

# Bioinformatics analysis of SARS-CoV-2 infection-associated immune injury and therapeutic prediction for COVID-19

Haomin Zhang<sup>a</sup>, Haoran Chen<sup>b</sup>, Jundong Zhang<sup>a</sup>, Ximeng Chen<sup>c</sup>, Bin Guo<sup>d</sup>, Peng Zhi<sup>a,b</sup>, Zhuoyang Li<sup>b</sup>, Geliang Liu<sup>b</sup>, Bo Yang<sup>a</sup>, Xiaohua Chi<sup>e</sup>, Yixing Wang<sup>f</sup>, Feng Cao<sup>g,\*</sup>, Jun Ren<sup>h,\*</sup>, Xuechun Lu<sup>a,\*</sup>

## Abstract

**Background:** Severe acute respiratory syndrome coronavirus 2 is a highly contagious viral infection, without any available targeted therapies. The high mortality rate of COVID-19 is speculated to be related to immune damage.

**Methods:** In this study, clinical bioinformatics analysis was conducted on transcriptome data of coronavirus infection.

**Results:** Bioinformatics analysis revealed that the complex immune injury induced by coronavirus infection provoked dysfunction of numerous immune-related molecules and signaling pathways, including immune cells and toll-like receptor cascades. Production of numerous cytokines through the Th17 signaling pathway led to elevation in plasma levels of cytokines (including *IL6*, *NF-κB*, and *TNF-α*) followed by concurrent inflammatory storm, which mediates the autoimmune response. Several novel medications seemed to display therapeutic effects on immune damage associated with coronavirus infection.

**Conclusions:** This study provided insights for further large-scale studies on the target therapy on reconciliation of immunological damage associated with COVID-19.

**Keywords:** Bioinformatics, Coronavirus, COVID-19, Drug prediction, Immune injury

HZ and HC contributed equally to this article.

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

<sup>a</sup>Department of Hematology, the Second Medical Center of the China PLA General Hospital & National Center for Clinical Medicine of Geriatric Diseases, Beijing, China, <sup>b</sup>Management School, Shanxi Medical University, Taiyuan, China, <sup>c</sup>Chinese Journal of Hepatobiliary Surgery, Beijing, China, <sup>d</sup>Department of Personnel, Cardiovascular Disease Hospital of Shanxi Province, Taiyuan, China, <sup>e</sup>Department of Pharmacy, China PLA Special Medical Center, Beijing, China, <sup>f</sup>Department of Internal Medicine of Traditional Chinese Medicine, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China, <sup>g</sup>Department of Cardiology, the Second Medical Center of the China PLA General Hospital, Beijing, China, <sup>h</sup>School of Pharmacy, University of Wyoming College of Health Sciences, Laramie, WY, USA

\* Corresponding author. Address: Department of Hematology, the Second Medical Center of the China PLA General Hospital & National Center for Clinical Medicine of Geriatric Diseases, Beijing 100853, China. E-mail address: luxuechun@126.com (X. Lu); School of Pharmacy, University of Wyoming College of Health Sciences, Laramie, WY 82071, USA. E-mail address: jren\_aldh2@outlook.com (J. Ren); Department of Cardiology, the Second Medical Center of the China PLA General Hospital Beijing, 100853, China. E-mail address: fengcao8828@163.com (F. Cao).

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## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly contagious virus, leading to a poor prognosis and high mortality rate.<sup>[1]</sup> Symptoms vary following coronavirus infections, although most patients present clinical complaints of fever, fatigue, respiratory symptoms, gastrointestinal symptoms, and chest imaging changes (Table 1). Most patients have a good prognosis, but some severe cases may develop acute respiratory distress syndrome or septic shock, leading to fatality.<sup>[2]</sup> At present, clinical treatment strategies for SARS-CoV-2 infections are mainly organ function support and prevention of complications. The lack of specific and target antiviral treatments remains a challenge, and there are no effective therapeutics for critically ill patients. A drug relocation study was conducted based on molecular docking technology using a coronavirus crystal structure. However, the predicted outcome suggested a single target compound that cannot be credited for all clinical problems, especially for SARS-CoV-2 infection-related immune damage. In fact, the omics impact of SARS-CoV-2 infection on human health is closely related to prognosis, clinical manifestation, and efficacy. Existing literature has confirmed that changing the microenvironment and epigenetics, as well as regulating the associated immune signaling pathways are effective ways to treat coronavirus infection.<sup>[3-6]</sup> Hence, this study took advantage of SARS-CoV-2 transcriptome data from the GEO database to conduct integrated multi-omics analysis to screen out molecules such as *IL6* and *TNF* that are associated with immune damage during SARS-CoV-2 infection. Signaling pathways such as Th17 signaling, T cell

**Table 1**

**Comparison of Symptoms in Different Subtypes of Coronavirus Infections<sup>[31–33]</sup>**

Symptoms	SARS	SARS-CoV-2
Systemic symptoms	Fever; fatigue; septic shock; metabolic acidosis; coagulation dysfunction	Chest pain; unconsciousness; muscle aches
Respiratory symptoms	Joint/systemic soreness; headache; chest pain	cough
Gastrointestinal symptoms	Rapid breathing; respiratory distress	Nasal congestion; runny nose; sore throat
Circulatory system symptoms	Diarrhea, nausea, vomiting	
Chest X-ray, CT imaging	Arrhythmia; heart failure; abnormal myocardial enzymes	Multiple ground glass shadows on both lungs, infiltrates, lung consolidation
	Extensive ground-glass or consolidation of both lungs	

SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CT, computed tomography.

receptor signaling, and B cell receptor signaling pathway may play important roles in SARS-CoV-2 infection-associated immune damage. Our study found that drugs such as Polygonum cuspidatum, etanercept, nevirapine, famciclovir, cidofovir, and fluvastatin may exert anticoronavirus effects through targeting molecules such as IL6, TNF, and IL1β and simultaneously regulate the immune system. This study attempted to offer direction and guidance for future basic and clinical research, in particular drug development against COVID-19.

**Materials and methods**

**Data**

Data employed in this study were downloaded from the GEO database, with detailed information provided in Table 2. The Impute package in Bioconductor was applied

to normalize the acquired expression profiles, in order to normalize data of individual sample.<sup>[7]</sup> Meanwhile, the annotation packet in Bioconductor was employed to annotate the data, and probes were corresponding to the gene. Finally, use the biomaRt software package to standardize gene names.<sup>[8]</sup>

**Differential expression analysis**

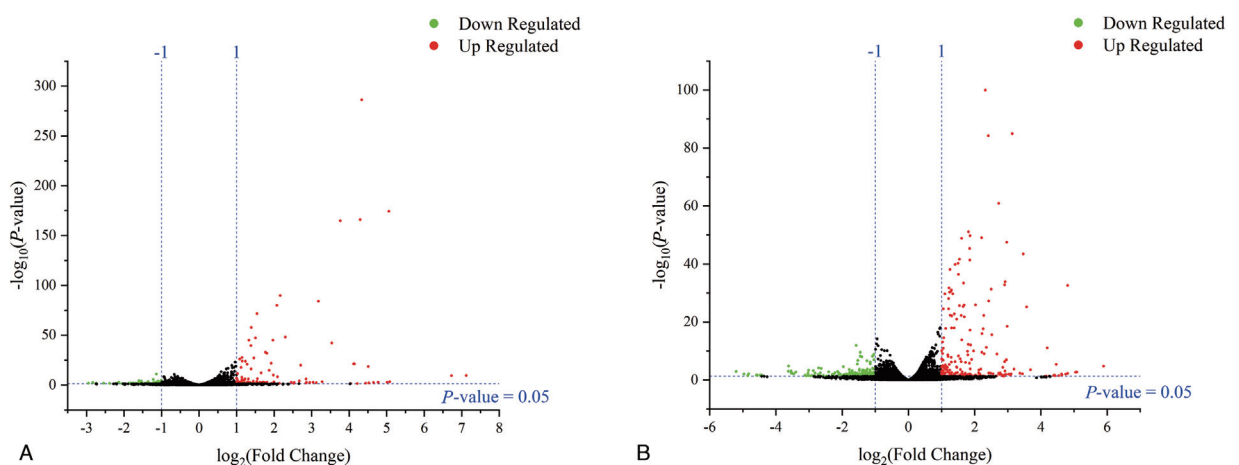
According to the experimental description and sample type, data were divided into four groups: NHBE cell line (Human Bronchial Epithelial Cells) infection and control group, A549 cell line (adenocarcinomic human alveolar basal epithelial cells) infection and control group. For different cell lines, a differential analysis was performed on the transcriptome data of SARS-CoV-2 infected and control cell line. The Limma software package in

**Table 2**

**Data Source Information for Transcriptome Analysis**

Data number	Species	Platform	Cell line	Cases	Treatment	Reference
GSE147507	Homo	GPL18573	NHBE	3	SARS-CoV-2 infected	<sup>[34]</sup>
			A549	3	SARS-CoV-2 infected	
			NHBE	3	Control	
			A549	3	Control	

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.



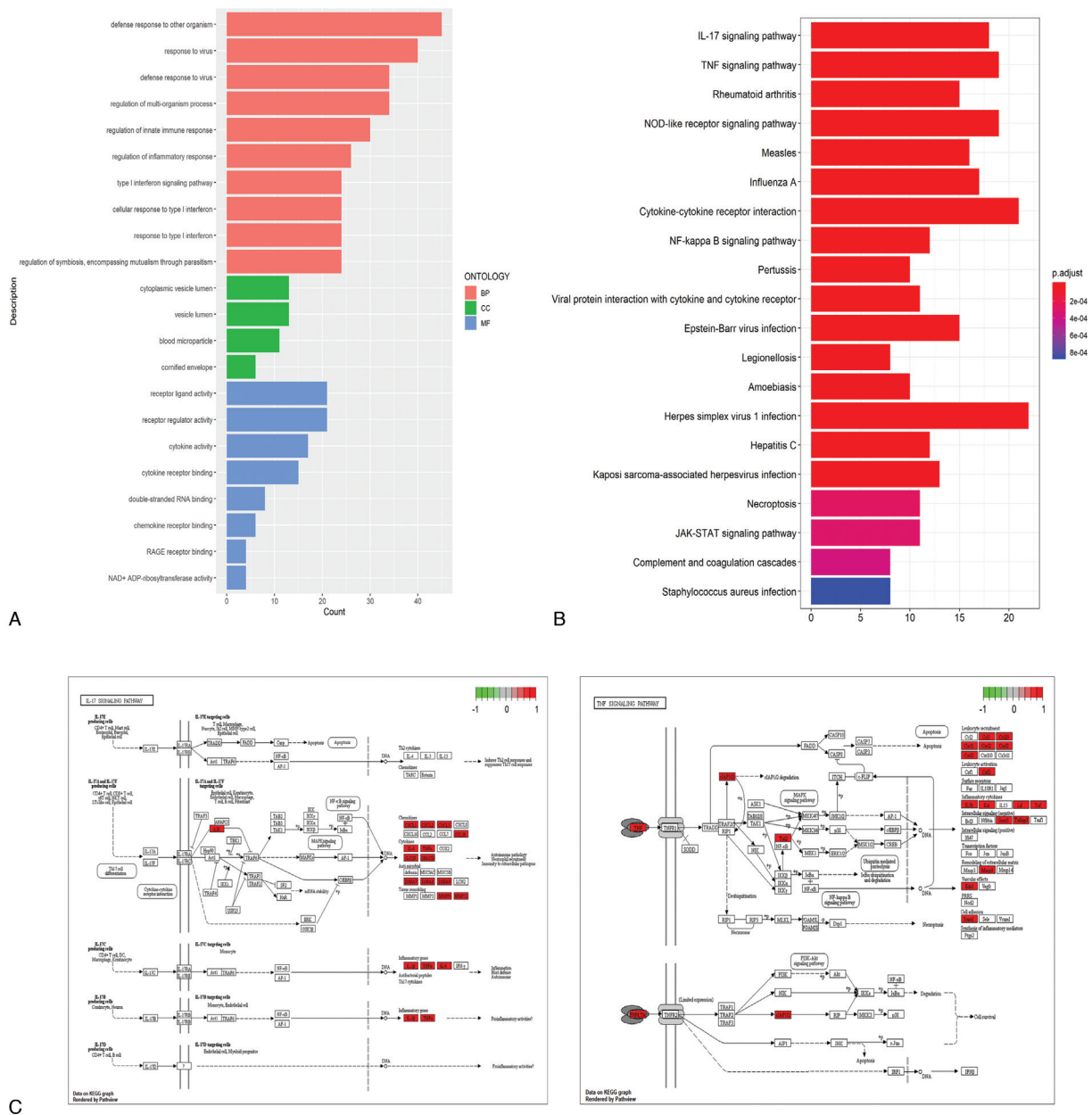
**Figure 1.** Difference analysis of transcriptome data of SARS-CoV-2 infected cell lines. The threshold was set as FDR < 0.05, Log<sub>2</sub>FC > 1. Red indicates up-regulated, whereas green indicates down-regulated. FDR, false discovery rate; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Bioconductor was used to screen the differential genes in transcriptome data of SARS-CoV-2 infected and control cell line. First, a preliminary screening was performed using  $P < 0.05$ , and thereafter the Bonferroni algorithm was used to correct original  $P$  values of the false discovery rate (FDR) and calculate fold change (FC).<sup>[9]</sup> In this study, the screening criteria for differential genes were  $FDR < 0.05$  and  $|\text{Log}_2\text{FC}| > 1$ . Subsequently, results of the preliminary screening were sorted out, the intersection genes with opposite regulatory effects in the differential genes of the NHBE and A549 groups were

removed, and gene expressions with same regulatory effects of the differential genes of the NHBE and A549 groups were averaged using the R program. Thus, characteristic expression gene of SARS-CoV-2 infection was obtained.

**Enrichment analysis and screening of key genes**

The clusterProfiler software package in R is a cluster analysis tool commonly used in bioinformatics.<sup>[10]</sup> It includes the Gene Ontology (GO)<sup>[11]</sup> and Kyoto



**Figure 2.** Enrichment analysis results and immune-related signaling pathways. (A) GO enrichment analysis results. The x-axis represents the number of genes enriched in each GO, and the y-axis represents the GO name. (B) The top 20 KEGG enrichment analysis results. The x-axis represents the number of genes enriched in the pathway, and the y-axis represents the pathway name. (C) Some important signaling pathways are shown, wherein red indicates the up-regulated genes and green indicates the down-regulated genes among the differential genes. The displayed pathways included *TNF* signaling pathway and *IL 17* receptor signaling pathway that were speculated to be closely related to the increase in plasma cytokines after coronavirus infection and pulmonary fibrosis in severe patients. Go, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes 1 and Genomes.

Encyclopedia of Genes and Genomes (KEGG) (<http://www.genome.jp/kegg/pathway.html>)<sup>[12]</sup> analyses. Here, SARS-CoV-2 characteristic expression genes screened above were clustered separately, and clustering results were preliminarily screened with  $P < 0.05$  as the threshold. Subsequently, based on symptoms of COVID-19 patients, immune-related signaling pathways were screened, and differential genes enriched in these signaling pathways were extracted as key genes for further analysis. Finally, results were visualized using the R language.<sup>[13]</sup>

### Construction of key gene protein-protein interaction network

A STRING v9.1 (<http://www.stringdb.org/>) open-source database was used to construct a protein-protein interaction (PPI) network.<sup>[14]</sup> The reliability threshold in this study was set at  $>0.7$ . Cytoscape software (<http://cytoscape.org/>) was used to build the PPI network.<sup>[15]</sup> In a PPI network, each node represents a gene, protein, or other molecule, and the connection between nodes represents the interaction between biological molecules. PPI networks can be used to identify interactions between proteins encoded by key genes that are closely related to immune damage associated with SARS-CoV-2 infection. Core nodes are proteins with important biological functions, which are determined by calculating the number of connections between proteins and whether each node is directly connected to another specific node while providing rankings. In this study, cytohubba software was used to screen the core nodes, which yielded the top 10 genes as the output.<sup>[16]</sup>

### Drug association analysis

Based on the theories of “systems biology” and “comparative functional genomics”, we designed an “Integrated Multi-omics Analysis” algorithm, which was used to establish a clinical bioinformatics analysis platform named Epi-precision Medicine Prediction Platform (EpiMed) that included human functional genomics big data of all diseases, 9000 commonly employed clinical drugs, 1000 Chinese medicines, and nearly 100,000 compounds. The platform was used to perform multi-omics association analysis of the abovementioned key genes to identify drugs or compounds that can effectively regulate the immune damage associated with SARS-CoV-2 infection among known compounds, commonly used drugs in clinical practice and traditional Chinese medicine. First, negative correlation outcomes were selected to indicate any drug with therapeutic effects, based on a correlation coefficient  $> |0.1|$ . A  $P$  value of  $<0.05$  was set as the threshold for screening.

## Results

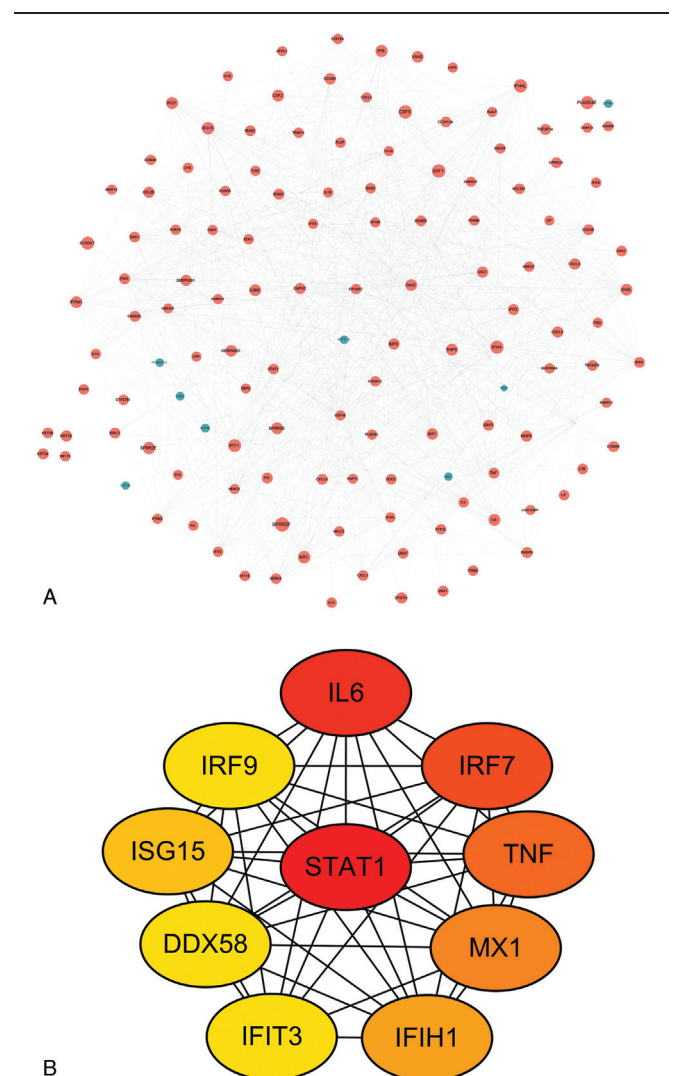
### The transcriptome signature of SARS-CoV-2 infected cell line

Differential gene screening threshold was set to  $FDR < 0.05$  and  $\text{Log}_2FC > 1$ . A total of 154 differentially expressed genes were screened, among which 114 were up-regulated and 40 were down-regulated in the HNBE

cell line group. A total of 128 differential genes were screened, of which 97 were up-regulated and 31 were down-regulated in the A549 cell line group (Fig. 1).

### Enrichment analysis and screening of immune-related genes

Next, enrichment analysis was performed on the sorted differential genes. GO analysis showed that the enrichment of these genes could be divided into three domains, including biological processes, cell components, and molecular function. In terms of biological processes, these genes are involved in viral infection, leukocyte proliferation and migration, intercellular signal transduction, and cytokine secretion. In terms of cellular components, most genes are related to endocytosis, exocytosis, vesicle synthesis, and secretion. In terms of molecular function, most of genes are related to the production and binding of cytokines, chemokines, and toll-like receptors (Fig. 2A).



**Figure 3.** Immune-damage-related gene PPI network and hub gene statistics. (A) Immune-damage-related gene PPI network. Red indicates up-regulated genes and blue indicate down-regulated genes. (B) The top 10 Hub gene statistics. PPI, protein-protein interaction.

**Table 3****Top 10 Correlation Analysis Results**

Name	Type	Correlation Coefficient	P Value	Therapeutics Molecular Targets
Polygonum cuspidatum	Herb	-0.748	<0.05	MYH7, TNF
Etanercept	Drug	-0.584	<0.05	CFD, THRSP, ADIPOQ, TMEM45B, OGN, DCN, HLF, ZNF212, ITGB5, DGAT2, SCD, FMO1, WFDC12, PSME1, MAFF, TUBB6, NOD2, MXD1, SERPINA3, IFITM3, NAMPT, HBEGF, ZFP36, LY6D,S100A4, TGM1, APOL1, CFB, IL1B, APOD, GBP3, NT5C3A, GBP2, NMI, UBE2L6, ATF3, ALDH1A3,C15orf48, MX1, OASL, CCL2, IL6
Houttuynia cordata	Herb	-0.396	<0.05	CCL2, STAT1, TNF, SOCS1
Nevirapine	Drug	-0.314	<0.05	LY6E, FASN,C15orf48, TIMP1, POLE4, COX7C, CHURC1, PSMA5, CENPWCUEDC1, MARCKSL1, LYRM1, DYNLL1, CFD, NT5C3A, OSTC,S100A4, SPATS2L,S100A10, GDF15, IGFBP6, SCD, CFB, SERPINA3, ISG15, ALDH1A3, MANF, ASS1, IFITM3, PPA1
Patrinia scabiosaefolia	Herb	-0.208	<0.05	IL1B, TNF
Famciclovir	Drug	-0.204	<0.05	CTSF, STAT2, PYCARD,C1QB, MARCKSL1, NFE2, LGALS3BP, PSMA5, CHD4, UBL3, PSMB9, IRF7, ITPA, SPL40,S100A4, PSME1, NEDD4, TNF
Cidofovir	Drug	-0.186	<0.05	OSTC, DSP,S100A10, SMOC1, TIMP1, SNRPF, FAM102B, CKS1B, DYNC112, ALDH1A3, GADD45A, IRF9,S100A14, MAMDC2, IFITM3,S100A4, IL1B, ISG15, TUBB6, PLAU, ASS1, CENPW, CSTA, GGCT,C1orf198, NAA20, IGFBP3, MX1, DYNLL1
Natural alpha interferon	Drug	-0.152	<0.05	COX7C, OAS1, DYNLL1, PARP12, LY6E, OAS3, PSMA5, ISG20, PALLD, IRF9, LAP3, PSMB8, TAP1, IFIT3, SNRPF, ISG15, APOL1, PML, PSMB9, DSP, IFITM3, TREX1, TRIM5, LGALS3BP, IGFBP6, IFIT2, EIF2AK2, NAMPT, CCL2, ADAR, PLAU, S100A10, OGFR, RSAD2, OASL, UBE2L6, MX1, PSME1, XAF1, AIDA, IFI35, OAS2, HERC6, DDX58, NMI, STAT1, IFIH1, IRF7
Ritonavir	Drug	-0.149	<0.05	CXCL10, ITPA, CYP2E1, Gadd45a, AIF1, IGFBP3, CTSF, GDFL5, LGALS3, PSME1, GATM, NR2F2, UBL3, RAB11B, C1QB, IRF7, NEDD4, PDK2, ADA, FMO1
Forsythia	Herb	-0.129	<0.05	TNF, BAX, PRTN3

KEGG analysis showed that these identified genes were mainly concentrated in immune-related pathways, infection-related signaling, immune factor secretion, binding, signal transduction, and other signaling pathways as well as cardiac function-related pathways (Fig. 2B and C). First, a threshold of  $P < 0.05$  was set to preliminarily screen 51 enrichment results, and subsequently 36 immune-related signaling pathways were further screened according to the immune-related clinical manifestations of SARS-CoV-2, among which 254 genes were extracted as key genes.

### Construction of key gene protein-protein interaction network

The PPI network was constructed for 254 key genes involved in 36 key pathways using the STRING online software. A total of 134 key genes coding various proteins were revealed in the PPI network. Figure 3A shows the interaction among these proteins. The interaction network was further analyzed, and core genes were screened according to the number of interacting gene connections. STAT1, IL6, IRF7, TNF, MX1, IFIH1, IFIT3, DDX58, ISG15, and IRF9 were the top 10 most abundantly expressed genes in the PPI network. The graphic display of statistical results is shown in Figure 3B.

### Screening of potential therapeutic drugs

Based on the theories of “systems biology” and “comparative functional genomics”, we designed an “Integrated Multi-omics Analysis” algorithm, which was used to establish a clinical bioinformatics analysis platform that

would include human functional genomics big data of all diseases, 9000 commonly used clinical drugs, 1000 Chinese medicines, and nearly 100,000 compounds – such system was named EpiMed. A total of 5000 negative correlation results were obtained using this platform for association analysis. According to the correlation coefficient  $> 0.1$  and  $P < 0.05$ , a total of 200 drugs with potential therapeutic effects were screened for negative correlation results. As shown in Table 3, the top 10 drugs were selected and were listed below.

### Discussion

Using clinical bioinformatics analysis of coronavirus transcriptome data, the salient findings from our study showed that coronavirus affects expression patterns of numerous genes, especially immune-related molecules, such as *STAT1*, *IL6*, *IRF7*, *TNF*, *MX1*, *IFIH1*, *IFIT3*, *DDX58*, *ISG15*, and *IRF9*. Previous evidence has confirmed that the abnormal expression of molecules such as *IL6* and *TNF* is closely related to the elevation of plasma cytokines in patients with coronavirus infection<sup>[17–19]</sup> (Fig. 4), which is consistent with our results. In addition, from the results of enrichment analysis, we speculated that changes in the expression levels of these molecules are also closely related to cardiac dysfunction, neurological symptoms, and metabolic and coagulation dysfunction in patients with coronavirus infection (Fig. 5). Further study on these signaling pathways should reveal the molecular mechanisms of the clinical manifestations and complications associated with immune impairment in coronavirus infection. The essence of coronavirus infection





development. Although researchers have identified several anticoronavirus drugs that target molecules and signaling pathways such as ACE2 or interferon signaling, they possess limited clinical utility and cannot resolve all clinical problems.<sup>[22,23]</sup> In this study, clinical bioinformatics was used to conduct a multi-omics association analysis of immune-related molecules following SARS-CoV-2 infection, in order to identify drugs that not only resist coronavirus infection but also regulate coronavirus-related immune abnormalities. Chang et al.<sup>[24]</sup> found that *Polygonum cuspidatum* could be used as an anti-SARS virus therapy. Chu et al.<sup>[25]</sup> demonstrated that ritonavir displayed therapeutic benefits against the SARS virus. The aforementioned results are in line with the results of our current study. Although other predicted drugs have not yet been confirmed, it may be predicted from correlation targets that these drugs can regulate the expression of immune-related molecules such as *TNF*, *IL1B*, *IL6*, and *CXCL10*. Their therapeutic effects and mechanisms need to be explored in future studies.

With the advance of clinical bioinformatics and the accumulation of coronavirus infection-related immunogenomic data, future studies are required to fully reveal the mechanism of immune damage following coronavirus infection. In this context, drug development should highly target and significantly inhibit coronavirus infection-related immune damage. Since 2000, we have been using clinical bioinformatics to study the diagnosis, treatment, and prognosis of blood diseases. During 20 years of clinical bioinformatics research based on “systems biology” and “functional genomics” theories of innovative design of the “integration of multiple omics analysis” algorithm, we have established the EpiMed. Using this platform, we reported six innovative treatment systems, proposed and optimized treatment schemes for aging myelodysplastic syndrome, severe aplastic anemia and refractory/recurrent malignant tumors, and successfully predicted new anti-HBV drugs.<sup>[26–30]</sup> This method is also applicable to prediction and screening of anticoronavirus epigenetic precision therapy drugs.

In summary, SARS-CoV-2 is capable of influencing the entire genome. This omics effect can lead to immunological disorders and provoke release of numerous cytokines that attack and damage human body. Moreover, it can suppress the antiviral response of the immune system and make the microenvironment of human body conducive to the survival of coronavirus in vivo. Given these noted changes, the clinical manifestations of SARS-CoV-2 infection may be complicated. The treatment is difficult with poor prognosis. Our present work showed that examining SARS-CoV-2 infection from a genome-wide perspective should further deepen our understanding of the pathogenesis of coronavirus mortality, thus clarifying the mechanism of SARS-CoV-2 infection-related immune damage, and providing a new direction for subsequent clinical treatment and medical research.

### Conflicts of interest statement

Jun Ren is an Associate Editor of *Emergency and Critical Care Medicine*. The article was subject to the journal's

standard procedures, with peer review handled independently of this Associate Editor and their research groups. The authors declare no conflict of interest.

### Author contributions

Haomin Zhang, Haoran Chen, Jundong Zhang, and Bin Guo participated in the performance of the research and data analysis. Ximeng Chen, Peng Zhi, Zhuoyang Li, Geliang Liu, Bo Yang, Xiaohua Chi, and Yixing Wang participated in the data analysis. Feng Cao and Xuechun Lu participated in the study design and the writing of manuscript, respectively. Jun Ren participated in the writing of manuscript.

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### Ethical approval of studies and informed consent

Not applicable.

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None.

### Availability of data and materials

The datasets used and/or analyzed for supporting the findings of this study are available from the corresponding authors upon reasonable request.

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