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Prediction of potential therapeutic drugs against SARS-CoV-2 by using Connectivity Map based on transcriptome data

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Abstract. – OBJECTIVE: Transcriptome data related to severe acute respiratory syndrome-related coronavirus 2 (a novel coronavirus discovered in 2019, SARS-CoV-2) in GEO database were downloaded. Based on the data, influence of SARS-CoV-2 on human cells was analyzed and potential therapeutic compounds against the SARS-CoV-2 were screened.

MATERIALS AND METHODS: R package "DESeq2" was used for differential gene analysis on the data of cells infected or non-infected with SARS-CoV-2. The "ClusterProfiler" package was used for GO functional annotation and KEGG pathway enrichment analysis of the differentially expressed genes (DEGs). A protein-protein interaction (PPI) network of the DEGs was constructed through STRING website, and the key subset in the PPI network was identified after visualization by Cytoscape software. Connectivity Map (CMap) database was used to screen known compounds that caused genomic change reverse to that caused by SARS-CoV-2.

RESULTS: By intersecting DEGs in two datasets, a total of 145 DEGs were screened out, among which 136 genes were upregulated and 9 genes were downregulated in SARS-CoV-2-infected cells. Functional enrichment analyses revealed that these genes were mainly associated with the pathways involved in viral infection, inflammatory response, and immunity. The CMap research found that there were three compounds with a median_tau_score less than -90, namely triptolide, tivozanib and daunorubicin.

CONCLUSIONS: SARS-CoV-2 can cause abnormal changes in a large number of molecules and related signaling pathways in human cells, among which IL-17 and TNF signaling pathways may play a key role in pathogenic process of SARS-CoV-2. Here, three compounds that may be effective for the treatment of SARS-CoV-2 were screened, which would provide new options for improving treatment of patients infected with SARS-CoV-2.

Key Words:
Novel coronavirus, Differential gene analysis, Functional enrichment analysis, Connectivity Map, Drug identification.

Introduction

According to the latest data of World Health Organization (WHO), the severe acute respiratory syndrome-related coronavirus 2 (a novel coronavirus discovered in 2019, SARS-CoV-2) has spread to 216 countries, with the number of confirmed cases exceeding 20 million and deaths over 800 thousand (https://covid19.who.int). SARS-CoV-2 as a member of the *coronaviridae* was firstly detected in Wuhan, China, in December 2019, and it spread rapidly to a worldwide pandemic within the next two to three months. Most patients infected with SARS-CoV-2 (Coronavirus disease 2019, COVID-19) only present with mild symptoms such as dry cough, sore throat and fever, and most of them can recover spontaneously[1]. Some severe and critically severe patients will develop various life-threatening complications, such as acute respiratory distress syndrome, septic shock, multi-organ failure and even death[2]. Angiotensin-converting enzyme 2 (ACE2) is a receptor for SARS-CoV-2 entering host cells, and it is widely expressed in hu-

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man cells, such as mucosal cells of respiratory tract, bronchial epithelial cells, and pulmonary epithelial cells[3]. This is one of the reasons for the widespread of SARS-CoV-2. With the current rapid spread of the epidemic, there are still no approved antiviral drugs or vaccines available to treat and prevent COVID-19. Treatment methods are very limited and only symptomatic treatment based on the experience of clinicians is available. Therefore, it is crucial to find new and effective therapeutic drugs against SARS-CoV-2.

Connectivity Map (CMap), jointly developed by Massachusetts Institute of Technology (MIT) and Harvard Medical School, provides a biological database based on connections among small molecule drugs, genes and diseases by using differential gene-expression profiles of human cells treated with small molecules[4]. With the assistance of the gene-expression profiles from the CMap, researchers can identify drugs that are highly associated with disease, infer major chemical structures of most drug molecules, and outline possible mechanisms of drug action. In recent years, increasing studies have used the CMap to mine potential therapeutic drugs for various diseases. For example, Xiao et al[5] applied gene expression profiling combined with the CMap database to study the molecular mechanisms and potential drugs for Hirschsprung disease, and they found that several compounds can counteract the damage caused by the disease. Liu et al[6] searched on the CMap using gene expression profiles from liver and hypothalamus, and they successfully discovered that Celastrol is an effective leptin sensitizer that can significantly reduce the obesity of leptin resistant mice. In addition, the CMap has been successfully applied to screen novel candidate therapeutic compounds for diseases such as cancer, Alzheimer's disease and osteoporosis[7-9]. All the above studies indicate that the CMap database has great application potential in exploring new therapeutic drugs for diseases. So far, there have been no reports on drugs for SARS-CoV-2 by using the CMap database. Therefore, this study aimed to screen potential therapeutic drugs for SARS-CoV-2 through differential gene analysis and research on the CMap database.

In this study, in order to identify potential therapeutic drugs for SARS-CoV-2, we explored the molecular differences between SARS-CoV-2-infected cells and blank control cells, on the basis of related transcriptome data in Gene Expression Omnibus (GEO) database. First, we identified differentially expressed genes (DEGs) between SARS-CoV-2-infected and blank control cells and analyzed changes of related molecular pathways in SARS-CoV-2-infected cells. Next, the CMap database was used to search for compounds that may cause genomic changes opposite to the changes caused by SARS-CoV-2, so as to identify novel and potentially effective drugs with antiviral properties. The drug candidates identified in this study may provide new directions for improving treatment of COVID-19.

Materials and Methods

Data Acquisition and Preprocessing

Gene expression data and sample information of GSE150392 and GSE147507 Series6 datasets were obtained from GEO database (https://www.ncbi.nlm.nih.gov/geo/). GSE150392 dataset included SARS-CoV-2-infected human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) (n=3) and blank control hiPSC-CMs (n=3). GSE147507 Series6 dataset included SARS-CoV-2-infected A549 cells (human lung adenocarcinoma cells) (n=3) and blank control A549 cells (n=3). Hoffmann et al[10] showed that the low expression of viral receptor ACE2 in A549 cells may lead to a low infection rate of SARS-CoV-2. Thus, the A549 cells in this study were with overexpressed ACE2.

DEGs Screening

R package "DESeq2"[11] was used to identify the DEGs between SARS-CoV-2-infected cells and blank control cells. The threshold was set as $|logFC|>1.5$ and $padj<0.05$. Volcano maps of the DEGs were drawn using R package "ggplot2"[12]. Intersections of down-regulated or up-regulated genes between the two datasets were taken, respectively, to obtain DEGs in both datasets. The overlapping DEGs were selected for follow-up analysis.

Functional Enrichment Analysis of the Overlapping DEGs

Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using R package "clusterProfiler"[13]. The threshold was set as $padj<0.05$. The enrichment results were visualized by using R package "enrichplot".

Protein-Protein Interaction (PPI) Network Construction and Functional Subset Analysis

To analyze the interaction between the above-mentioned genes, the STRING website (https://string-db.org/) was used to construct a PPI network based on the above DEGs, with the medium confidence of 0.4. Cytoscape[14] software was used to visualize the PPI network, and its plug-in MCODE[15] was used to analyze the functional subnetwork. ClueGO plug-in[16] was used for GO functional annotation and KEGG pathway enrichment analysis of the genes in the subnetwork.

Screening of Potential Drugs Against SARS-CoV-2

The CMap database contains expression profiles of more than 7,000 genes and 1,309 chemicals[4]. By comparing expression profiles of the gene set of a certain disease with known gene set in the database, the most relevant drug-disease-gene group will be calculated statistically, and then drugs related to the disease will be identified[4]. In this study, first, the online website CMap (https://clue.io/) was used to search for compounds that may cause genomic changes similar or opposite to the changes caused by SARS-CoV-2. By analyzing drug molecules related to the DEGs in SARS-CoV-2-infected cells, the drugs that pose opposite effect on genomic changes to SARS-CoV-2 were screened out.

Results

Results of Differential Gene Analysis

Differential gene analysis was performed on the data of SARS-CoV-2-infected cells and blank control cells. The results exhibited that a total of 3,157 DEGs were obtained in GSE150392 dataset, of which 1,408 genes were up-regulated and 1,749 genes were down-regulated in SARS-CoV-2-infected cells (Figure 1A, Differential Gene List in **Supplementary Table I**). While in GSE147507 Series6 dataset, a total of 731 DEGs were detected in SARS-CoV-2-infected cells, with 645

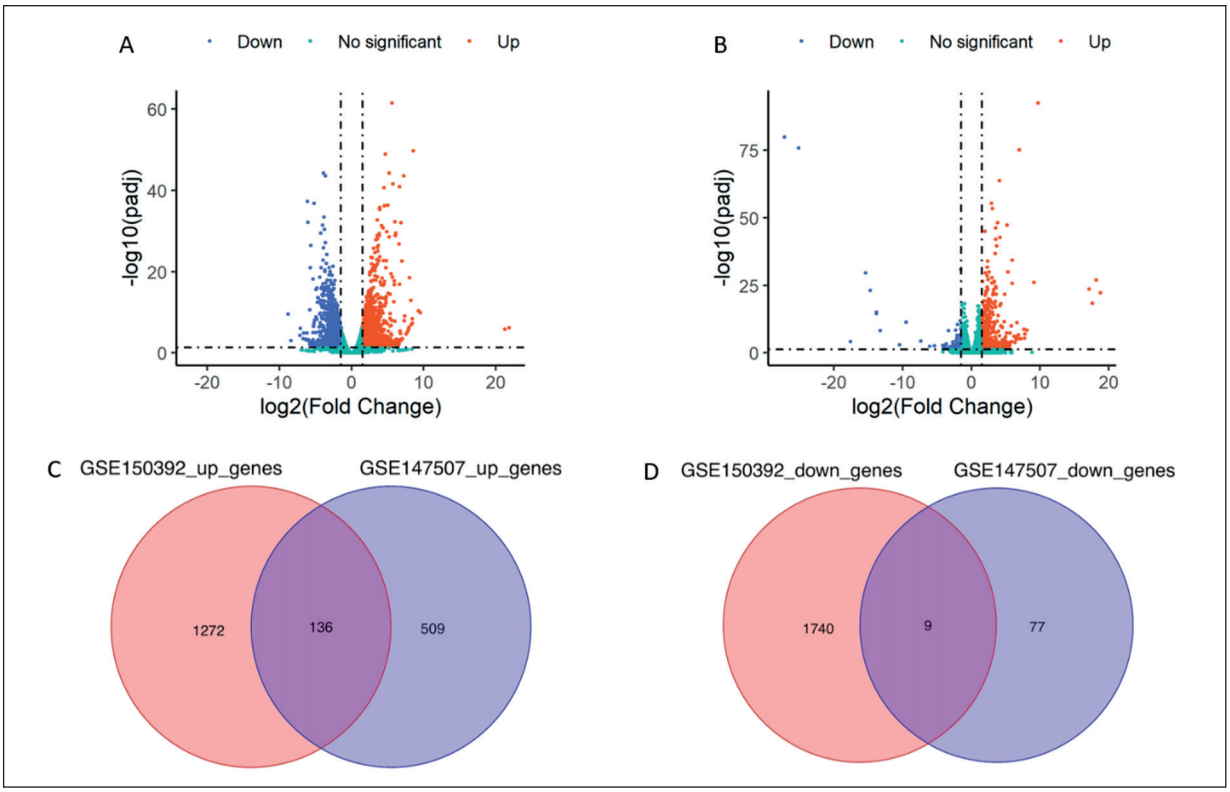

Figure 1. Volcano maps of differential gene analysis and Venn diagrams. **A**, Volcano map of DEGs in GSE150392 dataset; **B**, Volcano map of DEGs in GSE147507 dataset; **C**, Venn diagram shows the intersected up-regulated genes in the two datasets; **D**, Venn diagram shows the overlapping down-regulated genes in the two datasets.

up-regulated genes and 86 down-regulated genes (Figure 1B, **Supplementary Table II**). By intersecting the down-regulated genes or up-regulated genes between the two datasets, respectively, 145 overlapping DEGs were finally acquired, including 136 up-regulated genes (Figure 1C) and 9 downregulated genes (Figure 1D).

Results of Functional Analysis of DEGs

To analyze the function of the overlapping DEGs, we performed GO functional annotation and KEGG pathway enrichment analysis. GO functional analysis showed that the DEGs were mainly enriched in biological processes such as response to lipopolysaccharide, response to molecule of bacterial origin, regulation of leukocyte differentiation. The most enriched molecular functions were mainly DNA-binding transcription activator activity-RNA polymerase II-specific, cytokine activity, cytokine receptor binding and chemokine activity, etc. (Figure 2A, Table I). KEGG enrichment analysis displayed that the 145 genes were mainly concentrated in tumor necrosis factor (TNF) signaling pathway, IL-17 signaling pathway, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, NF-κB signaling pathway, and some bacterial and viral infection-related pathways (Figure 2B, Table II). The above results suggested that SARS-CoV-2 infection could cause changes in various signaling pathways and biological processes in human cells, especially in the pathways related to inflammatory response and immunoregulation.

PPI Network Construction and Functional Subset Selection

A PPI network of the 145 DEGs was constructed via the STRING website (https://string-db.org/), and 534 pairs were finally obtained (Figure 3A, **Supplementary Table III**). From the network, it was revealed that IL6, JUN, FOS, CCL2, EGR1, NFKB1, ICAM1, NFKBIA, ATF3 and CXCL1 had a relatively high node degree (Figure 3B). A functional subset with a high score in the network was screened by MCODE plug-in, including 29 genes such as IL6, JUN, FOS, CCL2, NFKB1, ICAM1, NFKBIA, CXCL1, PTGS2, IRF1, etc. (Figure 3C). This subset contained eight of the top ten ranked genes in node degree, suggesting that this subset may play an important role in the PPI network.

Enrichment Analysis for the Genes in the Functional Subset

GO and KEGG enrichment analyses were carried out for the genes in the subnetwork to further reveal the function of the key genes in the PPI network. GO functional analysis revealed that these genes were mainly concentrated in cellular response to lipopolysaccharide, chemokine activity, cellular response to interleukin-6, and regulation of type I interferon production, etc. (Figure 4A). KEGG pathway analysis indicated that these genes were mainly involved in IL-17 signaling pathway and TNF signaling pathway (Figure 4B). Both IL-17 and TNF are important cytokines involved in inflammatory response, and they are closely related to clinical response such as cytokine storm in patients with COVID-19.

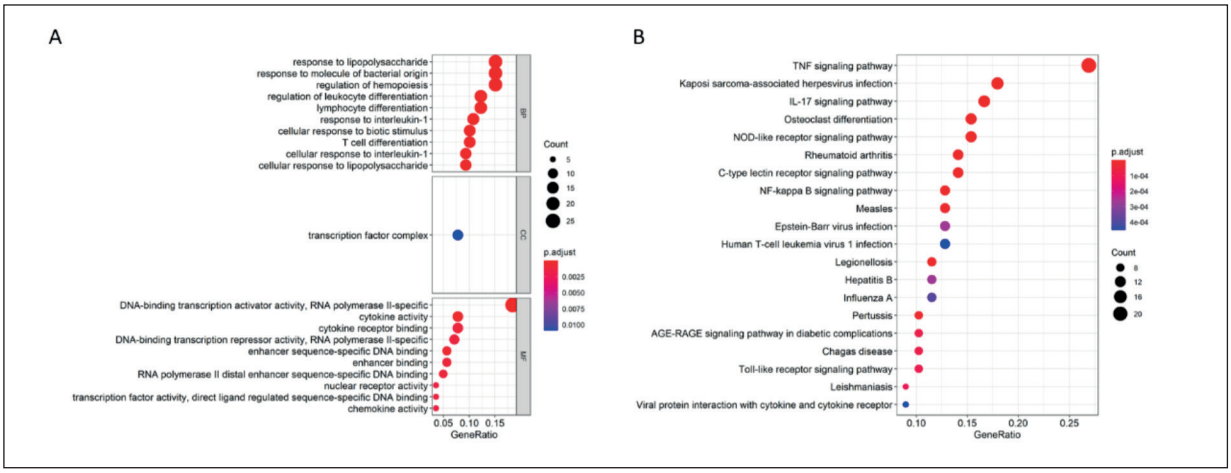

Figure 2. GO annotation and KEGG enrichment analysis of the overlapping DEGs. **A**, The most enriched GO terms of the 145 DEGs in SARS-CoV-2-infected cells; **B**, The most activated KEGG pathways of the 145 DEGs in SARS-CoV-2-infected cells.

Table I. Top 10 enriched GO terms of the 145 DEGs from three aspects.

GO Accession	GO Terms	Gene count	*p*.adjust
BP			
GO:0032496	Response to lipopolysaccharide	21	1.59E-10
GO:0002237	Response to molecule of bacterial origin	21	1.69E-10
GO:1902105	Regulation of leukocyte differentiation	17	2.13E-08
GO:0070555	Response to interleukin-1	15	3.24E-08
GO:1903706	Regulation of hemopoiesis	21	3.35E-08
GO:0071347	Cellular response to interleukin-1	13	4.46E-07
GO:0030098	Lymphocyte differentiation	17	5.22E-07
GO:0071216	Cellular response to biotic stimulus	14	1.03E-06
GO:0030217	T cell differentiation	14	1.14E-06
GO:0071222	Cellular response to lipopolysaccharide	13	1.30E-06
CC			
GO:0005667	Transcription factor complex	11	0.010877811
MF			
GO:0001228	DNA-binding transcription activator activity, RNA polymerase II-specific	26	3.43E-13
GO:0005125	Cytokine activity	11	0.000257112
GO:0001158	Enhancer sequence-specific DNA binding	8	0.000556917
GO:0035326	Enhancer binding	8	0.000950417
GO:0000980	RNA polymerase II distal enhancer sequence-specific DNA binding	7	0.000956191
GO:0005126	Cytokine receptor binding	11	0.00104346
GO:0001227	DNA-binding transcription repressor activity, RNA polymerase II-specific	10	0.001179341
GO:0004879	Nuclear receptor activity	5	0.001312257
GO:0098531	Transcription factor activity, direct ligand regulated sequence-specific DNA binding	5	0.001312257
GO:0008009	Chemokine activity	5	0.001449537

GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function; p.adjust, adjusted *p*-value.

These results implied that IL-17 signaling pathway and TNF signaling pathway were important in SARS-CoV-2-related abnormal immune status and inflammatory response.

Screening of Potential Therapeutic Drugs

The CMap database was used to search for compounds that could cause genomic changes contrary to the changes caused by SARS-CoV-2.

Table II. Top 20 enriched KEGG pathways of the 145 DEGs.

Pathway ID	KEGG Terms	Gene count	*p*.adjust
hsa04668	TNF signaling pathway	21	8.00E-20
hsa04657	IL-17 signaling pathway	13	3.62E-10
hsa05323	Rheumatoid arthritis	11	7.00E-08
hsa05167	Kaposi sarcoma-associated herpesvirus infection	14	9.21E-08
hsa04380	Osteoclast differentiation	12	9.21E-08
hsa05134	Legionellosis	9	9.21E-08
hsa04625	C-type lectin receptor signaling pathway	11	1.01E-07
hsa04064	NF-kappa B signaling pathway	10	1.21E-06
hsa04621	NOD-like receptor signaling pathway	12	2.93E-06
hsa05133	Pertussis	8	1.14E-05
hsa05162	Measles	10	1.37E-05
hsa04933	AGE-RAGE signaling pathway in diabetic complications	8	7.77E-05
hsa05142	Chagas disease	8	8.32E-05
hsa04620	Toll-like receptor signaling pathway	8	8.93E-05
hsa05140	Leishmaniasis	7	0.00010809
hsa05169	Epstein-Barr virus infection	10	0.00026837
hsa05161	Hepatitis B	9	0.00026977
hsa05164	Influenza A	9	0.00039114
hsa05166	Human T-cell leukemia virus 1 infection	10	0.00045159
hsa04061	Viral protein interaction with cytokine and cytokine receptor	7	0.00045159

KEGG, Kyoto Encyclopedia of Genes and Genomes; *p*.adjust, adjusted *p*-value.

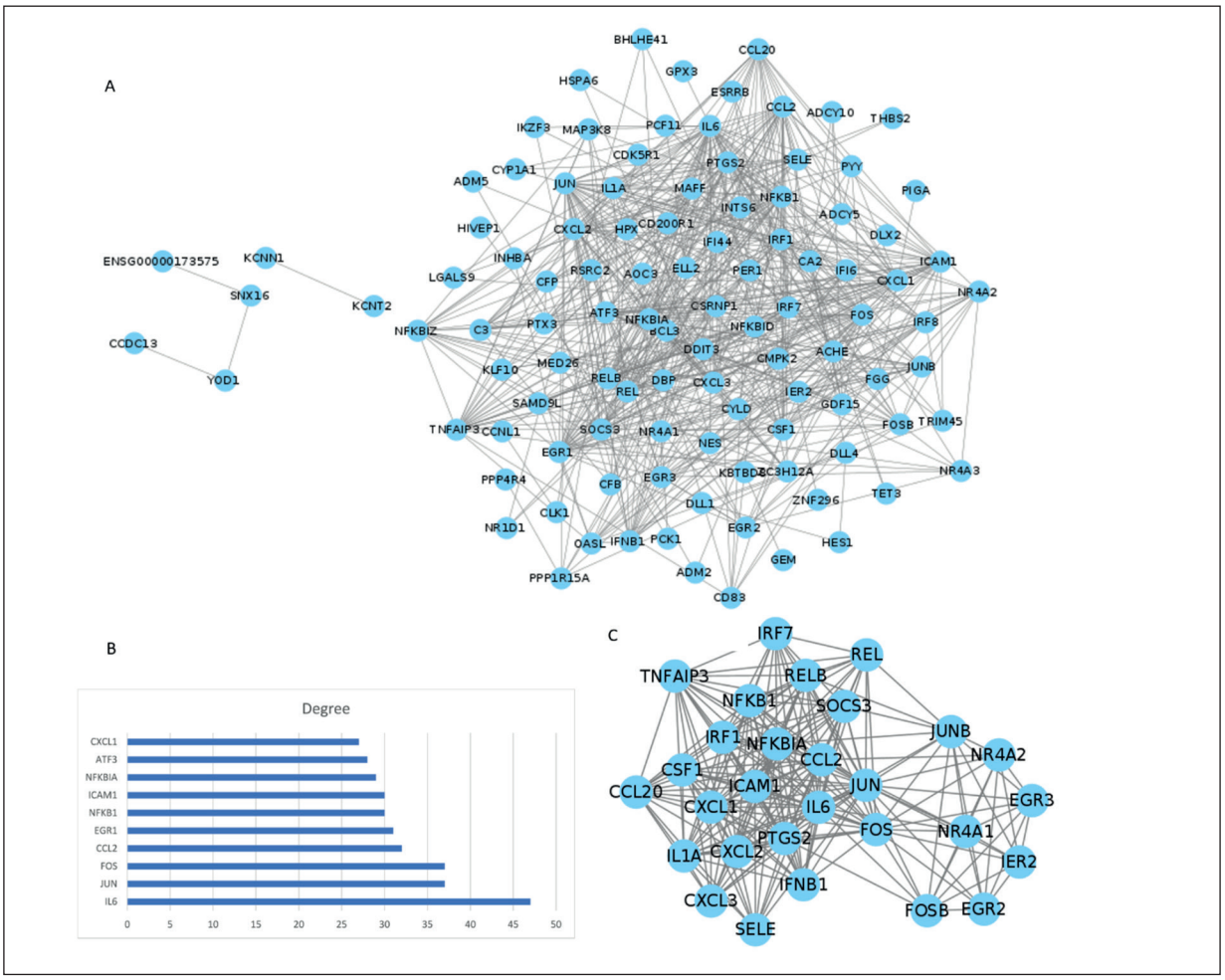

Figure 3. The PPI network based on the 145 DEGs. **A**, The PPI network of the 145 DEGs; **B**, Bar chart of the ten genes with the highest node degree in the PPI network; **C**, The primary subnetwork searched by MCODE.

Compounds with a negative median_tau_score were selected, and part of predicted drugs were listed in Table III. It was found that three compounds, namely triptolide, tivozanib and daunorubicin, were with a median_tau_score less than -90 (Figure 5). In addition, among the 9 drugs with a median_tau_score less than -80, ribavirin has been clinically used as an antiviral drug to treat COVID-19, which suggests that the analytical method used in this study is reliable. Although other predictive drugs have not been confirmed, we found through literature review that some of them have strong anti-inflammatory effects, and their therapeutic effects along with mechanisms are expected to be explored in subsequent in-depth studies. For instance, triptolide can exhibit an anti-inflammatory effect in LPS-induced pulmonary inflammation by inhibiting TLR4-mediated NF-κB signaling pathway[17,18]. Besides, chromomycin-a3 can indirectly affect inflammatory response by inhibiting TNF signaling pathway[19].

Discussion

At present, the COVID-19 epidemic is in a severe situation throughout the world, with increasing numbers of confirmed cases and deaths. No vaccine or drug has been approved for the specific treatment of COVID-19[20,21]. Although anti-coronavirus drugs targeting molecules and signaling pathways such as ACE2 or interferon signaling pathway have been developed by some researchers, these drugs still have certain limitations in clinical efficacy[20,21]. Therefore, exploration of novel and effective treatments is critical to the treatment and containment of COVID-19.

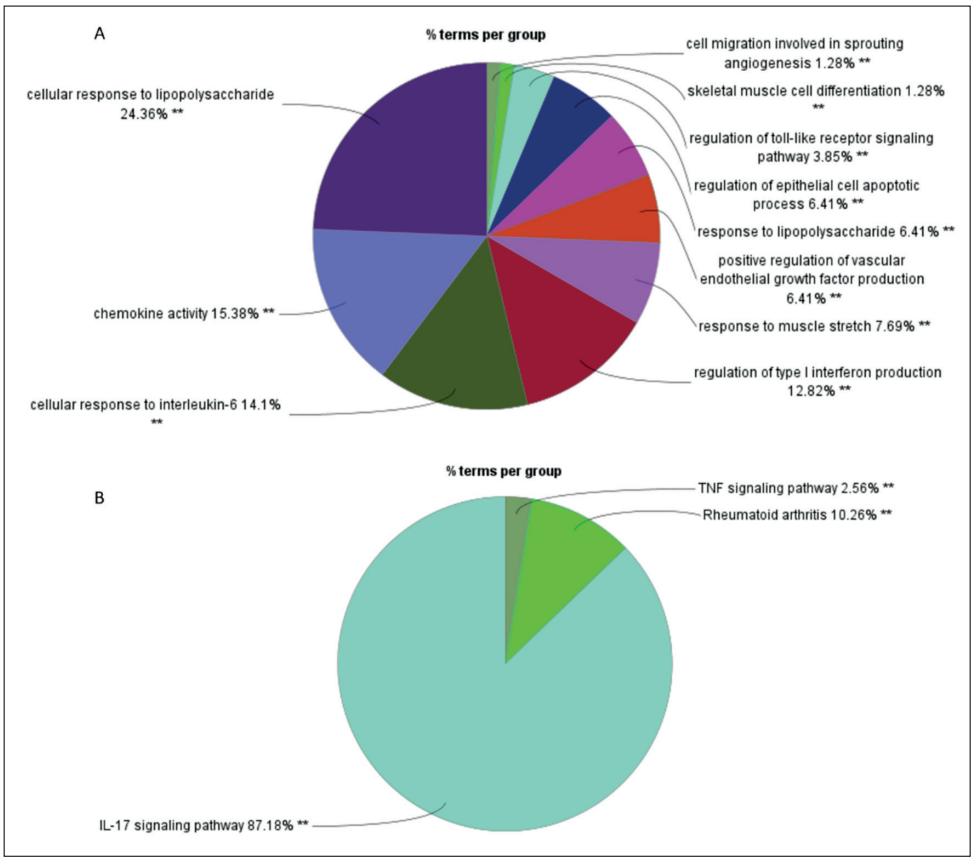

Figure 4. Functional analysis of the genes in the primary subnetwork. **A**, GO enrichment analysis; **B**, KEGG enrichment analysis.

Table III. Drugs with a median_tau_score less than -80 in the CMap database.

Name	Description	Target	Median_tau_score
Triptolide	RNA polymerase inhibitor	CYP2C19, RELA FLT1,	-97.5
Tivozanib	VEGFR inhibitor, KIT inhibitor, tyrosine kinase inhibitor	FLT4, KDR, KIT, PDGFRA, PDGFRB	-92.12
Daunorubicin	RNA synthesis inhibitor, topoisomerase inhibitor, DNA synthesis inhibitor, radical formation stimulant	TOP2A, TOP2B	-91.55
Chromomycin-a3	DNA binding		-87.22
NVP-AUY922	HSP inhibitor	HSP90AA1, HSP90AA2, HSP90AB1	-85.26
PD-0325901	MEK inhibitor, MAP kinase inhibitor, protein kinase inhibitor	MAP2K1, MAP2K2	-83.66
Atorvastatin	HMGCR inhibitor, dipeptidyl peptidase inhibitor, tumor necrosis factor expression inhibitor	HMGCR, DPP4, AHR, CYP3A5, FASLG	-80.63
Dasatinib	KIT inhibitor, src inhibitor, Bcr-Abl kinase inhibitor, ephrin receptor inhibitor, PDGFR tyrosine kinase receptor inhibitor, yes kinase inhibitor, Abl kinase inhibitor, Bruton's tyrosine kinase (BTK) inhibitor, discoidin domain containing receptor Inhibitor, lymphocyte specific tyrosine kinase inhibitor, tyrosine kinase inhibitor	ABL1, FYN, LCK, SRC, KIT, YES1, BCR, EPHA2, LYN, PDGFRB, ABL2, BTK, DDR1, DDR2, PDGFRA, STAT5B	-80.58
Ribavirin	Antiviral guanosine ribonucleoside analog, IMPDH inhibitor, inosine monophosphate dehydrogenase inhibitor	IMPDH1, ADK, ENPP1, IMPDH2, NT5C2	-80.33

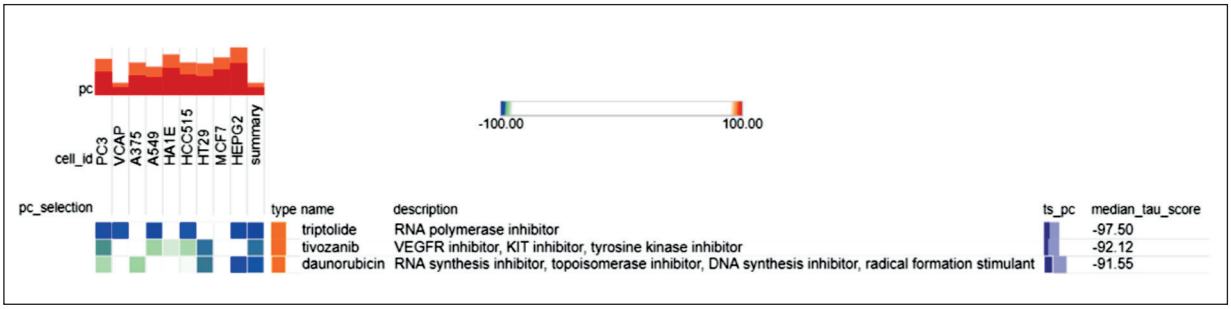

Figure 5. Heat map of the results predicted by the CMap database.

In this study, we analyzed DEGs between SARS-CoV-2-infected cells and blank control cells using two datasets from GEO database. By intersecting the DEGs between the two datasets, a total of 145 DEGs were obtained, including 136 up-regulated genes and 9 down-regulated genes. GO functional annotation and KEGG pathway analysis displayed that these 145 genes were mainly enriched in the pathways involved in viral infection, inflammatory response and immunoregulation, suggesting that these pathways might play a key role in the process of cell infection with SARS-CoV-2. These results are basically consistent with previous studies[20,22]. The results also suggest that the infection process of SARS-CoV-2 is not only the interaction between virus and body or susceptible cells, but also the genome-wide influence of the virus on a variety of molecules, signaling pathways and transcriptional regulation at the molecular level after entering the body.

To further explore the pathogenesis of SARS-CoV-2, study on intermolecular interaction may help better understand its nature underlying disease occurrence. Here, we used the STRING database and Cytoscape software to draw a PPI network of the overlapping DEGs between the two GEO datasets. MCODE and ClueGO plugins were used to find the functional subnetwork, and the genes in the subnetwork were subjected to functional enrichment analysis. It was observed that the genes in the subset were mainly related to IL-17 signaling pathway, TNF signaling pathway and so on. The IL-17 family is a subset of cytokines composed of IL-17A-F and plays a critical role in immunoregulation, acute and chronic inflammatory response[23]. TNF as an important cytokine can induce activation of various intracellular signaling pathways and play an important role in cell apoptosis, cell survival, inflammatory response and immunoregulation[24,25]. These results pointed out that SARS-CoV-2 may affect cell survival and apoptosis, inflammatory response and chemotaxis of varying degrees. In-depth research on the above molecules and signaling pathways is conducive to revealing its molecular mechanism.

In addition, we screened several known compounds that elicited genomic changes contrary to the changes caused by SARS-CoV-2 by using the CMap database. Among them, there were 9 drugs with the median_tau_score less than -80, including triptolide, tivozanib, daunorubicin, chromomycin-a3, NVP-AUY922, PD-0325901, atorvastatin, dasatinib and ribavirin. While the median_tau_score of triptolide, tivozanib and daunorubicin was less than -90. Ribavirin is currently recommended for treatment of COVID-19, suggesting that the analytical method used in this study is correct and reliable. Triptolide is the main active constituent in extracts of *Tripterygium wilfordii*, characterized by multiple pharmacological activities such as anti-inflammation, immunoregulation, anti-proliferation and pro-apoptosis[26]. Currently, triptolide has been widely used in treatment of inflammatory diseases, autoimmune diseases, organ transplantation and even tumors[26]. A study revealed that triptolide not only can directly induce apoptosis of tumor cells, but also can indirectly promote cell apoptosis by inhibiting NF-κB signaling pathway[26]. Triptolide has also been reported to play an anti-inflammatory role in LPS-induced pulmonary inflammation by inhibiting TLR4-mediated NF-κB signaling pathway[17,18]. While for tivozanib and daunorubicin, they have only been studied in kidney cancer, leukemia and other tumor-related diseases so far. These studies indicate that triptolide candidate screened in this study may play an anti-inflammatory role in treatment of COVID-19, and may be a new treatment option, but the specific efficacy requires further study.

Conclusions

To sum up, by using the public datasets in GEO database, we analyzed the changes in related molecules and pathways caused by SARS-CoV-2 infection. Potential therapeutic drugs were obtained through differential gene analysis coupled with the research on the CMap database. Among the potentially active drugs, triptolide has been confirmed to have an anti-inflammatory effect on pulmonary inflammation and is expected to be a potential drug for treatment of COVID-19.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Consent for Publication

All authors consent to submit the manuscript for publication.

Availability of Data and Materials

The data used to support the findings of this study are included within the article. The data and materials in the current study are available from the corresponding author on reasonable request.

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Authors' Contribution

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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