Tobacco Smoking Increases the Lung Gene Expression of ACE2, the Receptor of SARS-CoV-2

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On March 11, 2020, the World Health Organization declared the Coronavirus Disease 2019 (COVID-19) outbreak a pandemic. As of April 20th, 2020, laboratories have confirmed 2,470,410 COVID-19 cases and caused 169,794 deaths in 213 counties, areas, or territories. COVID-19 is caused by a new type of pathogenic coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is phylogenetically similar to SARS-CoV, with approximately 80% identity between genomes (1). SARS viruses affect the respiratory tract and cause acute respiratory response through the same cell-entry receptor, angiotensin-converting enzyme 2 (ACE2), which is the only experimentally confirmed SARS-CoV-2 receptor. SARS-CoV-2 infection also employs activation of the spike proteins found on the surface of the virus for cellular entry. The best candidates for priming spike proteins are two host-cell enzymes called Furin and TMPRSS2 (1). In the current severe global emergency, it is imperative to identify potential risk factors for effective prevention and care, such as cigarette smoking, which is a substantial risk factor for various important bacterial and viral infections.

Methods

We evaluated a comprehensive set of transcriptomic datasets to investigate the associations of smoking with ACE2, FURIN and TMPRSS2 gene expression in lung tissues. Two datasets were generated using normal lung tissues from patients with Lung Adenocarcinoma: a Caucasian RNA-seq dataset from The Cancer Genome Atlas TCGA (n=48) (2) and an Asian RNA-seq dataset from Gene Expression Omnibus (GEO) GSE40419 (n=74) (3). We included three polyethnic microarray datasets of gene expression of healthy small airway epithelium (SAE) samples (from GSE63127 (n=230) (4), GSE19667 (n=116) (5) and GSE5058 (n=24) (6)) and a large airway epithelium (LAE) samples from GSE7895 (n=104) (7). Also,
we analyzed three microarray datasets of samples derived from healthy and chronic obstructive pulmonary disease (COPD) patients, including SAE samples from current smokers (from GSE5058 (6), n=26), bronchial airway epithelium (BAE) samples from current and former smokers (GSE37147 (8), n=238), and lung samples from Caucasian patients (n=438) that underwent lung cancer surgery at the Institut universitaire de cardiologie et de pneumologie de Québec (IUCPQ) (9). RNA-seq datasets were generated with the Illumina HiSeq platform and microarray datasets were generated with Affymetrix arrays. All the data were de-identified and study approval has been stated in original studies. A total of 1286 assay results were evaluated. We considered the Fragments Per kilobase per million mapped reads (FPKM) for RNA-seq data and robust multi-array average (RMA) values for microarray data to represent normalized gene expression. All data were log\(^2\) transformed to improve normality.

Smoking status (never, former and current smokers) was identified based on self-reported smoking history. Association test was performed using a linear model with log\(^2\) ACE2, FURIN, or TMPRSS2 gene expression as the dependent variable and smoking status or COPD status as an independent variable. Meta-analysis was performed by pooling effect size and standard error estimated from each study using a random effects model. Age and sex were included as covariates. Although we did not observe significant associations of age and sex with the expression of ACE2 and FURIN, we found a negative correlation between TMPRSS2 expression and age in some of the datasets. Data management, statistical analyses, and visualizations were performed using R 3.6.1.

A single-cell RNA-seq dataset (GSE131391) (10) was also analyzed. This study profiled bronchial epithelial cells, single ALCAM+ epithelial cells and CD45+
white blood cells from six never and six current smokers. Sequencing read counts in single cells were downloaded, and subsequent data analyses, including data normalization, high variable feature selection, data scaling, dimension reduction, and cluster identification, were performed using the Seurat 3.0 package. We used SCANNER for data visualization and cell type identification.

Results

We identified upregulation of pulmonary ACE2 gene expression in ever-smokers compared to non-smokers in all datasets, irrespective of tissue subset or COPD status (Fig. 1). Meta-analysis showed ever-smoking significantly and substantially increased pulmonary ACE2 expression by 25% (p-value=1.4×10^{-16}, Fig. 1). Similarly, smoking status defined by never, former and current smokers was also significantly associated with ACE2 pulmonary expression in meta-analysis (beta=0.14, p-value=2.0×10^{-6}, Fig. 1). The significant smoking effect on ACE2 pulmonary expression identified in this study may suggest an increased risk for viral binding and entry of SARS-CoV and SARS-CoV-2 in lungs of smokers. FURIN was also upregulated by smoking, but to a lower extent compared to ACE2. TMRPSS2 gene expression in lung was not associated with smoking (Fig. 1).

We also evaluated the effect of COPD on gene expression. Stratifying data by smoking status, we observed a trend (beta=0.08, p-value=0.07) for higher ACE2 levels in COPD patients, but the results were not consistent across datasets (Fig. 1). In the IUCPQ data, ACE2 expression was upregulated in COPD patients (p=0.0006), but the effect was attenuated after adjustment for smoking status (p=0.03).

We further evaluated the effect of smoking on ACE2 pulmonary expression in single bronchial epithelial cells from six never and six current smokers. We found smoking remodels cells in the bronchial epithelium with a loss of club cells and
extensive hyperplasia of goblet cells. ACE2 gene is mainly expressed in goblet cells in smokers and club cells in never smokers (Fig. 2). This result is consistent with a very recent study that found the highest ACE2 expression in Alveolar Type II cells (which derive from club cells) and in a transient secretory cell type in subsegmental bronchial branches (11). This may indicate that smokers have a risk of COVID-19 infection complications based on ACE2 expression profiles, which could contribute to variations in infection susceptibility, disease severity, and treatment outcome.

Despite a significant increase in the prevalence of electronic cigarettes (E-cig), no studies have yet included information about single (E-cig only), or dual users (E-cig and tobacco). The mechanisms underlying tobacco-related upregulation of ACE2 pulmonary expression are unknown; among many others, the degrees of smoking effects to the infection susceptibility and clinical manifestations are unknown; further mechanistic studies are needed. Despite currently limited knowledge, this study indicates that smoking could be a risk factor for COVID-19 by affecting ACE2 expression and provides valuable information for identifying and stratifying more susceptible populations.
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Figure

Figure 1. Forest plots for smoking effect on *ACE2*, *FURIN*, and *TMPRSS2* pulmonary gene expression. Non-smoker/never-smoker and ever-smoker including current smoker and former smoker were identified in each original study based on self-reported smoking history. For each gene, the top panel shows the comparison of ever-smoker and non-smoker groups, the middle panel shows the association of *ACE2* gene expression with smoking status (never-, former- and current-smoker), and the bottom panel shows the comparison of COPD and healthy groups, stratified by smoking status. For each study, the estimated effect size and 95% confidence intervals are plotted. The size of square boxes is proportional to weights which were estimated by the standard ‘inverse-variance’ method for random-effects models in meta-analysis. TCGA, The Cancer Genome Atlas; IUCPQ, “Institut universitaire de cardiologie et de pneumologie de Québec”; SAE, small airway epithelium; LAE, large airway epithelium; BAE, bronchial airway epithelium.

Figure 2. *ACE2* expression in single-cell transcriptomics of bronchial epithelium cells from never and current smokers.

The tSNE plot of single-cell transcriptome profiles from never smokers and current smokers is showed. *ACE2* expression is shown by red stars. Cell types were identified based on gene expression of markers (10). Confirming the original study, we observed smokers showed a remodeled cell composition in bronchial epithelium with a loss of club cells and extensive hyperplasia of goblet cells.
| Study                  | Tissue                  | Effect size (95% CI) | Sample size | P-value |
|-----------------------|-------------------------|----------------------|-------------|---------|
| ACE2                  |                         |                      |             |         |
| Ever-smoker vs Non-smoker |                         |                      |             |         |
| GSE7895               | healthy LAE             | 0.09 (-0.06, 0.25)   | 104         | 0.23    |
| GSE83127              | healthy SAE             | 0.29 (0.20, 0.38)    | 230         | 3e-09   |
| GSE19687              | healthy SAE             | 0.27 (0.14, 0.39)    | 116         | 6e-05   |
| GSE56058              | healthy SAE             | 0.14 (-0.15, 0.43)   | 24          | 0.38    |
| TOGA                  | normal lung             | 0.30 (-0.20, 0.80)   | 48          | 0.24    |
| GSE40419              | normal lung             | 0.33 (-0.25, 0.90)   | 74          | 0.27    |
| IUCPO                 | normal lung             | 0.32 (0.14, 0.55)    | 194         | 0.0067  |
| IUCPO                 | COPD lung               | 0.27 (-0.10, 0.83)   | 244         | 0.15    |
| Meta-analysis         |                         | 0.25 (0.19, 0.31)    | 1034        | 1.4e-16 |
| Smoking status (never, former, current) |                     |                      |             |         |
| GSE7895               | healthy LAE             | 0.08 (0.01, 0.15)    | 104         | 0.036   |
| TOGA                  | normal lung             | 0.17 (-0.08, 0.41)   | 48          | 0.2     |
| GSE40419              | normal lung             | 0.17 (-0.20, 0.54)   | 74          | 0.37    |
| IUCPO                 | normal lung             | 0.22 (0.11, 0.33)    | 194         | 0.0018  |
| IUCPO                 | COPD lung               | 0.19 (0.06, 0.31)    | 244         | 0.002   |
| Meta-analysis         |                         | 0.14 (0.08, 0.19)    | 664         | 2e-06   |
| COPD vs Healthy, smoking stratified |                 |                      |             |         |
| GSE37147              | current BAE             | 0.02 (-0.15, 0.18)   | 99          | 0.85    |
| GSE37147              | former BAE              | 0.15 (0.05, 0.24)    | 139         | 0.0037  |
| GSE56058              | current SAE             | -0.27 (-0.35, 0.00)  | 26          | 0.06    |
| IUCPO                 | current lung            | 0.16 (-0.04, 0.37)   | 96          | 0.12    |
| IUCPO                 | former lung             | 0.09 (-0.03, 0.00)   | 303         | 0.13    |
| IUCPO                 | never lung              | 0.16 (-0.10, 0.40)   | 39          | 0.38    |
| Meta-analysis         |                         | 0.08 (-0.01, 0.16)   | 664         | 0.071   |
| FURIN                 |                         |                      |             |         |
| Ever-smoker vs Non-smoker |                         |                      |             |         |
| GSE7895               | healthy LAE             | 0.08 (-0.10, 0.26)   | 104         | 0.41    |
| GSE83127              | healthy SAE             | 0.05 (-0.06, 0.16)   | 230         | 0.38    |
| GSE19687              | healthy SAE             | 0.10 (-0.05, 0.25)   | 116         | 0.2     |
| GSE56058              | healthy SAE             | 0.32 (-0.05, 0.69)   | 24          | 0.11    |
| TOGA                  | normal lung             | 0.18 (-0.13, 0.54)   | 48          | 0.35    |
| GSE40419              | normal lung             | 0.09 (-0.13, 0.31)   | 74          | 0.43    |
| IUCPO                 | normal lung             | 0.12 (-0.02, 0.28)   | 194         | 0.1     |
| IUCPO                 | COPD lung               | 0.21 (-0.06, 0.47)   | 244         | 0.13    |
| Meta-analysis         |                         | 0.10 (0.04, 0.16)    | 1034        | 0.002   |
| COPD vs Healthy, smoking stratified |               |                      |             |         |
| GSE37147              | current BAE             | 0.01 (-0.08, 0.09)   | 104         | 0.87    |
| GSE37147              | former BAE              | -0.01 (-0.07, 0.04)  | 139         | 0.66    |
| GSE56058              | current SAE             | -0.29 (-0.10, 0.88)  | 26          | 0.15    |
| IUCPO                 | current lung            | 0.14 (-0.03, 0.30)   | 96          | 0.11    |
| IUCPO                 | former lung             | 0.07 (-0.01, 0.15)   | 303         | 0.091   |
| IUCPO                 | never lung              | -0.01 (-0.31, 0.29)  | 39          | 0.94    |
| Meta-analysis         |                         | 0.02 (-0.02, 0.07)   | 664         | 0.24    |
| TMPRSS5               |                         |                      |             |         |
| Ever-smoker vs Non-smoker |                         |                      |             |         |
| GSE7895               | healthy LAE             | 0.04 (-0.11, 0.19)   | 104         | 0.58    |
| GSE83127              | healthy SAE             | 0.09 (0.02, 0.15)    | 230         | 0.0066  |
| GSE19687              | healthy SAE             | 0.06 (-0.02, 0.17)   | 116         | 0.12    |
| GSE56058              | healthy SAE             | -0.02 (-0.20, 0.17)  | 24          | 0.87    |
| TOGA                  | normal lung             | 0.10 (-0.31, 0.51)   | 48          | 0.64    |
| GSE40419              | normal lung             | 0.14 (-0.59, 0.87)   | 74          | 0.71    |
| IUCPO                 | normal lung             | 0.07 (-0.08, 0.22)   | 194         | 0.36    |
| IUCPO                 | COPD lung               | -0.10 (-0.42, 0.22)  | 244         | 0.53    |
| Meta-analysis         |                         | 0.07 (0.03, 0.11)    | 1034        | 0.0021  |
| Smoking status (never, former, current) |                     |                      |             |         |
| GSE7895               | healthy LAE             | 0.07 (0.00, 0.13)    | 104         | 0.06    |
| TOGA                  | normal lung             | 0.00 (-0.21, 0.20)   | 48          | 0.98    |
| GSE40419              | normal lung             | 0.08 (-0.39, 0.55)   | 74          | 0.73    |
| IUCPO                 | normal lung             | 0.02 (-0.08, 0.11)   | 194         | 0.72    |
| IUCPO                 | COPD lung               | 0.03 (-0.08, 0.14)   | 244         | 0.53    |
| Meta-analysis         |                         | 0.04 (0.00, 0.09)    | 664         | 0.071   |
| COPD vs Healthy, smoking stratified |             |                      |             |         |
| GSE37147              | current BAE             | 0.07 (-0.01, 0.16)   | 99          | 0.1     |
| GSE37147              | former BAE              | 0.02 (-0.05, 0.08)   | 139         | 0.56    |
| GSE56058              | current SAE             | -0.04 (-0.28, 0.19)  | 26          | 0.72    |
| IUCPO                 | current lung            | 0.11 (-0.08, 0.29)   | 96          | 0.28    |
| IUCPO                 | former lung             | -0.04 (-0.13, 0.08)  | 303         | 0.45    |
| IUCPO                 | never lung              | 0.14 (-0.18, 0.45)   | 39          | 0.4     |
| Meta-analysis         |                         | 0.03 (-0.02, 0.07)   | 664         | 0.23    |

Figure 1
