

Research Paper

Severe acute respiratory syndrome coronavirus (SARS-CoV)-2 infection induces dysregulation of immunity: *in silico* gene expression analysis

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Abstract

Highly pathogenic coronaviruses (CoVs) induce acute respiratory distress syndrome, and the severe acute respiratory syndrome coronavirus (SARS-CoV)-2 has caused a pandemic since late 2019. The diversity of clinical manifestations after SARS-CoV-2 infection results in great challenges to diagnose CoV disease 2019 (COVID-19). There is a growing body of published research on this topic; however, effective medications are still undergoing a long process of being assessed. In the search for potential genetic targets for this infection, we applied a holistic bioinformatics approach to study alterations of gene signatures between SARS-CoV-2-infected cells and mock-infected controls. Two different kinds of lung epithelial cells, A549 with angiotensin-converting enzyme 2 (ACE2) overexpression and normal human bronchial epithelial (NHBE) cells, were infected with SARS-CoV-2. We performed bioinformatics analyses of RNA-sequencing in this study. Through a Venn diagram, Database for Annotation, Visualization and Integrated Discovery, Gene Ontology, Ingenuity Pathway Analysis, and Gene Set Enrichment Analysis, the pathways and networks were constructed from commonly upregulated genes in SARS-CoV-2-infected lung epithelial cells. Genes associated with immune-related pathways, responses of host cells after intracellular infection, steroid hormone biosynthesis, receptor signaling, and the complement system were enriched. Dysregulation of the immune system and malfunction of interferon contribute to a failure to kill SARS-CoV-2 and exacerbate respiratory distress in severely ill patients. Current findings from this study provide a comprehensive investigation of SARS-CoV-2 infection using high-throughput technology.

Key words: coronavirus, SARS-CoV-2, COVID-19, interferon, immune system

Introduction

Coronaviruses (CoVs) are RNA viruses that cause diseases related to the respiratory system in both humans and animals [1]. Currently, several CoV strains have been reported, including HCoV-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKU1, which infect humans and induce respiratory tract infections

[2]. These CoVs can cause less-pathogenic endemic diseases in infected hosts. However, in 2003, a severe pandemic occurred resulting in more than 8422 cases and about 916 related deaths caused by another strain of CoV, namely severe acute respiratory syndrome (SARS)-CoV [3-6]. In 2012, another CoV strain,

namely the Middle East respiratory syndrome (MERS)-CoV, that infected humans, bats, and camels also caused more than 4494 cases and 858 deaths [7]. Most recently, in 2019, a new strain of CoV, the so-called 2019-novel coronavirus (2019-nCoV/SARS-CoV-2), is causing a global pandemic affecting almost every country, causing unaccountable losses from taking human lives to damaging the world's economy. As of Dec 18, 2020, according to statistical reports from the World Health Organization (WHO) (<https://www.who.int/emergencies/diseases/novel-coronavirus-2019>), over 73,996,237 laboratory-confirmed cases and 1,663,474 deaths worldwide were caused by SARS-CoV-2. This CoVs outbreak again corroborates that CoVs can definitely cause severe disease in humans once infected. Investigating suitable treatment approaches is peremptory; however, there are no clear or widely recognized standard methods for dealing with this infection currently.

According to previous research, A549 lung adenocarcinoma cells are not vulnerable to SARS-CoV or SARS-CoV-2 infection [8, 9]. A549 cells express dipeptidyl peptidase 4, which is the receptor for MERS-CoV [10]. A549 cells are human adenocarcinomatous alveolar type II cells, and they turned out to be susceptible to SARS-CoV-2 after overexpressing angiotensin-converting enzyme 2 (ACE2) receptors [8]. Normal human bronchial epithelial (NHBE) cells are normal epithelial cells that are commonly used as *in vitro* lung models [11, 12]. ACE2 receptors are an entry point for CoVs, and ACE2 expression was detected in NHBE cells [13]. These cells are highly beneficial for studying CoV infections and developing therapeutic strategies in experimental subjects.

Over the last decade, high-throughput technologies have emerged as major players in producing huge amounts of output data for multipurpose research from genomics to proteomics. These technologies allow researchers to rapidly acquire hundreds to several thousand gene expression profiles in each experiment [14-17]. Leveraging these tools and publicly available datasets for SARS-CoV-2, we attempted to use a high-throughput approach to search for predictive markers and therapeutic strategies for CoVs. We integrated multiple bioinformatics tools for whole-profile gene expressions of SARS-CoV-2-infected human lung epithelial cells. We analyzed RNA sequencing (RNA-Seq) data and explored genetic signatures associated with SARS-CoV-2-infected ACE2-expressed-A549 and NHBE cells. In addition, downstream regulatory pathways were predicted. The candidate genes were evaluated as the potentially

therapeutic targets for SARS-CoV-2 infection. The present study can provide essential evidence of regulatory networks in SARS-CoV-2-infected disease.

Materials and Methods

Bioinformatics and high-throughput database analyses

We acquired RNA-Seq data of ACE2-expressed-A549 and NHBE cells with SARS-CoV-2 infection from the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO), with accession number GSE147507 [18] and analyzed those data with the CLC Genomics Workbench (<https://digitalinsights.qiagen.com>). To acquire gene symbols, gene IDs were mapped to Ensembl features using the biomaRt package vers. 2.26.1 and Gene Ontology (GO)-Elite platforms. The clustering of genes was based on their messenger (m)RNA expression profiles using pheatmap vers. 1.0.12 of R software [19-23]. Signals were processed and normalized as we previously described [24-32]. The Database for Annotation, Visualization, and Integrated Discovery (DAVID, vers. 6.8) was used for clustering analyses of genes of interest. This clustering algorithm uses biological functions, signaling pathways, and associated diseases.

In order to study acute infection of SARS-CoV-2, ACE2-expressed-A549 and NHBE cells were infected with SARS-CoV-2 and mock controls for 24 h. Differential expressions of genes were sequenced on the Illumina NextSeq 500 system. We set infected cells versus mock controls to >2.0 for both ACE2-expressed-A549 and NHBE cells as cutoff points. Differentially expressed genes (DEGs) were calculated, and P values of <0.05 were considered significant. The list of significantly different genes was imported to the gene ontology (GO) database to construct biological processes and associated diseases [33]. To construct the biological regulatory networks of targeted genes, Gene Set Enrichment Analysis (GSEA) software was used for enrichment [34]. A P value of <0.05 was selected as the cutoff point for the enrichment analysis.

Pathway and network enrichment analyses

To further investigate the signal pathways related to SARS-CoV-2-infected cells, an Ingenuity pathway analysis (IPA) was used with data input as the list of DEGs with significant differences from SARS-CoV-2-infected human lung epithelial cells. The P value (Benjamini-Hochberg) of <0.05 was selected as the cutoff for statistically significant differences.

Results

GSEA of SARS-CoV-2-infected human lung epithelial cells

We attempted to identify DEGs in SARS-CoV-2-infected cells compared to mock-infected controls. Experiments were conducted on ACE2-expressed-A549 and NHBE cells. Statistically significantly upregulated genes were analyzed with numerous bioinformatics tools, including a Venn diagram for the intersection of two studies, DAVID for associated functions, GO Elite for biological processes, IPA for regulated networks, and GSEA for biological regulation (Figure 1).

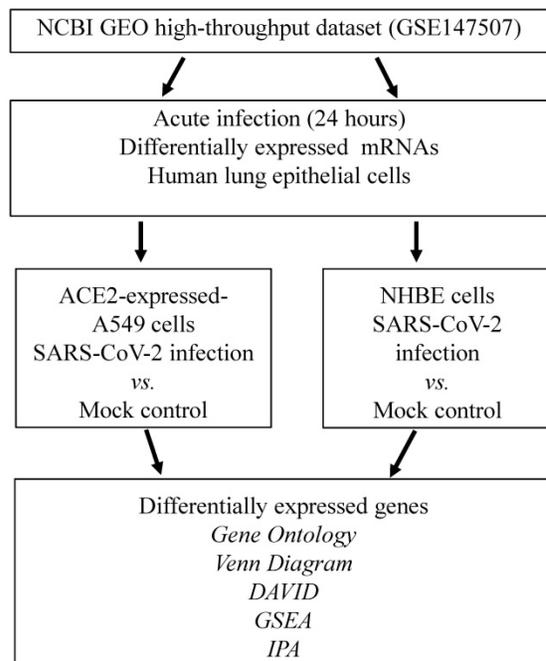


Figure 1. Schematic workflow illustrating the study design. Severe acute respiratory syndrome coronavirus (SARS-CoV)-2-infected ACE2-expressed-A549 and normal human bronchial epithelial (NHBE) cells were acquired from the GSE147507 dataset in the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) databases. Highly expressed genes were merged in a Venn diagram analysis. Shared genes from the two datasets were analyzed by the DAVID, GO, IPA, and GSEA. Abbreviation: ACE2, angiotensin-converting enzyme 2; DAVID, Database for Annotation, Visualization, and Integrated Discovery; GO, gene ontology; GSEA, Gene Set Enrichment Analysis; IPA, ingenuity pathway analysis; NHBE cells, normal human bronchial epithelial cells; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

GO analysis of SARS-CoV-2-infected human lung epithelial cells

To investigate cellular responses of SARS-CoV-2-infected lung epithelial cells, we collected gene expression values from the GSE147507 dataset. Comparing SARS-CoV-2-infected NHBE cells and mock-infected controls showed two clusters of genes. Genes in group 1 were upregulated in SARS-CoV-2-infected NHBE cells, and the associated pathways were enriched according to the GO analysis

(Figure 2A). The most significant pathways of upregulated genes were "immune system process ($P = 9 \times 10^{-22}$)", "defense response ($P = 8 \times 10^{-19}$)", "response to molecules of bacterial origin ($P = 1 \times 10^{-12}$)", and "cytokine activity ($P = 1 \times 10^{-12}$)". Cellular responses of SARS-CoV-2-infected A549 cells transduced with a vector expressing human ACE2 were analyzed with the same method, and enriched pathways are shown in Figure 2B. Data revealed that the most important pathways of upregulated genes were "DNA binding ($P = 1 \times 10^{-24}$)", "response to virus ($P = 1 \times 10^{-23}$)", "regulation of RNA metabolic process ($P = 3 \times 10^{-20}$)", and "regulation of immune system process ($P = 3 \times 10^{-18}$)". These results are consistent with recent COVID-19 research, which demonstrated an association of SARS-CoV-2 infection with immune response in patients [35]. Furthermore, in the progression and development of this infectious disease, declines in circulating natural killer cell levels were also linked to the disease severity [36].

Mutual gene signatures in ACE2-expressed-A549 and NHBE cells after SARS-CoV-2 infection

There were 1000 upregulated genes in SARS-CoV-2-infected NHBE cells compared to mock-infected controls, and 1263 genes in SARS-CoV-2-infected ACE2-expressed-A549 cells compared to the controls (Figure 3A). There were 194 genes in common. We imported these 194 genes into the IPA platform to explore characteristic signatures of gene expressions in these two lung epithelial cell lines. The highest enriched common pathways were "interferon (IFN) signaling ($P = 1.58 \times 10^{-11}$)", "role of pattern recognition receptors in recognition of bacteria and viruses ($P = 2.82 \times 10^{-10}$)", and "airway pathology in chronic obstructive pulmonary disease ($P = 1.86 \times 10^{-9}$)" (Figure 3B, Table 1). Details of IFN signaling are shown in Figure 3C, such as extracellular IFN- α/β interacting with IFN- α receptor 1/2 (IFNAR1/2), and extracellular IFN- γ being associated with IFN- γ R α/β . Activation of intracellular signal transducer and activator of transcription (STAT) signaling initiates transcription of downstream genes.

GSEA of SARS-CoV-2-infected cells

Leveraging public databases such as Hallmark and KEGG, we verified the importance of the enriched pathways from the shared 194 upregulated genes between SARS-CoV-2 infected A549 and SARS-CoV-2-infected NHBE cells relative to mock-infected controls (Figure 2). Several immune-related networks were enriched, including acute inflammation, chemokine, chemotaxis,

neutrophil migration, response to IFN, and interleukin (IL)-6-related signaling pathways (Figure 4). Some diseases associated with dysregulation of the immune system were also positively correlated, including systemic lupus erythematosus, graft versus host disease, and allograft rejection (Figure 5). Host

responses after SARS-CoV-2 infection were also enriched, e.g., regulation of viral genome replication, *Leishmania* infection, recognition receptor activity, steroid hormone biosynthesis, cell adhesion molecules, toll-like receptor (TLR) signaling pathway, and the complement system (Figure 6).

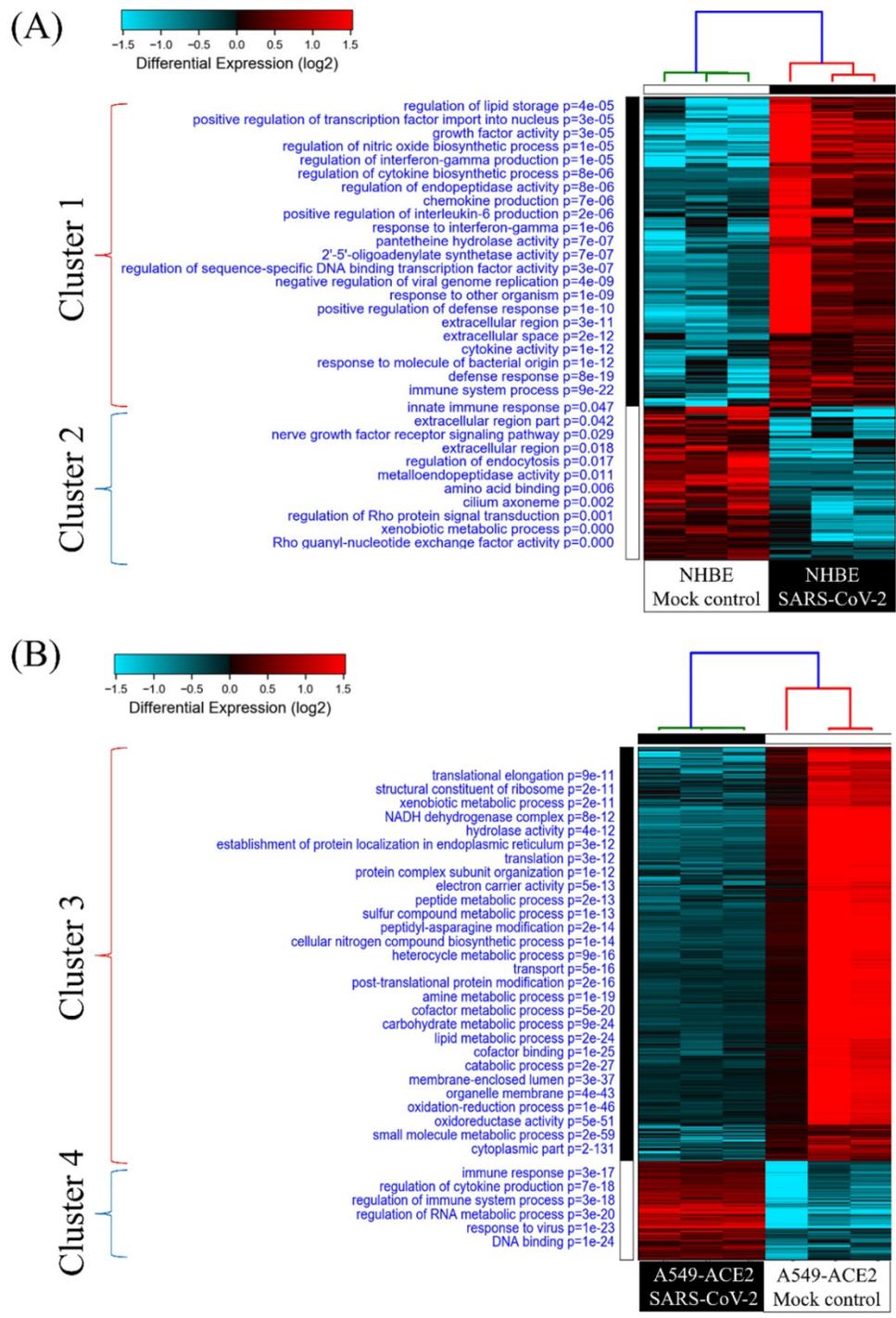


Figure 2. Heatmap visualization of enriched gene ontology (GO) from severe acute respiratory syndrome coronavirus (SARS-CoV)-2-infected lung epithelial cells compared to mock-infected control group. (A) Comparison between SARS-CoV-2-infected and mock-infected normal human bronchial epithelial (NHBE) cells. Upregulated genes were analyzed by a GO enrichment analysis for associated pathways. Cluster 1 pathways were derived from upregulated genes in SARS-CoV-2-infected cells, and cluster 2 pathways were of downregulated genes. (B) Comparison between SARS-CoV-2-infected and mock-infected A549 cells transduced with a vector expressing human ACE2. Upregulated genes were analyzed by a GO enrichment analysis for associated pathways. Cluster 3 pathways were derived from downregulated genes in SARS-CoV-2-infected cells and cluster 4 from upregulated genes.

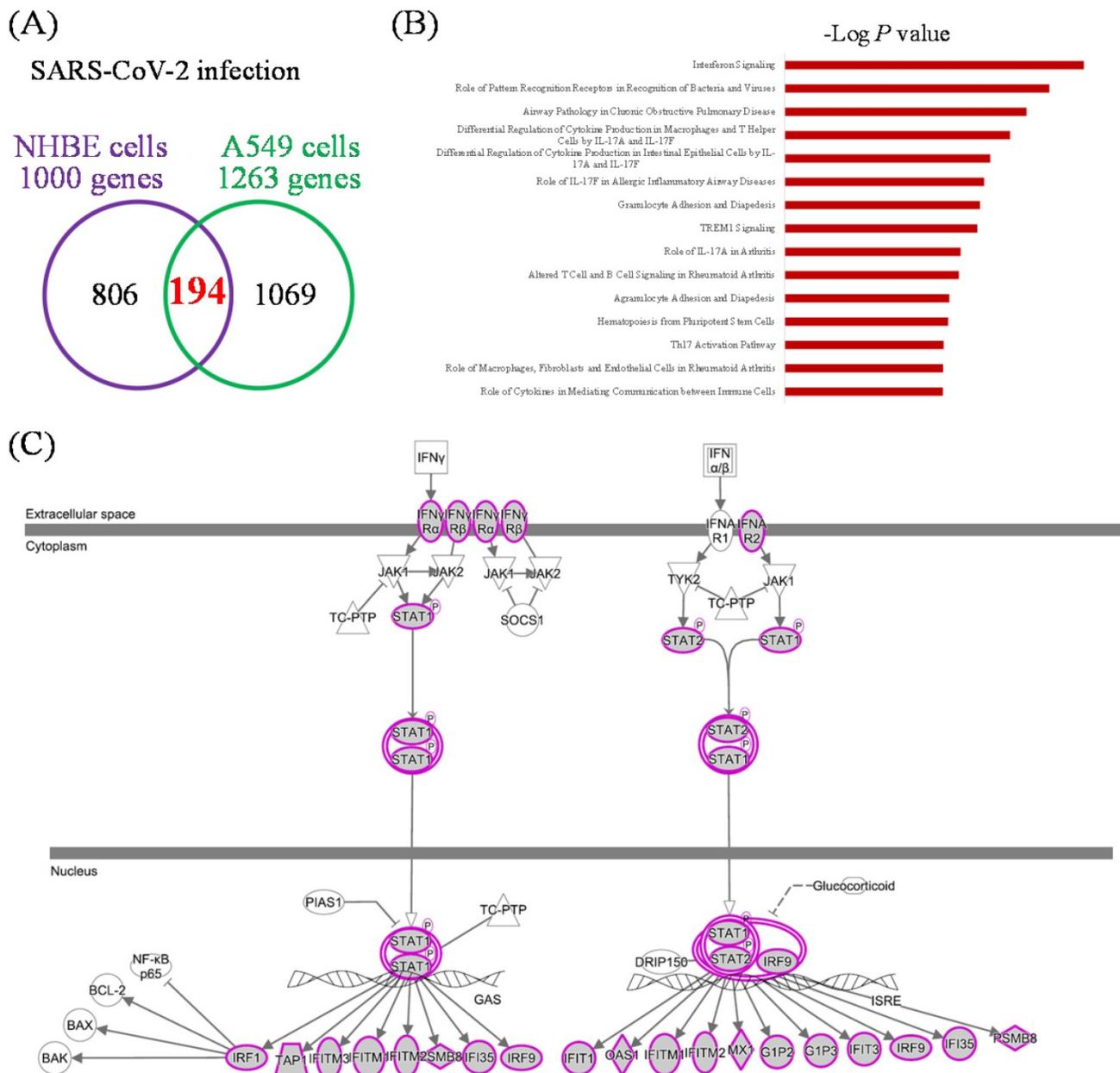


Figure 3. Common expressed genes, pathways, and networks from severe acute respiratory syndrome coronavirus (SARS-CoV)-2-infected lung epithelial cells. (A) Differentially highly expressed genes from two kinds of lung epithelial cells with SARS-CoV-2 infection were merged in a Venn diagram. (B) Ingenuity Pathway Analysis (IPA) software was used to analyze shared upregulated genes of ACE2-expressed-A549 and normal human bronchial epithelial (NHBE) cells. SARS-CoV-2-associated pathways and networks are listed with the negative logarithmic form of the *P* value. (C) Detailed pathway map of "interferon signaling".

Discussion

The COVID-19 pandemic has induced a global health crisis with tremendous impacts on humans. Many researchers have focused on this disease; however, there are currently still numerous unsolved problems. In the present study, we used two cell models from different human lung epithelial cell lines (ACE2-expressed-A549 and NHBE cells) and used them to compare SARS-CoV-2-infected and mock-infected cells. We concentrated on a 24-h model study of cellular responses after SARS-CoV-2

infection. Our results showed activation of immune-related networks, especially IFN signaling. Immune cell migration and chemotaxis were also upregulated. Increased expression of disease-associated genes were detected, including those correlated with immune-dysregulated diseases. The current data provide a comprehensive understanding of acute SARS-CoV-2 infection, and dysregulation of the immune system might be a future treatment target.

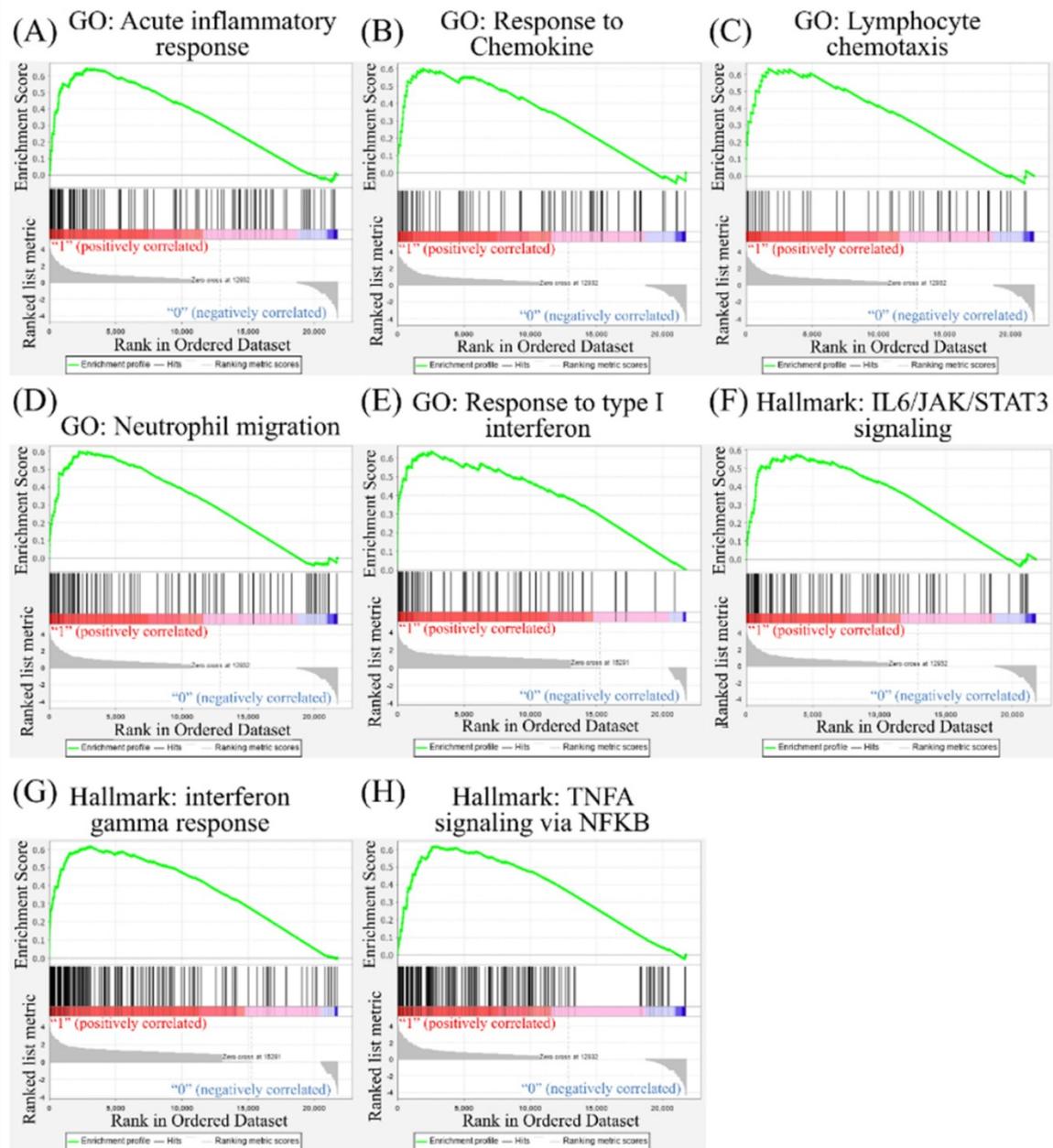


Figure 4. Gene Set Enrichment Analysis (GSEA) showing enriched immune-related pathways in severe acute respiratory syndrome coronavirus (SARS-CoV)-2 infected cells. The Gene Ontology (GO) and Hallmark platforms were used to analyze upregulated genes from SARS-CoV-2-infected ACE2-expressed-A549 and normal human bronchial epithelial (NHBE) cells relative to mock-infected controls. An enrichment score threshold of >0 was set for upregulation. In the middle portion of each figure, members of each gene set are represented as a single bar. For positive correlations highlighted in red, bars shown before the peak score contributed the most to that score, whereas for negative correlations highlighted in blue, bars shown right after the peak score contributed the most to that score. In the last portion of each graph, the ranking metric shows the correlation of the gene set with either the first or second phenotype. (A) Acute inflammatory response. (B) Response to chemokines. (C) Lymphocyte chemotaxis. (D) Neutrophil migration. (E) Response to type I interferon. (F) Interleukin (IL)-6/Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) signaling. (G) Interferon-gamma response. (H) Tumor necrosis factor- α (TNFA) signaling via nuclear factor- κ B (NFKB).

CoVs were reported to be correlated with cytokine storms and inflammation in previous studies [37, 38]. Serum levels of IL family members are elevated in patients experiencing a cytokine storm, including IL-6, IL-1, IL-1 β , tumor necrosis factor (TNF), and C-C chemokine ligand 2 (CCL2) [39, 40]. Targeting these cytokines should control complications due to the cytokine storm; however, results of a clinical trial were equivocal [37]. Other

cytokines may also play roles in SARS-CoV-2 infection. The accumulation of transforming growth factor (TGF)- β in the lungs induces dysregulation of coagulation and fibrinolytic pathways, resulting in lung fibrosis [41]. Expressions of IFN regulatory factor (IRF)-1, IL-6, IL-8, and IL-18 in lung tissues were elevated in acute respiratory distress syndrome [42, 43]. SARS-CoV-2 relies on ACE2 and transmembrane serine protease 2 (TMPRSS2) to enter cells [44].

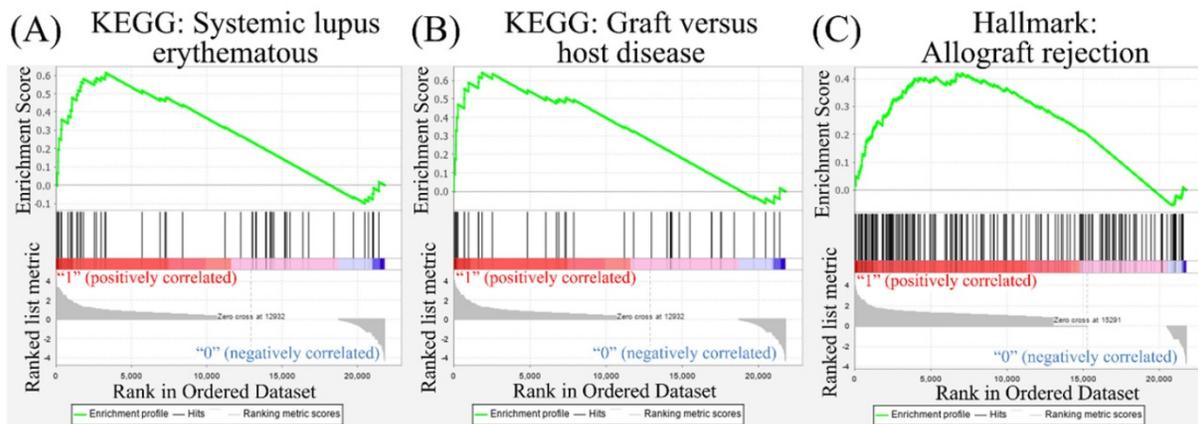


Figure 5. Disease associated with dysregulation of the immune system in a Gene Set Enrichment Analysis (GSEA). The Kyoto Encyclopedia of Genes and Genomes (KEGG) and Hallmark platforms were utilized to analyze upregulated genes from severe acute respiratory syndrome coronavirus (SARS-CoV)-2-infected ACE2-expressed-A549 and normal human bronchial epithelial (NHBE) cells. SARS-CoV-2-infected cells were compared to mock-infected controls. (A) Systemic lupus erythematosus. (B) Graft versus host disease. (C) Allograft rejection.

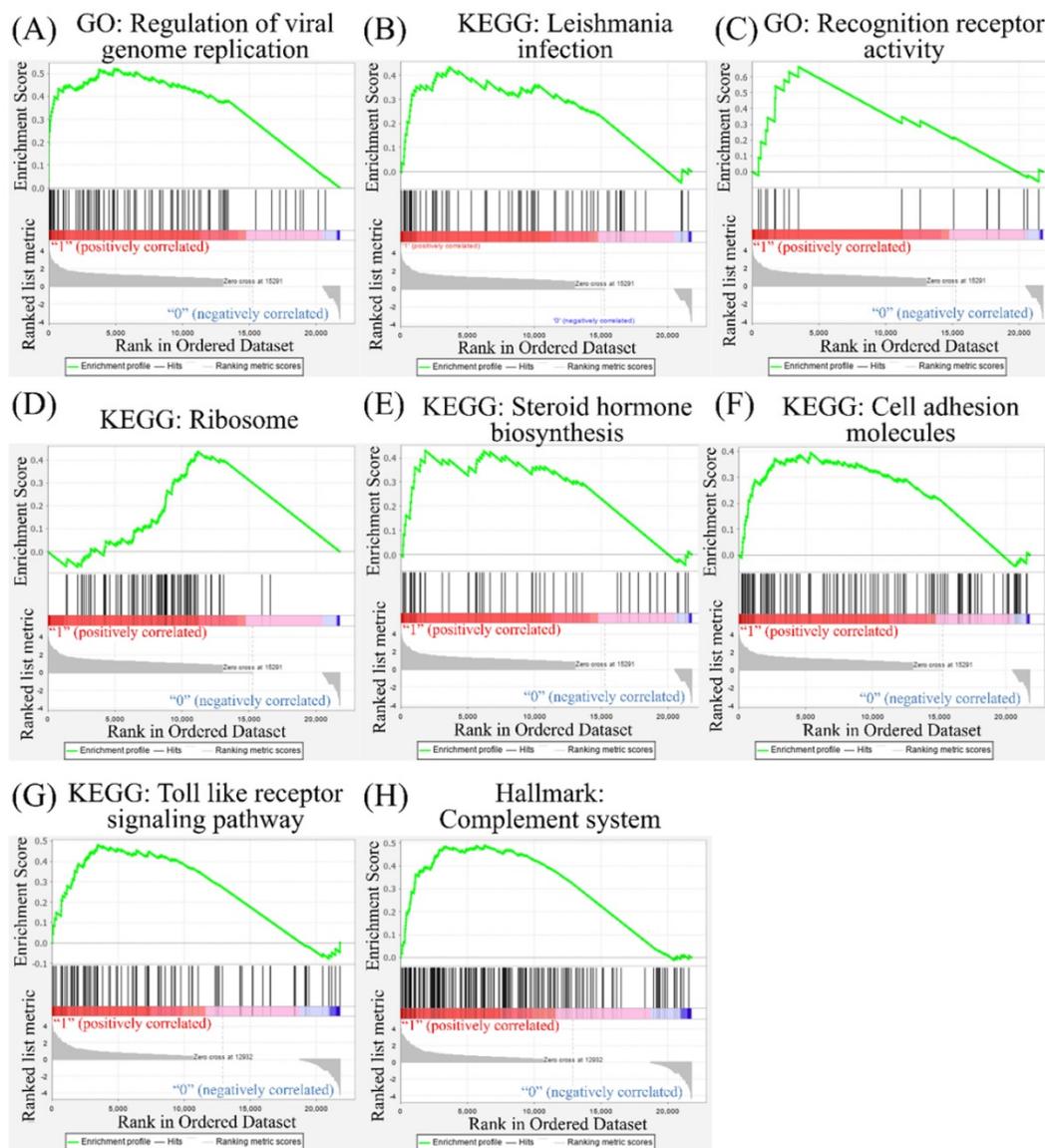


Figure 6. Other pathways in the Gene Set Enrichment Analysis (GSEA). The Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO), and Hallmark platforms were utilized to analyze upregulated genes from severe acute respiratory syndrome coronavirus (SARS-CoV)-2-infected ACE2-expressed-A549 and normal human bronchial epithelial (NHBE) cells compared to mock-infected controls. (A) Regulation of viral genome replication. (B) *Leishmania* infection. (C) Recognition of receptor activity. (D) Ribosomes. (E) Steroid hormone biosynthesis. (F) Cell adhesion molecules. (G) Toll-like receptor signaling pathway. (H) Complement system

Table 1: Ingenuity Pathway Analysis (IPA) of potential interactions among upregulated genes of ACE2-expressed-A549 and normal human bronchial epithelial (NHBE) cells infected for 24 h with severe acute respiratory syndrome coronavirus (SARS-CoV)-2 compared to a control group in the GSE147507 dataset.

Canonical Pathways	P value	Molecules
Interferon Signaling	1.58E-11	IFI35,IFI16,IFIT1,IFIT3,IFITM1,IFITM3,ISG15,MX1,OAS1
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	2.82E-10	CSF2,IL1A,IL6,IRF7,LIF,LTB,NFKB2,NOD2,OAS1,OAS2,OAS3,PTX3,TNF
Airway Pathology in Chronic Obstructive Pulmonary Disease	1.86E-09	CCL2,CCL20,CSF2,CXCL1,CXCL3,IL1A,IL23A,IL6,LIF,LTB,TNF
Differential Regulation of Cytokine Production in Macrophages and T Helper Cells by IL-17A and IL-17F	7.24E-09	CCL2,CSF2,CSF3,CXCL1,IL6,TNF
Differential Regulation of Cytokine Production in Intestinal Epithelial Cells by IL-17A and IL-17F	3.80E-08	CCL2,CSF2,CSF3,CXCL1,IL1A,TNF
Role of IL-17F in Allergic Inflammatory Airway Diseases	6.31E-08	CCL2,CSF2,CXCL1,CXCL10,IL6,MMP13,NFKB2
Granulocyte Adhesion and Diapedesis	8.91E-08	CCL2,CCL20,CSF3,CXCL1,CXCL10,CXCL11,CXCL2,CXCL3,IL1A,MMP13,TNF
TREM1 Signaling	1.12E-07	CCL2,CSF2,CXCL3,IL6,NFKB2,NOD2,STAT5A,TNF
Role of IL-17A in Arthritis	4.47E-07	CCL2,CCL20,CXCL1,CXCL3,MMP13,NFKB2,NFKBIA
Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	5.13E-07	CSF2,IL1A,IL23A,IL6,LTB,NFKB2,RELB,TNF
Agranulocyte Adhesion and Diapedesis	1.15E-06	CCL2,CCL20,CXCL1,CXCL10,CXCL11,CXCL2,CXCL3,IL1A,MMP13,TNF
Hematopoiesis from Pluripotent Stem Cells	1.26E-06	CSF2,CSF3,IL1A,IL6,IL7,LIF
Th17 Activation Pathway	1.82E-06	CCL20,CSF2,IL23A,IL6,IRAK2,NFKB2,SOCS3
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	1.91E-06	CCL2,CSF2,FRZB,IL1A,IL6,IL7,IRAK2,LTB,MMP13,NFKBIA,SOCS3,TNF,WNT3A
Role of Cytokines in Mediating Communication between Immune Cells	1.95E-06	CSF2,CSF3,IL1A,IL23A,IL6,TNF
IL-23 Signaling Pathway	2.29E-06	CSF2,IL23A,NFKB2,NFKBIA,SOCS3,TNF
IL-17A Signaling in Gastric Cells	2.29E-06	CCL20,CXCL1,CXCL10,CXCL11,TNF
Hepatic Cholestasis	3.24E-06	CSF2,CYP27A1,IL1A,IL6,IRAK2,LIF,LTB,NFKB2,NFKBIA,TNF
TNFR2 Signaling	5.01E-06	BIRC3,NFKB2,NFKBIA,TNF,TNFAIP3
Activation of IRF by Cytosolic Pattern Recognition Receptors	7.76E-06	IL6,IRF7,ISG15,NFKB2,NFKBIA,TNF
Role of Hypercytokinemia/hyperchemokineemia in the Pathogenesis of Influenza	8.32E-06	CCL2,CXCL10,IL1A,IL6,TNF
HMGB1 Signaling	8.51E-06	CCL2,CSF2,IL1A,IL6,LIF,LTB,NFKB2,RND1,TNF
IL-9 Signaling	9.55E-06	BCL3,NFKB2,SOCS3,STAT5A,TNF
IL-17A Signaling in Fibroblasts	1.29E-05	CCL2,IL6,NFKB2,NFKBIA,NFKBIZ
Dendritic Cell Maturation	1.55E-05	CSF2,IL1A,IL23A,IL6,LTB,NFKB2,NFKBIA,RELB,TNF
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	1.78E-05	BIRC3,CSF2,FRZB,IL1A,IL6,IL7,MMP13,NFKBIA,TNF,WNT3A
IL-17A Signaling in Airway Cells	1.91E-05	CCL20,CXCL1,CXCL3,IL6,NFKB2,NFKBIA
IL-10 Signaling	2.69E-05	IL1A,IL6,NFKB2,NFKBIA,SOCS3,TNF
Neuroinflammation Signaling Pathway	3.63E-05	BIRC3,CCL2,CXCL10,GABRR2,IL6,IRAK2,IRF7,NCF1,NFKB2,PLA2G4E,TNF
Toll-like Receptor Signaling	4.47E-05	IL1A,IRAK2,NFKB2,NFKBIA,TNF,TNFAIP3
Atherosclerosis Signaling	5.62E-05	CCL2,IL1A,IL6,MMP13,NFKB2,PLA2G4E,TNF
TNFR1 Signaling	6.31E-05	BIRC3,NFKB2,NFKBIA,TNF,TNFAIP3
Systemic Lupus Erythematosus In B Cell Signaling Pathway	8.51E-05	CSF2,IFIT3,IL1A,IL6,IRF7,ISG15,LIF,LTB,NFKB2,TNF
Lymphotoxin Receptor Signaling	1.00E-04	CXCL1,LTB,NFKB2,NFKBIA,RELB
Role of IL-17A in Psoriasis	1.38E-04	CCL20,CXCL1,CXCL3
Hematopoiesis from Multipotent Stem Cells	1.38E-04	CSF2,CSF3,IL7
Death Receptor Signaling	1.45E-04	BIRC3,NFKB2,NFKBIA,PARP12,PARP9,TNF
Antioxidant Action of Vitamin C	2.14E-04	CSF2,NFKB2,NFKBIA,PLA2G4E,STAT5A,TNF
Hepatic Fibrosis Signaling Pathway	2.88E-04	CCL2,IL1A,IRAK2,MMP13,MYLK3,NCF1,NFKB2,NFKBIA,RND1,TNF,WNT3A
IL-17 Signaling	5.89E-04	CCL2,CXCL1,CXCL10,CXCL11,IL6
IL-6 Signaling	7.08E-04	IL1A,IL6,NFKB2,NFKBIA,SOCS3,TNF
FAT10 Cancer Signaling Pathway	7.08E-04	IL6,NFKB2,NFKBIA,TNF
Communication between Innate and Adaptive Immune Cells	7.94E-04	CSF2,CXCL10,IL1A,IL6,TNF
Crosstalk between Dendritic Cells and Natural Killer Cells	8.71E-04	CSF2,IL6,LTB,NFKB2,TNF
Hepatic Fibrosis / Hepatic Stellate Cell Activation	9.12E-04	CCL2,CXCL3,IL1A,IL6,MMP13,NFKB2,TNF
Role of JAK family kinases in IL-6-type Cytokine Signaling	1.35E-03	IL6,SOCS3,STAT5A
Apoptosis Signaling	1.45E-03	BCL2A1,BIRC3,NFKB2,NFKBIA,TNF
PPAR Signaling	1.66E-03	IL1A,NFKB2,NFKBIA,STAT5A,TNF
Glucocorticoid Receptor Signaling	1.82E-03	CCL2,CSF2,CXCL3,IL6,NFKBIA,NPPA,POU2F2,STAT5A,TNF
Induction of Apoptosis by HIV1	1.95E-03	BIRC3,NFKB2,NFKBIA,TNF

ACE2 is a human IFN-stimulated gene in lung type II pneumocytes [45]. The immune response with type I IFN (IFN- α/β) is one kind of innate immunity against the virus. However, CoVs are capable of interrupting IFN responses by viral proteins [46, 47]. In our study, we used GSEA, GO, KEGG, and Hallmark platforms, and revealed that responses to IFN- α/γ were upregulated in SARS-CoV-2-infected ACE2-expressed-A549 cells (Figure 2). By merging upregulated genes in NHBE and ACE2-expressed-A549 cells, IFN signaling was found to be the most

crucial pathway (Figure 3). In other platforms, a positive correlation between responses to type I IFN or IFN- γ or IL-6 and SARS-CoV-2 infection were detected with other cytokines (Figure 4). Gene signatures of acute SARS-CoV-2-infected cells were similar to those of diseases induced by dysregulation of the immune system (Figure 5). Dysregulated immune system may restrain the killing ability of IFN signaling and SARS-CoV-2 survive in these conditions. Modulating the host response to stable homeostasis is an important part of treating

SARS-CoV-2 infection.

According to a previous study, the androgen receptor is required for transcription of the *TM6SS2* gene [48, 49], which is the protease for spike proteins on CoVs [50]. In the present study, positive correlations of steroid hormone biosynthesis with SARS-CoV-2 infection were detected (Figure 6). We also recognized responses of host cells after SARS-CoV-2 infection, including regulation of viral genome replication, *Leishmania* infection, and recognition receptor activity. The protozoan *Leishmania* is a genus of parasites, and *Leishmania* infection induces high expression of IL-17 [51]. Besides, recognition receptor activity is the cellular response after binding to specific receptors. Binding of SARS-CoV-2 to membranous ACE2 receptors of host cells initiates specific downstream actions that support the survival of CoVs [29]. Meanwhile, upregulation of cell adhesion molecules, TLR signaling pathways, and the complement system was also discovered. Binding of SARS-CoV-2 with TLRs induces the release of pro-inflammatory cytokines, including IL-1 β and IL-6 [52, 53]. The complement system is one of the essential components of innate immunity after viral infection. Activation of the complement system contributes to a dysregulated inflammatory response, and recent reports presumed the complement system to be a target for treating severe illness due to COVID-19 [54, 55]. Our bioinformatics analysis supported most of that research. The cellular response is complicated after SARS-CoV-2 infection, and a comprehensive understanding of these networks will help us find effective therapeutic agents.

Collectively, the present study focused on the response of lung epithelial cells after SARS-CoV-2 infection. Dysregulation of the immune system, production of steroid hormones, alteration of cell adhesion molecules, and a maladaptive complement system all contribute to clinical sequelae of lung fibrosis and respiratory distress. The current findings from our study could contribute to the battle against SARS-CoV-2 using high-throughput methods, which could shorten the time consumed for target screening and provide an underlying mechanism via a network enrichment analysis. These pathways and networks could provide potential targets for prospective experimental studies related to SARS-CoV-2 treatment.

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Competing Interests

The authors have declared that no competing interest exists.

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