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MAJOR ARTICLE

ACE2 Expression Is Increased in the Lungs of Patients With Comorbidities Associated With Severe COVID-19

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Patients who died from COVID-19 often had comorbidities, such as hypertension, diabetes, and chronic obstructive lung disease. Although angiotensin-converting enzyme 2 (ACE2) is crucial for SARS-CoV-2 to bind and enter host cells, no study has systematically assessed the ACE2 expression in the lungs of patients with these diseases. Here, we analyzed over 700 lung transcriptome samples from patients with comorbidities associated with severe COVID-19 and found that *ACE2* was highly expressed in these patients compared to control individuals. This finding suggests that patients with such comorbidities may have higher chances of developing severe COVID-19. Correlation and network analyses revealed many potential regulators of ACE2 in the human lung, including genes related to histone modifications, such as *HAT1*, *HDAC2*, and *KDM5B*. Our systems biology approach offers a possible explanation for increased COVID-19 severity in patients with certain comorbidities.

Keywords. angiotensin converting enzyme 2; COVID-19; SARS-CoV-2; KDM5B.

Recent studies of the epidemiological characteristics of coronavirus disease 2019 (COVID-19) have revealed that severe infection is more likely in people with an existing chronic medical condition. Two independent studies of infected populations in Wuhan, China found that approximately half the subjects with COVID-19 had an existing comorbidity [1, 2]. In a study of 1099 patients across mainland China, 38.7% of patients with comorbidities progressed to severe infection [3] and in a study of 52 inpatients in Wuhan, 67% of patients with comorbidities died [2]. The most common comorbidities reported in these studies were hypertension, diabetes, cerebrovascular disease, chronic obstructive lung disease, and coronary heart disease [1–3]. Other comorbidities such as carcinoma, chronic kidney disease, chronic liver disease, digestive system disease, and nervous system disease have also been reported in patients with COVID-19 [1, 2, 4]. A better understanding of the link between these conditions and COVID-19 infection is required to inform improved treatment and prevention interventions.

The molecular mechanism responsible for the increased disease severity in patients with these comorbidities is not fully understood, but previous studies suggest a role for angiotensin-converting enzyme 2 (ACE2) [5]. ACE2 is a membrane protein required for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to bind and enter cells [6–8]. After binding, viral entry is facilitated by activation of the viral spike glycoprotein and cleavage of the C-terminal segment of ACE2 by proteases like TMPRSS2 and FURIN that are readily expressed in lung tissue [9–11]. ACE2 is only moderately expressed in healthy lung tissue compared to the heart, kidneys, and testes [12], but staining of lung tissue sections from adults with pulmonary hypertension has revealed increased ACE2 protein in the endothelium of pulmonary arteries compared to healthy controls [13]. A comprehensive analysis of single-cell RNA sequence (RNA-seq) datasets revealed that *ACE2* was coexpressed with *TMPRSS2* within ileal absorptive enterocytes, nasal goblet secretory cells, and lung type 2 pneumocytes [14]. *ACE2* upregulation has also been observed in animal models of liver fibrosis [15]. However, the reason for this upregulation remains unclear, and a link to other COVID-19 comorbidities has not been determined.

Here, we showed that expression of the gene encoding the ACE2 receptor in lung tissue is upregulated in diseases reported to be comorbidities associated with severe COVID-19. We also used systems biology approaches, including coexpression analysis, meta-analysis, and network analysis, to determine a potential cause of the *ACE2* upregulation. From this analysis, we found that *ACE2* expression could be regulated by enzymes that modify histones, including KDM5B. This identification of a common molecular mechanism of increased COVID-19 severity in patients with diverse comorbidities could direct the development of interventions to reduce the infection risk and disease severity in this population.

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METHODS

Literature Curation

Relevant scientific literature related to key COVID-19 comorbidities was retrieved from PubMed on 16 March 2020 using the query terms "pulmonary hypertension," "chronic obstructive pulmonary disease," "hypertension," "smoking," "pulmonary fibrosis," and "asthma." For terms returning more than 100 000 papers ("hypertension," "smoking," and "asthma"), only the most recent 100 000 papers were analyzed. Abstracts were annotated to identify all genes, diseases, and species appearing in the title or abstract using the PubTator Central application programming interface [16]. This open source tool uses *TaggerOne* for disease annotations, *GNormPlus* for gene annotations, and *SR4GN* for species annotations [16]. The data were filtered to retain only papers containing a human species annotation. Annotation of the abstract text identified 6 relevant disease medical subject heading (MeSH) terms: "autoimmune diseases," "cardiovascular diseases," "familial primary pulmonary hypertension," "hypertension," "hypertension, pulmonary," and "renal insufficiency, chronic," as shown in Figure 1. Next, every possible combination of gene and disease annotation within the title and abstract of each paper was generated. Only gene-disease associations supported by at least 4 documents, and those with a proximity less than or equal to the median sentence length of the paper section were retained.

Gene identifiers (IDs) were converted to gene symbols using the *biomaRt R* package [17, 18], and disease IDs were converted to disease MeSH terms using the Entrez Programming Utilities to query the Entrez database provided by the National Center for Biotechnology Information. The data were then further filtered to retain disease MeSH terms relevant to reported clinical COVID-19 comorbidities [3]. Redundant terms were collapsed using fuzzy string matching. The final gene-disease data set was used to generate a network utilizing *Gephi* software where the nodes were genes and diseases, and the edge weight was determined by the number of analyzed papers containing the gene-disease combination [19].

Meta-analysis

We manually curated Gene Expression Omnibus (GEO) repository (https://www.ncbi.nlm.nih.gov/geo/) on 16 March 2020 to find lung transcriptome datasets related to "pulmonary arterial hypertension" (PAH), "chronic obstructive pulmonary disease" (COPD), and "smoking." Author-normalized expression values and metadata from these datasets were downloaded using the *GEOquery* package [20]. We performed differential expression analyses between patients with a disease and the control

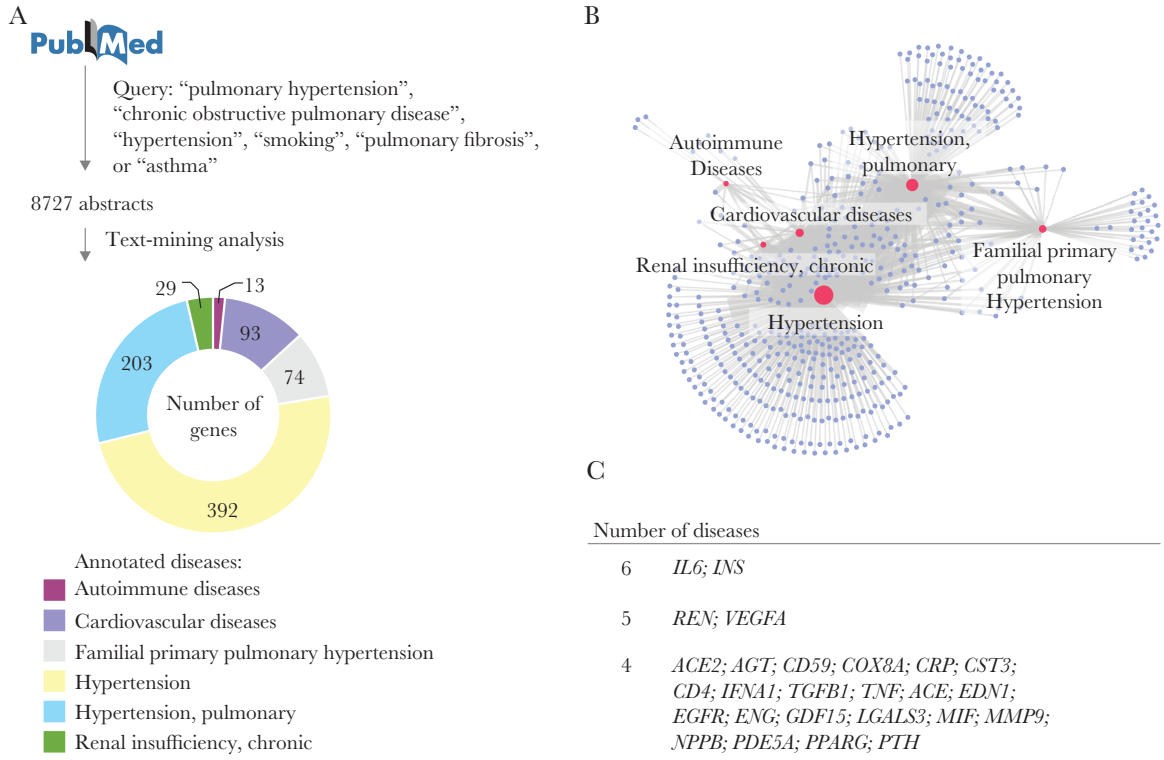

Figure 1. Literature curation of genes associated with key COVID-19 comorbidities. *A*, Text-mining of 8727 abstracts identified 6 relevant disease medical subject heading (MeSH) terms associated with a total of 804 genes. The number of genes associated with each disease MeSH term in at least 4 abstracts is shown in the pie chart. *B*, The knowledge-based network of COVID-19 comorbidities. The network shows the diseases (red nodes) and genes (purple nodes) from (*A*). The edges represent an association between a disease and a gene. The size of the nodes is proportional to its degree. *C*, Genes associated with 4 or more COVID-19 comorbidities.

individuals (Supplementary Table 1) using the *limma* package [21]. The gene symbol for each probe was obtained from the annotation file [22]. Probes that matched the same gene symbol were collapsed by taking the one with the lowest *P* value. Meta-analysis was performed with the *MetaVolcanoR* package [23] by combining the *P* values using the Fisher method. To adjusting for multiple comparisons, we calculated the false discovery rate (FDR) to identify the differentially expressed genes (FDR < 0.05). For enrichment analyses, we utilized the *EnrichR* tool [24] with the GO Biological Process 2018 and BioPlanet 2019 databases. We then selected pathways with a *P* value adjusted for multiple comparisons lower than 0.05. The network was created in *Cytoscape* [25].

Author-normalized fragments per kilobase of transcript per million mapped reads (FPKM) expression values of *ACE2* gene in COPD patients and in subjects with normal spirometry were downloaded from GEO (accession ID, GSE57148). A single *t* test was performed between COPD patients and controls ($P = .000359$).

Pearson correlation between the expression of *ACE2* and all other genes in each of the 7 lung transcriptome studies was performed. The *P* values were then combined using the Fisher method, and an FDR correction was applied to adjust for multiple comparisons.

For the epigenetics analysis, we run the *EnrichR* tool [24] with the ENCODE and ChEA consensus transcription factors from ChIP-X and Epigenomics Roadmap databases on the genes negatively or positively correlated with *ACE2*. Pathways with a *P* value adjusted for multiple comparisons lower than 10^{-10} were selected. We utilized the *Encode Roadmap* browser (http://www.roadmapepigenomics.org/) from the Roadmap Epigenomics Project database [26] to identify the H3K27ac, H3K4m1, and H3K4m3 markers of histone acetylation and methylation with the corresponding *P* values in the Lung of ENCODE donor STL002 (Roadmap alias E096).

Ethical approval was not applicable as we utilized publicly available data.

RESULTS

To identify the genes highly associated with key comorbidities of severe COVID-19 [1, 3], we mined all relevant scientific literature of these human diseases. Specifically, over 8000 abstracts were gathered from PubMed by querying titles and abstracts for the terms "pulmonary hypertension," "chronic obstructive pulmonary disease," "hypertension," "smoking," "pulmonary fibrosis," or "asthma" (Figure 1A). Several relevant terms, such as "autoimmune diseases" and "cardiovascular diseases" were excluded from the PubMed query because of the breadth of literature published in these fields. Annotation of the abstract text identified 6 relevant disease MeSH terms: "autoimmune diseases," "cardiovascular diseases," "familial primary pulmonary hypertension," "hypertension," "hypertension, pulmonary," and "renal insufficiency, chronic" [3] (Figure 1A). Our text-mining analysis revealed 804 genes highly associated with 1 or more COVID-19 comorbidities (Figure 1B). Among those genes, 26 were associated with 4 or more diseases (Figure 1C). Although *ACE2* was known to be related to "cardiovascular diseases," "familial primary pulmonary hypertension," "hypertension, pulmonary," and "hypertension," none of the articles containing this gene-disease association studied how *ACE2* expression was altered in the lungs of patients with these diseases.

Based on the list of key comorbidities of severe COVID-19 [1, 3], we searched for lung transcriptome datasets available at the GEO repository. We identified 7 lung transcriptome studies of patients with either COPD or PAH, as well as smoking volunteers compared to nonsmoking volunteers, which were downloaded and used in our meta-analysis (Supplementary Table 1). For each study, we performed differential expression analysis between patients and control individuals (Supplementary Table 1). By combining the *P* values obtained in all the 7 comparisons, we were able to identify 1740 and 938 genes that were, respectively, up- and downregulated in the disease (Figure 2A). Enrichment analysis using these differentially expressed genes revealed several pathways associated with inflammatory processes, metabolism, and endoplasmic reticulum stress. Among the pathways enriched with downregulated genes, were the "vasculogenesis" and "regulation of Notch signaling pathway" (Figure 2B). The "viral life cycle" pathway, which describes the processes utilized by viruses to ensure survival and to attach and enter the host cells, was enriched with upregulated genes (Figure 2B). *ACE2* was included in this pathway, as well as 25 other genes (Figure 2C), including *RAB1A*. Rab GTPases are involved in the replication of many viruses infecting humans [27] but have not been associated with SARS-CoV-2 life cycle yet. Genes encoding both *TMPRSS2*, which is required for SARS-CoV-2 S protein priming [5], and *FURIN*, which cleaves SARS-CoV-2 spike glycoprotein [28], were not differentially expressed in most of the lung transcriptome. However, both genes were highly expressed in lung (data not shown), suggesting that the levels of ACE2 may be the limiting factor for viral infection.

We then investigated whether the gene encoding the ACE2 receptor was specifically upregulated in the lungs of patients having one of these morbidities (Figure 3A). In a lung RNA-seq dataset (Supplementary Table 1), we compared *ACE2* expression between patients with COPD and subjects with normal spirometry [29]. Again, the expression of *ACE2* was significantly upregulated in the disease compared to controls (Figure 3B). In fact, *ACE2* was significantly upregulated in 6 of 7 lung transcriptome studies (Figure 3C), suggesting that patients who have COPD or PAH, and even people who smoke, may have higher chances of developing severe COVID-19.

Coexpression analyses can provide useful insights about the functional role of genes and their regulatory mechanisms [30]. We performed Pearson correlation between the

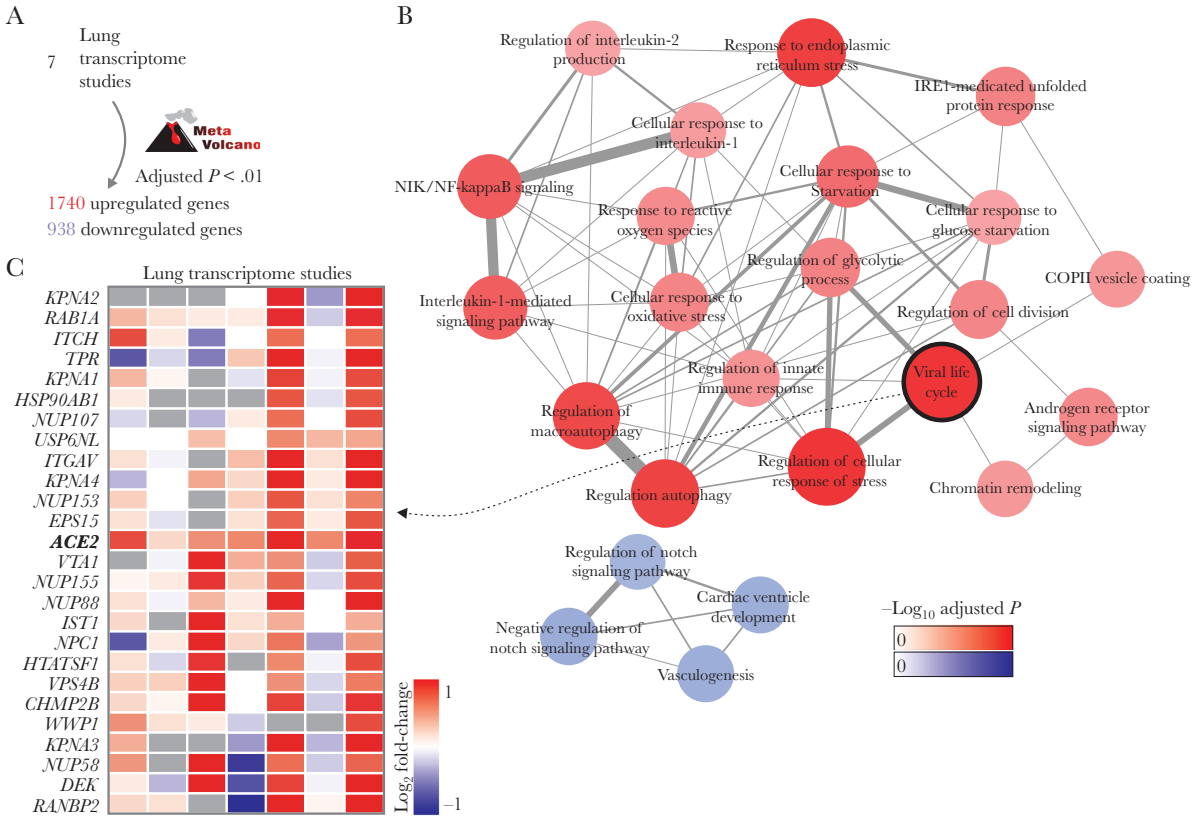

Figure 2. Meta-analysis of lung transcriptomes of patients with COVID-19 comorbidities. *A*, Meta-analysis of 7 differential expression analyses. MetaVolcano tool was used to combine the *P* values of 7 studies (Supplementary Table 1) and to identify the differentially expressed genes (false discovery rate < 0.01). *B*, Pathway enrichment analysis. Pathways from the GO Biological Process 2018 database with adjusted *P* value < .05 were selected to create the network. The width of edges is proportional to the number of genes shared by 2 pathways (nodes). The size and color of nodes are proportional to the $-\log_{10}$ adjusted *P* value. *C*, Genes from the viral life cycle pathway that were upregulated in human diseases. The colors in the heat map represent the $\log_2$ fold-change between patients and control individuals.

expression of *ACE2* and all other genes in each of the 7 lung transcriptome studies (Supplementary Table 1), combined the *P* values using the Fisher method, and applied an FDR correction (Figure 4A). This approach identified 544 and 173 genes with positive and negative correlation with *ACE2*, respectively (Figure 4A). Several of these genes were related to histone modifications, such as *HAT1*, *HDAC2*, *KDM5B*, among others (Figure 4A). Among the positively correlated genes, we identified *ADAM10*, which regulates ACE2 cleavage in human airway epithelia [31], and *TLR3*, which plays a key role in the innate response to SARS-CoV and MERS-CoV infection [32].

Pathway enrichment analysis revealed that several of the genes positively associated with *ACE2* were regulated by KDM5B, and by specific histone acetylation (H3K27ac) and histone methylation (H3K4me1 and H3K4me3) (Figure 4B). In fact, KMD5B demethylates lysine 4 of histone H3 (ie, H3K4) and is involved in transcriptional regulation and DNA repair [33]. We then checked in the Roadmap Epigenomics Project database [26] to see whether the *ACE2* locus contained ChIP-seq information for these histone markers. In the human lung, peaks for H3K4me1 and H3K4me3, as well as H3K27ac, were identified in the *ACE2* locus (Figure 4C), suggesting that *ACE2* may be epigenetically regulated in the lung.

DISCUSSION

We showed here that patients with comorbidities that have very distinct mechanisms have increased expression of *ACE2* in the lungs. Although our findings did not include COVID-19 infection data, we suggest that the higher expression of *ACE2* in the lungs is associated with higher chances of developing the severe form of COVID-19, by facilitating the SARS-CoV-2 entry into lung cells during the infection. In fact, COVID-19 patients classified as severe cases displayed higher viral loads in nasopharyngeal swab samples during the early stages of disease onset compared to mild patients [34].

The current diabetes pandemic [35] could be worsening the SARS-CoV-2 pandemic by increasing the comorbidities associated with severe COVID-19. As we did not find lung transcriptome samples from patients with type 2 diabetes, we could not directly test whether *ACE2* expression

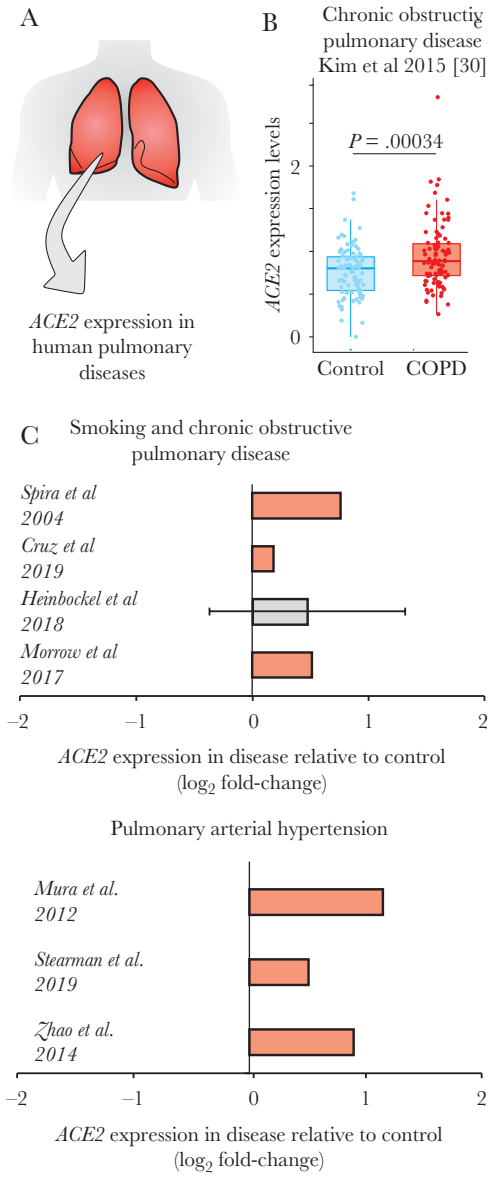

Figure 3. *ACE2* is upregulated in patients with lung diseases. *A,* Analysis of *ACE2* expression in lung transcriptome datasets of patients with human pulmonary diseases. *B, ACE2* expression in patients with chronic obstructive pulmonary disease (COPD). The boxplot on the right shows the difference between COPD patients (red dots) and control individuals (blue dots). Student *t* test *P* value is given. *C, ACE2* is upregulated in patients with COVID-19 comorbidities. Each bar represents the $\log_2$ expression fold-change between patients and control individuals. The error bars indicate the 95% confidence interval. Bars in red represent a *P* value <.05 and in grey a nonsignificant *P* value. The original studies are indicated and can be found in Supplementary Table 1.

is increased in patients with diabetes compared to healthy controls. However, our text-mining approach revealed that interleukin-6 (IL-6) and *INS* genes were associated with all the diseases we searched. The *INS* gene encodes the insulin hormone, and insulin is associated with the NAD-dependent histone deacetylase sirtuin 1 (SIRT1) [36]. We found that *SIRT1* was upregulated in the lung of patients with severe COVID-19 comorbidities in 4 of 7 studies (data not shown). Clarke et al [37] have demonstrated that, under conditions of cell energy stress, SIRT1 can epigenetically regulate *ACE2*. Others too have shown that nonsteroidal anti-inflammatory drugs may inhibit the SIRT1 deacetylase activity [38], which in turn could impact *ACE2* expression.

The "viral life cycle" pathway that was enriched with upregulated genes in patients with severe COVID-19 comorbidities contains several genes in addition to *ACE2* that could be potentially important for SARS-CoV-2 cell cycle and invasion/attachment. These include *RAB1A* gene, whose product promotes the replication of vaccinia virus [39]. Also, RAB1A is important for herpes simplex virus 1 secondary envelopment [40] and is required for assembly of classical swine fever virus particles [41]. It is possible that SARS-CoV-2 utilizes RAB1A as well.

The fact that *ACE2* gene is located in the X chromosome, and initial findings show that older men with comorbidities are more likely to be have severe COVID-19 compared to women [1], indicate that *ACE2* expression in the lung may be sex biased. Although no significant sex difference was found in the activity of ACE2 in mouse lung [42], in rats, the levels of ACE2 were dramatically reduced with aging in both sexes, but with significantly higher ACE2 expression in old female rats than male [43].

Although the mechanisms by which *ACE2* is upregulated in patients with severe COVID-19 comorbidities were not addressed, our analysis may shed some light on the subject. Among the genes whose expression was positively correlated with *ACE2*, we detected genes associated with epigenetic regulation of gene transcription. For instance, HAT and HDAC modulate chromatin and DNA condensation by changing histone acetylation status, thus permitting gene transcription. This could occur in lung tissue, facilitating *ACE2* expression, as observed during lung cancer and COPD.

KDM5B is associated with hepatitis B virus infection [44]. In breast cancer cells, blockage of KDM5 triggers a robust interferon response that results in resistance to infection by DNA and RNA viruses [45]. This finding suggests that KDM5 demethylases are potential targets for preventing SARS-CoV-2 infection.

COVID-19 may kill between 5.6% and 15.2% of people infected with SARS-CoV-2 [46]. Drug treatments that lower this mortality rate may save many thousands of lives. Our systems biology approach offers putative gene targets for treating and preventing severe COVID-19 cases.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors,

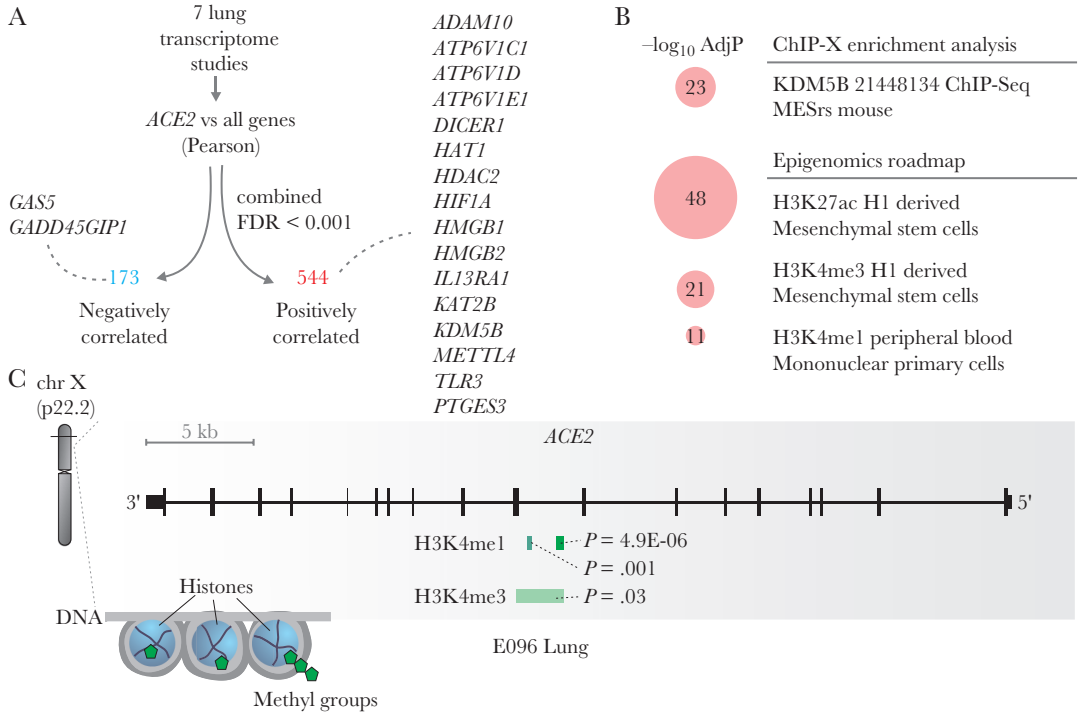

Figure 4. Insights into *ACE2* regulation in the lung. *A*, Genes whose expression is correlated with *ACE2* in the lung. Selected genes that were negatively (blue) or positively (red) correlated with *ACE2* are highlighted. *B*, Pathway enrichment analysis using the *ACE2*-positively correlated genes. Pathways from the ChIP-X Enrichment Analysis and Epigenomics Roadmap databases with adjusted *P* value $<10^{-10}$ were selected. The size of the circles is proportional to the $-\log_{10}$ adjusted *P* value of the enrichment. *C*, *ACE2* locus contains markers of histone acetylation and methylation. The plot was modified from the WashU EpiGenome Browser using E096 lung. The peaks corresponding to each histone modification and the *P* values of the markers are indicated. Abbreviations: AdjP, adjusted *P* value; FDR, false discovery rate.

so questions or comments should be addressed to the corresponding author.

Notes

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Author contributions. B. G. G. P., A. E. R. O., Y. S., L. J., A. N. A. G., R. L. T. O., R. C., and H. I. N. performed the analyses. J. P. S. P. and H. I. N. interpreted the results. All authors helped with the writing of the manuscript.

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