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INVITED COMMENTARY

Effects of cigarette smoking on SARS-CoV-2 receptor ACE2 expression in the respiratory epithelium†

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†Invited Commentary for Liu *et al*. Overexpression of the SARS-CoV-2 receptor ACE2 is induced by cigarette smoke in bronchial and alveolar epithelia. *J Pathol* 2021; **253:** 17–30.

Abstract

Due to the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic, the world is currently facing high morbidity and mortality rates as well as severe disruption to normal societal and social structures. SARS-CoV-2 uses the ACE2 receptor for cellular entry. In a recent publication of *The Journal of Pathology*, Liu and coworkers highlight the effects of cigarette smoking on ACE2 expression in the respiratory epithelium. The authors studied the effects of acute cigarette smoke exposure in a murine model and confirmed their findings in human lung tissues and gene expression datasets. Their findings demonstrate that cigarette smoking increases ACE2 expression specifically at the apical surface of the airway epithelium. Smoking cessation resulted in lower ACE2 expression, with implications for attenuating the risk of transmission of the virus. The role of ACE2 expression in the development of COVID-19 symptoms is still under investigation, with conflicting results from experimental models on the role of ACE2 expression in SARS-CoV-2-induced lung injury. In this commentary, we highlight the implications and limitations of the study of Liu *et al* as well as future therapeutic strategies directed towards ACE2.

Keywords: SARS-CoV-2; ACE2; cigarette smoke; COVID-19; airway epithelium

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Commentary

Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses that infect a large range of vertebrates, primarily bats but also other mammals including pigs, cows, and chickens. In humans, coronaviruses tend to cause mild to moderate upper respiratory tract infections. However, in the past two decades, there have been outbreaks of severe, novel, human pathogenic coronaviruses that are phylogenetically distinct from common coronaviruses. These strains exhibit stronger virulence, are quickly passed from human to human, and can produce mild to severe symptoms that can result in death. In December 2019, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was determined as the distinctive coronavirus responsible for an outbreak of fatal atypical pneumonia in Wuhan, Hubei province, China. As of 15 December 2020, the WHO Coronavirus Disease-19 (COVID-19) dashboard reported over 71 million individuals tested positive for SARS-CoV-2 infection and over 1.6 million deaths due to COVID-19. In response to the COVID-19 pandemic, the medical and scientific communities have swiftly changed their focus to COVID-19-related research, resulting in an overwhelming number of scientific articles being published on SARS-CoV-2 on a daily basis. The coronaviral genome encodes four major structural proteins: the spike, nucleocapsid, membrane, and envelope proteins [1]. As shown in Figure 1, the spike protein is responsible for facilitating entry of SARS-CoV and SARS-CoV-2 into human cells, and requires priming through cleavage by the serine protease TMPRSS2 to allow fusion of the viral and cellular membranes. The entry receptor utilized by the spike protein is angiotensin-converting enzyme 2 (ACE2) [2].

ACE2 is a type I transmembrane metallocarboxypeptidase that converts angiotensin II to the vasodilatory

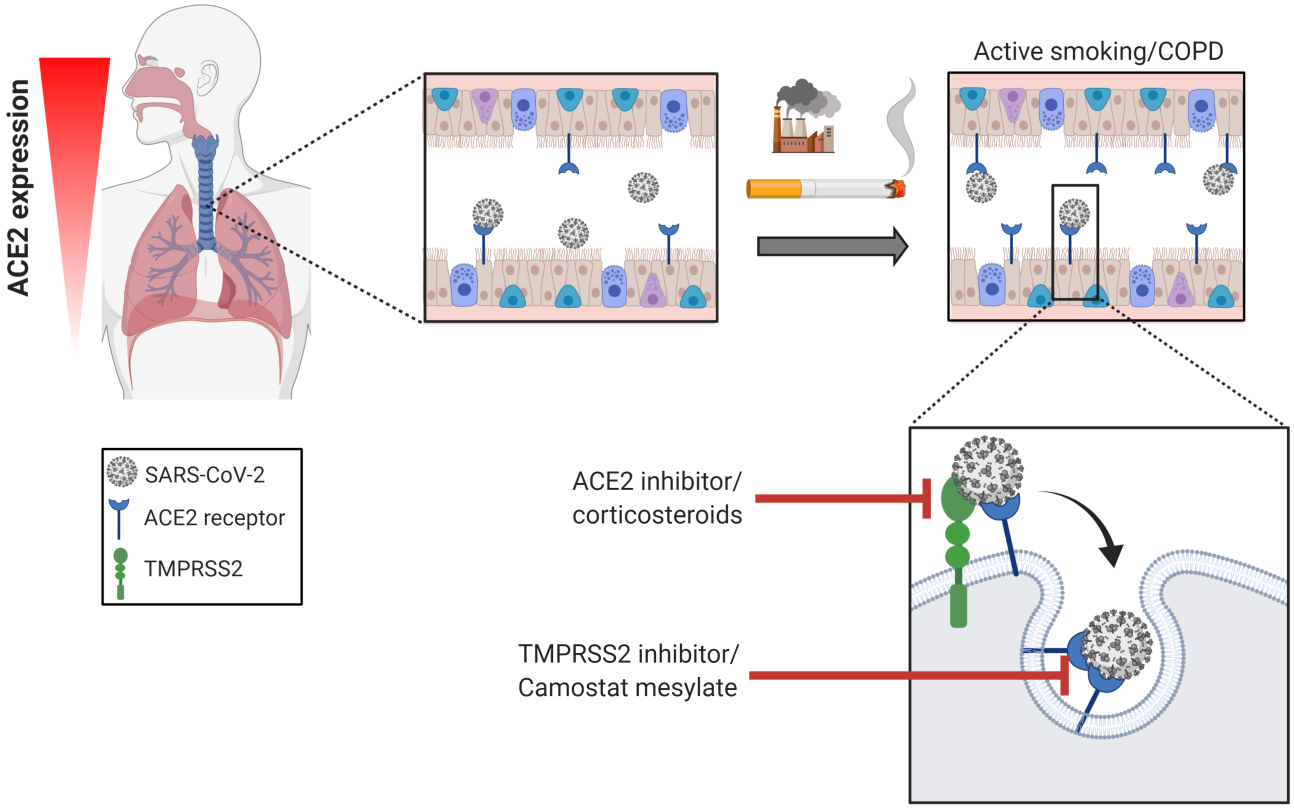

Figure 1. Schematic overview of ACE2 expression in relation to SARS-COV-2 infection in the airways. ACE2 expression is highest in the upper respiratory system and gradually decreases in the lower airways. SARS-COV-2 enters airway epithelial cells by anchoring to the ACE2 receptor, following priming through cleavage by the serine protease TMPRSS2 to allow fusion of the viral and cellular membranes. Several external factors including cigarette smoking and air pollution (NO_2, particulate matter) increase the expression of ACE2. Potential COVID-19 treatments are aimed at reducing ACE2 and TMPRSS2 expression or effectiveness, using ACE2 inhibitors, corticosteroids, TMPRSS2 inhibitors or camostat mesylate.

peptides angiotensin-(1–9) and angiotensin-(1–7), which are thought to negatively regulate the renin–angiotensin system (RAS). ACE2 is known to be highly expressed in vascular endothelial cells, renal tubular epithelium, and Leydig cells in the testes, and is also found in the heart and intestinal tissues as well as the lung [3]. Severe SARS-CoV-2 infections manifest as bilateral lower-lung pneumonias with diffuse alveolar damage that can progress to acute respiratory distress syndrome (ARDS). Therefore, there has been much research on virus entry within the lung. A number of studies using existing gene expression and single-cell RNA-sequencing data were able to confirm gene expression of *ACE2* and *TMPRSS2* within the human lung epithelium [4–7]. Specifically, the highest *ACE2* expression has been observed in the upper airways (nasal epithelium), with expression declining from the conducting ciliated epithelium to the respiratory airways, with the lowest expression in the distal lung alveolar type (AT) II pneumocytes [8]. These data suggest that SARS-COV-2 infection of the susceptible nasal epithelium most likely leads to aspiration-mediated virus exposure into the lower lung. High airway epithelial expression of ACE2 in at-risk individuals may thus contribute to susceptibility for the virus and poor prognosis.

ACE2 can be regulated by different factors. *In vitro* studies have shown that stimulation with interferons and activation of the deacetylase and anti-aging molecule sirtuin-1 increase ACE2, while stimulation with angiotensin II and interleukin (IL)-4 decrease ACE2 expression [3]. In addition to endogenous mediators, environmental factors can also influence the expression of ACE2. It has been shown that cigarette smoke and air pollutants such as nitric oxide (NO_2) and particulate matter increase ACE2 in the respiratory tract [9]. Also, the relationship between ACE2, smoking, and chronic obstructive pulmonary disease (COPD) has been studied. In a study of three independent cohorts of small airway epithelial brushings, it was found that current (but not former) smokers and patients with COPD have higher ACE2 expression [10]. In a second study using lung tissue specimens from 134 subjects, immunohistochemistry demonstrated elevated levels of ACE2 protein in the bronchial epithelium and alveolar tissue in current smokers with and without COPD [11].

To further test the effect of smoking on ACE2 *in vivo*, in a recent publication in *The Journal of Pathology*, Liu *et al* investigated ACE2 expression in different compartments of the respiratory system in relation to oxidative stress and hypoxia markers, using a mouse model and human data on both protein and gene expression [12].

In their mouse model of emphysematous manifestations, Liu *et al* observed increased protein levels of ACE2 in the bronchial epithelium upon whole-body cigarette smoke exposure (five cigarettes, four times a day) for 4–8 months. In contrast, ACE2 decreased in the alveolar epithelium, which was explained by a decrease in the percentage of SPC^+ cells upon smoke exposure. The authors report an increase in ACE2 specifically in ciliated bronchial epithelial cells as well as in club cells, although co-localization with the club cell marker CC10 was not confirmed. Whether cigarette smoke exposure also affects the number of club or ciliated cells in this model remains to be studied. In the conducting airways, higher ACE2 levels correlated with oxidative stress markers. These markers were also increased in alveolar epithelium, where no correlation with ACE2 was observed. In line with the murine data, the authors observed higher ACE2 in bronchial epithelium from non-malignant lung tissue of both current and former smokers compared with never smokers, which was related to pack-years as well as to specific oxidative stress and hypoxia markers. These data were confirmed in gene expression datasets, with higher *ACE2* in lung tissue of smokers compared with never smokers, without further analysis of subgroups of high/low pack-years. In both lung tissue and gene expression datasets, ACE2 was lower in individuals who had ceased smoking for less than 10 years. In contrast to the murine data, staining in human lung tissue revealed higher ACE2 in alveolar type II cells from smokers. *In vitro*, the hypoxia marker HIF-1α was able to induce ACE2 expression.

The findings of Liu *et al* may have important implications. The initial studies and public information suggest that the risk of COVID-19 may be higher among smoking populations, and smoking cessation may decrease susceptibility to SARS-CoV-2 infection. However, some caution must be taken. While cigarette smoking is a well-known inducer of oxidative stress, the data of Liu *et al* may especially support a role for hypoxia in ACE2 expression. Further, it is still unclear how cigarette smoke-induced oxidative stress in the airways, alveoli, or both, would affect the susceptibility to develop severe COVID-19 symptoms. There is also the concern that smoking has many well-documented health risks for the general population. Thus, it is more pertinent to use this information for the development of therapeutics that modify ACE2 expression.

To date, there are conflicting results from experimental models, with data to support a protective role of ACE2 in SARS-CoV spike protein-mediated lung injury [13], while SARS-CoV-2 has been shown to induce severe respiratory injury in transgenic mice overexpressing ACE2 [14]. Upon binding of SARS-CoV-2, ACE2 is downregulated, which interferes with downstream activation of RAS, thus inhibiting its anti-inflammatory and anti-fibrotic actions [15]. Therefore, co-expression studies with ACE2 and TMPRSS2 will be imperative, as this protease is crucial for viral entry and transmission. Sungnak *et al* have recently reported that *ACE2* and *TMPRSS2* are both expressed at the gene level in nasal epithelial cells, which may thus act as reservoirs for the virus [6]. Nawijn and Timens proposed that virus infection-mediated interferon upregulation in the upper airways may lead to a rapid increase of ACE2 expression in the lower airways and lung parenchyma, increasing the infection risk and viral spreading across the respiratory mucosa [16]. These new findings could greatly impact the development of effective therapies for COVID-19, as indicated in Figure 1. For instance, anti-ACE-2 antibodies could be used to block SARS-CoV-2 binding to the receptor. Additionally, TMPRSS2 inhibitors could be used to prevent SARS-CoV-2 entry into host cells. Camostat mesylate, a serine protease inhibitor, has been used to treat chronic pancreatitis in Japan and is currently undergoing phase 1/2 trial testing in the United States. Furthermore, various endocrine regulators as well as corticosteroids have been proposed for COVID-19 treatment, due to their ability to downregulate ACE2 [17–19]. Since additional host molecules have been proposed to facilitate viral entry or ACE2 shedding, including CD147, CD26/DPP4, GRP78, cathepsin L, and ADAM17, it will be important to assess the effects of lifestyle and environmental factors on these factors. Together, these studies may provide promising targets towards novel therapeutic strategies for COVID-19.

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Author contributions statement

All the authors were involved in drafting, revision and final approval of the manuscript.

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