Impact of Comorbidities on the Expression of SARS-CoV-2 Viral Entry-Related Genes

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Abstract

Comorbidities such as diabetes, chronic lung disease, and cardiovascular disease are highly prevalent in patients with Coronavirus Disease 2019 (COVID-19) and associated with worse outcomes. However, whether these conditions contribute directly to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virulence or simply worsen outcomes through independent mechanisms and reflect the general disease burden of the population remain unclear. As an important aspect of virulence, viral entry mechanisms have been proposed which require host cell membrane-bound angiotensin-converting enzyme 2 (ACE2) and type II transmembrane serine proteases (TTSPs), such as transmembrane serine protease isoform 2 (TMPRSS2). Herein, we survey expression data of SARS-CoV-2 entry-related genes from 69 differential expression datasets from patients suffering from a variety of reported common comorbidities and across infection-relevant organ systems to understand their potential role in morbidity and mortality of COVID-19. Indeed, expression levels of entry-related genes are modulated by the disease state of the organ systems analyzed. Expression levels were lower in some comparisons such as type 2 diabetes in whole blood and peripheral blood mononuclear cells (PBMCs). However, many of these genes were consistently upregulated in cancer, a history of smoking, asthma in bronchial epithelium and chronic lung disease in pulmonary tissues. Additionally, increased expression was found in select sets of hypertension, type 1 diabetes, cardiomyopathy and chronic kidney disease (CKD) in other infection-relevant tissues. These findings may be particularly relevant to the significant morbidity and mortality seen in COVID-19 and may suggest novel therapeutic targets to influence outcomes in patients infected with SARS-CoV-2.

Introduction

Comorbidities such as diabetes, chronic lung disease, and cardiovascular disease are highly prevalent in patients with COVID-19 and associated with worse outcomes [1, 2]. However, whether these conditions contribute directly to SARS-CoV-2 virulence or simply worsen outcomes through independent mechanisms and reflect the general disease burden of the population remain unclear [2, 3]. Furthermore, clinical and experimental evidence has demonstrated that in addition to the lungs, SARS-CoV-2 infection of other target organ systems such as heart, kidney, and blood may have important deleterious consequences which can potentially compromise organ function and compound disease burden in COVID-19 patients [4, 5].

The spike (S) protein of SARS-CoV and SARS-CoV-2 is a key facilitator for host cell entry through its binding to host cell membrane-bound ACE2 [6]. Therefore, the impact of the modulation of ACE2 and related renin-angiotensin-aldosterone system genes on COVID-19 has been an area of interest [3] and were included in the current study. Additionally, after binding, cleavage of the S protein is necessary for S protein-mediated membrane fusion which drives viral entry into host cells. This proteolytic activity may be cathepsin-L dependent and occur upon pH change in cellular endosomes, or may occur through the action of membrane bound serine proteases at the host cell membrane surface or within vesicles [7]. Additionally, viral entry mechanisms have been proposed which involve cleavage of ACE2 itself by membrane bound serine proteases leading to increased viral entry [7]. In fact, the importance of serine proteases in a viral entry mechanism may be emphasized by the success of serine protease inhibition in vitro [6]. However, while this and current mechanistic studies have focused on the proteolytic activity of TMPRSS2 and human airway trypsin-like protease (HAT, also referred to as TMPRSS11D) additional TTSPs are hypothesized to have similar extracellular cleavage activity [8] and were included in the current study. Furthermore, the influence of comorbidities on these genes is unknown. Thus, in the current study we examined the influence of comorbidities on the expression of key renin-angiotensin-aldosterone system and protease genes which may prime the cell entry mechanisms for SARS-CoV-2 across various organ systems.

Methods

In the current study, we examined the expression levels of SARS-CoV-2 entry-related genes in target organ systems including pulmonary, renal, cardiac, and PBMCs from 2032 patients across a variety of common comorbidities including hypertension (n=94 hypertensives vs. n=61 normotensives), diabetes (n=131 diabetic vs. n=101 normoglycemic), obesity (n=56 obese vs. n=58 healthy weight), chronic lung disease (n=200 asthmatic vs. n=106 non-asthmatic and Chronic Obstructive Pulmonary Disease (COPD) n=94 vs. healthy tissue n=42) and cardiovascular disease (ischemic n=36 and dilated n=91 cardiomyopathy vs. healthy tissue n=69), as well as other common pathologies such as CKD and cancer. Differential gene expression was curated from genetic data deposited in the National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine, Gene Expression Omnibus (GEO) DataSets and the European Molecular Biology Laboratory (EMBL), European Bioinformatics Institute (EBI). Exhaustive queries in these databases in the form of “[tissue] and [hypertension, diabetes, obesity, smoking, asthma]” were performed using the NCBI and

iLINCS website (ilincs.org) and all hits were considered. DataSeries not containing disease state vs. healthy controls for the tissues concerned were excluded and common off-target hits such as cancer were included. Differential Expression analysis was performed using the GEO2R (NCBI) interactive web tool, and the iLINCS integrative web platform for the analysis of the Library of Integrated Network-Based Cellular Signatures (LINCS). The expression values in healthy tissues are reported as logarithm to base 2 of the fold change (Log2FC) as described in the GEO DataSet (GDS3113) and visualized by Morpheus (Broad Institute). For comorbidity disease condition data, expression levels are described in log2FC in relation to each unaffected control group specific to each DataSeries and heatmaps were generated in GraphPad Prism (GraphPad Software Inc.). All log2FC values outside of the -2 to 2 range are shown as either -2 or 2. DataSeries in this analysis were as follows with GSEXXXX referring to NCBI-GEO and E-XXXX-XXXX referring to EMBL-EBI:

Pulmonary Tissues: (1, GSE18965; 2, GSE41649; 3, GSE2125; 4, E-GEOD-63142; 5, E-GEOD-63142; 6, E-GEOD-19187; 7, E-GEOD-19187; 8, E-GEOD-44077; 9, GSE19804; 10, E-GEOD-43458; 11, E-GEOD-60052; 12, GSE5057; 13, GSE3320; 14, GSE2125; 15, E-MTAB-6040; 16, GSE21411; 17, GSE21411);

Renal Tissues: (1, GSE104948; 2, GSE28345; 3, GSE104954; 4, GSE28360; 5, GSE104948; 6, GSE30528; 7, GSE104954; 8, GSE30529; 9, GSE66494; 10, GSE12682; 11, GSE20602; 12, GSE32591; 13, GSE32591; 14, GSE46699; 15, GSE46699; 16, GSE46699; 17, GSE46699);

Cardiac Tissues: (1, GSE1145; 2, GSE67492; 3, GSE42955; 4, GSE3585; 5, GSE116250; 6, GSE3586; 7, GSE120836; 8, GSE116250; 9, GSE42955; 10, GSE1145; 11, GSE26887; 12, GSE10161;

Blood and PBMCs: (1, GSE24752; 2, GSE75360; 3, GSE75360; 4, GSE48424; 5, GSE69601; 6, E-TABM-666; 7, GSE9006; 8, GSE55098; 9, GSE9006; 10, GSE23561; 11, GSE131793; 12, GSE42057; 13, GSE59019; 14, GSE16032; 15, GSE15072; 16, GSE37171; 17, GSE15072; 18, GSE37171; 19, GSE87493; 20, GSE69039; 21, GSE32575; 22, GSE23561; 23, GSE66360).

Results

In healthy human tissues, there is a diverse transcription of renin-angiotensin-aldosterone system related genes (ACE, ACE2, and AGTR1) as well as proteases (ADAM17, TMPRSS1-5, TMPRSSD, TMPRSSE, and TMPRSS15) which may prime the cell entry mechanisms for SARS-CoV-2 across various organ systems. Consistent with other published DataSets we note that while some tissues (colon, heart, kidney, liver, lung,

etc.) show high levels of expression, others (adrenal gland, bone marrow, ovary, etc.) show minimal expression (Figure 1A). Additionally, we examined the impact of a variety of common comorbidities on the expression of these genes in select organ systems (pulmonary, renal, cardiac, and blood tissues) based on their relevance to infection and expression levels at baseline. Expression data displayed in each comorbidity heatmap is displayed from greatest (left) to least (right) expression of ACE2.

In pulmonary tissues (Figure 1B) we see the greatest increase in ACE2 in cancer with substantial increases in nearly all of the TTSPs, which is consistent with their role in tumor cell proliferation, motility and invasion [8]. Additionally, samples from patients with a history of smoking showed increases in ACE2 in both small and large airways, consistent with recent findings [9]. While expression levels in the context of pre-existing asthma appeared to be largely unaffected in pulmonary tissues on average, there were more pronounced increases in bronchial compared to nasal epithelium.

In renal tissues (Figure 1C), we found the greatest expression of ACE2 in obesity. Similar to what has been seen in pulmonary tissues, a history of smoking or cancer associated with an increase in ACE2 as well as slight increases in TTSPs in renal biopsy. Hypertensives had increases in ACE2, TMPRSS1 and TMPRSS4 in renal cortical and tubulointerstium, but not glomerular or medullar samples. CKD resulted in the greatest diversity in modulation of these genes, however, with consistent increases in TMRPSS4 in samples from both tubular and glomerular origin.

In cardiac tissues (Figure 1D), we found the greatest increases in ACE2 in patients who had experienced heart failure with pre-existing diabetes or patients with aortic stenosis. While cardiomyopathies resulted in variable expression levels, increases in ACE2 were found in left ventricle (LV) tissues while decreases were found in right ventricle (RV) tissues. On average, slight increases were found for many TTSPs.

In blood (Figure 1E), remarkable increases in all selected genes were found in patients with coronary artery disease. Hypertension and chronic lung pathologies resulted in slight, but consistent increases in most of the selected genes including ACE2 and many of the TTSPs. In contrast to the results seen in renal tissues, obesity did not appear to modulate the expression of any of these genes in circulating immune cells. Notably, increased expression levels were found in the context of type 1 diabetes, while decreases were apparent in

type 2 diabetes in whole blood and PBMCs. Lastly, highly variable modulation was found in the context of CKD with or without hemodialysis.

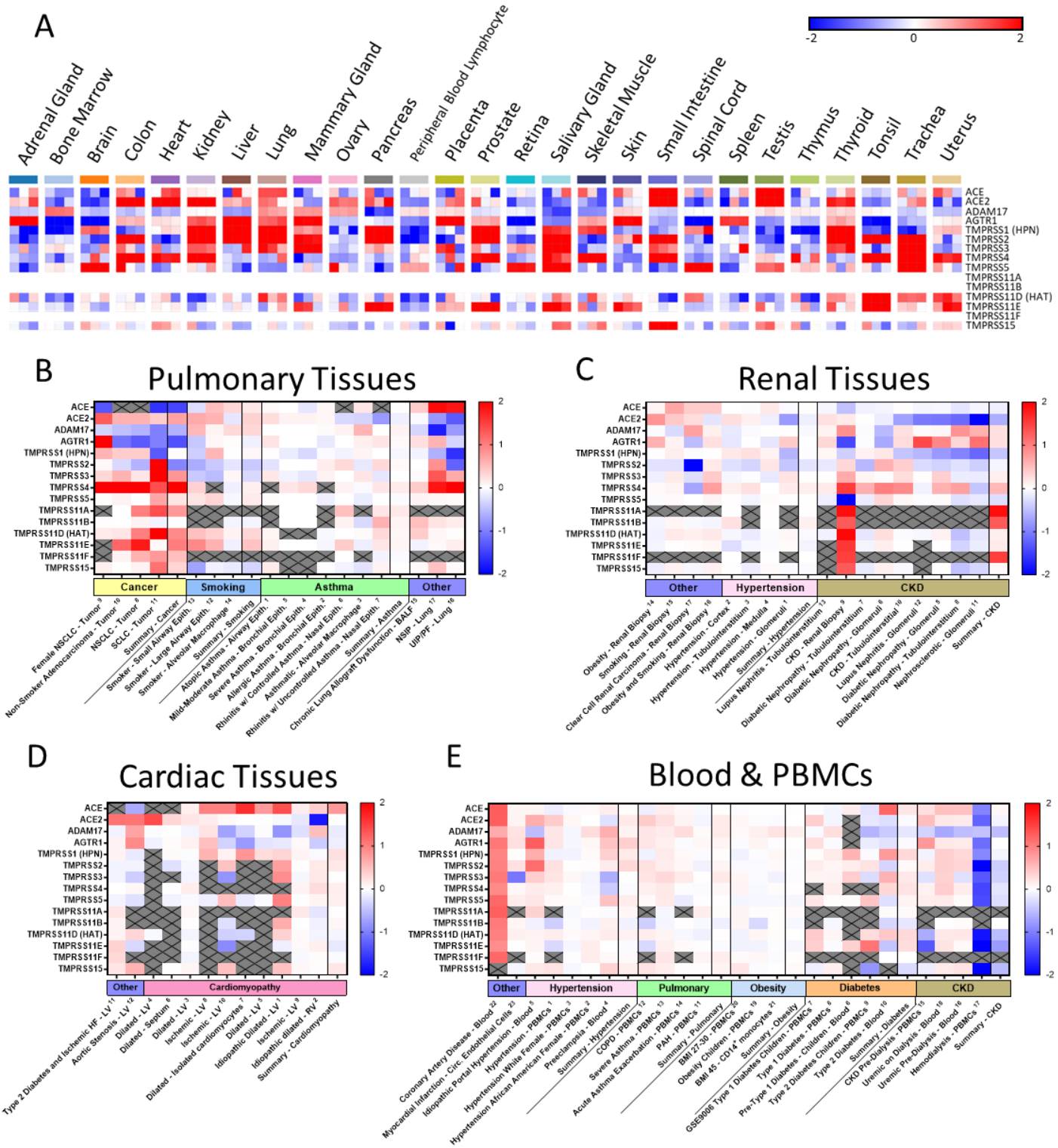

Figure 1. SARS-CoV-2 viral-entry gene expression across healthy and diseased human tissues. Expression of select genes related to SARS-CoV-2 viral-entry in healthy volunteers across 27 different body

sites. Expression is displayed as Log2FC, n=3 (A). Expression of select SARS-CoV-2 viral-entry genes in pulmonary (B), renal (C), cardiac (D), and blood tissues (E) across 69 DataSets representing various common comorbidities. Values are displayed as Log2FC in comparison with respective unaffected control samples from each DataSet. Comorbidity groups and each DataSet within are sorted from greatest (left) to least (right) expression of ACE2. All heatmaps are set to a scale of -2 (blue) to 2 (red). BALF = Bronchoalveolar lavage fluid; BMI = body mass index; CKD = chronic kidney disease; COPD = chronic obstructive pulmonary disease; HF = heart failure; LV = left ventricle; NSCLC = non-small cell lung carcinoma; NSIP = non-specific interstitial pneumonia; PBMCs = peripheral blood mononuclear cells; PAH = pulmonary arterial hypertension; RV = right ventricle; SCLC = small cell lung carcinoma; UIP/PF = usual interstitial pneumonia/idiopathic pulmonary fibrosis.

Discussion

Investigations since the SARS-CoV outbreak in 2002-2003 and especially more recently have focused on the expression of ACE2 and its relationship to viral entry [6]. In a recent thorough report, ACE2 and transmembrane serine protease isoform 2 (TMPRSS2) expression was mapped across various body sites in normal healthy tissue by single cell RNA sequencing [10]. However, a more complete model of viral entry for SARS-CoV and SARS-CoV-2 describe a potential role for TMPRSS2, HAT, and potentially other TTSPs [7]. Because interest in TTSPs seems to have blossomed only within the last decade, their appearances on micro array technologies and therefore their appearances in these data are limited. However, understanding the modulation of the expression of these genes across various organ systems and in the context of common comorbidities should provide us with a more complete understanding of the potential impact of these comorbidities on viral proliferation. Increases in the expression of these genes as suggested by this data in common comorbidities across tissues such as hypertension, cancer and a history of smoking may help to partially explain their association with higher morbidity and mortality in COVID-19. In other comorbidities, such as obesity, and diabetes or in those with tissue specificity such as in cardiomyopathies and chronic lung disease, the lack of consistent alteration across tissues may suggest a mechanism for tropism of the virus. To be sure, while SARS-CoV and SARS-CoV-2 virulence is driven by factors outside of viral entry, it may be important to understand the influence of the varied expression of these genes among target organ systems and across comorbidities as demonstrated.

In conclusion, expression levels of SARS-CoV-2 viral-entry related genes in patients suffering from common comorbidities such as hypertension, cancer, a history of smoking, obesity, diabetes, cardiomyopathies, or chronic lung or kidney disease may be increased in target organ systems and be capable of directly contributing to infection. This represents an important step in designing effective therapeutic and preventative strategies to improve outcomes in vulnerable populations.

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