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Angiotensin Converting Enzyme 2: A Double-Edged Sword

Running Title: *Wang et al.; ACE2: A Double-Edged Sword*

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Angiotensin converting enzyme 2 (ACE2) has garnered much attention given the current COVID-19 pandemic as the cellular receptor for Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). ACE2 was discovered twenty years ago based on approaches searching for ACE homologues and was initially cloned from human heart failure ventricular and lymphoma cDNA libraries.[1] Since then, two major functions have been identified for ACE2 as (1) an endogenous counter-regulator of the renin-angiotensin system (RAS), (2) a cellular receptor for SARS-CoV and SARS-CoV-2 viruses.

ACE2 is ubiquitously expressed with highest levels detected in the cardiovascular (CV) system, gut, kidneys and lungs. In the CV system, ACE2 is expressed in cardiomyocytes, epicardial adipose tissue, cardiac fibroblasts, vascular smooth muscle and endothelial cells.[1, 2] ACE2 is a type I transmembrane protein that functions as a monocarboxypeptidase with a catalytically active ectodomain exposed to the circulation that hydrolyzes various peptides, including angiotensin II (Ang II) and angiotensin I (Ang I) generating angiotensin 1-7 (Ang 1-7) and angiotensin 1-9, respectively.[1] A soluble form of ACE2 can be released from the membrane through proteolytic cleavage mediated by ADAM17 resulting in loss of ACE2 protection against tissue RAS and increased plasma ACE2 activity, a known marker of adverse prognosis in patients with CV disease.

The discovery of ACE2 introduced an alternative protective arm, ACE2/Ang 1-7/Mas receptor axis, to counterbalance the more renowned pathogenic ACE/Ang II/AT_1 receptor axis that predominates in disease states due to RAS overactivation (Figure A). Cleavage of Ang I by ACE generates Ang II, which is the primary effector peptide of the ACE/Ang II/AT_1 receptor axis, triggering potent vasoconstriction, inflammation, cell proliferation, hypertrophy, fibrosis and tissue remodelling. ACE2 cleaves Ang II into the cardioprotective Ang 1-7, which acts

through Mas receptors to counterbalance the detrimental effects of Ang II signalling. Therefore, ACE2 protects against RAS-induced injuries through two processes (1) degrading Ang I and Ang II to limit substrate availability in the adverse ACE/Ang II/AT_1 receptor axis, and (2) generating Ang 1-7 to increase substrate availability in the protective ACE2/Ang 1-7/Mas receptor axis.

Loss-of-function experiments using ACE2 knockout mice and ACE2 inhibitors have revealed increased susceptibility to myocardial infarction, hypertension and Ang II-induced myocardial hypertrophy, microvascular complications, inflammation, fibrosis, diastolic and systolic dysfunction and oxidative stress.[1, 2] Importantly, partial loss of ACE2, as seen in human hearts explanted from patients with heart failure and dilated cardiomyopathy, is sufficient to enhance the susceptibility to heart disease.[1] Conversely, gain-of-function experiments with recombinant ACE2, overexpression of ACE2, and supplemental Ang 1-7 have shown protective roles in various models of CV disease including hypertension, diabetes and heart failure with preserved ejection fraction (HFpEF).[1, 2] Pharmacological antagonists of the RAS, such as ACE inhibitors and ARBs, protect the CV system partly by increasing ACE2 levels in disease states. Clinical trials with intravenous infusion of recombinant human ACE2 (rhACE2) in patients with pulmonary arterial hypertension and acute lung injury reported immediate decreases in plasma Ang II/Ang 1-7 ratios reflecting ACE2 functions and its therapeutic effects.

Binding and entry of both SARS-CoV and SARS-CoV-2 into human cells is facilitated by the interaction between receptor-binding domain (RBD) of the S1 subunit on viral spike glycoproteins with the ectodomain of ACE2.[3] Endocytosis of ACE2 alongside viral particles into endosomes reduces surface ACE2 expression which represents an initial insult towards ACE2-mediated tissue protection. Of particular concern are the positive feedback pathways in place to

facilitate further down regulation of ACE2 expression following the initial endocytotic event, perpetuating tissue damage and imbalance of the tissue RAS from SARS-CoV-2 infections (Figure B). Viral entry is also facilitated by ADAM17 activity, which is upregulated by SARS-CoV, a process dependent on the ACE2 cytoplasmic domain. Upregulation in ADAM17 protease activity perpetuates loss of ACE2 from the cell surface, resulting in a shift away from the protective ACE2/Ang 1-7/Mas receptor axis towards the disease state and accumulation in Ang II. Ang II further upregulates ADAM17 activity in a well characterized positive feedback loop leading to the shedding of its regulator, ACE2, through the AT_1 receptors and downstream ERK/p38 MAP kinase signaling pathways as a sequela to SARS-CoV-2 receptor binding. Furthermore, ADAM17 also mediates the liberation of membrane bound precursors of TNFα, IFN-γ, and IL-4 pro-inflammatory cytokines into the circulation, giving rise to its alternative name, tumor necrosis factor converting enzyme (TACE). These cytokines, namely IL-4 and IFN-γ, downregulate cell surface expression of ACE2 and reduce ACE2 mRNA levels leading to another pathway for ACE2 loss from SARS-CoV-2-induced systemic and tissue inflammation.

In lung injury, deregulation of RAS through downregulation of ACE2 increases vascular permeability, pulmonary edema and severity of injury in SARS-CoV infections though actions of Ang II that are attenuated by AT_1 receptor blockade. In post-mortem autopsy samples of heart tissue from patients that succumb to SARS, increased myocardial fibrosis, inflammation and reduced myocardial ACE2 expression have been reported, along with detectable viral SARS-CoV genome, providing suggestive evidence for myocardial injury from SARS-CoV.[4] Despite the predominance of respiratory symptoms, acute cardiac and kidney injuries, myocarditis, arrhythmias, and gut and liver abnormalities occurs in COVID-19 patients[5], consistent with the widespread expression of ACE2. The loss of ACE2-mediated protection from the CV systems

following SARS-CoV-2 infection could contribute to the CV eventss observed in COVID-19 patients.[5]

Recombinant human ACE2 has entered into clinical trial in a cohort of 24 patients in China. Systemic delivery of rhACE2 (0.4 mg/kg i.v. twice a day for seven days) will hopefully sequester viral SARS-CoV-2 particles in the circulation preventing their interaction and subsequent internalization through endogenous ACE2 receptors while also activating the systemic protective axis of the RAS.

In summary, the bifunctional role of ACE2 as a "double-edged sword" turns off the RAS system and leads to beneficial effects but also mediates unique susceptibility to lung and CV disease in COVID-19 patients by serving as the SARS-CoV-2 receptor. The ACE2 double-edged sword can be carefully wielded to provide potential novel therapeutics for CV disease but also for COVID-19. Moreover, the long-term sequelae of COVID-19 survivors and their possible increased risk for lung and CV disease requires careful monitoring and follow-up informed by knowledge of ACE2 biology.

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Figure Legend.

Figure: Role of ACE2 in the renin-angiotensin system (RAS) and proposed mechanism for SARS-CoV-2-induced downregulation of cell surface ACE2 expression. (A) ACE2 balances the two axes of the RAS, increased ACE2 promotes the protective ACE2/Ang 1-7/Mas receptor axis and loss of ACE2 results in a shift toward diseased states characterized by overactivity in the ACE/Ang II/AT_1 receptor axis. (B) Viral spike glycoprotein of SARS-CoV-2 interacts with cell surface ACE2 and becomes internalized together through endocytosis, resulting in decreased surface ACE2 expression. The endocytic event upregulates ADAM17 activity, which cleaves ACE2 from the cell membrane, perpetuating the loss of ACE2 from tissue RAS. Loss of ACE2 leads to accumulation of angiotensin II, which through AT_1 receptors also upregulates ADAM17, resulting in further cleavage of cell surface ACE2. Soluble recombinant human ACE2 (rhACE2) is a promising therapeutic for SARS-CoV-2 through its ability to (1) sequester viral particles to prevent their interaction and subsequent entry through cell surface ACE2 (2) limit activities of angiotensin II and increase levels of protective angiotensin 1-7.