner and Aronson 2020). In-vitro experiments showed that hydroxychloroquine could inhibit SARS-CoV-2 at a lower concentration (Yao et al. 2020). A wealth of clinical trials has shown no significant changes in patient mortality, need for intubation, or virus-negative conversion at 28 days after hydroxychloroquine administration (Rosenberg et al. 2020). As of now, there are more than 200 ongoing trials of hydroxychloroquine for the treatment of COVID-19 (Sanders et al. 2020). In a WHO-sponsored international clinical trial, on 17 June 2020, it was announced that the hydroxychloroquine section of the Solidarity trial was discontinued due to concerns about the safety of hydroxychloroquine and its associated adverse cardiac events, as it had failed to reduce mortality in hospitalized COVID-19 patients (Gadebusch Bondio and Marloth. 2020; Rosenberg et al. 2020). Should we deny hydroxychloroquine completely based on this statement? In response to the decision to withdraw hydroxychloroquine from the Solidarity trial, WHO stated that this decision applies only to hospitalized COVID-19 patients and does not affect the study evaluation on the use of hydroxychloroquine in other non-hospitalized patients, or the evaluation of preexposure and postexposure prevention of COVID-19 (Gadebusch Bondio and Marloth 2020). Besides, a considerable amount of literature has demonstrated the antiviral role of hydroxychloroquine and its important immunomodulatory properties, such as its use in systemic lupus, arthritis, and other autoimmune diseases (Rosenberg et al. 2020). Hydroxychloroquine can inhibit lysosomal activity and autophagy, lowering antigen processing and presentation (Gies et al. 2020). Further, it interferes with toll-like receptor signaling and nucleic acid cytoplasmic sensors, thereby inhibiting cell activation and reducing the secretion of type 1 interferon and inflammatory cytokines (Gies et al. 2020). Considering its antiviral and anti-inflammatory properties, we presumed that hydroxychloroquine makes sense for the prevention and treatment of COVID-19, even though the immunomodulatory effects

of hydroxychloroquine may sometimes be harmful. For example, as an antagonist of *TLR7* and *TLR9*, it weakens the host's immune response to the virus and reduces the production of interferon, thus providing an opportunity for the virus entry into the body and to replicate (Sakata et al. 2018; Gies et al. 2020). This duality may also explain the divergence in recent studies on hydroxychloroquine. It is worth noting that hydroxychloroquine may be useful, but more research is needed to reveal its mechanism of action. However, combining multiple drugs to balance it's antiviral and immunomodulator may be a reliable way to treat COVID-19. Targeting *CXCL10*, lopinavir/ritonavir has received considerable attention because of the beneficial effects in some cases, which have long been used to treat AIDS. However, lopinavir/ritonavir did not provide significant benefit in patients with severe COVID-19, according to the results of a recent clinical trial (Costanzo et al. 2020).

Besides, as an RNA-dependent RNA synthase inhibitor, remdesivir may be an ideal drug in this fight against the epidemic. As announced on May 1, 2020, the FDA granted emergency use authorization (EUA) for research on remdesivir as a potential antiviral against COVID-19 (Nabil et al. 2020). In an early clinical trial, patients administered with remdesivir exhibited a shorter recovery time than those given placebo (Costanzo et al. 2020). Despite these considerable advances, the implementation of individualized treatment regimens in different populations and at different stages of the disease remains elusive. Thus, the implementation of specific treatment guidelines will require more extensive and standardized randomized clinical trials of these drugs.

To obtain more information about the regulatory relationship between the hub gene and the three coronavirus-related receptors (ACE2, TMPRSS2, and BSG), we constructed a network of these genes and their targeted miRNAs. The network may provide new insights for COVID-19 treatment strategies. For example, some researchers documented that miR-200c-3p could be upregulated via the NF-κB pathway to reduce ACE2 levels, this elevated angiotensin II levels and subsequently led to lung injury (Liu et al. 2017). miR-146a-5p, one of the miRNAs targeting BSG, could enhance the replication of infectious bronchitis virus (one kind of coronavirus which infects chickens) at the early stage of infection by downregulating IL-1 receptor-associated kinase-2 (IRAK2) and TNF receptor superfamily member 18 (TNFRSF18) (Liu et al. 2018). Through this miRNA–gene network, we could obtain more reliable information about the molecular mechanisms of COVID-19.

Due to the incomplete information in the database and the differences in drug action, we did not find the genes that interacted with the following 5 drugs (arbidol, chloroquine diphosphate, ribavirin, favipiravir, and remdesivir). Thus, future studies are needed to clarify the specific mechanisms of their action. In the epidemic, there have been numerous studies on these drugs which have demonstrated their potential in managing COVID-19. For instance, as a non-nucleoside broad-spectrum antiviral drug, arbidol has been shown to effectively inhibit the replication of SARS-CoV-2 (Zhu et al. 2020). Also, darunavir, an antiviral drug of the protease inhibitor, was able to inhibit the virus, in vitro (Costanzo et al. 2020). Favipiravir which belongs to the RNA dependent RNA polymerase (RdRp) inhibitor and is a broad-spectrum anti-influenza virus drug, has recently been shown to exert significant *in vitro* inhibitory effects against novel coronavirus (Du and Chen 2020). Chloroquine diphosphate is mainly used for treating malaria and rheumatic diseases. Notably, previous studies documented that chloroquine diphosphate has a broad-spectrum antiviral effect and can effectively inhibit SARS-CoV-2 infection at the cellular level (Ferner and Aronson 2020).

Currently, most of the drug choices are based on previous experience in the treatment of SARS, MERS, or other new influenza viruses, thus active symptomatic support therapy remains the focus of clinical treatment. Screening based on drug targets and their mechanisms is still the key to developing new drugs. The present study provides comprehensive bioinformatics information of genes that are potential targets and candidate drugs for COVID-19. This information may help to better understand the relevant molecular targeting mechanisms of this disease. However, further explorations are required to evaluate the biological functions of these biomarkers and drugs in the pathogenesis of the disease.

**Author contributions** All the authors contributed significantly to this work. LW and GK conceived and supervised the study; ST and WC drafted the manuscript; ST wrote the manuscript; HX and LZ made manuscript revisions. Thanks to all authors for their immense contributions to this article.

**Funding** This work was supported by the Key Research and Development Program of Hunan Province (Grant No. 2020SK3011).

## Compliance with ethical standards

**Conflict of interest** Siyou Tan, Wenyan Chen, Hongxian Xiang, Gaoyin Kong, Lianhong Zou, and Lai Wei declare that they have no conflict of interest.

## References

Bandettini WP, Kellman P, Mancini C, Booker OJ, Vasu S, Leung SW, Wilson JR, Shanbhag SM, Chen MY, Arai AE (2012) MultiContrast Delayed Enhancement (MCODE) improves detection of subendocardial myocardial infarction by late gadolinium enhancement cardiovascular magnetic resonance: a clinical validation study. J Cardiovasc Magn Reson 14:83

Benito-Martin A, Peinado H (2015) FunRich proteomics software analysis, let the fun begin! Proteomics 15:2555–2556

Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman WH, Pages F, Trajanoski Z, Galon J (2009) ClueGO: a cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics 25:1091–1093

Biswas C, Zhang Y, DeCastro R, Guo H, Nakamura T, Kataoka H, Nabeshima K (1995) The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. Cancer Res 55:434–439

Chen X, Ji ZL, Chen YZ (2002) TTD: therapeutic target database. Nucleic Acids Res 30:412–415

Chen Z, Mi L, Xu J, Yu J, Wang X, Jiang J, Xing J, Shang P, Qian A, Li Y et al (2005) Function of HAb18G/CD147 in invasion of host cells by severe acute respiratory syndrome coronavirus. J Infect Dis 191:755–760

Chow KT, Gale M Jr, Loo YM (2018) RIG-I and other RNA sensors in antiviral immunity. Annu Rev Immunol 36:667–694

Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M (2020) The cytokine storm in COVID-19: an overview of the involvement of the chemokine/chemokine-receptor system. Cytokine Growth Factor Rev 53:25–32

Costanzo M, De Giglio MAR, Roviello GN (2020) SARS-CoV-2: recent reports on antiviral therapies based on lopinavir/ritonavir, darunavir/umifenovir, hydroxychloroquine, remdesivir, favipiravir and other drugs for the treatment of the new coronavirus. Curr Med Chem 27:4536–4541

Cotto KC, Wagner AH, Feng YY, Kiwala S, Coffman AC, Spies G, Wollam A, Spies NC, Griffith OL, Griffith M (2018) DGIdb 3.0: a redesign and expansion of the drug-gene interaction database. Nucleic Acids Res 46:D1068–D1073

Danser AHJ, Epstein M, Batlle D (2020) Renin-angiotensin system blockers and the COVID-19 pandemic: at present there is no evidence to abandon renin-angiotensin system blockers. Hypertension 75:1382–1385

Du YX, Chen XP (2020) Favipiravir: pharmacokinetics and concerns about clinical trials for 2019-nCoV infection. Clin Pharmacol Ther 108:242–247

Erard F, Ryffel B (2008) Toll like receptor—potential drug targets in infectious disease. Infect Disord Drug Targets 8:221–231

Ferner RE, Aronson JK (2020) Chloroquine and hydroxychloroquine in covid-19. BMJ 369:m1432

Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Minguez P, Bork P, von Mering C et al (2013) STRING v9.1: protein–protein interaction networks, with increased coverage and integration. Nucleic Acids Res 41:D808–D815

Gadebusch Bondio M, Marloth M (2020) The "Historic Study" SOLIDARITY-research's answer to the Sars-CoV-2 Pandemic. NTM 28:219–225

Gies V, Bekaddour N, Dieudonne Y, Guffroy A, Frenger Q, Gros F, Rodero MP, Herbeuval JP, Korganow AS (2020) Beyond antiviral effects of chloroquine/hydroxychloroquine. Front Immunol 11:1409

Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, Feldt T, Green G, Green ML, Lescure FX et al (2020) Compassionate use of remdesivir for patients with Severe Covid-19. N Engl J Med 382:2327–2336

Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A et al(2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181:271–280 (**e278**)

Ibrahim ZA, Armour CL, Phipps S, Sukkar MB (2013) RAGE and TLRs: relatives, friends or neighbours? Mol Immunol 56:739–744

Liu Q, Du J, Yu X, Xu J, Huang F, Li X, Zhang C, Li X, Chang J, Shang D et al (2017) miRNA-200c-3p is crucial in acute respiratory distress syndrome. Cell Discov 3:17021

Liu H, Yang X, Zhang ZK, Zou WC, Wang HN (2018) miR-146a-5p promotes replication of infectious bronchitis virus by targeting IRAK2 and TNFRSF18. Microb Pathog 120:32–36

Meng J, Xiao G, Zhang J, He X, Ou M, Bi J, Yang R, Di W, Wang Z, Li Z et al (2020) Renin-angiotensin system inhibitors improve the clinical outcomes of COVID-19 patients with hypertension. Emerg Microbes Infect 9:757–760

Moreno-Eutimio MA, Lopez-Macias C, Pastelin-Palacios R (2020) Bioinformatic analysis and identification of single-stranded RNA sequences recognized by TLR7/8 in the SARS-CoV-2, SARS-CoV, and MERS-CoV genomes. Microbes Infect 22:226–229

Nabil A, Uto K, Elshemy MM, Soliman R, Hassan AA, Ebara M, Shiha G (2020) Current coronavirus (SARS-CoV-2) epidemiological, diagnostic and therapeutic approaches: an updated review until June 2020. EXCLI J 19:992–1016

Oliviero A, de Castro F, Coperchini F, Chiovato L, Rotondi M (2020) COVID-19 pulmonary and olfactory dysfunctions: is the chemokine CXCL10 the common denominator? . Neuroscientist 0:1073858420939033

Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, Vilo J (2019) g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res 47:W191–W198

Rosenberg ES, Dufort EM, Udo T, Wilberschied LA, Kumar J, Tesoriero J, Weinberg P, Kirkwood J, Muse A,DeHovitz J et al (2020) Association of treatment with hydroxychloroquine or azithromycin with in-hospital mortality in patients with COVID-19 in New York State. JAMA 323:2493–2502

Sakata K, Nakayamada S, Miyazaki Y, Kubo S, Ishii A, Nakano K, Tanaka Y (2018) Up-regulation of TLR7-mediated IFN-alpha production by plasmacytoid dendritic cells in patients with systemic lupus erythematosus. Front Immunol 9:1957

Sanders JM, Monogue ML, Jodlowski TZ, Cutrell JB (2020) Pharmacologic treatments for coronavirus disease 2019 (COVID-19): a review. JAMA 323:1824–1836

Shahzadi S, Shabana, Chaudhry M, Arooj I, Hasnain S (2016) A single-nucleotide polymorphism in C12orf43 region is associated with the risk of coronary artery disease in a Pakistani cohort. Biochem Genet 54:676–684

Shi D, Chen M, Liu L, Wang Q, Liu S, Wang L, Wang R (2020) Anti-influenza A virus mechanism of three representative compounds from Flos Trollii via TLRs signaling pathways. J Ethnopharmacol 253:112634

To EE, Broughton BR, Hendricks KS, Vlahos R, Selemidis S (2014) Influenza A virus and TLR7 activation potentiate NOX2 oxidase-dependent ROS production in macrophages. Free Radic Res 48:940–947

Treister A, Pico AR (2018) Identifier mapping in cytoscape. F1000Res 7:725

Tsan MF, Gao B (2007) Pathogen-associated molecular pattern contamination as putative endogenous ligands of Toll-like receptors. J Endotoxin Res 13:6–14

Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD (2020) Renin-angiotensin-aldosterone system inhibitors in patients with Covid-19. N Engl J Med 382:1653–1659

van der Made CI, Simons A, Schuurs-Hoeijmakers J, van den Heuvel G, Mantere T, Kersten S, van Deuren RC, Steehouwer M, van Reijmersdal SV, Jaeger M et al (2020) Presence of genetic variants among young men with severe COVID-19. JAMA 324:663–673

Wang D, Tian L, Shi C, Wei YX, Wang H, Liu TT, Gong M, Zhang YW, Yu RG, Wu XH (2020) Network pharmacology-based prediction of the active ingredients and mechanism of Shen Gui capsule for application to coronary heart disease. Comput Biol Med 122:103825

Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, Liu X, Zhao L, Dong E, Song C et al (2020) In vitro antiviral activity and projection

of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). Clin Infect Dis 71:732–739

Ye Q, Wang B, Mao J (2020) The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. J Infect 80(6):607–13

Yurchenko V, Constant S, Bukrinsky M (2006) Dealing with the family: CD147 interactions with cyclophilins. Immunology 117:301–309

Zhang YF, Huang Y, Ni YH, Xu ZM (2019) Systematic elucidation of the mechanism of geraniol via network pharmacology. Drug Des Devel Ther 13:1069–1075

Zhang P, Zhu L, Cai J, Lei F, Qin JJ, Xie J, Liu YM, Zhao YC, Huang X, Lin L et al (2020) Association of inpatient use of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers with mortality among patients with hypertension hospitalized with COVID-19. Circ Res 126:1671–1681

Zhu Y, Shao Y, Qu X, Guo J, Yang J, Zhou Z, Wang S (2019) Sodium ferulate protects against influenza virus infection by activation of the TLR7/9-MyD88-IRF7 signaling pathway and inhibition of the NF-kappaB signaling pathway. Biochem Biophys Res Commun 512:793–798

Zhu Z, Lu Z, Xu T, Chen C, Yang G, Zha T, Lu J, Xue Y (2020) Arbidol monotherapy is superior to lopinavir/ritonavir in treating COVID-19. J Infect 81:e21–e23