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High expression of ACE2 in the human lung leads to the release of IL6 by suppressing cellular immunity: IL6 plays a key role in COVID-19

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Abstract. – OBJECTIVE: The pathogenesis of coronavirus disease 2019 (COVID-19) remains clear, and no effective treatment exists. SARS-CoV-2 is the virus that causes COVID-19 and uses ACE2 as a cell receptor to invade human cells. Therefore, ACE2 is a key factor to analyze the SARS-CoV-2 infection mechanism.

MATERIALS AND METHODS: We included 9,783 sequencing results of different organs, analyzed the effects of different ACE2 expression patterns in organs and immune regulation.

RESULTS: We found that ACE2 expression was significantly increased in the lungs and digestive tract. The cellular immunity of individuals with elevated ACE2 expression is activated, whereas humoral immunity is dampened, leading to the release of many inflammatory factors dominated by IL6. Furthermore, by studying the sequencing results of SARS-CoV-2-infected and uninfected cells, IL6 was found to be an indicator of a significant increase in the number of infected cells. However, although patients with high expression of ACE2 will release many inflammatory factors dominated by IL6, cellular immunity in the colorectum is significantly activated. This effect may explain why individuals with SARS-CoV-2 infection have severe lung symptoms and digestion issues, which are important causes of milder symptoms.

CONCLUSIONS: This finding indicates that ACE2 and IL6 inhibitors have important value in COVID-19.

Key Words:
COVID-19, ACE2, IL6, Cellular immunity, Inflammatory factors.

Introduction

Coronavirus is a single-stranded positive RNA virus that infects various vertebrate hosts[1]. The novel coronavirus (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) epidemic spread rapidly worldwide, triggering a global health crisis[2]. SARS-CoV-2 causes coronavirus disease 2019 (COVID-19) and belongs to the genus *Betacoronavirus* (β-CoV)[1]. SARS-CoV-2 and severe acute respiratory syndrome coronavirus (SARS-CoV) share 79.5% sequence homology, with angiotensin-converting enzyme 2 (ACE2) as the receptor, and SARS-CoV-2 enters the cell similarly to SARS-CoV[3]. Therefore, ACE2 is an important target for COVID-19. The coronavirus can activate excessive and maladjusted host immune responses, which may cause acute respiratory distress syndrome (ARDS)[4]. Autopsy analysis of patients with COVID-19 and ARDS showed that cytotoxic T cells were overactivated and that the concentration of cytotoxic particles was elevated[5]. Reports describing the immunological characteristics of critically ill COVID-19 patients indicated that excessive activation of humoral immune pathways, including the interleukin (IL)-6 pathway, is a key mediator of respiratory failure, shock and multiple organ dysfunction[5]. Coomes et al[6] have shown that, in COVID-19 patients, the IL6 levels are significantly increased and are associated with adverse clinical outcomes. Because ACE2 and immune-related regulators play

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crucial roles in the infection and occurrence of COVID-19, determining the relationship between them is important. To study this subject, we analyzed the RNAseq data of SARS-CoV-2-infected and uninfected cell lines in GSE147507 and found that COVID-19 causes the expression of many immune factor-related genes; one of them, IL6, showed a significant difference. Thus, IL6 plays a key role during COVID-19. To further determine why SARS-CoV-2 uses ACE2 as the receptor, we included the sequencing results of 9,783 different organs and analyzed the effects of different ACE2 expression patterns on organs and immune regulation to find the target and use this large data set to facilitate the development of prevention and control measures.

Materials and Methods

Data Sets

GEO (https://www.ncbi.nlm.nih.gov/geo/) is a public knowledge base that stores high-throughput functional genomic data in the form of microarrays and other analyses, and we obtained GSE147507 from the GEO database expression profiles. The GSE147507 database contains SARS-CoV-2 infection samples and control samples based on the GPL18573 platform [Illumina NextSeq 500 (*Homo sapiens*)]. From the UCSC Xena project (HTTPS:/xenabrowser.net/datapages/), we downloaded the GTEX RNA-SEQ gene expression profile data set [RNAseq by expectation maximization (RSEM) standardization] of 31 normal human tissues, including the sequencing results of 9,783 different organs.

Screening of Differentially Expressed Genes

The RSEM method was used to normalize the level 3 transcriptome data of the data set and logarithmically convert all the gene expression values. After normalizing the quantile, the approximate data were normally distributed[7]. In this study, the R package limma was used to analyze the gene expression data of infected and uninfected patients meeting the mRNA requirements of $p < 0.01$, FDR < 0.01 and Log 2 fold change (FC) | >1.5; there were two further studies, in which p indicates that hypothesis testing is very important, and FDR is the control index of the hypothesis testing error rate. As an indicator of the selected differentially expressed gene, the number of false rejections is proportional to the number of invalid hypotheses rejected. FC is usually used to study the degree of change from the initial value to the final value. In this study, the ratio of the gene expression value of tumor tissues to the gene expression value of normal tissues is also called the difference multiple. To facilitate visual comparison, a volcano map of differentially expressed genes was constructed in R[8].

Gene Ontology and Pathway Enrichment Analysis

Gene set enrichment analysis (GSEA) was performed using GSEA v4.0.3 software (http://software.broadinstitute.org/gsea/index.jsp). The enrichment score was compared with the results of 1,000 randomly arranged genomes to obtain the p-value and evaluate statistical significance[9].

Enumeration of Hematopoietic Cell Subsets from Gene Expression Profiles

By studying the gene expression data of the normal population, the relative proportion of 22 different genotypes was calculated by the CIBERSORT immune infiltration algorithm to analyze the penetration of immune cells. For the GTEX data set, voom (observation-level variation model) was used to convert the RNA-seq data, and the count data were converted to a value closer to the microarray results[10]. The 22 cell types counted by the CIBERSORT algorithm included B cells, T cells and natural killer (NK) cells. CIBERSORT is a type of deconvolution algorithm that uses a set of data that is considered the least expressed amount of each cell type, corresponding to the data representing the expression value of reference genes (547 genes in the "feature matrix")[11]. CIBERSORT uses Monte Carlo sampling to obtain the p-value of each sample deconvolution and provides the interval measurement of the results. Gene expression data sets are available using standard annotation files and uploaded to the CIBERSORT website (http://cibersort.stanford.edu/). The algorithm uses the default feature matrix to run 1,000 times. We drew a heatmap to show the infiltration of immune cells in different individuals. By analyzing the correlation among immune cells in different groups, the related heatmap was drawn. To explore the differences in immune infiltrating cells in different groups, we drew a violin chart to display them directly.

WGCNA Co-Expression Network Construction

Gene expression data (mRNA SEQ data) were obtained by analyzing the GTEX database. In total, 22,804 genes were identified in each sample. We performed ANOVA, sorting from large to small. We calculated the SD value of each gene, sorted them from large to small and then selected the first 5,000 genes for weighted correlation network analysis (WGCNA).

The WGCNA data package in R language software was used to construct the expression data formal map of these 5,000 genes and construct a gene co-expression network[12]. The adjacency of functions in the WGCNA software package was used for unified analysis, and the adjacency matrix was constructed by calculating the Pearson correlation among all gene pairs in the sample analyzed. In our study, soft threshold parameters were used to ensure the accuracy of scale-free networks. To further identify the functional modules in the co-expression network of these 5,000 genes, we used the adjacency matrix to calculate the topological overlap measure (ToM), which represents the overlap in the shared neighborhood.

Identification of Clinically Significant Modules

We studied and analyzed the correlation between MEs and phenotypes to understand the relevant modules. The log 10 transformation of the *p*-value (GS = LGP) in the linear regression of gene expression and phenotype information was defined as gene significance (GS). Additionally, module significance (MS) was defined as the GS mean of all genes studied in the module. Generally, among the modules selected, the module with the highest absolute value of MS is considered the module related to the expression level of ACE2.

PPI Network Construction of Key Module Genes

Core genes can be highly connected with the key points in related modules with important and representative functions. We studied the top 30 core genes in the modular network as preparatory genes for research and analysis. The string data set is a type of network biological resource with authority that can reveal the interaction among proteins to obtain accurate function of the real protein[13]. The prepared core genes were uploaded to the string database for the protein interaction network framework, and the cut-off value was set to 0.4. The significant modules with strong protein-protein linkage were calculated and analyzed. The default parameters were as follows: degree cut ≥ 2, node cut ≥ 2, k-core ≥ 2, and maximum depth = 100. $p < 0.05$ was considered statistically significant.

Statistical Analyses

Pearson's χ^2 test was used to count the differences in ACE2 expression in different sexes and IL6 expression in different ACE2 expression groups. IBM SPSS statistical software version 22.0 and R version 5.3.0 (R Foundation) were used for statistical analysis. The *p*-value was two-sided, and $p < 0.05$ was considered statistically significant.

Results

SARS-CoV-2-Infected Cells Can Cause a Storm of Inflammatory Factors Dominated by IL6

We compared the differentially expressed genes and enriched signaling pathways in GSE147507-infected and uninfected cells. The expression of inflammation-related factors in infected cells increased significantly, including that of IFNB1 (logFC = 9.667475; p = 3.36E-06), IFNL3 (logFC = 9.234619556; p = 9.05E-06), IFNL2 (logFC = 8.312059088; p = 3.52 E-05), IFNL1 (logFC = 7.498878295 p = 5.91E-05), IFNL4 (logFC = 6.452675842; p = 0.000548969), ZBP1 (logFC = 6.071254311; p = 0.000783647), CH25H (logFC = 5.931967285; p = 1.65 E-05), CXCL11 (logFC = 5.693455357; p = 3.02E-05), CXCL10 (logFC = 5.53526306; p = 7.65E-06), and IL6 (logFC = 5.290455576; p = 8.67E-09). Figure 1A shows that the significant difference in the IL6 level is the largest, indicating the important role of IL6 during infection. The infected cells were enriched in pathways related to inflammatory factors and pathways closely related to immune cell activation (Figure 1B-F).

Infection With SARS-CoV-2 Causes the Enrichment of Immune Cells, Such As T Lymphocytes

From the above pathway enrichment, we found that COVID-19 may be correlated with immune cell activation. To this end, we predicted the enrichment of immune cells through the related expression of immune cell genes. We found that infection caused plasma cells, follicular helper T

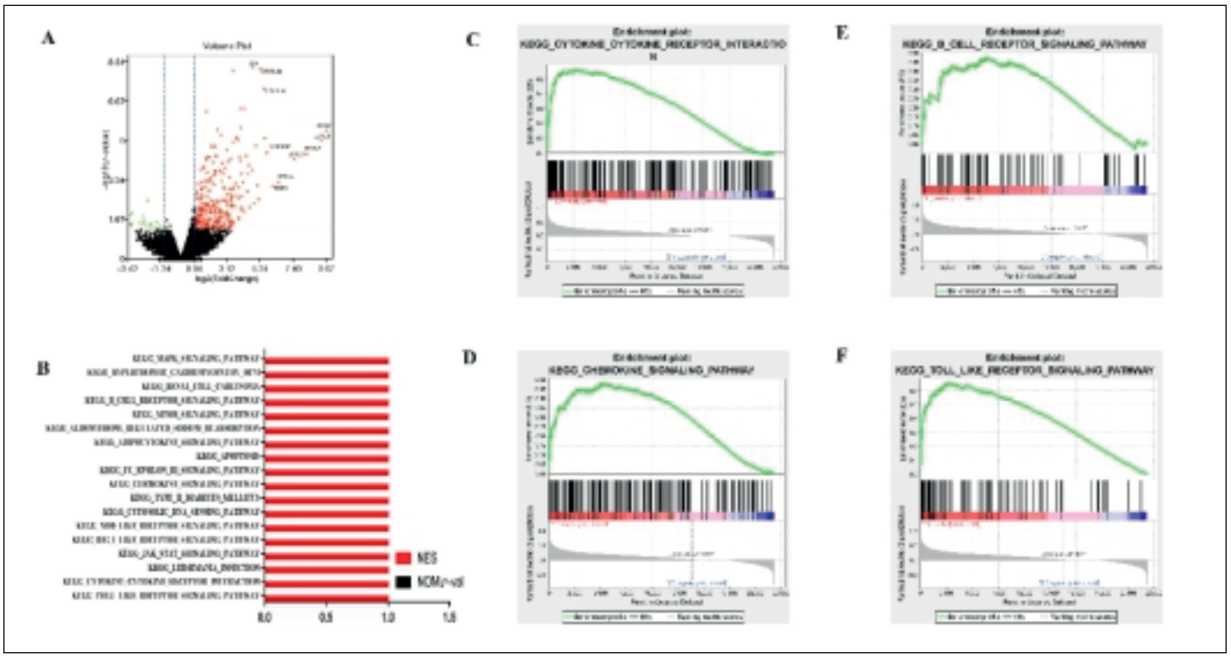

Figure 1. A, Volcano map of differential gene expression in COVID-19 and non-infection. **B,** Functional enrichment and pathway analysis of the COVID-19 and non-infection groups. **C,** Cytokine receptor interaction pathway in COVID-19. **D,** Chemokine signalling pathway in COVID-19. **E,** B-cell receptor signalling in COVID-19. F TOLL-like receptor signalling pathway in COVID-19.

cells, and M0 macrophages to decline in number but increased the number of regulatory T cells (Tregs), resting dendritic cells, and activated mast cells (Figure 2A-C). The decline in the number of follicular helper T cells and rise in the number of Tregs indicate that infection can suppress cellular immunity, further allowing the infection to spread. The decrease in M0 macrophages and increase in activated mast cells can promote the release of related inflammatory factors, thereby destroying the body and organs.

ACE2 Expression Is Inconsistent in Different Organs

The ACE2 receptor is the main mechanism of COVID-19 pathogenesis. Analysis of the expression of ACE2 in different organs of the human body revealed the COVID-19-susceptible parts. We analyzed 9,783 RNAseq results from different organs. The expression level of ACE2 differed between men and women. However, the digestive and respiratory tracts of men and women displayed significant upregulation of ACE2 expression, and its expression in the male reproductive system was significantly increased (Figure 2A and B). ACE2 exhibited the highest level in the digestive tract (Figures 3 and 4A). Regarding sex differences, ACE2 expression in women's blood was higher than that in men's blood, and ACE2 expression in the brain, breast, esophagus, heart and skeletal muscle in men was significantly higher than that in women (Figure 4B). This result may also explain why the incidence in men is significantly higher than that in women.

Increased ACE2 Expression in Normal Human Lungs Has An Important Relationship with IL6

To build a WGCNA network, the lung data from the GTEx database were downloaded. Using R for background correction and normalization, the same pre-processing was performed on the original data. R package annotation matching between probes and gene symbols was performed to remove probes matching multiple genes. For genes matching multiple probes, the median was used as the final expression value. We calculated the SD value of each gene, sorted them from large to small, and then, selected the first 5,000 genes for WGCNA. Cluster analysis of the 5,000 genes was carried out using the fastcluster function of the WGCNA package (Figure 5A). The selection of

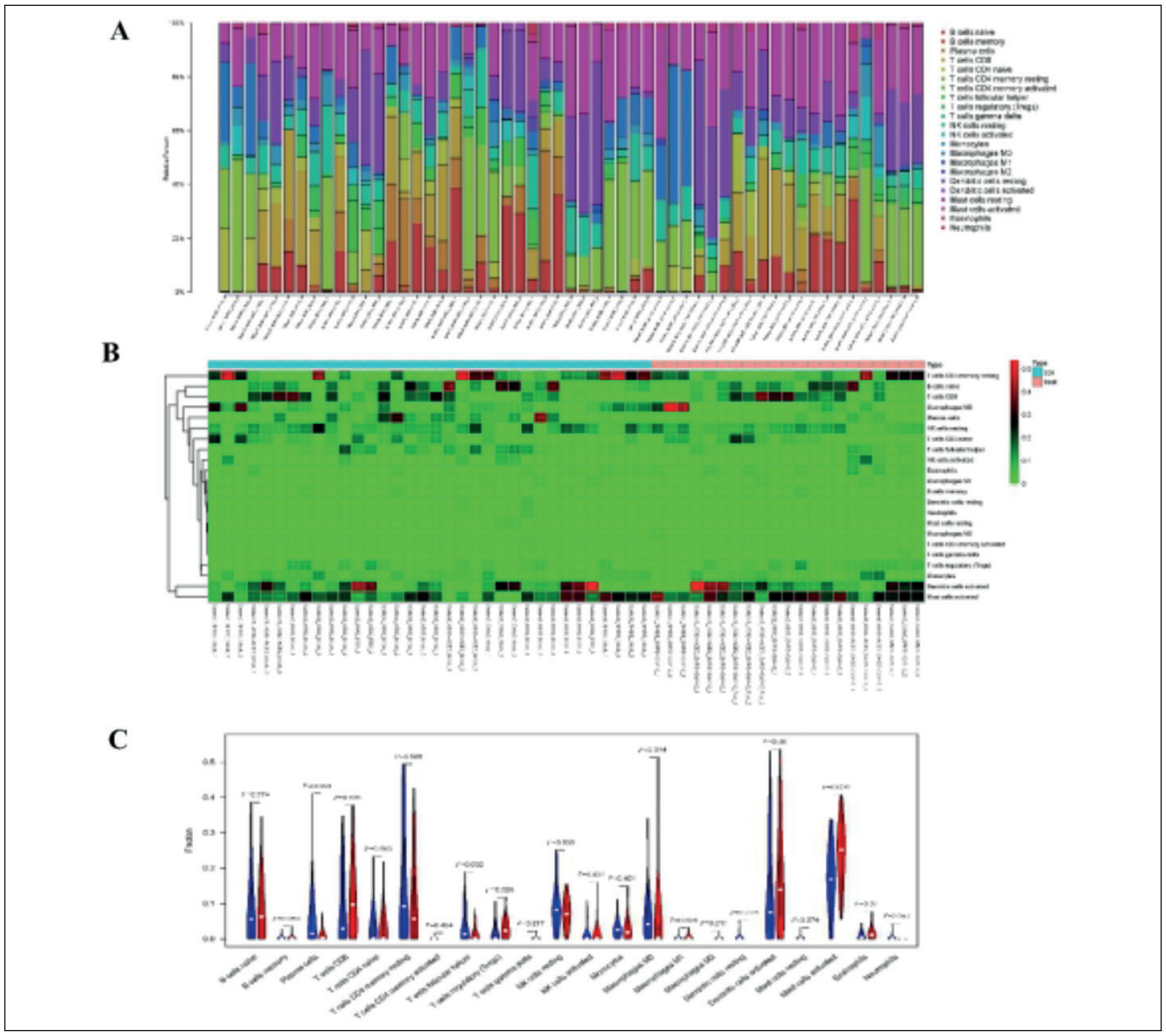

Figure 2. A, Proportion of immune cell subsets in the COVID-19 and non-infection groups. **B,** Heatmap of different immune cell subsets in the COVID-19 and non-infection groups. **C,** Violin map of the significant differences between the COVID-19 and non-infection groups.

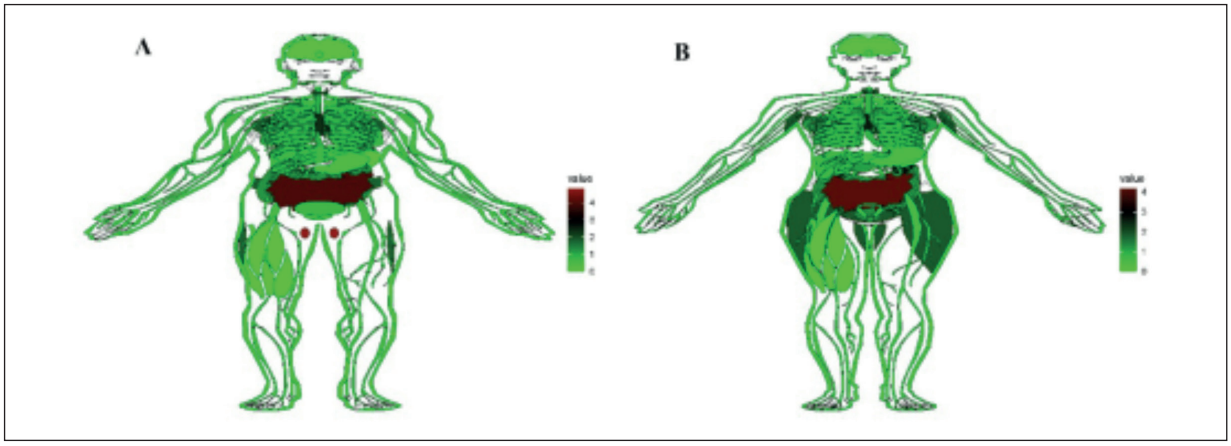

Figure 3. RNA expression of ACE2 in normal tissues. **A,** ACE2 expression in different tissues from men. **B,** ACE2 expression in different tissues from women.

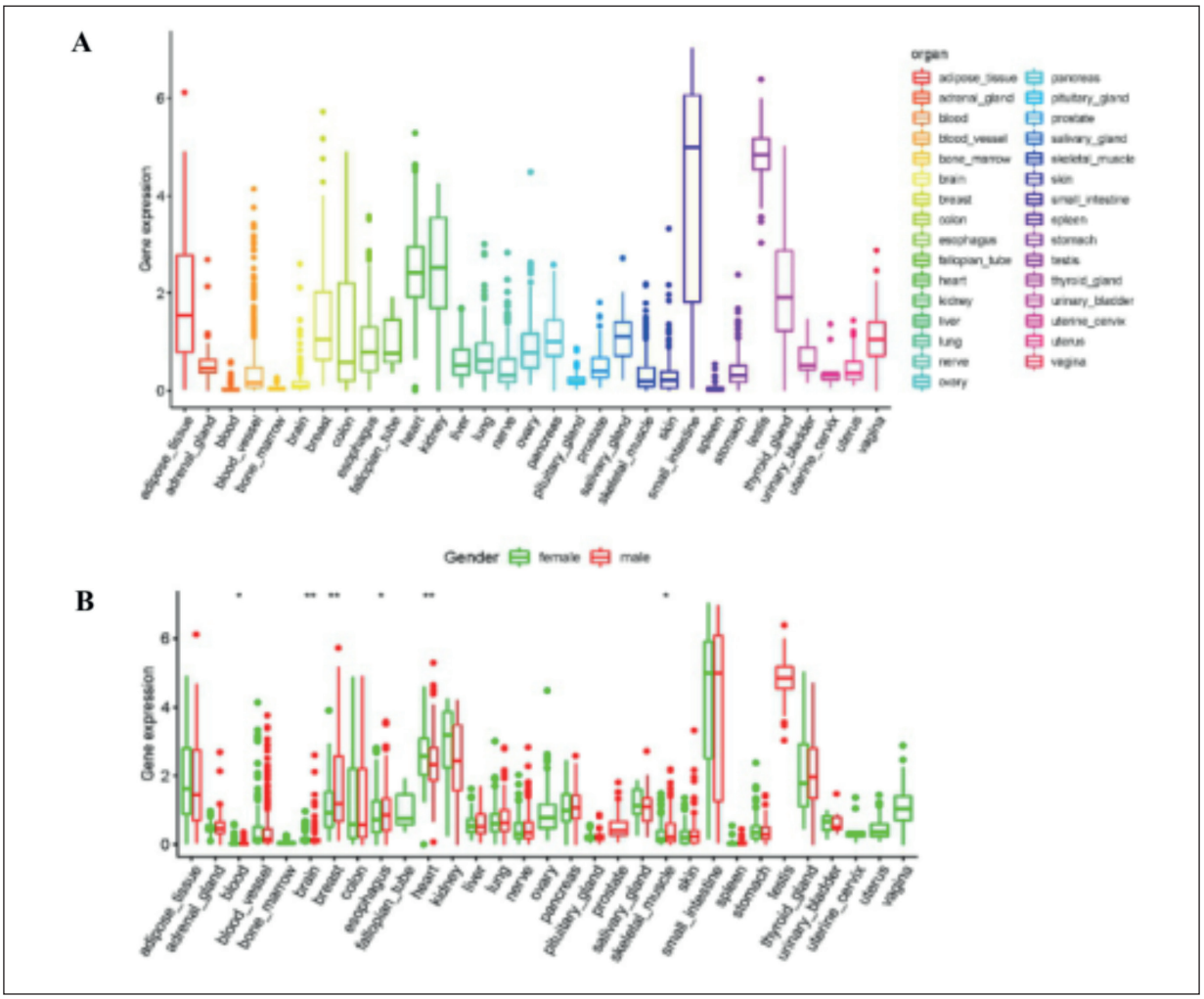

Figure 4. A, Comparison of the ACE2 expression levels across 31 human tissues in GTEx. **B,** Comparison of ACE2 expression levels between men and women.

the soft threshold power is an important part of the network model. By analyzing the network topology under 1~20 threshold weights, the relative balance scale independence and average connectivity of the WGCNA were determined. As shown in the figure, the power values of 5B and 5C were selected as the minimum power values (0.9) of the scale-free topological fit index to generate 5,000 gene hierarchical clustering trees. We set multithreads to 0.25 to merge similar modules (Figure 5D) and generate three modules (Figure 5E). The gene statistics of each module are shown in Table I. The genes that could not be included in any module were placed in the grey module and excluded from the subsequent analysis.

We analyzed the interaction among the three modules and constructed the network heat map (Figure 5F). Each module was independent from each other, and each module was highly independent from each other, with relatively independent gene expression. Additionally, the characteristic genes were calculated and clustered according to their correlation with exploring the co-expression similarity of all modules (Figure 5G). We found that the three modules are mainly divided into two clusters. Turquoise was positively correlated with high ACE2 expression. The protein interaction of the turquoise gene set was submitted to STRING, and the binding confidence interval of truncation value was set as 0.4. In Plugin Molecular Complex Detection (MCODE), significant models with strong protein-protein connections were calculated and selected, with default parameters (degree cut≥2, node score cut≥2, K-core≥2, and

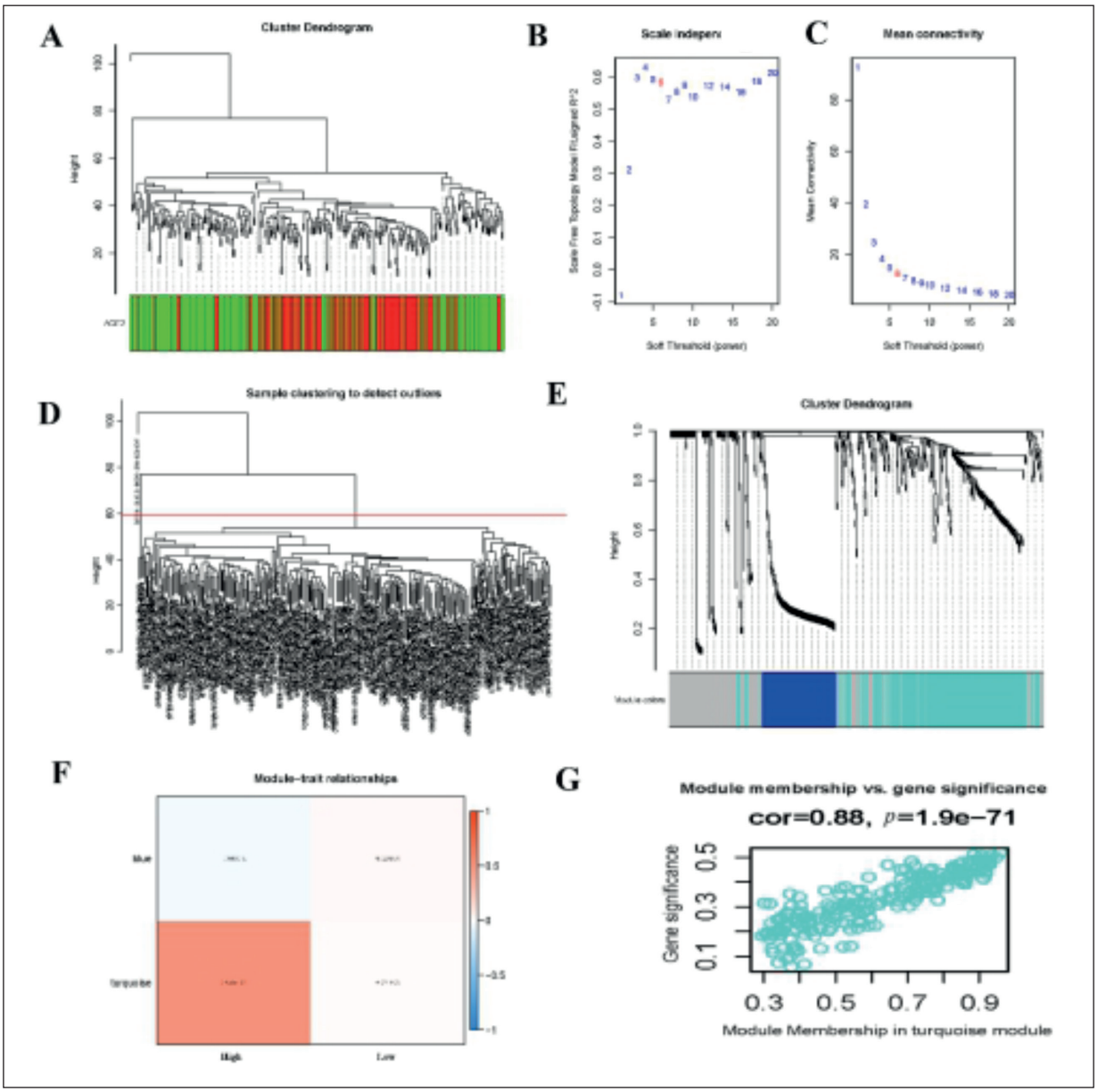

Figure 5. WGCNA of low and high ACE2 expression in the human lung. **A,** Clustering was based on the GTEx expression data. The top 5,000 genes with the highest SD values were used for WGCNA. The colour intensity was proportional to expression status (ACE2 low and high). **B,** Analysis of the scale-free fit index for various soft-thresholding powers (β). **C,** Analysis of the mean connectivity for various soft-thresholding powers. Overall, 4 is the power value with the best fit. **D,** Cluster dendrogram of module eigengenes. **E,** Cluster dendrogram of genes in GTEx. Each branch in the figure represents one gene, and every colour below represents one co-expression module. **F,** Heatmap of the correlation between module eigengenes and a high ACE2 expression status. The turquoise module was the most positively correlated with high ACE2 expression. G Scatter plot of module eigengenes in the turquoise module.

Table I. Gene statistics in each module of the ACE2 status of the lung.

Module	Genes
Blue	97
Grey	183
Turquoise	217

maximum depth =100). A p-value < 0.05 indicated statistical significance. The nodal degree candidate genes were sequenced, and the core genes were selected for further analysis. Figure 6A and B shows hub genes in turquoise. IL6 was the dominant gene, indicating it plays the most important role in ACE2 expression.

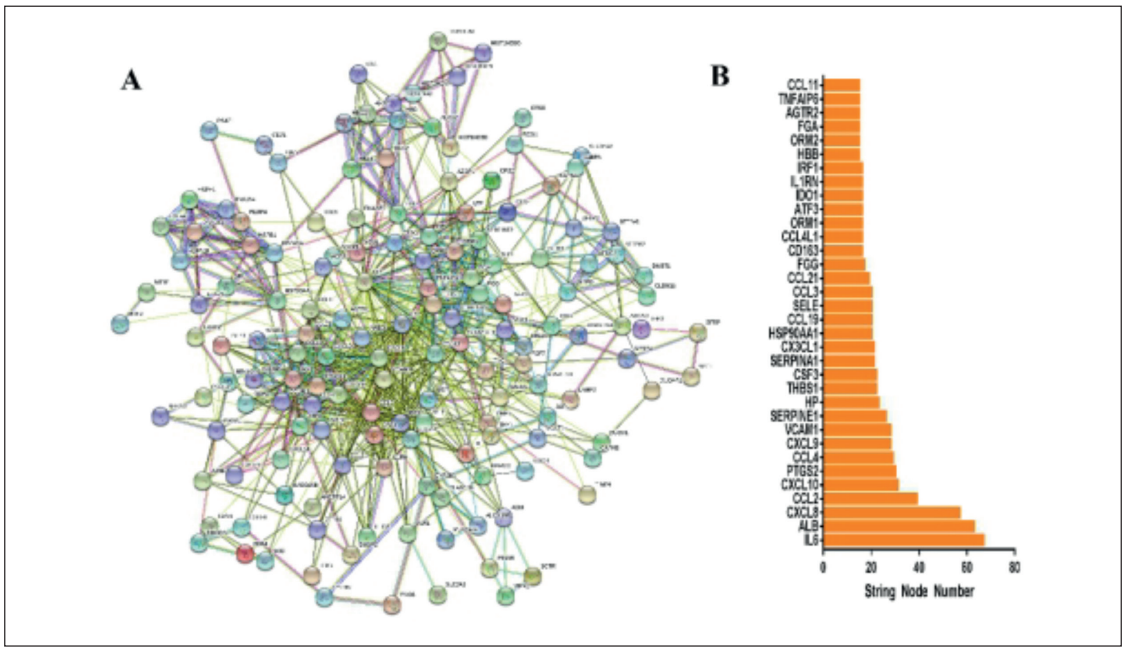

Figure 6. A, Top hub genes in the turquoise module of the human colorectum. **B,** Number of node strings of hub genes in the turquoise module. Edges represent protein-protein associations. Cambridge blue: from curated databases. Violet: experimentally determined. Green: neighbourhood. Red: gene fusions. Blue: gene co-occurrence. Reseda green: text mining. Black: co-expression. Lilac: protein homology.

Increased Expression of ACE2 Can Downregulate Cellular Immunity and Increase Humoral Immunity in the Lung

The lung is the main infection site of COVID-19, and the ACE2 receptor is the main target. COVID-19 can release many immune factors and suppress cellular immunity. To study the effect of high ACE2 expression on lung immune-related cells, we compared the proportion of immune cell subsets in the different ACE2 expression groups and found that the ACE2 high-expression group had plasma cells, M0 macrophages, M2 macrophages, resting dendritic cells, and neutrophils that were significantly more abundant than those in the low-expression group. CD8 T cells, Tregs and resting mast cells were significantly less abundant in the ACE2 high-expression group than in the low-expression group. People with high expression of ACE2 may not only be easily susceptible to COVID-19 but also have lower cellular immunity and develop excessive release of inflammatory mediators (Figure 7).

High Expression of IL6 in the Lung, Colorectum and Skin Is Associated With High Expression of ACE2

IL6 is the core inflammatory factor released after infection with COVID-19. The IL6 expression level was the highest in the normal lung (Figure 8A and B). However, the relationship between ACE2 and IL6 has not been thoroughly studied. We divided the cohort into the high and low ACE2 expression groups and compared IL6 expression in these two groups. High ACE2 expression was correlated with high IL6 expression in the lung, colorectum and skin. The lung represents the respiratory system, the colorectal represents the digestive system, and the skin communicates with the outside world. The increased ACE2 expression in these systems may be related to the release of inflammatory factors. This finding also revealed that individuals with high ACE2 expression may be more prone to inflammatory factor storms.

Increased ACE2 Expression in Normal Human Colorectal Tissue Is Associated With IL6 But Can Lead to the Upregulation of Cellular Immunity

Similarly, we used the WGCNA method to identify the core genes of ACE2 with different expression levels in the colorectum (Table II). IL6 was still the core gene with high ACE2 expression (**Supplementary Figure 1** and **Supplementary Figure 2**). By analyzing the expression of ACE2 and the enrichment of immune cells, we

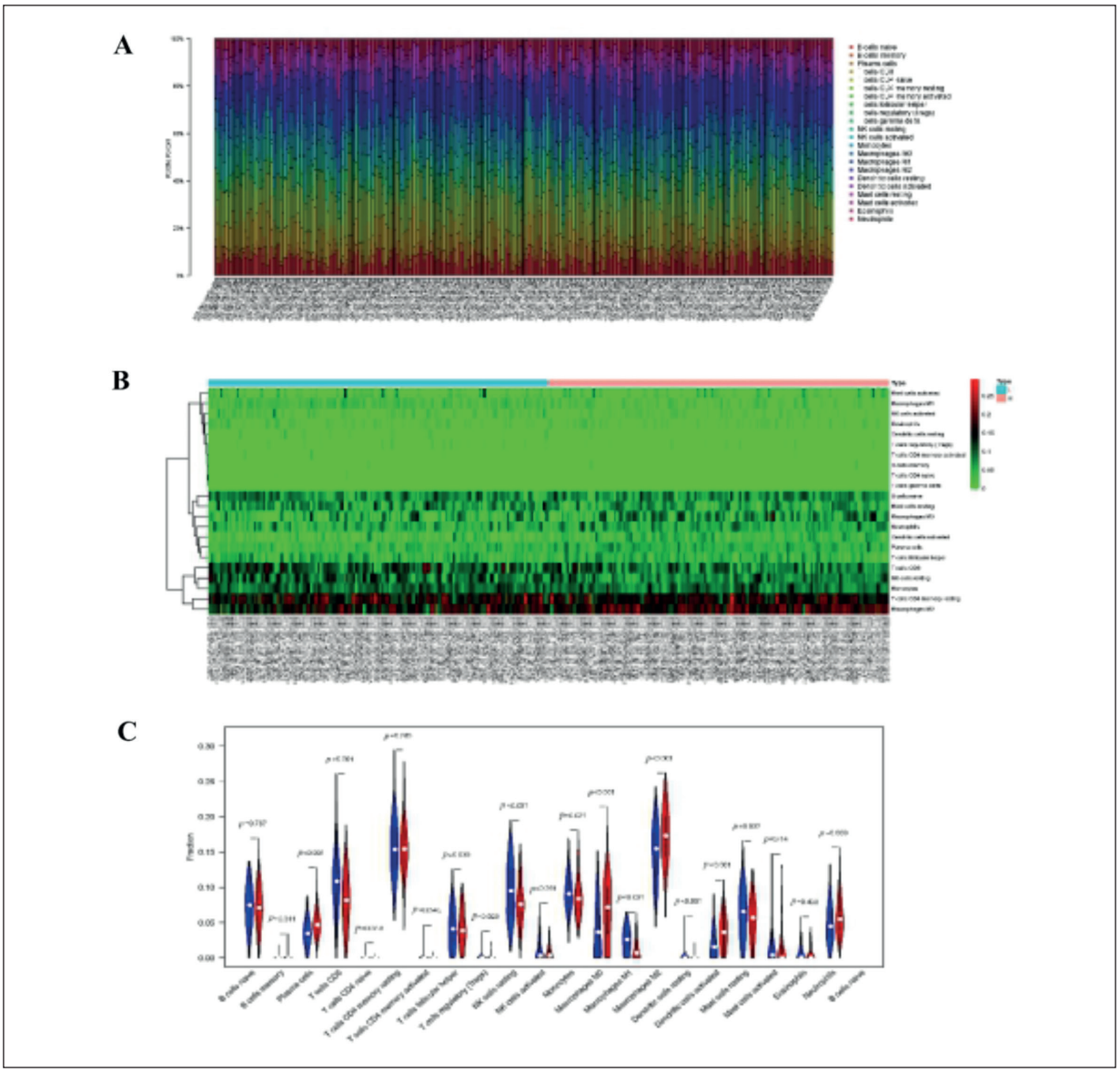

Figure 7. A, Proportion of immune cell subsets in the low and high ACE2 expression groups in the human lung. **B**, Heatmap of different immune cell subsets in the low and high ACE2 expression groups. **C,** Violin map of the significant differences between the low and high ACE2 expression groups.

Table II. Gene statistics in each module of the ACE2 status of the colon and small intestine.

Module	Genes
Blue	486
Brown	131
Green	41
Grey	92
Turquoise	778
Yellow	126

found that, in the colorectal group, the number of memory B cells, plasma cells, CD8 T cells, resting memory CD4 T cells, activated memory CD4 T cells, T follicular helper cells, Tregs, and resting NK cells was significantly higher in the low-expression group. The number of activated NK cells, monocytes, M0 macrophages, M1 macrophages, M2 macrophages, resting dendritic cells, resting mast cells, activated mast cells, eosinophils, and neutrophils were significantly lower in the high-expression population than in the low-expression population. This finding contrast

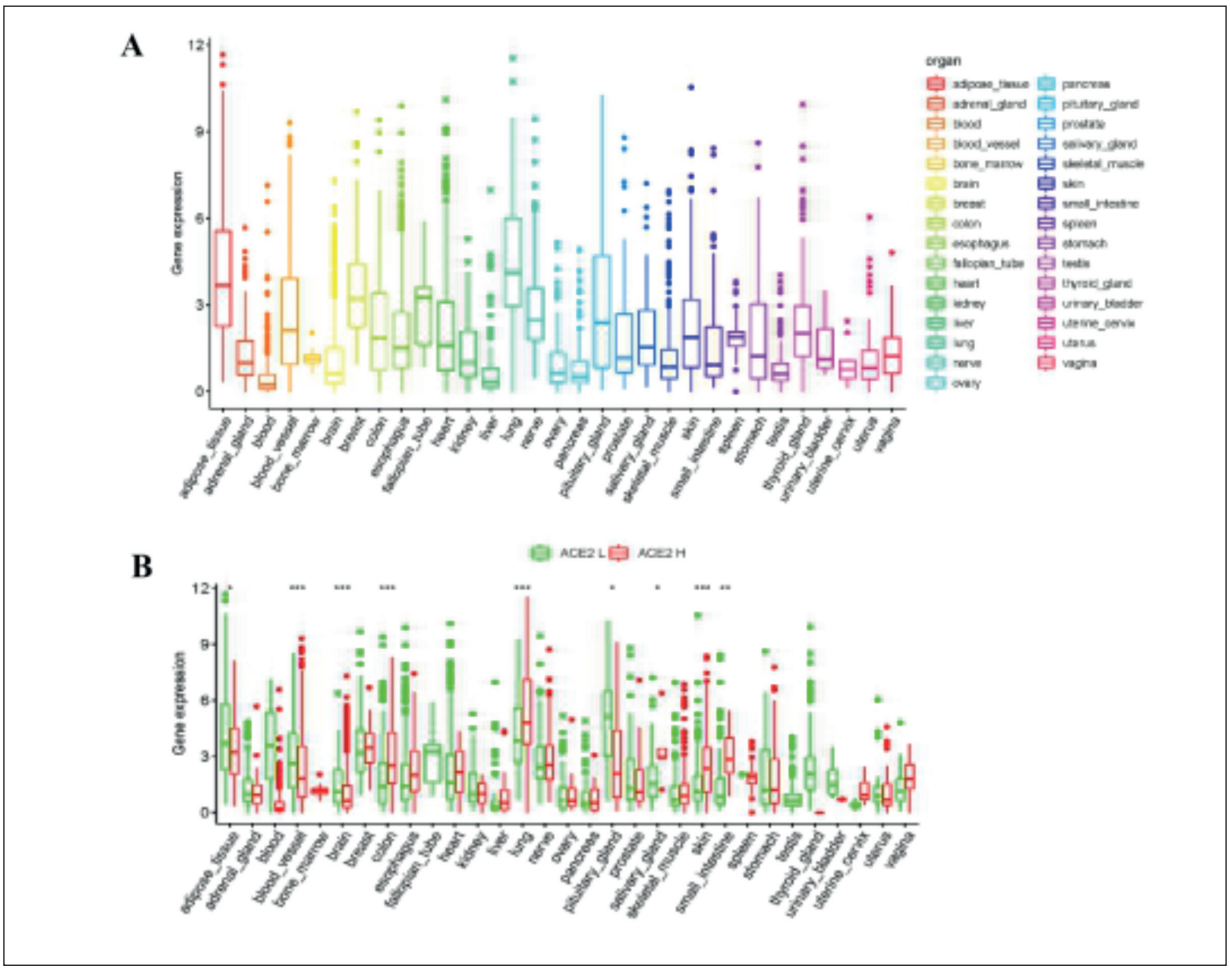

Figure 8. A, Comparison of IL6 expression levels across 31 human tissues in GTEx. **B,** Comparison of IL6 expression levels between the low and high ACE2 expression groups.

that in the lungs, showing more evident immune cell enrichment (Supplementary Figure 3). In the colorectal group, the cellular immunity of the population with high ACE2 expression increased significantly, and the humoral immunity decreased significantly. This finding can also explain why most patients with COVID-19 develop severe pneumonia but rarely die due to digestive tract manifestations. Although studies have shown that viruses in the digestive tract can cause related symptoms, patients with severe digestive tract infections and mortality are rare.

Although IL6 Expression Is Significantly Increased in Individuals with High ACE2 Expression in the Skin, Immune-Related Enrichment Is Not Observed

Through WGCNA of skin ACE2 in high-expression and low-expression populations (Table III), no association was found between ACE2 and related genes (Supplementary Figure 4). Through correlation analysis, immune enrichment was not significantly different regarding ACE2 expression in the skin and the enrichment of immune-related cells (Supplementary Figure 5). This result shows that the skin is not affected by the virus. We analyzed the relationship between ACE2 and IL6 expression in 13 organs of

Table III. Gene statistics in each module of the ACE2 status of the skin.

Module	Genes
Blue	38
Brown	34
Grey	233
Turquoise	84

the human body and found that ACE2 expression in the respiratory system and digestive system was higher than that in other organs, but the immune enrichment of the lung and colorectal systems was correlated, precisely because of the lung. ACE2 is highly expressed in this compartment, the cellular immunity of the high-expression population is low, and the humoral immunity is strong, thus causing severe pneumonia after viral infection. However, the digestive system showed the opposite manifestation. The digestive system already has strong immunity; although ACE2 is highly expressed as a virus receptor, the digestive system can kill the virus through cellular immunity.

Discussion

In severe SARS patients, the mortality rate is very high[14]. Two severe coronaviruses have caused severe respiratory syndrome in the past 100 years: SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV)[15,16]. However, effective treatment or a vaccine against the fatal respiratory syndrome caused by SARS-CoV-2 is lacking, other than control measures. Why is this virus so infectious and lethal? This problem has been troublesome. Fortunately, we found evidence that ACE2 is one of the host receptors required for SARS-CoV group members (including SARS-CoV-2) to enter the cell[1]. This is a unique feature of the virus, but it has caused new concerns. Why do these viruses choose ACE2 as their receptor? Angiotensin II (Ang II) is a small linear peptide composed of 8 amino acids and is the main effector of the renin-angiotensin system (RS). Ang II is the ligand for two main receptors – Ang II 1 receptor (AT1R) and AT2R – which are specific G protein-coupled type 1 and 2 receptors on the surface of target cells. Ang II participates in regulating biological functions, such as vasomotion, water-salt balance, inflammatory responses, cell proliferation, and apoptosis, through the above two receptors[17]. To this end, we analyzed the expression of ACE2 in the normal population. Additionally, we analyzed the impact of high ACE2 expression and low ACE2 expression on the human body to unravel this mystery.

When we analyzed the core genes of individuals with high or low expression of ACE2 in the lungs, we found that IL6 is the target of differences in this group of people. IL6 is a cytokine of the chemokine family. IL6 targets many cells, including macrophages, hepatocytes, resting T cells, activated B cells, and plasma cells[18]. Thus, individuals with high ACE2 expression are more likely to develop a storm of IL6-based inflammatory factors. The clues regarding how the increased levels of IL6 in ARDS affect immunity come from experimentally induced viral pulmonary infection, in which IL6 may have background protection or aggravating effects, including modulating the infection severity, survival rate and tissue remodeling. However, in general, the data for coronavirus family members are limited. In human epithelial cells, SARS-CoV induces more IL6 than influenza A virus and parainfluenza 2 virus but induces less soc3 expression than other viruses, suggesting that the IL6 response to this virus may be more exaggerated[19]. We also analyzed the RNAseq data of COVID-19-infected and uninfected cell lines in GSE147507 for the first time and found that the difference in IL6 expression was the most significant. Individuals with high ACE2 expression are also more likely to produce more IL6. Michot et al[20] reported on the limited findings of the IL6 inhibitor tocilizumab in COVID-19 treatment. Anti-IL6 receptor inhibitor treatment can reduce the risk of progression to SARS by reducing cytokine storms in the lungs of COVID-19 patients[20]. However, this finding was from one case report. Aziz et al[6] included 8 related studies in a systematic review and found that, in patients with COVID-19, the IL6 levels were significantly increased and associated with adverse clinical outcomes. In the preliminary study, the use of tocilizumab to inhibit IL6 seemed effective and safe[6]. We found that, in addition to high ACE2 expression 0in the lungs, high ACE2 expression in the colorectum and skin is related to IL6. However, why do patients present with severe pulmonary symptoms rather than gastrointestinal and skin symptoms?

Presently, very limited information exists concerning the innate immune status of the host with SARS-CoV-2 infection. In a report of 99 cases investigated in Wuhan, an increase in total neutrophils (38%), a decrease in total lymphocytes (35%), an increase in serum IL6 levels (52%), and an increase in c-reactive protein levels (84%) were observed[3]. Another report from Wuhan[21] showed that, in 41 patients in the intensive care unit, the total neutrophils increased and total lymphocytes decreased compared with those in non-intensive care patients, with a significant difference. Neutropenia and lymphope-

nia are also related to the severity of the disease and death. These clinical features suggest the possibility that a highly proinflammatory state is involved in disease progression and severity. An early increase in the level of proinflammatory cytokines in the serum was also observed in SARS-CoV and MERS-CoV infections, suggesting similar cytokine storm-mediated disease severity[22]. SARS-CoV directly infects macrophages and T cells, a key feature of SARS-CoV pathogenesis. It remains unclear whether SARS-CoV-2 infects any immune cells[23]. Cellular immunity has always been a great weapon for killing viruses. We found that, in the lungs of the normal population with high ACE2, cellular immunity is significantly lower than that in the ACE2 low-expression population, but humoral immunity is higher than that in the low-expression population. This related mechanism has not been researched and clarified, but individuals with high ACE2 expression are not only susceptible to COVID-19 but also more likely to develop immunodeficiency and further spread the virus. This finding may also be an important reason why COVID-19 is more likely to invade people with high ACE2 expression.

Although the typical manifestations of this infection, such as fever, cough and pneumonia, are well understood, the incidence of typical gastrointestinal symptoms (such as diarrhea, in the range of 1%-3.8%) reported by earlier studies is lower[24]. Several articles reported on gastrointestinal symptoms, detection of the virus in feces, and potential pathophysiology, including the expression of viral receptors in the gastrointestinal tract[25]. In approximately 50% of COVID-19 cases, SARS-CoV-2 was present in stool samples and the intestinal mucosa of infected patients, suggesting that intestinal symptoms may be caused by intestinal epithelium-expressed ACE2 facilitating cell invasion, and the gastrointestinal tract may be another route of infection. In more than half of patients, SARS-CoV-2 RNA in stool samples remained positive for an average of 11 days after the clearance of respiratory samples[26]. Our research also found that ACE2 expression in the digestive tract was significantly increase. In patients with high ACE2 expression, the IL6 content was also significantly increased. However, why do viruses easily invade the digestive tract, such as the colorectum, but very few patients die due to digestive tract infections? This issue has not been resolved. Our study found for the first time that, in the group with increased colorectal ACE2 expression, cellular immunity was significantly higher than that in the group with low ACE2 expression, while humoral immunity was lower than that in the group with low ACE2 expression. This finding also explains why patients susceptible to COVID-19 have evident pulmonary symptoms but have milder digestive symptoms because the digestive tract can produce sufficient cellular immunity against viruses.

Conclusions

Our study found for the first time that individuals with elevated ACE2 expression in the lungs have upregulated humoral immunity and are prone to develop an inflammatory cytokine storm dominated by IL6 and that the cellular immunity of these individuals is decreased significantly. IL6 is the cytokine that changes most significantly during COVID-19. This result also explains why the population susceptible to COVID-19 is more likely to develop severe lung infections and injuries, ultimately leading to breathing difficulties. However, because the digestive tract has higher ACE2 expression, it is more susceptible to COVID-19 but has stronger cellular immunity. Thus, the digestive tract releases inflammatory factors and causes damage at the same time, but it also has sufficient immunity to fight the virus, possibly explaining why digestive tract symptoms are mild. Together, these findings indicate that ACE2 and IL6 inhibitors have important value in COVID-19.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Declaration of Funding Interests

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