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Dysregulation of ACE2 Expression and Function in Co-morbid Disease Conditions Possibly Contributes to COVID-19 Complication Severity

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Abbreviation list:
ARDS, Acute Respiratory distress syndrome; ACE, Angiotensin converting enzyme; AngII, Angiotensin II; AT1R and AT2R, Angiotensin receptor 1 and 2; ARBs, Angiotensin receptor blockers; BDNF, brain-derived neutrophic factor; JNK, c-Jun N-terminal kinase; CVD, Cardiovascular disease; catL, cathepsin L; CNS, central nervous system; CXCL1, chemokine (C-X-C motif) ligand; COVID-19, coronavirus infectious disease 2019; ADAM16, Disintegrin and metallopeptidase domain 16; ERK, extracellular signal-regulated kinase; ICU, intensive care unit; IFNγ, Interferon gamma; IL, interleukin; LPS, lipopolysaccharides; M1, macrophage; mTOR, mammalian target of rapamycin; MMP-9, matrix metallopeptidase-9; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant-1; NF-κB, nuclear factor kappa-light-chain enhancer of activated B cells; PPARγ, Peroxisome proliferator-activated receptors gamma; PIKfyve, Phosphoinositide Kinase, FYVE-Type Zinc Finger Containing; PCOS, polycystic ovarian syndrome; PH, Pulmonary hypertension; RAS, Renin-angiotensin system; Th, T-helper; TGF-β, Transforming growth factor beta; TMPRSS2, Transmembrane protease serine 2; TrkB, Tropomyosin receptor kinase B; TNFα, Tumor necrosis factor alpha; TACE, tumor necrosis factor alpha converting enzyme; TPC2, Two pore segment channel 2; VEGF, Vascular endothelial growth factor.
Abstract

ACE2 has emerged as a double agent in the COVID-19 ordeal being physiologically protective yet virally conducive. The identification of ACE2 in as many as 72 tissues suggests that extra-pulmonary invasion and damage is likely, which indeed has already been demonstrated by cardiovascular and gastro-intestinal symptoms. On the other hand, identifying ACE2 dysregulation in patients with co-morbidities may offer insight as to why COVID-19 symptoms are often more severe in these individuals. This may be attributed to a pre-existing pro-inflammatory state that is further propelled with the cytokine storm induced by SARS-CoV-2 infection or the loss of functional ACE2 expression as a result of viral internalization. Here, we aim to characterize the distribution and role of ACE2 in various organs in order to highlight the scope of damage that may arise upon SARS-CoV-2 invasion. Furthermore, by examining the disruption of ACE2 in several co-morbid diseases, we offer insight into potential causes of increased severity of COVID-19 symptoms in certain individuals.

Significance Statement:

Cell surface expression of ACE2 determines the tissue susceptibility for COVID-19 infection. Co-morbid disease conditions altering ACE2 expression could increase the patient’s vulnerability for the disease and its complications directly through modulation of viral infection, or indirectly through alteration of inflammatory status.
1. Introduction

1.1 The renin-angiotensin system:

The renin-angiotensin system (RAS) plays a key role in regulating the normal physiology and pathogenesis of cardiovascular diseases (CVD). RAS mainly consists of a series of enzymatic reactions, with renin being the rate limiting enzyme and angiotensin II (AngII), generated by local RAS, being the major component acting on angiotensin receptor 1 and 2 (AT$_1$R, AT$_2$R). Overstimulation of the ‘classic’ RAS pathway involving the activation angiotensin converting enzyme 1 (ACE1/AngII/AT$_1$R) results in vasoconstriction, inflammation, sodium and water retention, and oxidative stress generation, thus increasing sympathetic nervous system activity, cardiovascular fibrosis and hypertrophy. The view of RAS has changed with the identification of an ACE homolog, ACE2 (Donoghue et al., 2000). A more complex signaling network mediating protective physiological effects along with the detrimental ones has now been recognized. ACE2 degrades both AngI and AngII into Ang(1-9) and Ang(1-7), respectively. As such, another arm of RAS, ACE2/Ang1-7, and ACE2/Ang1-9, emerged and is reported to act as a physiological antagonist to counteract RAS over-activation, though its full physiological role is still not completely understood (Santos and Ferreira, 2007; van Twist et al., 2014).

1.2 Impact of RAS imbalance on various organs

Of relevance to SARS-CoV-2 infection, lungs are rich in RAS components including ACE1/ACE2. Imbalance in RAS pathways in favor of ACE1 exacerbates pulmonary tissue damage leading to acute respiratory distress syndrome (ARDS) (Marshall, 2003). AngII promotes pulmonary vasoconstriction, inflammation, as well as fibrosis (Uhal et al., 2012). Studies have also linked both AT$_1$/AT$_2$R with AngII-induced lung fibrosis via a mitogen-activated protein kinase (MAPK) pathway (Marshall et al., 2000). Further, AngII stimulates Transforming growth factor beta (TGF-β) and collagen expression in fibroblasts and mediates alveolar epithelial apoptosis in mice via c-Jun N-terminal kinase (JNK) phosphorylation (Uhal et al., 2007; Uhal et al., 2011).

On the other hand, the kidney is the main source of renin, the enzyme initiating RAS cascade. Renal AT$_1$R expression is important in regulating baseline blood pressure and the local renal RAS activation underlies hypertension-induced renal damage (Crowley et al., 2006). AngII induces a local immuno-inflammatory response leading to glomerulosclerosis, interstitial fibrosis, albuminuria and renal failure (Park et al., 2007). A direct link between stimulation of RAS/AT$_1$, NADPH oxidase, reactive oxygen species and renal remodeling was reported (Nistala et al., 2008). As well, AngII initiates fibrosis and inflammation in kidney, through stimulation of TGF-β1 signaling (Chen et al., 2012) and triggering extracellular signal-regulated kinase (Erk)/p38MAPK/Smad3 and Rho kinase pathways (Ruperez et al., 2005; Ruster and Wolf, 2011).

Furthermore, RAS exerts additional cardiovascular control through its activity in the brain. Brain-RAS is a central modulator of blood pressure and sympathetic nervous system (Zucker et al., 2014). AngII/AT$_1$R pathway activates the brain-derived neurotrophic factor/Transmembrane protease serine 2 (BDNF/TrkB) signaling inducing p38MAPK-dependent reduction of voltage gated potassium current, and sympathetic excitation (Becker et al., 2017; Becker et al., 2015). Additional pathways involved in AngII-induced increase in blood pressure and sympathetic activity include Rho kinase activation and reduction of Peroxisome proliferator-activated receptors gamma (PPARγ) DNA-binding as well as downregulation of ACE2 (Pellegrino et al.,
Higher RAS-driven sympathetic activity increases metabolic rate by activation of brown adipose tissues and brown adipose tissue thermogenesis (Young et al., 2015).

On the immune system, RAS stimulation is found to induce inflammation. Briefly, AngII/AT₁R stimulation induces macrophage infiltration, increased nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) signaling, reactive oxygen species production and inflammatory cytokine generation (Guo et al., 2011). Further, AngII via AT₁R and AT₂R, activates T-cells, which in turn increase superoxide and tumor necrosis factor alpha (TNF-α) production (Hoch et al., 2009). Different T-helper (Th) cells contribute to the detrimental cardiovascular effect of AngII by releasing multiple cytokines. Th1 and Th17 secrete interferon (IFNγ) and IL-17 respectively, participating in AngII-induced hypertension. On the other hand, Th2-produced IL-4 has a role in cardiac fibrosis (Kamat et al., 2015; Madhur et al., 2010; Peng et al., 2015). Moreover, AngII-stimulated secretion of the pro-inflammatory IL-22 from Th22 is involved in AngII-induced hypertension and endothelial dysfunction (Ye et al., 2017).

As such, dysregulation of the RAS system is heavily involved in the pathogenesis of cardiovascular diseases (CVDs) including hypertension and heart failure (Simões e Silva et al., 2013; Sparks et al., 2014). In fact, most of the deleterious symptoms of CVDs, like excessive vasoconstriction, inflammation, fibrosis, hypertrophy and fluid retention, are primarily mediated by the ACE1/AngII/AT₁R axis (Patel et al., 2016b). RAS components like ACE1 and AT₁ exist throughout the heart and vasculature, and their upregulation is involved in pathological phenotypes of cardiovascular diseases (Gonzalez et al., 2019; Patel et al., 2016b). This was demonstrated by the in vitro treatment of cardiomyocytes with AngII, triggering myocyte hypertrophy, cardiac fibroblast proliferation and collagen synthesis. Conversely, inhibition of ACE2 in cardiomyocytes in vivo, reverses the hypertrophy and fibrosis related to remodeling and heart failure (Patel et al., 2016b). Moreover, inhibition of this system mitigates the morbidity and death rate among these patients (Zaman et al., 2002).

### 1.3 ACE2 as the protective arm of the RAS system

The ability of ACE2 to lend physiological protection stems from its various functions including metabolizing AngI to Ang1-9 reducing ACE1 substrate, breaking AngII down reducing the AT₁R activation, and generating Ang1-7 responsible for inducing cardioprotective effects (Patel et al., 2016b). Although the ACE2/Ang1-7 axis is responsible for anti-hypertensive effects, much of the activity of this system does not directly cause vasodilation, but rather entails other mechanisms, which pre-dominantly include down regulation of pro-inflammatory responses (Crackower et al., 2002). ACE2 activity decreases inflammation by maintaining the balance in RAS activity (Nehme and Zibara, 2017). In pathologies associated with increased RAS activation, e.g. hypertension, heart failure and diabetes, ACE2 activity confers some protection by counteracting the effect of AngII activation possibly by reducing cellular apoptosis and oxidative stress (Bernardi et al., 2016; Chang et al., 2011; Shenoy et al., 2010). Specifically, stimulating ACE2 activity decreased the levels of TNF-α, IL-1β, and IL-6; in addition to increasing the expression of anti-inflammatory cytokine IL-10 in an animal model of autoimmune myocarditis (Sukumaran et al., 2011).

Significantly, ACE2 plays a key role in cellular entry for several coronaviruses, including SARS-CoV-2, and its wide distribution may support viral invasion and potential damage in susceptible organs (Chen et al., 2020c; Coutard et al., 2020; Hamming et al., 2004). Conversely, ACE2 activity is crucial in non-communicable disorders, such as auto-immune, cardiovascular,
metabolic and gastro-intestinal diseases, to mitigate many associated pro-inflammatory and pathogenic effects (Patel et al., 2016b; Perlot and Penninger, 2013; Simões e Silva et al., 2013; Zhang and Wada, 2007). Thus, inhibition of ACE2 as a therapeutic target against SARS-CoV-2 has been ruled out from the start. As such, ACE2 has emerged as the double-edged sword in the SARS-CoV-2 ordeal as it is both physiologically protective yet virally conducive (Zores and Rebeaud, 2020). Highlighting the distribution of ACE2 in the body will elucidate various tissue-specific symptoms that may arise upon viral infection. Moreover, identifying its dysregulation in patients with co-morbidities may offer insight as to why coronavirus infectious disease 2019 (COVID-19) symptoms are often more severe in these individuals.

2. ACE2 tissue distribution

ACE2 is a zinc metalloproteinase transmembrane protein characterized by having several domains- a signal peptide, a carboxy peptidase active site, a transmembrane domain, and a cytoplasmic domain (Donoghue et al., 2000; Haga et al., 2008). Importantly, it has been detected in cell culture media indicating that it likely also exists in an extracellular secreted form. The enzyme is abundantly found on the vascular endothelium, including capillaries, arterioles, arteries, venules and veins, which embed every organ (Hamming et al., 2004). Besides vessels, ACE2 is also majorly detected in the tissues of the lung, heart, kidney, and testis (Donoghue et al., 2000). It is present on cells which have direct contact with the environment, particularly type 1 and 2 alveolar epithelial pneumocytes of the lungs (Hamming et al., 2004). Hamming et al. were able to further characterize the distribution of ACE2-like immunoreactive material in approximately 72 different human tissues obtained from autopsy specimens including the brush border of the small intestine enterocytes and renal proximal tubular cells, cholangiocytes, the brain, the basolateral layer of the skin epithelium and epithelial cells of the oral and nasal mucosa and nasopharynx (Hamming et al., 2004). Interestingly, recent investigation correlates ACE2 expression levels with SARS-CoV-2 viral loads in swabs from different tissues in infected individuals (Sungnak et al., 2020; Zou et al., 2020).

3. The effect of co-morbid ACE2 dysregulation on COVID-19 associated symptoms

Many diseases are associated with impaired ACE2 activity in the implicated organs. One of the major corollaries of this impairment is an elevated pro-inflammatory response (Kangussu et al., 2019; Patel et al., 2016b). Ultimately, tissue injury and fibrosis may result. Patients with underlying diseases such as hypertension, diabetes and coronary heart diseases have been shown to have more severe COVID-19 symptoms (Guan et al., 2020; Huang et al., 2020). This may be attributed to the pre-existing pro-inflammatory state that is further propelled by the cytokine storm induced by SARS-CoV-2. Moreover, it can be also be a consequence of an interplay with RAS, and thus further loss of functional ACE2 units that are shed from the cell surface or downregulated following internalization after interaction with SARS-CoV-2 or (Gheblawi et al., 2020; Haga et al., 2008; Heurich et al., 2014a).

On the other hand, extra-pulmonary symptoms in infected individuals with no known underlying diseases are increasingly emerging in COVID-19 patients. Besides pneumonia, other symptoms associated with the virus span the intestinal tract, cardiovascular system, central nervous system and others (Fang et al., 2020; Zheng et al., 2020). We propose that the emergence of such
symptoms is related to the presence of ACE2 in these specific tissues. Conversely, the disruption of ACE2 in patients with underlying diseases may contribute to the severity of symptoms detected in these individuals. Therefore, it is important to highlight the distribution of ACE2 in various organs, its dysregulation in specific diseases and to monitor related symptoms in inflicted patients.

3.1 Pulmonary disease

3.1.1 The role of ACE2 in pulmonary diseases

Pulmonary hypertension (PH) in rats led to an enlarged right ventricle, interstitial fibrosis and increased pulmonary wall thickness (Ferreira et al., 2009). In tandem, renin, ACE, angiotensinogen, AT1R and pro-inflammatory cytokines were also elevated. These manifestations were precluded by treatment with a synthetic ACE2 activator (Ferreira et al., 2009). In a related study, rats, in which pulmonary fibrosis was induced, displayed reduced levels of ACE2, augmented collagen deposits, increased expression of TGF-β and other cytokines, as well as elevated AT1R expression (Shenoy et al., 2010). Moreover, lung injury in ACE2 knockout mice was associated with an exacerbated reduction of exercise capacity, further impairment of lung function, and increased lung fibrosis (Li et al., 2016). This phenotype was markedly attenuated by Ang1-7 or ACE2 overexpression. These findings emphasize the integral role of the ACE2 in pulmonary protection against inflammation and fibrosis. Similar findings were observed upon assessing the role of ACE2 in mouse lungs in situations of inhaled endotoxins (Sodhi et al., 2018). The loss of ACE2 expression induced an activation of des-Arg9 bradykinin/bradykininB1 receptor pathway leading to an increase in chemokines, macrophage inflammatory protein-2, TNF-α, neutrophil infiltration, thus exaggerating lung inflammation and injury (Sodhi et al., 2018). Even in chronic airway inflammatory conditions like asthma, Ang1-7 significantly reduced the number of auto-inflammatory-related leukocytes including macrophages, eosinophils and neutrophils (El-Hashim et al., 2012). Ang1-7 also reduced immune cell infiltration, fibrosis and goblet cell metaplasia which suggests airway remodeling. As such, it becomes plausible to assume that suppression of ACE2 activity/expression might exacerbate lung injury following viral invasion.

3.1.2 ACE2 and pulmonary symptoms in SARS-CoV-2 patients

ACE2 is highly expressed in the lungs (Letko et al., 2020), whereby 83% of ACE2-displaying pulmonary cells were alveolar cells implicating the alveoli as a reservoir for viral invasion (Li et al., 2020b). Recent investigation showed that while lung inflammatory disorders including asthma and chronic obstructive pulmonary disease are not associated with changes in ACE2 expression, smokers had increased pulmonary expression levels further confirming the susceptibility of these patient groups (Grundy et al., 2020; Li et al., 2020a). Interestingly, this is thought to occur as a consequence of nicotine-mediated stimulation of α7-nicotinic acetylcholine receptors (Leung et al., 2020). Conversely, others propose that some of the SARS-CoV-2 proteins might carry amino acid sequences similar to those of known neurotoxins blocking nicotinic acetylcholine receptors (Farsalinos et al., 2020b; Farsalinos et al., 2020c). This suggested a possible role for α7-nicotinic acetylcholine receptors as potential sites of interaction with SARS-CoV-2. As such, one might speculate that this interaction drives inflammatory cytokine production whereby infected lung macrophage will be deprived of the anti-inflammatory effect exerted by nicotine-mediated activation of α7-nicotinic acetylcholine receptors on their surface (Kloc et al., 2020). If this competitive relationship between SARS-
CoV-2 and nicotine on α7-nicotinic acetylcholine receptors is proven experimentally, it could potentially offer a molecular explanation for the clinical debate that nicotine, and hence tobacco smoking, might be protective against COVID-19 (Farsalinos et al., 2020a). On the other hand, the large surface area of the lungs favors their high susceptibility to inhaled viruses (Zhang et al., 2020a). Because of ACE2 abundance on pneumocytes and pulmonary vessels, massive SARS-CoV-2 entry and subsequent alveolar wall destruction is expected, contributing to the lung damage associated with COVID-19 (Hamming et al., 2004; Zhou et al., 2020b). Contrary to other coronaviruses, SARS-CoV-1 and SARS-CoV-2 have been found to decrease cell membrane bound ACE2, which in turn promotes severe acute respiratory complications (Haga et al., 2008; Ingraham et al., 2020; Kuba et al., 2005) further alluding to the paradoxical nature of ACE2 role in the course of pulmonary infections. However, other work reports a rebound in pulmonary ACE2 expression 48 hours post-infection that was correlated to inflammatory cytokine production (Li et al., 2020a) raising the question of why this induced increase in expression does not lead to the same beneficial effect associated with basal ACE2 expression/function.

Another factor counting to a much higher SARS-CoV-2 pulmonary infectivity is that the viral entry is mediated by cellular membrane proteases (Hoffmann et al., 2020). Transmembrane protease serine 2 (TMPRSS2) is expressed in the epithelial cells in human lungs and is involved in the regulation of airway surface liquid. Previous studies have identified TMPRSS2 as a key element in SARS-CoV pathogenesis (Matsuyama et al., 2010). TMPRSS2 enhances viral entry through cleavage of ACE2, and activation of cell membrane fusion by cleaving S protein (Heurich et al., 2014a). TMPRSS2 contributes to higher levels of cytokines and chemokines production after SARS-CoV infection. In TMPRSS2-knockout mice, the levels of monocyte chemoattractant-1 (MCP-1), IL-1α, IL-1β, and IL-12 in the lungs induced by SARS-CoV infection were much lower (Iwata-Yoshikawa et al., 2019). A new study using primary human airway epithelial cells reported that SARS-CoV-2 viral entry is highly dependent on TMPRSS2, where the inhibition of TMPRSS2 by camostat mesylate totally inhibited viral infection (Hoffmann et al., 2020). Camostat mesylate, currently used in Japan as an antiviral drug for other indications, could be useful in patients with COVID-19 (Kawase et al., 2012; Yamamoto et al., 2016; Zhou et al., 2015). Following the SARS-CoV-2 induced cytokine storm, patients usually suffer from signs of microvascular dilation, increase of pleural effusions and thickening and fibrotic streaks which could lead to subsequent progression into acute lung injury, ARDS and respiratory failure (Zhang et al., 2020b; Zhou et al., 2020c).

3.2 Cardiovascular disease

3.2.1 ACE2 and cardiovascular diseases

Chronic hypertension and myocardial infarction are associated with cardiac remodeling, a pathological process leading to heart failure and mortality (Grobe et al., 2007; Maaliki et al., 2019). The remodeling occurs following prolonged and enhanced production of AngII and inhibition of the ACE1/AngII/AT1R system confers protection from these effects. In fact, induced overexpression of ACE2 reverses cardiac remodeling (Grobe et al., 2007) in a manner mediated by Ang1-7, which specifically reduced myocyte hypertrophy and interstitial fibrosis without affecting blood pressure. Furthermore, overexpression of ACE2 prevents cardiac hypertrophy in hypertensive rats (Sriramula et al., 2011). In a related study, ACE2 mRNA expression was markedly reduced in rat models of hypertension and was associated with defects in cardiac contractility, increased AngII levels, and upregulation of hypoxia induced genes (Alaaeddine et al., 2019). Drugs used to treat hypertension, such as the AT1R blockers (ARBs),
upregulate myocardial expression of ACE2 and reduce levels of inflammatory markers like MCP-1, interleukins and NF-κB (Sukumaran et al., 2012). The ACE1/AngII/AT1R system also promotes atherosclerotic lesion formation, aneurysms, and proinflammatory cytokine secretion (Daugherty et al., 2000). ACE2 has been shown to counteract these effects. For example, knock out studies of ACE2 in mice led to plaque growth as well as an increase in several proinflammatory and atherogenic proteins like adhesion molecules, IL-6 and MCP-1 (Thomas et al., 2010).

3.2.2 ACE2 and CVD symptoms in COVID-19 patients

Cases of acute cardiovascular anomalies have been reported in patients with COVID-19 that were previously healthy. Patients show high levels of tropon, creatine kinase, arrhythmias as well as incidences of myocardial injuries which lead to higher rates of intensive care unit (ICU) admission and mortality (Guo et al., 2020; Inciardi et al., 2020; Wang et al., 2020a). Patients with high troponin levels also had high levels of C-reactive protein, suggesting an inflammatory pathway linked to the SARS-CoV-2-induced myocardial injury (Guo et al., 2020). Thus, myocardial injury seen could be attributed to SARS-CoV-2 direct viral entry as well as an increased production of cytokines that can result in decreased coronary blood flow as well as oxygen supply (Oudit et al., 2009). As well, similar to other respiratory infections (Milbrandt et al., 2009), COVID-19 patients demonstrate elevated D-dimer levels that were also associated with disseminated intravascular coagulation, severe symptoms, and higher risk of mortality (Guan et al., 2020; Zhou et al., 2020a). Contributary mechanisms could include a series of inflammatory-immunological reactions, which could directly contribute to atherosclerotic plaque rupture predisposing the patient to ischemia and thrombosis (Zhou et al., 2020a).

Significantly, ACE2-mediated viral endothelial entry could induce the vascular disorders and an accelerated coagulation in patients with COVID-19 (Gallagher et al., 2008). Indeed, SARS-CoV-2 was shown to infect endothelial cells in different vascular beds in COVID-19 patients (Varga et al., 2020). Treatment with clinical grade human recombinant ACE2 precluded SARS-CoV-2 infection in blood vessel organoids highlighting the importance of ACE2 in endothelial infection (Monteil et al., 2020). The endothelium plays a critical role in the regulation of the adherence of immune cells, capillary permeability as well as clotting and platelet activation, all of which could be altered by viral infection (Dalrymple and Mackow, 2014). Under normal conditions, vascular endothelial growth factor (VEGF) induces repair of vascular damage. Hanta virus, a virus affecting respiratory system, was shown to bind to VEGF and disengage the normal regulation of VEGF-induced permeability (Gavrilovskaya et al., 2012). A similar profile is encountered in patients with COVID-19. Indeed, serum VEGF levels were elevated in COVID-19 patients (Huang et al., 2020) possibly triggered by hypoxia and inflammatory changes and contributing to increased vascular permeability and pulmonary edema. Interestingly, increased ACE2 expression was shown to reduce VEGF production in vitro and in vivo (Cheng et al., 2016) ameliorating increased vascular permeability during lung injury (Yu et al., 2016) raising the question of whether SARS-CoV-2-induced endothelial ACE2 downregulation could underlie this observation. These findings highlight the importance of monitoring VEGF levels in patients with COVID-19 and also suggests a possibility of introducing treatment modalities to target VEGF responses. Bevacizumab, a humanized monoclonal anti-VEGF antibody, is currently being studied for a potential beneficial effect in reducing lung injury due to increased vascular permeability (Clinicaltrials.gov: NCT04275414) (ClinicalTrials.gov/ct2/show/NCT04275414, 2020).
On the other hand, accumulating evidence indicate an association between existing cardiovascular and metabolic disease with further progression of severe COVID-19 complications (Liu et al., 2020a; Rodriguez-Morales et al., 2020; Wehbe et al., 2020; Zhou et al., 2020a). Higher mortality rates as well as progression of ARDS were reported in patients with hypertension, diabetes, and coronary artery diseases (Wu et al., 2020; Wu and McGoogan, 2020). This could be attributed to stimulation of immuno-inflammatory pathway, dysregulation of RAS, and most importantly viral entry through upregulated ACE2 where previous work showed that patients with heart failure had a higher ventricular ACE2 expression levels (Zisman et al., 2003). Indeed, a recent study reported that COVID-19 patients with heart failure had higher cardiac ACE2 expression (Chen et al., 2020a). Specifically, higher ACE2 expression was observed in cardiac pericytes raising the possibility that cardiac injury could be initiated by microvascular dysfunction. Moreover, ACE2 expression in vascular endothelial cells could facilitate localized vascular injury and subsequent viral spread. As such, in situations of altered vascular function; vascular inflammation and/or increase in VEGF, SARS-CoV-2 vascular viral entry and viral spread could be aggravated.

### 3.3 Inflammatory disease: Obesity, diabetes, and auto-immune disorders

ACE2 activity has been detected in macrophages where its deficiency led to an increased production of AngII, pro-inflammatory cytokines like TNFα, MCP-1, IL-6 and matrix metalloproteinase-9 (MMP-9), and endothelial adhesion (Thatcher et al., 2011; Thomas et al., 2010). Importantly, overexpression of ACE2 by macrophages significantly reduced their MCP-1 production (Guo et al., 2008) and Ang1-7 treatment decreased macrophage inflammatory response (Souza and Costa-Neto, 2012). These findings further support the vital role of ACE2 in relieving the pro-inflammatory response and thus both low- and high-grade inflammatory conditions could affect the pathogenesis of COVID-19.

Interestingly, obesity is associated with increased RAS activity in adipose tissue (Yvan-Charvet and Quignard-Boulangé, 2011) together with increased macrophage infiltration (Weisberg et al., 2003). Adipose tissue macrophages in obesity tend to acquire macrophage (M1) polarization with increased expression of pro-inflammatory cytokines (Lumeng et al., 2007). Under such circumstances, adipose inflammation in obesity contributes to insulin resistance, glucose intolerance, and cardiovascular dysfunction (Elkhatib et al., 2019; Jiao and Xu, 2008). Interestingly, ACE2 deficiency enhanced M1 polarization of adipose tissue macrophages, increased adipose tissue inflammation, exacerbated the metabolic dysfunction and also worsened the associated cardiovascular function in diet-induced obesity (Patel et al., 2016a; Thatcher et al., 2012). Indeed, recent evidence implicate obesity as a risk factor for severe ARDS in COVID-19 patients (Simonnet et al.). Whereas no change in adipose tissue ACE2 expression levels was observed in obese patients compared to lean individuals (Pinheiro et al., 2017), the increased disease severity could be attributed to the overall inflammatory state resulting from an obesity-triggered systemic RAS imbalance (Engeli et al., 2005).

Moreover, functional and clinical evidence supports the role of vascular inflammation induced by metabolic diseases in vascular impairment and CVD (Assar et al., 2016; Ormazabal et al., 2018). Patients with type 2 diabetes mellitus have reduced levels of ACE2 expression in various organs, which correlate with inflammatory changes especially in the kidney (Bindom and Lazartigues, 2009). Along the same lines, high levels of VEGF were observed that were correlated with high fasting blood glucose and glycosylated hemoglobin (HbA1c) levels (Zhang
et al., 2018). Increases in VEGF reflected the severity of endothelial dysfunction in diabetic patients and may lead to microvascular complications. As well, higher levels of VEGF are associated with diabetes-induced central and peripheral neuropathy (Atif et al., 2017; Jeric et al., 2017; Tang et al., 2018). Collectively, vascular inflammation in patients with CVD and higher expression of VEGF in diabetic patients could be additional factors to enhance viral entry and viral spread which consequently increase COVID-19 severity in those patients. It is noteworthy that increased usage of VEGF inhibitors could induce cardiovascular and nephrological consequences, as such extreme care should be taken in treatment with such patients (Kikuchi et al., 2019). Moreover, it is noteworthy that patients who previously recovered from SARS-CoV-1 infection reported high blood pressure as well as altered metabolism 12 years later (Wu et al., 2017). Knowing the similarities between SARS-CoV-1 and -2, a similar profile in could also occur in patients with COVID-19.

ACE2 is also implicated in the autoimmune disease of rheumatoid arthritis. Mice models of antigen-induced arthritis portrayed elevated levels of neutrophils in the knee joints and treatments with Ang1-7 or its analogue, AVE0991, were able to reduce neutrophil migration in joints and peri-articular tissue (da Silveira et al., 2010). Likewise, the elevated levels of pro-inflammatory cytokines TNFα, chemokine (C-X-C motif) ligand (CXCL1) and IL-1β were reduced by Ang1-7 treatment together with hypernociception, the pain index of arthritis (da Silveira et al., 2010). Interestingly, this effect was not mediated by hypotensive or vasodilatory actions. Thus, Ang1-7 is able to mediate these effects by inhibiting local production of cytokines and, importantly, by hindering leukocyte-endothelium adhesion and rolling on the microvasculature of the knee joint. Importantly, Ang1-7 receptors have been detected on microvessels as well as leukocytes (Nie et al., 2009). These findings suggest that loss of ACE2 activity in arthritic patients may inflame symptoms and cytokine production. Significantly, a recent meta-analysis showed some indication that autoimmune diseases might be associated with an increased risk of COVID-19 severity and mortality (Liu et al., 2020b), while on the other hand, emerging reports indicate that SARS-CoV-2 infection precedes the development of various autoimmune disorders (Galeotti and Bayry, 2020). Yet in either case, the role of ACE2 expression or activity alterations has not been examined.

3.4 Renal disease

3.4.1 ACE2 in renal disease

ACE2 is abundantly expressed on the vascular endothelium supplying the kidneys and on the tubular and glomerular epithelium (Lely et al., 2004). Mice lacking ACE2 develop late onset nephrotic glomerulosclerosis (Oudit et al., 2006) and more profound diabetic renal injury (Wong et al., 2007), where ACE2 activity was shown to be protective by reducing local AngII levels (Batlle et al., 2012). Indeed, in a related study of diabetic mice, RAS imbalance involving ACE2 downregulation was found to be involved in renal damage mediated via elevated reactive oxygen species (Chang et al., 2011). Importantly, the renoprotective effects of ACE2 are not only restricted to chronic disorders. While renal ischemia and reperfusion trigger acute kidney injury via activation of inflammatory cascades involving increased migration of leukocytes and release of cytokines, treatment with the Ang1-7 analogue AVE0991 reduced renal injury and inhibited leukocyte migration in mice post-ischemia reperfusion (Barroso et al., 2012). These findings broadly categorize the ACE2 axis once again as anti-inflammatory. However, the role of ACE2/Ang1-7 appears to be rather complex and should be approached with caution as other studies have conversely demonstrated that this pathway may contribute to certain forms of kidney injury in diabetes including fibrogenesis and renal hypertrophy (Tikellis et al., 2008).
possibly affected by differences in disease stage, locally activated signaling pathways, and dosages of the activating or inhibiting agents used (Zimmerman and Burns, 2012).

3.4.2 ACE2 and renal symptoms in SARS-CoV-2 patients

Acute kidney injury was identified in up to 25% of patients admitted with COVID-19 (Fanelli et al., 2020) representing a higher mortality risk in these patients (Wilson and Calfee, 2020). A similar profile was seen in two other studies where patients showing progressive increases in blood urea nitrogen as well as creatinine had higher mortality rates (Chen et al., 2020b; Wang et al., 2020a). The mechanism behind this renal injury could be multifactorial. While ischemic injury and inflammatory cytokines are likely to contribute to renal damage, direct SARS-CoV-2 entry through ACE2 to the renal cells could still be a major factor. SARS-CoV-2 entry in a human kidney cell line (MDBK) required priming by both TMPRSS2 as well as the endosomal cysteine proteases Cathepsin B & L (Hoffmann et al., 2020). However, viral entry in ACE2 expressing HEK293 cells was reported to be mediated through endocytosis where other proteins as PIKfyve, two pore segment channel 2 (TPC2), and Cathepsin L were found essential for virus entry (Ou et al., 2020). Significantly, a recent study showed cellular damage and direct ultrastructural evidence of viral infection in proximal tubular epithelial cells in post-mortem examination of a COVID-19 patient with acute kidney injury (Farkash et al., 2020). Yet other studies argue against a causal relationship between COVID-19 infection and acute kidney injury (Wang et al., 2020b). Indeed, the same factors that contribute to SARS-CoV-2 infection severity and involve alteration of ACE2 activity/expression including age, obesity, and diabetes contribute to increased risk of acute renal dysfunction (Fanelli et al., 2020) making the establishment of causal relationships and the examination of a role for ACE2 difficult in this context.

3.5 The reproductive system

3.5.1 ACE2 in reproductive diseases

While ACE1, AngII, AT1R, AT2R, ACE2, Ang1-7 and Ang1-7 receptors are all involved in the reproductive physiology in both males and females, ACE2 was found to play an important role in the testis with expression specifically enriched in Leydig cells and cells in the seminiferous tubules (Donoghue et al., 2000; Pan et al., 2013). In males, ACE1/AngII and ACE2/Ang1-7 balance drives optimal male fertility including steroidogenesis, epididymal contractility and sperm cell function. Males with impaired spermatogenesis do not have detected levels of Ang1-7 or Ang1-7 receptors in seminiferous tubules as opposed to healthy males (Reis et al., 2010). Also, lower levels of ACE2, Ang1-7 and Ang1-7 receptors in the testis are correlated to severe impairment in spermatogenesis and lower testicular weight (Reis et al., 2010). ACE2 is specifically implicated in spermatogenesis due to its presence in Leydig cells which produce sex hormones. Moreover, ACE2 is being increasingly considered as a therapeutic mechanism to improve male fertility (Pan et al., 2013). As well, the expression of ACE2, Ang1-7 and Ang1-7 receptors in human ovaries is directly affected by gonadotropin suggesting an important role in ovarian physiology, follicular development and ovulation (Pereira et al., 2009; Reis et al., 2011). Collectively, disruption in ACE2 in male and female reproductive cells may contribute to reproductive impairments and polycystic ovarian syndrome (PCOS) respectively. Furthermore, its presence within the testes makes them vulnerable to possible damage by SARS-CoV-2.

3.5.2 ACE2 and reproductive impairment in SARS-CoV-2 patients
SARS-CoV-induced viral testicular tissue damage has been reported. SARS-CoV lead to germ destruction, loss of spermatozoon in seminiferous tubules as well as leukocyte infiltration (Xu et al., 2006). The possibility of viral orchitis following SARS-CoV-2 infection potentially leading to testicular damage and infertility remains high (Cardona Maya et al., 2020). As such, it is recommended that proper care is given to male patients with COVID-19 to guard against possible orchitis and follow up in males recovering from COVID-19 is equally important. Furthermore, exacerbation of PCOS symptoms in females may also need proper follow-up.

3.6 The digestive system

3.6.1 ACE2 and the digestive system

Although ACE2 is a key component of the RAS system, it has an independent role in the gut. ACE2 is necessary for the expression of transporters of neutral amino acids in the small intestine (Hashimoto et al., 2012; Perlot and Penninger, 2013). Furthermore, ACE2 has been shown to confer protection against colitis. This was also directly correlated with a reduced uptake of Tryptophan, one of the main functions of ACE2 in the gut. A reduction in Tryptophan results in a reduction in the mTOR pathway in the small intestine. This impairs the expression of antimicrobial peptides leading to an altered intestinal microbiome and thus an increased susceptibility to colitis (Perlot and Penninger, 2013). Hence, ACE2 has a key role in maintaining a protective healthy biome in the gut and its function involves an mTOR pathway.

3.6.2 ACE2 and digestive impairment in SARS-CoV-2 patients

Reports from Wuhan highlighted the enteric involvement of COVID-19. 2-10% of patients with COVID-19 initially presented to hospitals with gastrointestinal symptoms as diarrhea, abdominal pain, and vomiting (Chen et al., 2020b; Yang et al., 2020). Patients expressed symptoms 1-2 days prior to the development of respiratory symptoms and fever. Moreover, patients who received ICU care were more likely to report abdominal pain and anorexia (Wang et al., 2020a). The intestine represents one of biggest immune system compartments, highly vascularized and innervated. Previous studies reported that the gut impacts the viral immune and neuronal systems and also affects pulmonary function (Mowat and Agace, 2014; Shenoy et al., 2014). In fact, the progression of intestinal infection, inflammation and subsequent live virus emergence in the lungs suggests the development of sequential respiratory infection. Intestinal cells are highly permissive to coronaviruses regardless of the stage of cell differentiation suggesting a high sensitivity of the intestinal epithelial cells to these viruses (Cinatl et al., 2004; Zhou et al., 2017). Furthermore, ACE2 is expressed in the oral cavity, particularly in epithelial cells of the tongue. Also, lymphocytes in the oral cavity express ACE2 receptors in a similar density as those in the lungs (Xu et al., 2020). The stomach and the esophagus have not yet displayed infected epithelial cells. SARS-CoV-2 infection could have been initiated by eating raw food from the Wuhan market, the center of coronavirus outbreak. Yet, ACE2 being located in the basolateral membrane of the oral mucosa makes the viral uptake via this route less likely to be efficient. However, its presence on the apical surface of the enterocytes and the detection of the virus in stool samples provides a possible explanation and highlights the possibility of fecal to oral transmission as stated by previous studies (Yeo et al., 2020).

3.7 The bile duct

3.7.1 ACE2 and biliary impairment in SARS-CoV-2 patients

The possible involvement of hepatotoxicity in COVID-19 patients is questionable. Liver injury is found to be more prevalent in severe than in mild cases of COVID-19 and significant increases
in liver enzymes and bilirubin were reported in 20-37% of cases (Chen et al., 2020b; Huang et al., 2020). Taking this into account, it is not clear whether liver injury in COVID-19 patients is mainly due to direct entry of the virus into liver cells or induced by certain drugs following treatment. ACE2 has not been detected on hepatocytes, but rather on cholangiocytes of the bile ducts (Hamming et al., 2004). However, the role of ACE2 in liver disease is of great importance as it participates in the regulation of liver inflammation, tissue remodeling and liver fibrosis (Rajapaksha et al., 2019). The protective pathway of ACE2 is thought to be mediated through a significant reduction in AngII-induced fibrosis, TGF-β and NADPH oxidase (Rajapaksha et al., 2019). A study by Chai et al identified cholangiocyte-specific expression of ACE2, which could have been a possible route of SARS-CoV-2 entry and hence could lead to profound liver toxicity in patients with COVID-19 (Chai et al., 2020). Moreover, ACE2 expression and activity were shown to induced as a response to chronic liver injury (Osterreicher et al., 2009). As such, this begs the question of whether patients with chronic hepatic inflammatory conditions might be at a higher risk of SARS-CoV-2-induced liver damage. Subsequently proper monitoring of liver function should be undertaken to guard against liver injury in patients with COVID-19.

3.8 The nervous system

3.8.1 ACE2 and the brain

ACE2 is also found in the brain (Xu et al., 2011). The highest activity of ACE2 in the central nervous system is detected in the hypothalamus (Xu et al., 2011). AngII has been shown to reduce levels of ACE2 expression in cerebral and medullary astrocytes, though the exact role is still elusive. Conversely, ACE2 overexpression in the paraventricular nucleus (PVN) has been shown to directly impede AngII induced hypertension (Sriramula et al., 2011). As such, aside from the local neurogenic effects, even within the brain, ACE2 can impart cardioprotective roles.

3.8.2 ACE2 and CNS symptoms in SARS-CoV-2 patients

Of the most common atypical symptoms in patients with COVID-19 are the neurological signs which were among 45.5% of patients with severe cases (Mao et al., 2020). In this study headache (13.1%), dizziness (16.8%) and impaired consciousness (14.8%) were the most common signs of viral neuro-invasion. Other studies also reported the prevalence of neurological symptoms as well (Baig et al., 2020; Chen et al., 2020b; Wang et al., 2020a). Moreover, a new COVID-19 case was reported to have altered mental status that developed into hemorrhagic necrotizing encephalopathy (Poyiadji et al., 2020). Moreover, a sudden loss of smell and/or taste is common in patients infected with SARS-CoV-2 possibly occurring without any other typical symptoms highlights the central nervous system (CNS) involvement as a COVID-19 manifestation (Gautier and Ravussin, 2020). Previous studies detected SARS-CoV in the brain. The virus was administered via the intranasal route where it enters through the olfactory neurons and heavily infect several areas of the brain including piriform and infralimbic cortices, basal ganglia, and midbrain with first- or second-order connections with the olfactory bulb (Netland et al., 2008). Other sites, including those in the brainstem, were shown to have been possibly infected via the oral route. In this study SARS-CoV infection induced neuronal death in absence of encephalitis in mice. Moreover, SARS-CoV is thought to invade the peripheral nerve terminals then get access to the CNS via the synapse connected route (Li et al., 2012; Li et al., 2013). More studies have documented the neuro-invasion of other coronaviruses, where it was shown to spread through peripheral nerves into the medullary neurons involved in the peristaltic movement of the digestive system leading to vomiting (Li et al., 2020c). The transfer between nerves could be
through clathrin-coated mediated endocytosis/exocytosis pathway (Li et al., 2013). As for SARS-CoV-2, the latency period of the virus could be sufficient for neuroinvasion. Besides, severe development of COVID-19 could be linked to neuroinvasion and subsequent dysfunction in the cardiorespiratory center in the brainstem as reported elsewhere (Netland et al., 2008). On the other hand, further to viral entry to neurons via ACE2 receptors, brain ischemia could also take place as a result of downregulation of ACE2 expression (Simoes et al., 2017). As such, care should be taken to guide towards proper treatment and prevention of possible neuroinvasion of SARS-CoV-2 induced cardiorespiratory failure (Li et al., 2020c). Altered consciousness could be as devastating as respiratory distress syndrome.

4. ACE2 protein interactions and associated signaling pathways

4.1 ACE2 interacts with various proteins

It is well known now that SARS-CoV-2 and SARS-CoV share 76% amino acid identity and utilize ACE2 as a receptor for entry (Lu et al., 2020). The spike (S) proteins of coronaviruses are responsible for attachment to target cells and cellular invasion (Fehr and Perlman, 2015). Priming of SARS-CoV S proteins by host cell proteases is fundamental for proper viral uptake (Matsuyama and Taguchi, 2009; Simmons et al., 2005; Simmons et al., 2004). Several proteases have been previously reported to activate SARS-CoV S protein. Yet in the absence of proteases on the cell surface, SARS-CoV entry occurs through endosomal pathway where S protein is activated by endosomal proteases. However, when proteases are expressed on the cell surface, viral replication is shown to be 100 times higher (Matsuyama et al., 2005). In the latter case, S proteins will be activated and cleaved at S1/S2 and S2’ site. The cleavage at S2’ site exposes S2 domains and enable the partition of membrane by fusion peptide (FP) and internal fusion peptide (IFP) and facilitate viral entry (Lu et al., 2015).

Although ACE2 is the receptor for SARS-CoV-1 and SARS-CoV-2, there are major differences between infectivity, onset of symptoms, severity of symptoms and mortality rate. SARS-CoV-2 poses an immense challenge compared to the SARS pandemic of 2003. Whereas a total of 8,098 patients worldwide got infected with the first SARS-CoV, out of whom 774 had died (CDC, 2017), up to the time this manuscript was written, SARS-CoV-2 resulted in over 36 million infections and over one million deaths. While, 100% of SARS-CoV-1 patients had fever, only around 44% of SARS-CoV-2 individuals displayed elevated temperatures. Additionally, 3.8% SARS-CoV-2 patients have displayed diarrhea compared to the 22% of individuals infected with SARS-CoV-1 (Huang et al., 2020). One explanation for this difference is that the S protein of SARS-CoV-2 binds with greater affinity to ACE2 than SARS-CoV-1. The disparity in the furin-like S2’ cleavage site between SARS-CoV-1 and 2 may impart unique ACE2-protein interactions and signaling cascades that ultimately play a role in the differences noted between these two coronaviruses (Coutard et al., 2020). Because of these reasons, it is necessary to characterize established and potential interactions with the receptor.

On the apical membrane of intestinal enterocytes, ACE2 has been shown to colocalize with the broad neutral amino acid transporter B₀AT1 (SLC6A19) and CD13 (Jando et al., 2017). CD13 is a type II transmembrane zinc metalloprotease which catalyzes the removal of amino terminal amino acids from peptides. The expression of B₀AT1 directly depends on the co-expression of ACE2. B₀AT1 is also detected in the kidney proximal tubule in association with collectrin (TMEM27) which interestingly shares the same membrane anchoring domain of ACE2. In this context, ACE2 is considered a dimer and the interaction with B₀AT1 implicates that S protein is
able to bind to two sites. The collectrin-like membrane anchoring domain in ACE2 has been shown to enable the interaction with B0-AT1. Collectrin is considered a homolog of ACE2 (Zhang and Wada, 2007). Not only is it involved in amino acid uptake, but it has roles in development of renal epithelial cilia and insulin secretion. Importantly, it can interact with the SNARE complex via snapin. SNARE is indispensable for enabling the fusion of vesicles to their target intracellular compartments and directing vesicles to the extracellular membrane, as in the case of secreting insulin (Martens and McMahon, 2008). It is worth examining the role of ACE2 in similar processes, particularly in the context of viral uptake. Although B0-AT1 is not necessarily prominent in the lungs, the homology between ACE2 and collectrin may highlight similar roles for this receptor making it prudent to delineate it in the context of viral infection.

TMPRSS2 is a type II membrane serine protease that has been shown to cleave the S protein to allow efficient entry (Matsuyama et al., 2010). It should be noted that HAT is a similar protein that was shown to activate SARS-COV-1 but, to our knowledge, has not been confirmed in the context SARS-COV-2. Importantly, TMPRSS2 also can cleave the ACE2 receptor and this event is implicated in increased viral invasion. However, the processing of ACE2 does not have any effect on cleavage of the viral S protein itself. Increased expression of TMPRSS2 is directly associated with increased viral entry. As such, the association of TMPRSS2 with ACE2 spurs increased viral entry by cleavage of ACE2 and enhances S protein membrane fusion by direct cleavage of the S protein (Heurich et al., 2014b).

Cathepsin L is also a well-studied protease in SARS-COV infections. Contrary to TMPRSS2, cathepsin L (catL) is an endosomal and lysosomal pH dependent protease that process S protein after uptake. However, initial cleavage by TMPRSS2 is independent of catL and is in fact the main way of entry (Heurich et al., 2014b).

Combination of the S protein with ACE2 also triggers processing of ACE2 by ADAM16/TACE (disintegrin and metallopeptidase domain 16/tumor necrosis factor alpha converting enzyme). This leads to shedding of a functional ACE2 into extracellular space and promotes uptake of SARS-CoV-1. It is likely that a similar mechanism occurs upon S protein binding of SARS-CoV-2 (Heurich et al., 2014b).

Another protease that is worth examining is furin and its related proteins. The unique cleavage site on S2’ is postulated to interact with furin or furin-like proteases which are associated with more potent virulence. Hence, upon conducting a protein blast we detected proteins with considerable sequence homology that may also potentially interact with the S protein (Table 1).

4.2 Signaling Pathways Associated with SARS-CoV-2 entry

Not only is ACE2 the mode of entry of SARS-CoV-1 and -2 but it is also involved in its major symptom, the untreatable ARDS. MAPK pathways involving ERK, p38 and JNK are significantly activated and associated with the process of ARDS. Lipopolysaccharides (LPS)-induced lung damage is diminished upon inhibition of p38 and JNK. The activity of p38 in the lung is linked to pulmonary edema and fibrosis. In the context of the heart, it was shown that ACE2 can directly inhibit ERK thereby reducing cardiac fibrosis. We have previously highlighted the involvement of MAPK pathways in the manifestation of cardiovascular diseases like hypertrophy and atherosclerosis as well as the possible hijack of these pathways to assist SARS-CoV-2 host internalization and replication (Wehbe et al., 2020).

ACE2 present on microvascular endothelial cells has been shown to inhibit local ERK and JNK pathways thereby avoiding apoptosis and inflammation under LPS-induced inflammatory
conditions (Li et al., 2016). It is worth examining if inhibition of ACE2 to block viral entry, while infusion with Ang1-7, is sufficient to maintain the protective effect of ACE2 on various organs involved.

5. Concluding Remarks

SARS-CoV-2 invasion is proving to be a complex and multi-faceted issue, making it difficult to quell. Much of the complexity arises from the wide physiological distribution of its favored receptor, ACE2. Adding further insult to injury is the dual role of ACE2 in this viral dilemma. As we have demonstrated, the protective role of this receptor is important in mitigating several diseases and offering proper homeostasis. Potential therapeutic mechanisms should include ways to preserve its regulatory role, while shunting downstream events that may be hijacked for viral entry. For example, further investigation in protein-protein interactions and signaling pathways associated with ACE2 may offer various perspectives on how to subvert viral-induced uptake and organ injury.

Conflicts of Interest: None to declare

Authors contribution:

Wrote or contributed to the writing of the manuscript: Hammoud, Wehbe, Abdelhady, Kobeissy, Eid, and El-Yazbi
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### Table 1. Potential Proteins that may be involved in S protein processing and ACE2 interaction

<table>
<thead>
<tr>
<th>Protein Implicated in SARS-CoV-2 Entry</th>
<th>BLASTp Results in Human Protein Database</th>
<th>Sequence identity (filter: min ~ 50%)</th>
<th>Relevance (UniProKb Database)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furin Protease</td>
<td>Cadherin Related Family Member 4</td>
<td>55.6%</td>
<td>Expressed in Lung, Uterine Tube, Testis (Similar Pattern to ACE2 prominent distribution)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Single Pass Transmembrane Protein</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Isoform 7 of Tumor necrosis factor receptor superfamily member 25</th>
<th>50%</th>
<th>Interacts with receptor adapter protein TRADD and activates NFkB which are involved in inflammatory-induced apoptosis.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>May play a role in regulating lymphocyte homeostasis.</td>
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