

REVIEW

Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System

Celebrating the 20th Anniversary of the Discovery of ACE2

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ABSTRACT: ACE2 (angiotensin-converting enzyme 2) has a multiplicity of physiological roles that revolve around its trivalent function: a negative regulator of the renin-angiotensin system, facilitator of amino acid transport, and the severe acute respiratory syndrome-coronavirus (SARS-CoV) and SARS-CoV-2 receptor. ACE2 is widely expressed, including, in the lungs, cardiovascular system, gut, kidneys, central nervous system, and adipose tissue. ACE2 has recently been identified as the SARS-CoV-2 receptor, the infective agent responsible for coronavirus disease 2019, providing a critical link between immunity, inflammation, ACE2, and cardiovascular disease. Although sharing a close evolutionary relationship with SARS-CoV, the receptor-binding domain of SARS-CoV-2 differs in several key amino acid residues, allowing for stronger binding affinity with the human ACE2 receptor, which may account for the greater pathogenicity of SARS-CoV-2. The loss of ACE2 function following binding by SARS-CoV-2 is driven by endocytosis and activation of proteolytic cleavage and processing. The ACE2 system is a critical protective pathway against heart failure with reduced and preserved ejection fraction including, myocardial infarction and hypertension, and against lung disease and diabetes mellitus. The control of gut dysbiosis and vascular permeability by ACE2 has emerged as an essential mechanism of pulmonary hypertension and diabetic cardiovascular complications. Recombinant ACE2, gene-delivery of *Ace2*, Ang 1–7 analogs, and Mas receptor agonists enhance ACE2 action and serve as potential therapies for disease conditions associated with an activated renin-angiotensin system. rhACE2 (recombinant human ACE2) has completed clinical trials and efficiently lowered or increased plasma angiotensin II and angiotensin 1–7 levels, respectively. Our review summarizes the progress over the past 20 years, highlighting the critical role of ACE2 as the novel SARS-CoV-2 receptor and as the negative regulator of the renin-angiotensin system, together with implications for the coronavirus disease 2019 pandemic and associated cardiovascular diseases.

Key Words: cardiovascular diseases ■ coronavirus ■ dysbiosis ■ heart failure ■ renin-angiotensin system

Knowledge of the underlying biology and physiology of ACE2 (angiotensin-converting enzyme 2) has accumulated over the last 20 years since its discovery and has provided a major stimulus to further our understanding of the renin-angiotensin system (RAS).^{1–4} ACE2 has distinct roles ranging from catalytic activities with various substrates, as functional receptors for severe acute respiratory syndrome (SARS) coronaviruses (SARS-CoV), and as an amino acid transporter.^{5–8}

ACE2 functions as a master regulator of the RAS mainly by converting Ang (angiotensin) I and Ang II into Ang 1–9 and Ang 1–7, respectively.^{1,4} Both loss-of-function and gain-of-function approaches in experimental models of human diseases have defined a critical role for ACE2 in heart failure (HF), systemic and pulmonary hypertension (PH), myocardial infarction (MI), and diabetic cardiovascular complications.¹ Gut dysbiosis and altered gut permeability have emerged as an important mechanism

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Nonstandard Abbreviations and Acronyms

ACE	angiotensin-converting enzyme
ADAM-17	a disintegrin and metalloproteinase 17
Ang	angiotensin
ARB	AT ₁ R blocker
AT₁R	angiotensin II type 1 receptor
COVID-19	coronavirus disease 2019
CTGF	connective tissue growth factor
CVD	cardiovascular disease
HF	heart failure
IL	interleukin
MAPK	mitogen-activated protein kinase
MasR	Mas receptor
MCP-1	monocyte chemoattractant protein-1
MI	myocardial infarction
MMP	matrix metalloproteinase
NEP	neprilysin
PH	pulmonary hypertension
RAS	renin-angiotensin system
rhACE2	recombinant human ACE2
SARS	severe acute respiratory syndrome
SARS-CoV	SARS coronavirus
TACE	tumor necrosis factor- α converting enzyme
TMPRSS2	transmembrane protease serine 2
TNF-α	tumor necrosis factor- α

of disease controlled by the ACE2 axis in both vascular and lung diseases,^{9,10} as well as in diabetes mellitus.¹¹ Clinical and experimental studies support a physiological and pathophysiological role for ACE2 in cardiovascular disease (CVD), and increasing/activating ACE2 may elicit protective effects against hypertension and CVD, although this has yet to be proven clinically.^{1,12–14}

More recently, ACE2 has garnered widespread interest as the cellular receptor of SARS-CoV-2, the causative virus of the coronavirus disease 2019 (COVID-19) pandemic, which emerged from Wuhan, China, in late 2019.^{4,15,16} ACE2 offers protection in acute lung injury, suggesting that, although it facilitates viral entry at the epithelial surface, the ACE2/Ang 1–7 axis can be carefully manipulated to mitigate SARS-induced tissue injuries, which represents a potential target for therapeutic intervention.^{17,18} In experimental models of lung disease, catalytically active ACE2 alleviates pulmonary injury and vascular damage^{17,19} and prevent PH, decreased lung fibrosis, arterial remodeling, and improved right ventricular performance¹² due to a combination of direct action in the lungs and via the ACE2-dependent gut-lung axis.^{19,20} In 2 phase II clinical trials, administration of ACE2 was shown to reduce systemic inflammation and shifted the RAS

peptide balance away from Ang II toward Ang 1–7.^{21,22} Ongoing global efforts are focused on manipulating the ACE2/Ang 1–7 axis to curtail SARS-CoV-2 infection while affording maximal protective effects against lung and cardiovascular damage in patients with COVID-19. In this review, we summarize the diverse roles of ACE2, highlighting its role as the SARS-CoV-2 receptor and negative regulator of the RAS, and the implications for the COVID-19 pandemic. We also provide a framework for developing novel therapeutic strategies exploring the ACE2 pathway as it relates to CVD and COVID-19.

BASIC BIOCHEMISTRY

Discovery of ACE2

Following the initial and seminal discovery of renin in 1898 by Tigerstedt and Bergman, the RAS now encompasses a complex network of enzymes, peptides, and receptors (Figure 1).^{2,3,15,16,23–27} While many metalloproteinases cluster in small inter-related gene families (eg, the neprilysin [NEP] family), unusually, no human homolog of the vasoactive zinc-peptidase ACE (angiotensin converting enzyme) had been identified at the turn of the century. Almost simultaneously, in 2000, 2 independent approaches searching for such ACE homologs revealed the existence of a close relative of the ACE gene designated *ACEH2* or *ACE2*.³ *ACEH* was cloned from a human lymphoma cDNA library and the identical *ACE2* from a human HF ventricular cDNA library, the latter emphasizing a potential role for ACE2 in cardiovascular pathologies. Expression of the *ACE2* gene was initially established in the heart, kidney, and testis, but subsequent studies have shown a much broader distribution, including the upper airways, lungs, gut, and liver (Figure 2A). Sequence comparison of ACE and ACE2 strongly suggested that ACE2, like ACE, was an integral transmembrane protein (and ectoenzyme) with a transmembrane anchor close to the C-terminus (type I membrane protein). A close evolutionary relationship existed between the *ACE* and *ACE2* genes and it was presumed that the 2 proteins would have similar substrate specificities and involvement in the RAS.

As it turned out, important differences occur, particularly in the active site regions of the enzymes, such that the 2 enzymes counterbalance rather than reinforce each other's actions. Many subsequent studies over the next 20 years have revealed their inter-relationship, respective roles in the RAS, and multiple physiological and pathological actions from vasoactive peptide metabolism, importantly including not only Ang II but also apelin, to intestinal amino acid transport affecting innate immunity, to lung function and brain amyloid metabolism (converting A β 43 to A β 42, a substrate for ACE).^{1,4,28} Another unexpected twist in ACE2 biology was its identification in 2003 as the cell-surface receptor for the then

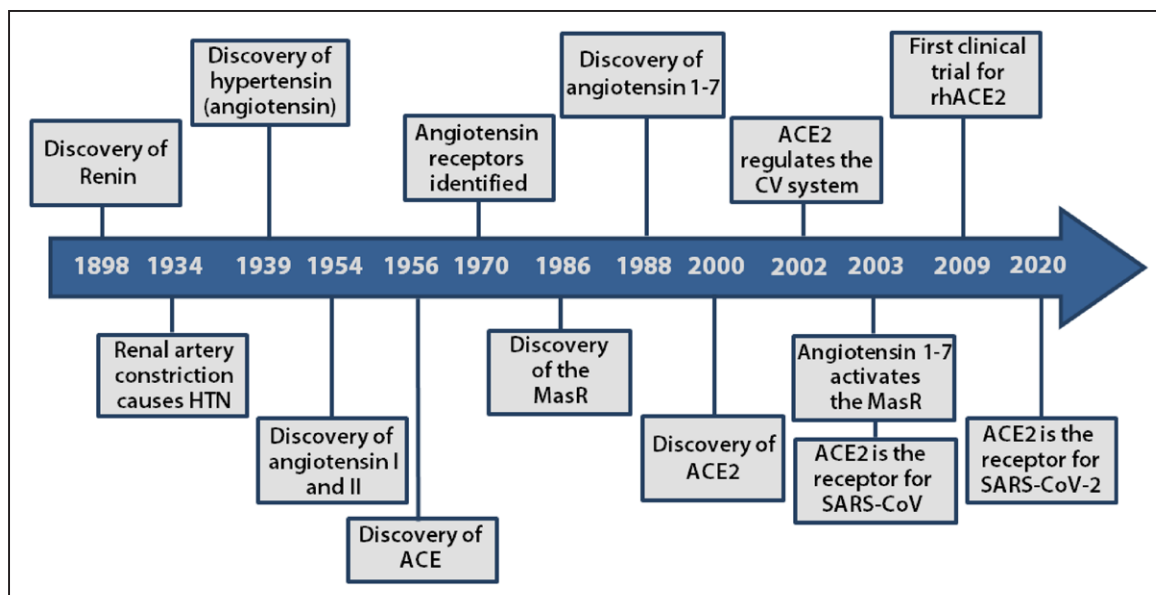


Figure 1. Historical timeline of discovery of the major renin-angiotensin system (RAS) components, including ACE2 (angiotensin-converting enzyme 2).

Renin was the first component of the RAS discovered following the finding that extracts from rabbit kidney produced pressor effects (Tigerstedt and Bergman, 1898). Constriction of the renal artery was then found to lead to hypertension (HTN), thus driving the discovery of hypertension and angiotensin (and later termed angiotensin; Goldblatt et al²⁷; Page and Helmer²⁴). Ang (angiotensin) was subsequently purified, and 2 forms were resolved: Ang I and Ang II. Therefore, the existence of a converting enzyme was predicted (ACE) and subsequently isolated and characterized (Skeggs et al²⁶). The counter-regulatory axis of RAS was then described, pioneered with the discovery of ACE2 by 2 independent research groups (Donoghue et al³; Tipnis et al²) and identification of the Ang1–7/Mas receptor axis (Santos et al¹⁰). The cardioprotective effects of ACE2 were discovered shortly after (Crackower et al²⁵). Studies have identified the ACE2 protease domain as the receptor for severe acute respiratory syndrome-coronavirus (SARS-CoV; Li et al⁵) and, more recently, as the SARS-CoV-2 receptor (Walls et al¹⁵; Yan et al¹⁶).

newly identified SARS-CoV that led to >8000 cases of SARS and almost 800 deaths,⁵ and as the receptor for SARS-CoV-2 that is currently devastating many countries worldwide.^{29,30}

The ACE2 Gene and Basic Biochemistry

Unlike the *ACE* gene, which is located on human chromosome 17, the 40kb *ACE2* gene is located on chromosome Xp22 and contains 18 exons, most of which resemble exons in the *ACE* gene. Whereas somatic ACE contains 2 active sites, ACE2 possesses only a single catalytic domain. Both ACE and ACE2 act as zinc metallopeptidases but of differing substrate specificities defining their distinct and counterbalancing roles in the RAS. Whereas ACE cleaves C-terminal dipeptide residues from susceptible substrates (a peptidyl dipeptidase), ACE2 acts as a simple carboxypeptidase able to hydrolyze Ang I, forming Ang 1–9 and Ang II to Ang 1–7 (Figure 2B). ACE2 does not cleave bradykinin, further distinguishing its specificity from that of ACE while it is also insensitive to conventional ACE inhibitors.^{2,28} The C-terminal domain of ACE2, which has no similarity with ACE, is a homolog of a renal protein, collectrin, which regulates the trafficking of amino acid transporters to the cell surface, endowing ACE2 with multiple and distinctive physiological functions. It is the multiplicity of physiological roles that ACE2 plays that has allowed it

to be hijacked by SARS-CoV-2 as a receptor, resulting in the COVID-19 pandemic.^{15,16} Structural studies have revealed the structures of both the SARS-CoV and much more recently, the SARS-CoV-2 in complex with ACE2 (Figure 2B).^{31,32} In the case of SARS-CoV-2, the major spike glycoprotein (S1) binds to the N-terminal region of ACE2. The knowledge of the biology and physiology of ACE2 accumulated over the last 20 years since its discovery should provide a major stimulus to understanding some of the key steps in SARS-CoV-2 infection and its ultimate prevention.

ROLE OF ACE2 IN COVID-19

COVID-19 Pandemic

On March 11, 2020, the World Health Organization declared the outbreak of SARS-CoV-2 a global pandemic, reporting community scale transmissions occurring in every continent outside Antarctica. Since then, the outbreak has escalated to well over one million cases and caused over 60 000 deaths worldwide by the start of April 2020. However, before the emergence of SARS-CoV in 2002, coronaviruses were conventionally viewed as inconsequential pathogens circulating in nature throughout various host and intermediate species that occasionally infected humans causing only mild upper respiratory tract infections and symptoms of the common cold.^{33–35} As such, to better understand the

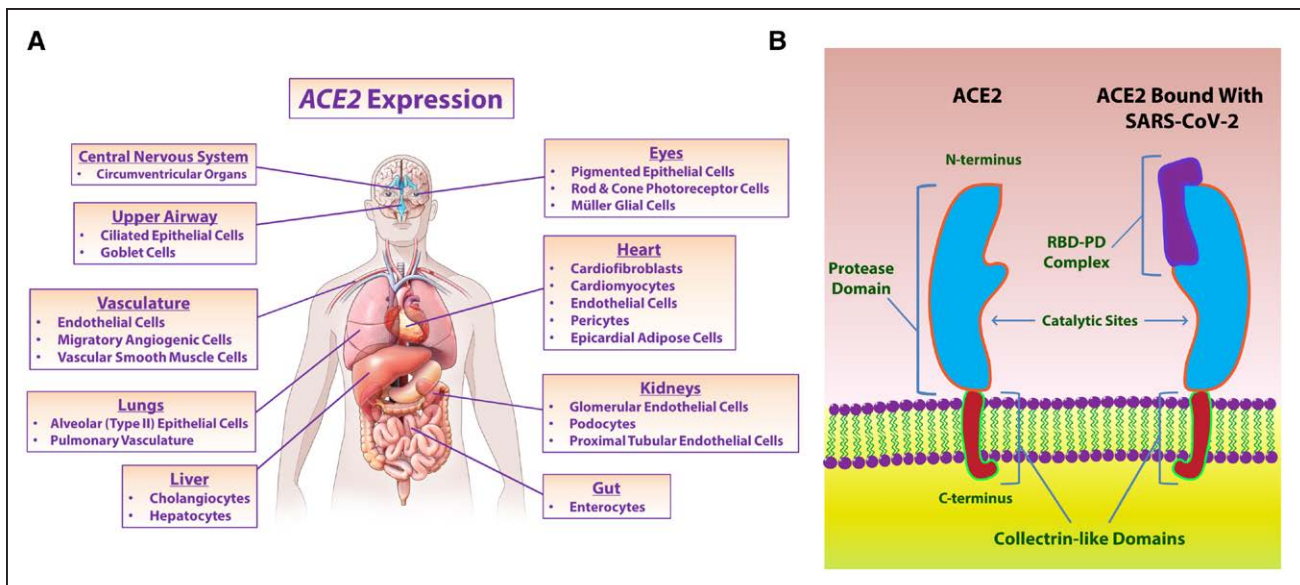


Figure 2. ACE2 (angiotensin-converting enzyme 2) expression throughout the body and schematic of ACE2 primary domains.

A, ACE2 is expressed in the vascular system (endothelial cells, migratory angiogenic cells, and vascular smooth muscle cells), heart (cardiofibroblasts, cardiomyocytes, endothelial cells, pericytes, and epicardial adipose cells) and kidneys (glomerular endothelial cells, podocytes and proximal tubule epithelial cells). ACE2 is also expressed and functions in the local RAS of the liver (cholangiocytes and hepatocytes), retina (pigmented epithelial cells, rod and cone photoreceptor cells and Müller glial cells), enterocytes of the intestines, circumventricular organs of the central nervous system, upper airway (goblet and ciliated epithelial cells), and alveolar (Type II) epithelial cells of the lungs and pulmonary vasculature. **B**, ACE2 has an extracellular facing N-terminal domain and a C-terminal transmembrane domain with a cytosolic tail. The N-terminal portion of the protein contains the claw-like protease domain (PD), while the C-terminal domain is referred to as the Collectrin-like domain. The receptor-binding domain (RBD) of severe acute respiratory syndrome-coronavirus (SARS-CoV-2) binds with the PD of ACE2, forming the RBD-PD complex distinct from the ACE2 catalytic site.

severity of global health risks posed by SARS-CoV-2 and optimize treatment for infected patients, we must recognize the role of ACE2 in SARS-CoV-2 pathogenesis. In addition to respiratory involvement, multiorgan dysfunction occurs in response to SARS-CoV-2 infections.^{36–38} While respiratory symptoms are predominant, acute cardiac and kidney injuries, arrhythmias, gut, and liver function abnormalities have all been documented in infected patients, suggesting myocardial, renal, enteric and hepatic damage in COVID-19. Similarly, SARS-CoV also resulted in systemic manifestations with damages to the heart, gastrointestinal, liver, kidney, and other tissues.^{39,40}

ACE2 as the Receptor for SARS-CoV-2

SARS-CoV-2 differs from the original SARS-CoV by 380 amino acid substitutions, which translates to differences in five of the six vital amino acids in the receptor-binding domain between the viral spike (S) protein with surface expressed human ACE2.⁴¹ Viral S-proteins are well established as a significant determinant of host tropism and represents a key target for therapeutic and vaccine development. Additionally, host cell proteases are important for SARS-CoV-2 entry and infection of cells as both S-proteins and ACE2 are proteolytically modified during the process. The binding affinity of SARS-CoV-2 with ACE2 seems stronger than SARS-CoV, with alterations in several amino acid residues allowing for enhanced

hydrophobic interactions and salt bridge formations, which may explain the considerably larger global influence of COVID-19 than the initial SARS.^{16,42} Moreover, SARS-CoV-2 has evolved to utilize a wide array of host proteases including cathepsin L, cathepsin B, trypsin, factor X, elastase, furin, and TMPRSS2 (transmembrane protease serine 2) for S-protein priming and facilitating cell entry following receptor binding.⁴³ So far, TMPRSS2 and cathepsin L/B mediates S-protein priming of SARS-CoV-2, and camostat mesylate, a serine protease inhibitor combined with cathepsin L/B inhibitor, E-64d blocked SARS-CoV-2 entry.⁴⁴ The entry of both SARS-CoV and SARS-CoV-2 into cells is facilitated by the interaction between viral S-protein with extracellular domains of the transmembrane ACE2 proteins, followed by subsequent downregulation of surface ACE2 expression (Figure 3).^{5,15,29} In a cohort of 12 COVID-19 patients, circulating Ang II levels were markedly elevated compared with healthy controls (linearly correlated with viral load), providing a direct link between tissue ACE2 downregulation with systemic RAS imbalance, and facilitating the development of multiorgan damage from SARS-CoV-2 infections.^{44,45} Potential therapeutic strategies may include preventing the binding of human ACE2 and SARS-CoV-2 by blocking the receptor-binding domain (RBD) of the viral S-protein. In addition to this receptor-binding domain blocking strategy, other possible treatment options may include localized use of

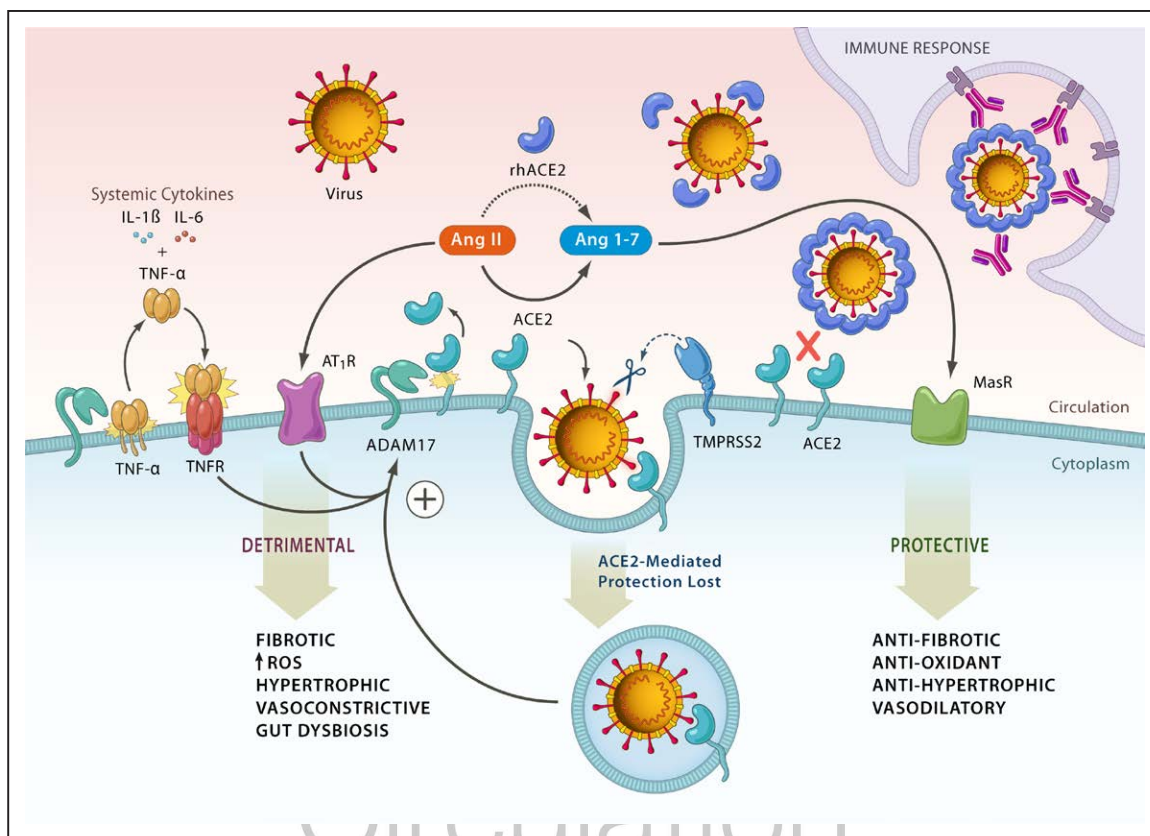


Figure 3. Role of ACE2 (angiotensin-converting enzyme 2) in the pathogenesis of coronavirus disease 2019 and the inflammatory response.

ACE2-mediated cardiovascular protection is lost following endocytosis of the enzyme along with severe acute respiratory syndrome-coronavirus (SARS-CoV-2) viral particles. Ang II (angiotensin II) levels elevate with increased activity of angiotensin 1 receptors (AT₁R) at the cost of ACE2/Ang 1–7 driven pathways leading to adverse fibrosis, hypertrophy, increased reactive oxygen species (ROS), vasoconstriction, and gut dysbiosis. ADAM17 (a disintegrin and metalloproteinase 17)-mediated proteolytic cleavage of ACE2 is upregulated by endocytosed SARS-CoV-2 spike proteins. Activation of the AT₁R by elevated Ang II levels also further increases ADAM17 activity. ADAM17 correspondingly also cleaves its primary substrate releasing soluble TNF- α (tumor necrosis factor- α) into the extracellular region where it has auto- and paracrine functionality. TNF- α activation of its tumor necrosis factor receptor (TNFR) represents a third pathway elevating ADAM17 activity. TNF- α along with systemic cytokines released due to SARS-CoV-2 infection and in conjunction with comorbidities such as diabetes mellitus and hypertension can lead to a cytokine storm. TMPRSS2 indicates transmembrane protease serine 2.

ACE2-derived peptides, small molecule inhibitors, ACE2 antibody or single chain antibody fragment against ACE2.

Cardiovascular Disease in Patients With COVID-19

In postmortem autopsy heart tissues from 20 patients who succumbed to SARS-CoV, 7 heart samples had detectable viral SARS-CoV genome, which was characterized by increased myocardial fibrosis, inflammation, and reduced myocardial ACE2 expression.⁴⁶ These patients also had a much more aggressive illness associated with earlier mortality. Additionally, bilateral pleural effusions were frequently observed during autopsy of SARS-CoV patients, further supporting the evidence of cardiac involvement. Individuals with preexisting diabetes mellitus, hypertension, and lung disease are at particular risk of COVID-19 infection^{37,47} and this is likely due to dysregulated RAS that occurs in these conditions.^{4,48} Significance of the SARS-CoV-2 infection in the

cardiovascular system is reflected through incidences of acute myocardial injury (elevated high sensitivity troponin I levels and/or new ECG/echocardiogram abnormalities), arrhythmias, cardiac arrest, sepsis, septic shock, viral myocarditis, and HF (elevated NT-proBNP levels, systolic dysfunction on cardiac magnetic resonance imaging).^{49–52} Further abnormalities from laboratory tests, including elevation in D-dimers reflective of increased thrombosis risk, may lead to acute coronary syndrome, and sustained increased inflammatory cytokines levels throughout the clinical course suggest ongoing systemic and tissue inflammation in patients with COVID-19.^{36,37,47}

Gut Dysbiosis and a Possible Link to Disease Progression in COVID-19 Patients

Ubiquitous expression of ACE2 throughout the luminal surface of the gastrointestinal tract, and most prevalently in enterocytes, may serve as a secondary site for enteric SARS-CoV-2 infection (Figure 2A). Leaky gastrointestinal

conditions in experimental models of human disease can be ameliorated and worsened with either the gain or loss of ACE2, respectively.^{8,11} Patients with COVID-19 also suffer from gastrointestinal discomfort and diarrhea, which may arise earlier than respiratory conditions concurrent with the detection of viral RNA in feces, as seen with previous coronavirus outbreaks.^{38,47,53–56} Moreover, common comorbidities of CVD, including diabetes mellitus and obesity, are known to affect the integrity of the gastrointestinal-blood barrier and result in gut dysbiosis, bacteremia, and systemic inflammation (Figure 4). Development of gastrointestinal leakage and gut dysbiosis have correspondingly been linked to the onset of PH through the gut-lung axis and is closely related to hyperactivation of the ACE/Ang II/AT₁R (angiotensin II type 1 receptor) axis from ACE2 loss.^{20,57} Continued viral production by host enterocytes perpetuates this situation and deteriorates conditions in the gut-lung axis.^{54,55} Evidence supports that SARS-CoV-2 infection potentially leads to degeneration of the gut-blood barrier leading to systemic spread of bacteria, endotoxins, and microbial metabolites likely affecting the host's response to COVID-19 infection and cumulating in multisystem dysfunction and septic shock.^{37,38,47} Enteric involvement and associated worsening in patient outcomes were documented from the initial SARS-CoV outbreak in the early 2000s. Fecal viral RNA was detected in up to 70% of patients with viral shedding from the gastrointestinal tract associated with a more aggressive clinical

course.^{54,55} In a separate study, SARS-CoV particles were detected within the cytoplasm and surface microvilli of apical enterocytes in the ileum and colon⁵⁵ while in patients with COVID-19, SARS-CoV-2 was detected in feces suggesting fecal-oral transmission.⁵⁸ As such, the gastrointestinal tract of SARS-CoV, and possibly SARS-CoV-2 patients, acts as a staging ground for sustained viral replication concurrent with disruption of the enteric ACE2 axis and adverse outcomes.^{39,47,54,55,59,60}

In addition to the direct impact of the virus on the microbiome, the predisposing disease states such as diabetes mellitus⁶¹ and pulmonary disease have their own adverse effects on the gut microbiome,^{62,63} which may be worsened by SARS-CoV-2 infection. Ang II-dependent hypertension in animal models⁶⁴ and humans is associated with gut dysbiosis, increased gut leakiness, and gut wall pathology.^{10,63,65,66} There is broad support for these observations in pulmonary diseases including PH, COPD, and asthma^{67,68} and in type 2 diabetes mellitus where dysbiosis characterized by decreased microbial richness and diversity, altered representation of bacterial metabolic pathways and modifications in the composition of Firmicutes (F) and Bacteroidetes (B).^{69–71} ACE2 disruption in biomedical models has shown us that gut dysbiosis is quite prevalent and that this change in microbial profiles can alter systemic pathways exacerbating diabetes mellitus and hypertension. We recently showed that ACE2 deficiency magnifies diabetes mellitus-induced dysbiosis¹¹ characterized by an increase

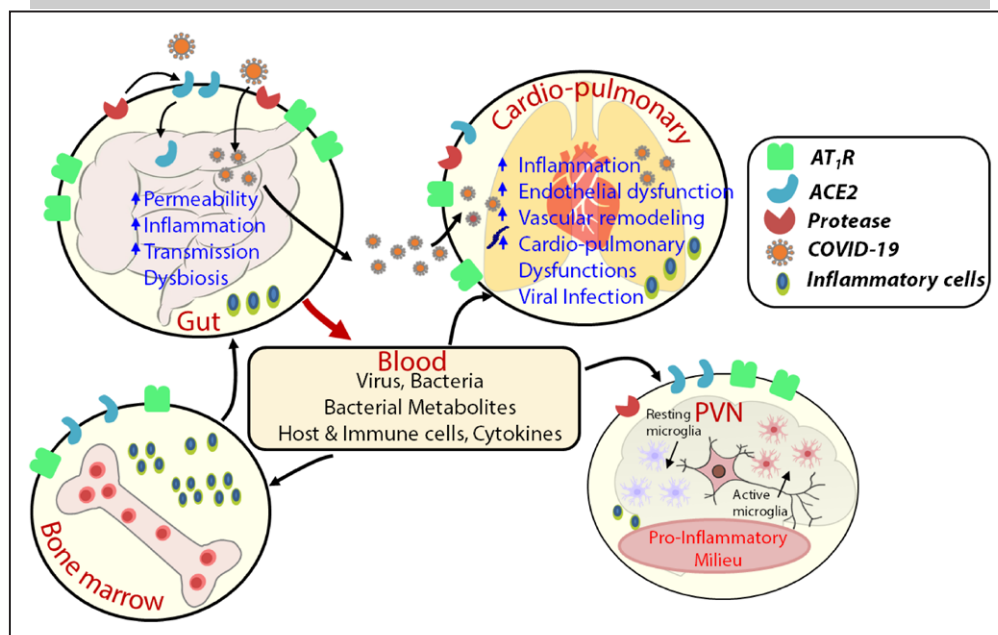


Figure 4. Link between ACE2 (angiotensin converting enzyme 2), gut dysbiosis, and cardiovascular disease.

Loss of ACE2 on the luminal surface of the gut alters microbiota profiles facilitating dysbiosis and disruption of the integrity of the epithelial barrier. Epithelial dysfunction provides a conduit for both gastrointestinal metabolites and bacterium passage into the vascular bed driving local and systemic inflammation, which may cumulate in hypertension and septic shock. ACE2 deficiency and gut dysbiosis predisposes to the development of pulmonary hypertension through the gut-lung axis. Severe acute respiratory syndrome coronavirus (SARS-CoV-2) enteric or pulmonary infection can further worsen the pathophysiology of the gut-lung axis through increased bacterial infiltration and inflammation in addition to worsened pulmonary function. AT₁R indicates angiotensin II type 1 receptor; and COVID-19, coronavirus disease 2019.

in peptidoglycan-producing bacteria and loss of gut barrier integrity in *Ace2*^{-/-}-Akita mice. We also identified a new role of bone marrow cells in the gut. In the *Ace2*^{-/-}-Akita or Akita mice, the disrupted gut barrier was associated with reduced levels of circulating angiogenic cells, hematopoietic cells with reparative function. Giving exogenous circulating angiogenic cells from wild-type mice corrected gut barrier dysfunction in *Ace2*^{-/-}-Akita or Akita mice. Thus, decreased enteric ACE2 expression from SARS-CoV-2 infection may similarly reduce circulating angiogenic cells and compromise the integrity of the endothelium and gut epithelium leading to dysbiosis. Further examination is required to validate this link and whether it is a direct or indirect effect of viral infection.¹¹

LINK BETWEEN ACE2, ADAM17, AND INFLAMMATION

Proteolytic Cleavage of ACE2 by ADAM-17

TNF- α (tumor necrosis factor- α) is a cytokine implicated in chronic inflammation, and its extracellular domain shedding and activation is driven by the membrane-bound protease coined TACE (TNF- α -converting enzyme), also known as ADAM-17 (A disintegrin and metalloproteinase 17).^{72,73} ADAM-17 is a type I transmembrane protein belonging to the adamalysin subfamily of Zn-dependent metalloproteases.⁷⁴ Following the discovery that ADAM-17 cleaves TNF- α , the substrate specificity of the enzyme has expanded to include various cytokines and receptors, many of which contribute to initiating and exacerbating inflammation.^{75,76} Importantly, ADAM-17 was also found to mediate proteolysis and ectodomain shedding of ACE2.⁷⁷ Enhanced ACE2 shedding resulting from RAS overactivation, and subsequent ADAM-17 upregulation drives pathogenesis in HF, atrial fibrillation, coronary artery disease, and thoracic aortic aneurysm.^{1,13,78,79} Ang II-mediated activation of AT₁R triggers a signaling cascade, which culminates in the activation of p38 MAPK (mitogen-activated protein kinase) and ADAM-17 phosphorylation by NADPH oxidase 2-induced reactive oxygen species formation.^{80,81} Phosphorylation enhances the catalytic activity of ADAM-17, thus increasing ACE2 shedding, resulting in loss of ACE2 at the membrane and impaired conversion of Ang II (into Ang 1–7), leading to RAS-mediated detrimental effects in a positive feedback cycle.^{77,82}

Importantly, depletion of ACE2 at the cell surface is a critical pathological outcome of SARS-CoV-2 infection. SARS-CoV-2 is endocytosed by cells in complex with ACE2; thus, the initial detrimental effects of viral infection begins with a loss of ACE2-mediated tissue protection.⁸³ ADAM-17 activity is upregulated upon binding of SARS-CoV to ACE2 and facilitates viral entry, while knockdown of ADAM-17 by siRNA severely attenuated SARS-CoV cellular entry.⁸⁴ The molecular mechanisms

of SARS-CoV and another human coronavirus that only causes mild respiratory symptoms, HNL63-CoV, were compared. Interestingly, although HNL63-CoV also utilizes ACE2 as a receptor for cellular entry, it does not induce ADAM-17 activation and ACE2 ectodomain shedding.^{84,85} Therefore, this study elucidates the unique role of ADAM-17 mediated shedding of ACE2 in SARS-CoV infectivity and may inform the disparity in severity between coronavirus subtypes. Furthermore, loss of membrane ACE2 promotes Ang II accumulation, which also activates ADAM-17 activity, thus perpetuating membrane shedding of ACE2, RAS overactivation, and inflammation.⁷⁷

ACE2, SARS-CoV-2, and Inflammation

The modulatory effects on the Ang II/AT₁R and Ang 1–7/MasR axes make ACE2 a plausible target in preventing and treating chronic inflammation and inflammatory diseases, as highlighted by the recent COVID-19 pandemic.²⁹ Patients with COVID-19 develop pneumonia with acceleration of injury in susceptible patients to multiple organ failure^{30,86} driven in part by an inflammatory cytokine storm and is a notable cause of death in patients who are critically ill.^{30,86} When the immune system is activated due to factors such as SARS-CoV-2 infection, there is an imbalance of Th17/Treg cell function and overactivation of immune cells, which secrete a large number of proinflammatory cytokines.^{17,87,88} Imbalance in the RAS system and the loss of ACE2 in patients with COVID-19 are further contributing factors to tissue and systemic inflammation.^{30,86} Lipopolysaccharide-induced acute lung injury decreased expression of ACE2, precipitated inflammatory injury, and upregulated expression of renin, Ang II, ACE, and AT₁ receptors.⁸⁹ After injection of rhACE2, lung function and pathological injury improved with attenuation of inflammation.⁸⁹ In addition, rhACE2 is beneficial and improves acute lung injury caused by SARS-CoV, acid inhalation, and sepsis.^{17,18,88}

Ace2 knockout (KO) mice showed very severe acute respiratory distress syndrome (ARDS)/acute lung injury pathology, increased vascular permeability, increased pulmonary edema, neutrophil accumulation, and deterioration of lung function compared with normal WT control mice.^{18,87} ACE deficiency partially rescued the severe phenotype of mice with a single mutation of *Ace2* in acute lung injury by further deletion of the *Ace* gene,¹⁷ suggesting that the balance of ACE2/ACE levels is the key to lung injury/lung protection during an inflammatory storm. ARBs (AT₁R blockers) induce ACE2, Ang 1–7, and Mas expression in line with the reduction of proinflammatory cytokines and induction of IL-10, an anti-inflammatory cytokine.⁸⁸ We showed that *Ace2* KO hypertensive mice exhibited enhancement of proinflammatory cytokines, IL-1 β , IL6, TNF- α , and chemokine (C-C motif) ligand 5 while administration of rhACE2

rescued Ang II-induced T-lymphocyte-mediated inflammation.^{90,91} Blockade of Mas receptor by D-Ala⁷-Ang 1–7 (A-779) completely inhibited the Ang 1–7 mediated anti-inflammatory effects while AVE 0991, the agonist of Ang 1–7 receptors, mimicked the actions of Ang 1–7.⁸⁸

PHYSIOLOGICAL ROLE OF ACE2

Negative Regulator of the RAS

Discovery of ACE2 resulted in a paradigm-changing concept in all aspects of the RAS. ACE2 is a monooxypeptidase that converts Ang I into a nonapeptide, Ang 1–9, and Ang II into a heptapeptide, Ang 1–7 (Figure 5A). This distinct enzymatic pathway for degradation of Ang I and Ang II negatively regulates RAS activation and mitigates the deleterious actions mediated by Ang II and AT₁R.¹ This is of particular significance in pathological conditions where the RAS is overstimulated. Ang 1–7 is a biologically active peptide whose vast array of effects are opposite to those attributed to Ang II.^{92–98} Furthermore, ACE2 can antagonize ACE-independent formation of Ang II, such as from mast cell chymase.^{13,99} In 2003, an endogenous orphan receptor, Mas receptor (MasR), was identified as the Ang 1–7 receptor, and A779, a MasR antagonist was shown to inhibit the majority of Ang 1–7 effects.^{94,100–103} Ang 1–9 has also shown beneficial biological effects via the AT₂R that result in cardioprotection.^{104–107} Thus, the ACE2/Ang 1–7/MasR axis has emerged as a physiological antagonist that counter-regulates the activated RAS.^{93,108–112} The cardioprotective effects of ACE2 taken together can be attributed to (1) degradation of Ang I to Ang 1–9, whereby limiting action of ACE on its substrate, (2) reducing Ang II detrimental effects through degradation of the peptide, and (3) formation of Ang 1–7 which exercises cardioprotective effects. Formation of Ang 1–7 is an important mechanism of ACE2 mediated protection, as antagonism of Ang 1–7 using A779 prevented beneficial effects of rhACE2 in murine model of systolic dysfunction.¹¹³ Diminished ACE2 activity results in activation of the Ang II/AT₁R axis, contributing to the increased progression of CVD. Elevated ACE2 level and activity result in the formation of Ang 1–9 and Ang 1–7, leading to protection against CVD (Figure 5A).

Interaction With Apelin Peptides

The apelin family of peptides act through the apelin receptors mediating protection against CVD.^{114,115} The X-linked *APLN* gene encodes a 77 amino acids prepro-apelin that is subsequently cleaved by endopeptidases to various bioactive peptides from 13 to 36 amino acids in length. CVD, including HF and hypertension, is characterized by an apelin deficient state in both human myocardium and plasma.^{116–118} Apelin KO mice exhibit increased infarct

size and systolic dysfunction following coronary ligation and reduced myocardial contractility concomitant with increased susceptibility to HF in pressure-overload models.^{119,120} Reduced myocardial *Ace2* mRNA and ACE2 protein levels in apelin KO mice, which were rescued by infusion of apelin-13, suggest a crucial regulatory role of apelin in *Ace2* gene expression.¹²¹ Apelin signaling through the apelin receptors specifically increased *Ace2* promoter activity leading to an increase in *Ace2* mRNA and protein.^{121–123} These effects are consistent with the ability of the pyr-apelin-13 peptide to negatively regulate Ang II-mediated superoxide production, myocardial hypertrophy, dysfunction, and fibrosis¹²³ and analogs of apelin-17 preventing abdominal aortic rupture in low-density lipoprotein receptor KO models induced by Ang II infusion.¹²⁴ However, ACE2, through its monooxypeptidase activity, cleaves and inactivates bioactive apelin peptides apelin-13 and apelin-36 through a negative feedback mechanism in the heart and vasculature (Figure 5B).^{28,125} Due to the short half-life of endogenous apelin peptides in the plasma, synthetic apelin peptide analogs resistant to ACE2 degradation and retaining their binding capability to endogenous apelin receptors elicit protection in the cardiovascular system are being explored as potential new therapies.^{114,124}

ACE2 As a Chaperone Protein for the Amino Acid Transporter, B⁰AT1 (SLC6A19)

B⁰AT1 is highly expressed in the intestines and kidneys with function in the absorption of neutral amino acids.¹²⁶ The ACE2-B(0)AT1 complex is assembled as a dimer of heterodimers, with the collectrin-like domain of ACE2 mediating homo-dimerization.¹⁶ ACE2 has a RAS-independent function, regulating intestinal amino acid homeostasis, expression of antimicrobial peptides, and the gut microbiome.⁸ ACE2 is necessary for the expression of the Hartnup transporter in the intestine, and the differential functional association of mutant B(0)AT1 transporters with ACE2 in the intestine regulates the phenotypic heterogeneity of human Hartnup disorder.¹²⁶

ROLE OF ACE2 IN CARDIOVASCULAR AND LUNG DISEASES

ACE2 and Heart Disease

Cardiovascular disease is the leading cause of death worldwide and a major public health concern. Heart disease is characterized by the activation of several signaling pathways associated with pathological hypertrophy and maladaptive ventricular remodeling. In the heart, ACE2 is localized to cardiomyocytes, cardiac fibroblasts, epicardial adipose tissue, and the coronary vascular endothelium^{77,127,128}; Ang 1–7/MasR is also present on cardiomyocytes, cardiac fibroblasts, and endothelial and

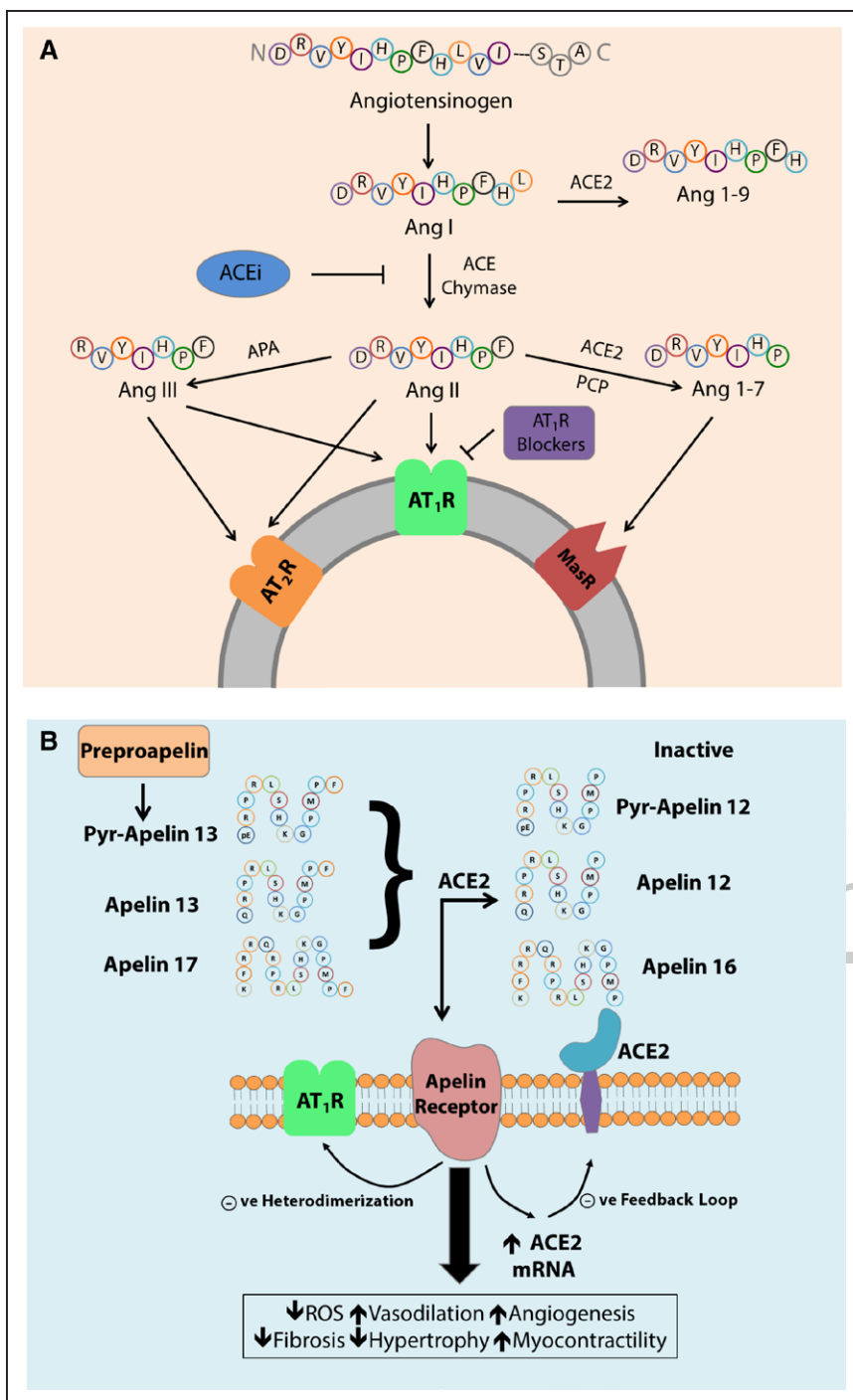


Figure 5. ACE2 (angiotensin converting enzyme 2) role in the renin-angiotensin system peptide cascade and its interaction with the apelinergic peptide system.

A. Angiotensinogen is processed by renin into Ang I (angiotensin I), which is further cleaved by ACE or mast cell chymase into Ang II. Ang II can go on to affect the cardiovascular system predominantly through the angiotensin type 1 receptor (AT₁R) or via the angiotensin type 2 receptor (AT₂R). Alternatively, Ang II can be degraded by the carboxypeptidase ACE2 or the PCP (prolyl carboxypeptidase) into Ang 1–7 (angiotensin 1–7). Ang 1–7 mediates protective effects throughout tissues which host the Mas receptor (MasR). Ang 1–7 can be further formed through the activity of ACE2 on Ang I forming Ang 1–9 which is then cleaved by either ACE or NEP (nephilysin). **B.** Stimulation of the apelin receptor by apelin peptides leads to cardiovascular protective effects while disrupting Ang II signaling by sequestration of the AT₁R through receptor heterodimerization. Apelin is inactivated by ACE2 cleavage of its C-terminal phenylalanine while stimulation of the apelin receptor promotes ACE2 mRNA transcription presenting apelin’s role as a positive regulator of ACE2. ROS indicates reactive oxygen species.

vascular smooth muscle cells.^{100,129–131} Genetic *Ace2* deletion resulted in exacerbation of Ang II-mediated cardiorenal fibrosis and oxidative stress in the heart and kidney of hypertensive mice while administration of rhACE2 (recombinant human ACE2) remarkably rescued the Ang II-induced hypertension, pathological hypertrophy, oxidant injury, and cardiac dysfunction.^{90,91}

Various ACE2 polymorphisms are linked to CVD.¹³² Post-MI remodeling and coronary artery disease are common causes of HF.^{1,133} Notably, MI increases *ACE2* mRNA expression in human, mice, and rat hearts,^{134,135}

whereas genetic ACE2 deletion results in worsening of MI-induced cardiac dysfunction, infarct size, MMP (matrix metalloproteinase)2/MMP9 activation, and extracellular matrix disruption.^{134,135} Loss of ACE2 leads to increased neutrophilic infiltration in the infarct and peri-infarct regions, resulting in upregulation of inflammatory cytokines, interferon- γ , IL (interleukin)-6, and the chemokine, MCP-1 (monocyte chemoattractant protein-1), as well as increased phosphorylation of ERK1/2 (extracellular signal-regulated kinase 1/2) and JNK1/2 signaling pathways, changes that were blocked with an

ARB ultimately resulting in improvement in myocardial function.¹³⁵ In contrast, overexpression of ACE2 and the action of Ang 1–7 ameliorates MI-induced cardiac remodeling.^{136,137} Importantly, heterozygote loss of ACE2, as seen in explanted human hearts from patients with dilated cardiomyopathy, was sufficient to increase susceptibility to heart disease.¹³⁸

HF with preserved ejection fraction is a proinflammatory state closely linked to obesity-related cardiac and microvascular dysfunction for which there are no approved therapies.^{128,139,140} Epicardial adipose tissue is a primary source of inflammatory cytokines that could have detrimental effects on the heart.¹³⁹ Loss of ACE2 increases macrophage polarization to proinflammatory M1-phenotype (alternatively activated, CD11c⁺) in epicardial adipose tissue from patients with HF with preserved ejection fraction, with decreases in polarization to anti-inflammatory, M2-phenotype macrophages, and worsening of HF with preserved ejection fraction in response to diet-induced obesity.¹³⁹ Importantly, Ang 1–7 decreased macrophage polarization in epicardial adipose tissue and preserved the cardiac function of obese *Ace2* KO mice.^{128,141} Ang 1–7 has potent anti-inflammatory effects in adipose tissue of obese type 2 diabetic mice and protects against diabetic cardiomyopathy and nephropathy.^{141–143} The ACE2/Ang 1–7 axis also promotes browning of adipose tissue leading to improved metabolic effects and weight loss, which can confer further benefits to the cardiovascular system.^{144,145}

ACE2 and Vascular Disease

Blockade of the deleterious arm of the RAS has been the mainstay of the therapeutic management of hypertensive individuals. An increase in ACE2 and the vasoprotective axis of the RAS by ACE inhibitors and angiotensin receptor blockers (ARBs) clearly reinforces this view (see section Pharmacological Antagonists of the RAS and ACE2 Expression below). Furthermore, increased ACE2 expression protects against hypertension, while ACE2 deficiency exacerbates hypertension. Renal *Ace2* expression is inversely related to blood pressure in experimental models of hypertension.²⁵ In the spontaneously hypertensive rat and stroke-prone spontaneously hypertensive rat, renal *Ace2* mRNA levels are reduced compared with normotensive Wistar-Kyoto rats.²⁵ These studies support the essential role of ACE2 in maintaining healthy blood pressure. Lentiviral overexpression of ACE2 results in increased expression of antihypertensive components of the RAS and attenuates elevated blood pressure.^{146,147} Pretreatment with rhACE2 prevented hypertension induced by Ang II and decreased plasma Ang II while increasing plasma Ang 1–7 levels.¹⁴⁸ ACE2 and ADAM17 were selectively knocked down from all neurons (AC-N), which revealed a reduction of inhibitory inputs to AC-N presympathetic neurons relevant for

blood pressure regulation. Mice with ACE2 selectively knocked down from Sim1 neurons in mice exhibited a blunted blood pressure elevation and preserved ACE2 activity during the development of salt-sensitive hypertension.¹⁴ The metalloproteinase ADAM17 is responsible for mediating ACE2 shedding from the cell membrane-bound domain, which can be promoted by Ang II, and release of ACE2 as a soluble form in plasma^{147,149} impairing brain ACE2 compensatory activity and thus contributing to the development of neurogenic hypertension.¹⁵⁰ Genetic *Ace2* deficiency is associated with the upregulation of putative mediators of atherogenesis and enhances responsiveness to proinflammatory stimuli suggestive of a key role of ACE2 in suppressing vascular inflammation and atherosclerotic disease.¹⁵¹ In addition, ACE2 inhibition blocks neuropeptide catestatin-mediated protective effects in the development of atherosclerosis in *ApoE*^{-/-} mice fed a high-fat diet.¹⁵²

ACE2 in Diabetic Cardiovascular Complications

The counter-regulatory role of the ACE2/Ang 1–7/MasR axis of the RAS has been well characterized in the progression of diabetic complications, including cardiovascular and kidney disease.^{1,153,154} Support for the importance of ACE2 in diabetes mellitus comes from its impact on diabetic complications wherein diabetes mellitus-induced vascular dysfunction is strongly associated with a shift in the RAS axis toward the profibrotic, proinflammatory arm of RAS with a reduction in the protective arm (Figure 6). Loss of the protective effects of the RAS is related to the regulation of tissue and circulating levels of Ang II and their sequelae in the context of diabetes mellitus.^{155,156} Alterations within the RAS are considered pivotal for the development of both diabetic micro and macrovascular complications.^{1,157}

The blockade of the proinflammatory and profibrotic arms of the RAS provides significant renoprotection in both experimental models of diabetes mellitus and in patients. While the loss of ACE2 worsens diabetic kidney injury,¹⁵⁸ rhACE2 is therapeutic in an animal model of diabetic nephropathy¹⁵³ and experimental Alport syndrome.¹⁵⁹ ACE inhibitors in T1D and angiotensin receptor blockade with losartan and irbesartan in T2D retard the progression of nephropathy.¹⁶⁰ In diabetic renal tubules, ACE2 gene expression is decreased by ≈50%, which would reduce Ang 1–7 formation and allow Ang II accumulation hence directly increasing the expression of TGF-β and CTGF (connective tissue growth factor), leading to tubulointerstitial fibrosis.¹⁶¹ RAS blockade retards renal damage and ACE inhibitor therapy, as mentioned above, resulting in a compensatory increase in ACE2, leading to renoprotection.⁹¹ Therefore, support for the loss of ACE2 contributing to vascular complications in diabetes mellitus comes from strong clinical and experimental evidence.¹

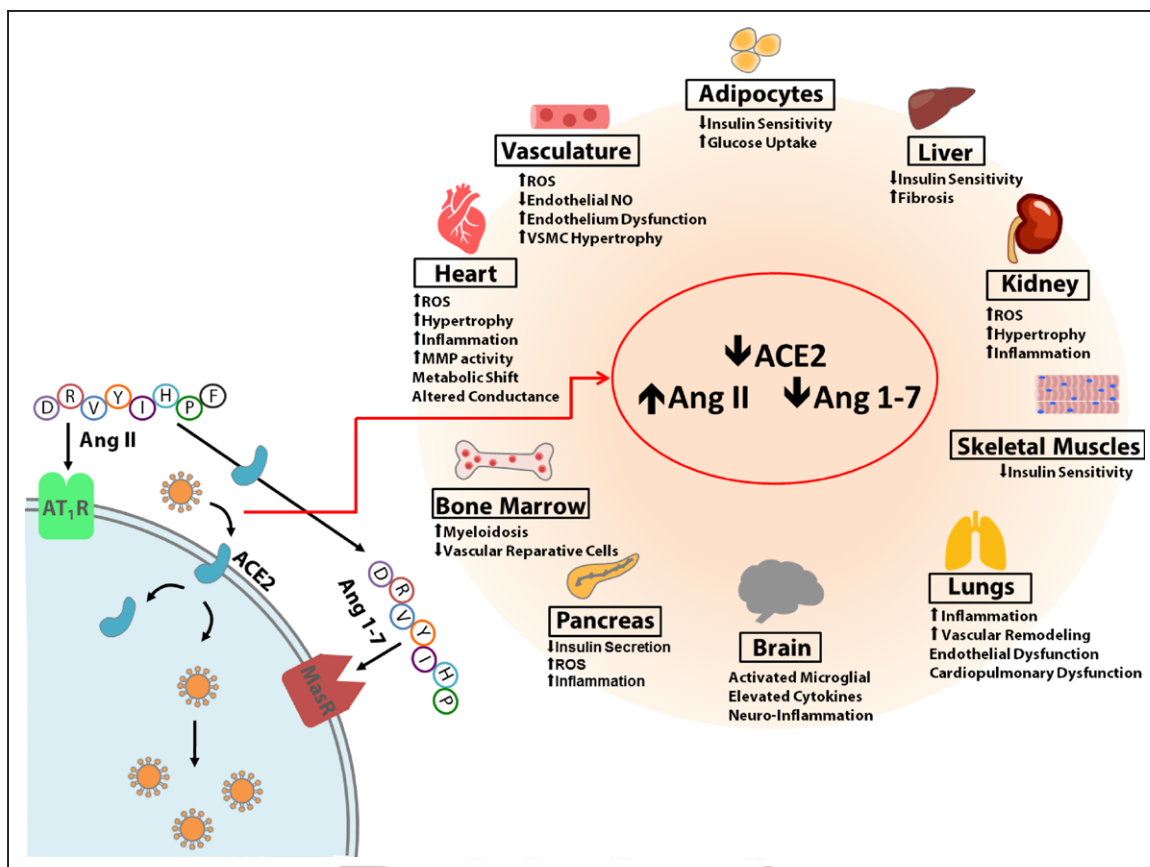


Figure 6. Loss of ACE2 (angiotensin converting enzyme 2) exacerbates diabetic cardiovascular complications via a multitude of disease mechanisms.

The loss of ACE2 action in diabetic states elevates Ang II (angiotensin II) and lowers Ang 1–7 levels in tissues and systemically. Increased Ang II/angiotensin II type 1 receptor (AT₁R) signaling drives multiple pathologies in various end-organs elevating reactive oxygen species (ROS) and promoting fibrosis, hypertrophy, and inflammation aggravated by the loss of the protective effects of Ang 1–7. Ang II stimulation also systemically alters metabolic profiles and modulates insulin sensitivity in affected tissues.

Retinopathy, the most common complication of diabetes mellitus and one of the leading causes of blindness in working-age adults is linked to activation of oxidative stress, profibrotic, and proinflammatory arm of the RAS, which can be effectively curtailed by the ACE2/Ang 1–7 axis in experimental models.^{162,163} Increased secretion of proinflammatory cytokines by bone marrow mesenchymal stem cells skews hematopoiesis toward the generation of an increased number of myelo-monocytic cells.¹⁶⁴ Target tissues of diabetic complications secrete CCL2 in response to high glucose-induced stress¹⁶⁵ facilitating the homing of CCR2⁺ cells to these regions and promoting the development of vascular complications.^{166–171} In addition to an increase in myeloidosis, diabetics with complications have reduced bone marrow–derived vascular reparative cells and circulating angiogenic cells (CD34⁺ cells).¹¹ Levels of ACE2 mRNA were also a significant predictor of the presence of microvascular disease in diabetic patients.¹⁷² Diabetic individuals who remained free of retinopathy despite >40 years of poor glycemic control had higher mRNA levels for genes of the vasoprotective axis (ACE2/Mas) compared with age,

sex, and glycemia-matched diabetics with retinopathy.¹⁷² In dysfunctional CD34⁺ cells from diabetic individuals, activation of the protective arm of RAS, by exposing the cells to Ang1–7 corrected their dysfunction by restoring bioavailable NO and reducing reactive oxygen species. Ang1–7 gene modification of CD34⁺ cells restored the in vivo vasoreparative function of these cells in a mouse retinal ischemia-reperfusion injury model.¹⁷² Moreover, intraocular administration of AAV-ACE2 or Ang1–7 reduced diabetes mellitus–induced retinal vascular leakage and inflammation, thus preventing retinopathy.¹⁶³

Patients with diabetes mellitus have a dysregulated RAS, which may influence their vulnerability to SARS-CoV-2. Guan et al examined 1099 individuals with confirmed COVID 19. Of these individuals, 173 had severe disease, and of this, 16.2% were diabetics.⁴⁷ Zhang et al studied 140 patients who were hospitalized due to the severity of their COVID-19 infection, of these individuals, 12% had diabetes mellitus. It is interesting to speculate why diabetics may be more at risk for SARS-CoV-2 infection than the general population, and this may be due to the reduced ACE2 levels that are typically observed in

the vasculature of diabetic individuals and diabetic animal models.¹⁷³ Indeed, loss of ACE2 was associated with marked gut dysbiosis, which was further worsened in a model with type 1 diabetes mellitus.¹¹

ACE2 and Lung Disease

Lung epithelial cells express high levels of ACE2, which positively correlates with airway epithelial differentiation.^{17,19,174} Involvement of ACE2 in ARDS, which is triggered by multiple diseases including SARS-CoV and SARS-CoV-2, has been established in multiple animal models.^{18,175} *Ace2* KO mice exhibit severe pathology of ARDS.^{17,19} Additional *Ace* deficiency, or treatment with AR1R blockers of *Ace2* KO mice rescues them from ARDS implicating the benefit of ACE2 and the critical balance of the protective versus proinflammatory and fibrotic axes of the RAS.¹⁸ These findings are consistent with evidence of a beneficial effect of rhACE2 on pulmonary blood flow and oxygenation in a pig model of lipopolysaccharide-induced ARDS.¹⁷⁶ Age-related loss of ACE2 in the lungs correlates with the increased mortality and worsened phenotype in elderly patients with COVID-19.¹⁷⁴

ACE2 has been implicated in acute lung injury by inducing an imbalance in the RAS. Evidence includes that in acute lung injury (1) a decrease in pulmonary ACE2 and an increase in Ang II levels occurs; (2) supplementation with ACE2 or inhibition of Ang II improves outcomes; and (3) a lack or decrease of pulmonary ACE2 aggravates viral-induced acute lung injury. ACE2 is also involved in PH and fibrosis.¹⁹ Increasing ACE2 activity using rhACE2 reduced bleomycin-induced inflammation and fibrosis, resulting in improved lung function and exercise capacity,¹⁹ and the ACE2 activator, DIZE, protects animals from PH and fibrosis.¹⁷⁷ Moreover, oral feeding of a bioencapsulated form of ACE2 protects and arrests the progression of PH.¹² Validation of this protective effect comes from a small human study that showed that PH is characterized by reduced ACE2 activity and supplementation of these individuals with rhACE2 improved pulmonary hemodynamics and reduced oxidative and inflammatory markers.²¹ Collectively, these studies unequivocally establish the conceptual framework that ACE2 is a central player in normal pulmonary function, and its imbalance leads to pulmonary diseases.

TARGETING ACE2 FOR THERAPEUTICS

Pharmacological Antagonists of the RAS and ACE2 Expression

Pathological neurohormonal activation of the RAS drives the development and progression of CVD. Current pharmacotherapies aim to achieve multilevel RAS inhibition through distinct modes of action. Although ACE2 is not

the direct cellular target of these therapies, *Ace2* gene transcription, translation, and ultimately catalytic activities are modified due to the intricate nature of the RAS. Blocking the ACE/Ang II/AT₁R axis through limiting the formation and actions of Ang II potentiates the effects of ACE2 as the endogenous RAS counter-regulator. The ARBS consistently increased *Ace2* mRNA expression, protein levels, and catalytic activities in the heart, kidneys, and thoracic aorta, but the translation to protein levels and activity differs between experimental models and tissues for ACE inhibitors (Table).^{77,178–185} Combination of lisinopril and losartan treatment in normotensive Lewis rats abolished the increase in *Ace2* mRNA levels observed individually but retained losartan induced rise in ACE2 activity in the heart.¹⁷⁹ Moreover, lisinopril in normotensive Lewis rats increased *Ace2* mRNA without affecting ACE2 activity in the heart, but the opposite was observed in the kidneys.^{179,180} These findings could be attributed to tissue-specific regulation of ACE2, as higher ACE2 protein levels were reported in the heart, but ACE2 activity was higher in the kidneys of Sprague-Dawley rats, adding to the complexity of the tissue RAS.¹⁸⁶ In type 1 diabetic Akita angiotensinogen-transgenic mice, dual RAS blockade with perindopril and losartan normalized disease-mediated reduction in kidney *Ace2* mRNA expression and protein levels.¹⁸⁷ These findings suggest that the accumulation of Ang II in pathological conditions contributes to the modulatory effects of RAS blockade on ACE2.

Ang II can regulate *ACE2* expression through the AT₁R. Healthy hearts and kidneys are characterized by high levels of *ACE2* mRNA and protein expression, with moderate expression of *ACE*.¹⁹¹ RAS overactivation in CVD increases AT₁R stimulation by Ang II, promoting ERK1/2 and p38 MAPK signaling pathways to downregulate *ACE2* while upregulating *ACE* expression.¹⁹¹ Activation of p38 MAPK upregulates ADAM17 activity though posttranslational phosphorylation of the cytoplasmic domain results in shedding of surface ACE2 in a positive feedback loop and could explain the observed effects of ARBs in increasing ACE2 protein levels and activity.^{77,80,81} Mechanisms behind the augmentation of *ACE2* mRNA levels by ACE inhibitors and ARBs require further characterization. Moreover, mineralocorticoid receptor antagonists increased *ACE2* mRNA expression and activity in samples from patients with chronic HF, wild-type mice, and rats to varying degrees among different tissues but not in the heart of a rat hypertensive disease model (Table).^{188–190} Spironolactone, a nonselective mineralocorticoid receptor antagonist, prevented the increase in both *ACE* and AT₁R mRNA levels, and the associated increase in AT₁R density from aldosterone signaling in cardiomyocytes.^{192,193} Activation of mineralocorticoid receptors also stimulates overlapping downstream signaling pathways with AT₁R, including the ERK1/2 and p38 MAPK pathways mentioned before.^{194,195} Blocking these signaling pathways contributes to the observed

Table. Pharmacological Regulation of the RAS and Their Effects on RAS Components, ACE2 Gene Expression, Protein Levels, and Cellular Activity

Pharmacological Agent	Experimental Model/Subject	Tissues	Observation
ACE inhibitors			
Lisinopril	Lewis rats	Heart	Decrease in plasma Ang II, increase in plasma Ang 1–7 and <i>Ace2</i> mRNA, but not cardiac ACE2 activity ¹⁷⁹
Enalapril	Coronary artery ligation in Sprague Dawley rats	Heart	Increased plasma and cardiac ACE2 activity, and cardiac <i>Ace2</i> mRNA levels 8 wk post-surgery ¹⁸³
Lisinopril	Transgenic Ren2 rats	Heart/Kidney	Decrease in plasma Ang II, increase in plasma Ang 1–7, cardiac and renal <i>Ace2</i> mRNA and activity ¹⁸²
Lisinopril	Lewis rats	Kidney	No change in kidney <i>Ace2</i> mRNA but increased ACE2 activity ¹⁸⁰
Angiotensin receptor blockers			
Losartan/ Olmesartan	Coronary artery ligation in Lewis rats	Heart	Increase in plasma Ang II, Ang 1–7 and <i>Ace2</i> mRNA 28 days post surgery ¹⁷⁸
Losartan	Lewis rats	Heart	Increase in plasma Ang II, Ang 1–7 levels, <i>Ace2</i> mRNA and cardiac ACE2 activity ¹⁷⁹
Irbesartan	C57BL/6 mice	Heart	Increase in cardiac <i>Ace2</i> mRNA, Irbesartan prevented Ang II-induced decrease in ACE2 protein levels ⁷⁷
Losartan	Transgenic Ren2 rats	Heart/Kidney	Increase in plasma Ang II, Ang 1–7, cardiac and renal <i>Ace2</i> mRNA and activity ¹⁸²
Telmisartan	C57BLKS/J mice	Kidney	Following 2 wk administration, increased ACE2 protein levels, and <i>Ace2</i> mRNA expression ¹⁸⁴
Irbesartan	C57BL/6 mice	Aorta	Treatment with irbesartan significantly augmented ACE2 protein levels and <i>Ace2</i> mRNA expression ¹⁸⁵
Olmesartan	Spontaneously hypertensive rats	Aorta	Increased plasma Ang II and Ang 1–7 levels, <i>Ace2</i> mRNA expression and ACE2 protein levels ¹⁸¹
Mineralocorticoid receptor blockers			
Spironolactone	Patients with heart failure	Monocyte-derived macrophage	Increase in ACE2 activity and ACE2 mRNA expression 1-month post-therapy ¹⁸⁸
Eplerenone	Balb/C mice	Heart/Kidney	Increase in cardiac ACE2 activity and <i>Ace2</i> mRNA expression but nonsignificant increase in the kidneys ¹⁸⁸
Eplerenone	Wistar rats	Heart	Prevented aldosterone induced reduction in cardiac <i>Ace2</i> mRNA expression ¹⁸⁹
Eplerenone	Dahl salt-sensitive hypertensive rats	Heart	No effect on cardiac <i>Ace2</i> mRNA expression and protein levels observed in DS rats ¹⁹⁰

ACE indicates angiotensin-converting enzyme; and RAS, renin-angiotensin system.

effect of mineralocorticoid receptor antagonist on ACE2 gene expression, surface protein levels, and activity.

Enhancing ACE2 Action

Promoting the ACE2/Ang 1–7/Mas signaling by rhACE2 or the Ang 1–7 receptors agonist AVE 0991 can have salutary therapeutic effects in CVD and lung disease from diverse etiologies.¹ The Ang 1–7 receptors agonist AVE 0991 has been shown to exert cardiorenal and pulmonary protective effects,⁸⁸ and treatment with rhACE2 improved the symptoms of acute lung injury, CVD, and kidney injury in various preclinical models.^{17,87,88,90} Maintaining ACE2 levels in patients with or predisposed to common CVD states such as diabetes mellitus, hypertension, and obesity wards off the advancement of these comorbidities in instances where the patient contracts SARS-CoV-2 by maintaining a level of ACE2/Ang 1–7/MasR negative counter-regulation.

rhACE2 functionally sequesters circulating viral particles to prevent S-protein interactions with endogenous ACE2,

while simultaneously regulating the systemic RAS may provide therapeutic benefits in COVID-19 and is moving into phase II clinical trials in Europe.¹⁹⁶ A potential limitation of rhACE2 is the restricted penetrance and activity against tissue RAS owing to its large molecular size. Pharmacological RAS blockade agents, ARBs, in particular, are capable of modulating both systemic and tissue RAS, and simultaneously increasing ACE2 expression and activity in experimental models. The direct implications of RAS inhibition in patients with COVID-19 with hypertension remain elusive, and clinical evidence is desperately needed to determine the relative benefits and risks associated with usage of these medications.¹⁹⁷ Nonetheless, introducing ARBs to patients already infected by SARS-CoV-2 may be an effective therapeutic option in addressing the viral-mediated RAS imbalance and is currently under investigation in several clinical trials (www.ClinicalTrials.gov number NCT04312009, NCT04311177, and NCT04318418).^{198–200}

Potential for ACE2 as a therapy is also facilitated by using the probiotic species *Lactobacillus paracasei* (LP), which can be engineered to express recombinant

proteins. Mice treated with the recombinant LP expressing the secreted ACE2 in fusion with the nontoxic subunit B of cholera toxin (acts as a carrier to facilitate transmucosal transport) showed increased ACE2 activities in serum and tissues and reduced diabetic retinopathy.²⁰¹ These results provide proof of concept for using bioengineered probiotic species as live vectors for delivery of human ACE2 with enhanced tissue bioavailability for treating diabetic complications but could potentially be repurposed for treating CVD and COVID-19 infection.

CONCLUSIONS

Since the discovery of ACE2 in 2000, tremendous progress has been made in elucidating its biochemical actions and fundamental role in CVD and, more recently, as the SARS-CoV-2 receptor. ACE2 is a dominant mechanism for negative regulation of the RAS by metabolizing Ang II into the beneficial peptide Ang 1–7, and this important biochemical and physiological property is being harnessed as a potential therapy in patients with HF. The activation of the RAS axis due to binding of SARS-CoV-2 to ACE2, leading to direct loss of ACE2 and indirectly via proteolytic processing and shedding, partly drives the systemic manifestations of COVID-19. Careful targeting of the RAS axes is needed in these patients to optimize their clinical outcomes, including the use of AT1 receptor blockers (ARB).

ARTICLE INFORMATION

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Disclosures

None.

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