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Short communication

Mechano-inflammatory sensitivity of ACE2: Implications for the regional distribution of SARS-CoV-2 injury in the lung

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ABSTRACT

The coronavirus disease (COVID-19) caused by SARS-CoV-2 can result in severe injury to the lung. Computed tomography images have revealed that the virus preferentially affects the base of the lung, which experiences larger tidal stretches than the apex. We hypothesize that the expression of both the angiotensin converting enzyme-2 (ACE2) receptor for SARS-CoV-2 and the transmembrane serine protease 2 (TMPRSS2) are sensitive to regional cell stretch in the lung. To test this hypothesis, we stretched precision cut lung slices (PCLS) for 12 h with one of the following protocols: 1) unstretched (US); 2) low-stretch (LS), 5% peak-to-peak area strain mimicking the lung base; or 3) high-stretch (HS), the same peak-to-peak area strain superimposed on 10% static area stretch mimicking the lung apex. PCLS were additionally stretched in cigarette smoke extract (CSE) to mimic an acute inflammatory exposure. The expression of ACE2 was higher whereas that of TMPRSS2 was lower in the control samples following LS than HS. CSE-induced inflammation substantially altered the expression of ACE2 with higher levels following HS than LS. These results suggest that ACE2 and TMPRSS2 expression in lung cells is mechanosensitive, which could have implications for the spatial distribution of COVID-19-mediated lung injury and the increased risk for more severe disease in active smokers and patients with COPD.

1. Introduction

The coronavirus disease (COVID-19) caused by SARS-CoV-2 can result in early hypoxemia due to injury to the lung (Bos et al., 2020; Gattinoni et al., 2020a), followed by acute respiratory distress syndrome (Gattinoni et al., 2020b; Ziehr et al., 2020) and multiple organ failure and death (Mokhtari et al., 2020). CT images revealed that the virus preferentially affects the base of the lung (Pan et al., 2020), which experiences larger stretches than the apex (Napadow et al., 2001). However, it is not known whether SARS-CoV-2 entry into alveolar epithelial type 2 (AET2) cells themselves (Hou et al., 2020), mediated by the angiotensin converting enzyme-2 (ACE2) and the transmembrane serine protease 2 (TMPRSS2) (Hoffmann et al., 2020), is mechanosensitive. This would be important as mechanical stretch has been shown to regulate basic processes in AET2 cells such as surfactant secretion (Wirtz and Dobbs, 1990), and more recently, ACE2 expression in vascular smooth muscle cells (Song et al., 2020). We hypothesized that expression of both the ACE2 and TMPRSS2 are sensitive to regional cell stretch in the lung.

2. Methods

Approved by Boston University Institutional Animal Care and Use Committee, male Sprague-Dawley rats were used to obtain precision cut lung slice (PCLS) samples from our recent study (Mondonedo et al., 2020). PCLS samples were stretched using a 12-well mechanical stretcher device designed to recreate physiologic stretch conditions *in vivo* (Mondonedo et al., 2020). Briefly, PCLSs were sinusoidally stretched in cell culture media for 12 h at 1 Hz (spontaneous breathing rate for rats) with one of the following stretch protocols: 1) unstretched (US); 2) low-stretch (LS: dynamic-only), 5% peak-to-peak area strain mimicking the lung base; and 3) high-stretch (HS: dynamic + static), 5% peak-to-peak area strain superimposed on 10% static area stretch mimicking the lung apex. PCLSs from $N = 8$ rats were either control (Cnt) or exposed to cigarette smoke extract (CSE; subtoxic concentration of 0.01 cig/ml) during stretching to simulate the acute inflammatory effects of cigarette smoking (Mondonedo et al., 2020). Western blots on equal amounts of protein were used to evaluate ACE2 and TMPRSS2 expressions (primary antibodies from Santa Cruz Biotechnology) from homogenates. Following quantitative densitometry, data were

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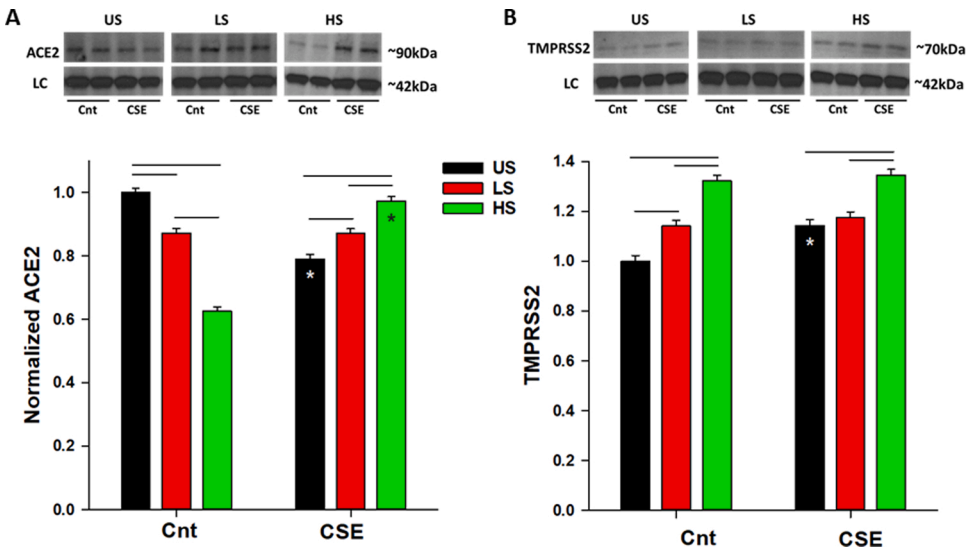

Fig. 1. ACE2 (A) and TMPRSS2 (B) protein levels in homogenized PCLS samples normalized with the mean of the unstretched (US) control (Cnt) samples measured at 3 different conditions (US: unstretched, LS: low stretch with 5% dynamic area strain, HS: high stretch with 5% dynamic area strain superimposed on 10% static strain) stretched for 12 h with or without CSE exposure. Error bars represent measurement variability in 8 independent PCLS samples from 8 rats. Western blots above graphs show representative examples from each stretch and treatment conditions. LC: loading control. Horizontal bars within Cnt or CSE denote statistically significant stretch dependence; * denotes differences due to CSE exposure compared to Cnt at the same stretch level.

statistically analyzed by one-way and two-way analysis of variance (ANOVA) (SigmaPlot).

3. Results and discussion

We found that the effect of stretch was highly significant for ACE2 ($p < 0.001$) within both the Cnt and CSE groups (Fig. 1A). There was no difference between the LS conditions (red bars) of the Cnt and CSE groups; however, CSE significantly affected the mechanosensitivity of ACE2 expression by increasing its level with HS to above the value with LS ($p < 0.001$). There was a significant stretch dependence for TMPRSS2 in both the Cnt and CSE groups ($p < 0.001$; Fig. 1B). However, there was no difference between the TMPRSS2 levels of the US and LS conditions in the CSE group. The effect of CSE was only significant in the US case ($p < 0.001$).

These findings support our hypothesis that ACE2 and TMPRSS2 are sensitive to stretch *in vitro*, which may suggest they would exhibit similar regional differences *in vivo* given the known spatial distribution of stretch magnitudes in the lung. The expression of both ACE2 and TMPRSS2 is stretch dependent, although TMPRSS2 expression exhibits a pattern with stretch that is opposite to that of ACE2 in the Cnt group. Moreover, ACE2 appears to be either downregulated in the presence of the larger static stretch at the apex, or, alternatively, its expression responds to the larger dynamic stretch experienced in the lung base. Although our experiments cannot differentiate between these possibilities, the mechanosensitivity of ACE2 in lung cells increases the susceptibility of the base of the lung to SARS-CoV-2, which may, in part, explain the characteristic spatial pattern of lung injury in COVID-19(6). Additionally, prone positioning has been utilized for patients with severe, symptomatic COVID-19 infection and changes the gravitational gradient within the lung parenchyma (Karpov et al., 2020). Indeed, a prolonged improvement in gas exchange after prone positioning of critically ill COVID-19 patients was found to be associated with reduced death rate and duration of mechanical ventilation (Scaramuzzo et al., 2021). It is also possible the redistribution of mechanical stresses following proning serves not only as a recruitment of alveolar regions but as a means to "protect" areas of at-risk lung by changing the regional stretch experienced.

We also observed that acute CSE exposure inverted the effects of stretch on ACE2. This could have implications for a potentially different pattern of injury and severity of SARS-CoV-2 among patients with COPD. Current smokers and patients with COPD have an increased risk of mechanical ventilation and worse outcomes as well as higher rate of ground-glass opacities and local patchy shadowing on CT chest imaging (Wu et al., 2020). Additionally, once smokers and COPD patients become infected, their risk for more severe disease increases (Lacedonia et al., 2021). Our results suggest that even a short-term exposure to CSE and the associated inflammation can significantly alter the baseline mechanosensitivity of ACE2 expression, which could potentially increase the overall available lung susceptible to viral infection, contributing to the clinical and radiologic observations above. Our prior experience showed that CSE increased the level of IL-1β in a stretch-dependent manner (Mondonedo et al., 2020), which would similarly exacerbate the effects of COVID-19 in patients with COPD.

In summary, these results provide the first evidence that ACE2 and TMPRSS2 expression in lung cells is mechanosensitive, a pertinent finding in light of the COVID-19 pandemic. Despite the limitations of the rat PCLS, these findings suggest mechanosensitive nature of ACE2 and TMPRSS2 expression could play a role in the spatial distribution of COVID-mediated lung injury, while the inflammatory effects of acute cigarette smoke exposure on this relationship may have further implications for active smokers and patients with COPD. Details of the underlying molecular mechanism and clinical implications warrant further investigation.

Author statement

EBS carried out biochemical analysis, contributed to concept, wrote paper; JRM carried out stretching experiments, wrote paper; BS formulated concept, wrote paper.

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