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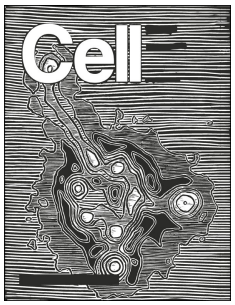

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Angiotensin Converting Enzyme 2 - at the Heart of the COVID-19 pandemic

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Abstract

ACE2 is the indispensable entry receptor for SARS-CoV and SARS-CoV-2. Because of the COVID-19 pandemic, it has become one of the most therapeutically-targeted human molecules in biomedicine. ACE2 serves two fundamental physiological roles: as an enzyme, it alters peptide cascade balance, and as a chaperone, it controls intestinal amino acid uptake. ACE2's tissue distribution, affected by comorbidities and sex, explains the broad tropism of coronaviruses and the clinical manifestations of SARS and COVID-19. ACE2-based therapeutics provide a universal strategy to prevent and treat SARS-CoV-2 infections, applicable to all SARS-CoV-2 variants and other emerging zoonotic coronaviruses exploiting ACE2 as their cellular receptor.

Introduction

Angiotensin-converting enzyme 2 (ACE2) serves as the indispensable entry receptor for multiple human coronaviruses, including hCoV-NL63, a virus associated with common-cold infections, and the severe acute respiratory syndrome coronaviruses, SARS-CoV and SARS-CoV-2, which caused the SARS epidemic in 2002-2003 and the COVID-19 pandemic, respectively. The concerted global effort to tackle SARS-CoV-2 made ACE2 one of the most researched human proteins.[1] Despite sharing a close evolutionary relationship with ACE, ACE2 has distinct functions and substrate specificity as a regulator of the renin-angiotensin system (RAS).[2,3] Individuals with pre-existing diabetes, hypertension, or pulmonary diseases are at elevated risk of severe COVID-19[4], likely as a compounded effect of the dysregulated canonical RAS and altered *ACE2* expression and ACE2 levels.[5,6] Ongoing efforts focus on manipulating the ACE2 axis to structurally and functionally curtail SARS-CoV-2 infections while protecting against multi-organ injuries. Understanding the precise role of ACE2 in COVID-19 is critical to designing specific and efficacious therapies. In this review, we summarize ACE2's diverse functions, highlighting its relationship with SARS-CoV-2 and the implications for COVID-19 disease sequelae. We provide a framework for developing universal therapeutic strategies exploring the ACE2 pathway and interfering with the spike-ACE2 interaction as a fundamental principle against all current and future SARS-CoV-2 variants.

Functions of ACE2

Following initial reports describing the ACE-homologue ACE2 in 2000, its biological function was first demonstrated in *Ace2* mutant mice showing that ACE2 controls heart function through inactivation of the RAS.[2,3,7] Comprehensive examination of the human transcriptome and numerous other mammalian species revealed expression of *ACE2* in specific cell types of the intestine, pancreas, kidney, adipose tissue, lung, heart, blood vessels, testes, and placenta, among others, which was corroborated by tissue immunohistochemistry and immunoblotting (**Fig. 1A**).[6,8-11] *ACE2* codes for an integral type I transmembrane protein that functions as a monocarboxypeptidase. ACE2's catalytic activity affects three central cardiovascular pathways: (i) the RAS pathway to hydrolyze angiotensin I (Ang I) and Ang II forming Ang-(1–9) and Ang-(1–7), respectively; (ii) the apelin pathway to form apelin-12/apelin-16 from apelin-13/apelin-17, and (iii) the kallikrein-kinin pathway involved in the breakdown and inactivation of des-Arg[9]

bradykinin (**Fig. 1B**).[12,13] Additional ACE2 substrates include amyloid β, angiotensin A, dynorphin A 1-13, β-casomorphin, neurotensin 1-8, or ghrelin[13-15], thereby expanding the role of ACE2 beyond classic cardiovascular peptide cascades to homeostasis of multiple organs. Although the essential *in vivo* role of ACE2 was initially unclear because of its proteolytic promiscuity, gene knockout studies demonstrated that ACE2 inactivates Ang II to maintain homeostasis in the RAS.[7] Moreover, loss of ACE2 in disease-permissive environments results in increased pathologies of the cardiovascular, pulmonary, or renal systems, accompanied by tissue injury and fibrosis, most of which result from enhanced local Ang II levels.[6]

ACE2 is an evolutionarily ancient protein, with ACE2-like carboxypeptidases even found in bacteria. One such bacterial ACE2-like protein is B38-CAP, which can catalyze the cleavage of Ang II into Ang-(1-7) and functionally alters the RAS in mammals.[16] Moreover, in evolutionary terms, ACE2 is a chimeric molecule that carries homologies to ACE in the catalytic domain and collectrin (Tmem27) in the transmembrane domain. ACE2 and collectrin reside in close proximity on the X-chromosome, with this juxtaposition observed throughout evolution.[17] Collectrin critically stabilizes multiple single transporters in the kidney required for amino acid reabsorption; thus, mutant mice and humans that carry mutations in collectrin or such transporters exhibit aminoaciduria.[17,18] ACE2 exerts a collectrin-like function in the intestine, where it forms a functional heterodimer coupled to the amino acid transporter B^0AT1 (*SLC6A19*) (**Fig. 1C**).[19] Thus, besides inactivating Ang II and cleaving other small peptides via its enzymatic carboxypeptidase function, ACE2, via its collectrin-domain, critically controls essential amino acid dietary uptake by stabilizing amino acid transporters at the brush border membranes of enterocytes.[18]

ACE2 regulation

Given the canonical role of ACE2 as a RAS modulator and receptor for SARS-coronaviruses, deciphering the molecular control of ACE2 is critical for understanding physiology and disease susceptibilities (**Fig. 2**). Based on the location of *ACE2* on the X-chromosome and its ability to escape X-gene inactivation, differential expression would be anticipated to contribute to sex-based differences in health and disease.[7,11,20] However, regulation of ACE2 is complex and controlled by gonadal steroids. Androgen receptor (AR) agonists, such as testosterone, upregulate *ACE2* expression[21], whereas estrogen downregulates *ACE2* expression (**Fig. 2**).[22] Furthermore, female mice exhibit reduced ACE2 activity in the heart and kidney compared to males through an

estrogen-dependent mechanism.[20] Aside from sex hormones, the apelin pathway transcriptionally induces *ACE2* expression through a feedback loop via nuclear translocation of GATA transcription factors (**Fig. 2**).[23] Nevertheless, *ACE2* transcriptional regulation remains incompletely understood representing an active research area.

DNA methylation and histone modifications mediate epigenetic control of the *ACE2* locus (**Fig. 2**). Aberrant DNA hypermethylation at CpG4 and CpG5 within the *ACE2* promoter, presumably promoting transcriptional repression, has been found in essential hypertension.[24,25] Histone post-translational modifications, such as lysine-specific histone demethylase 5B (*KDM5B)* that demethylates lysine 4 of histone 3 (H3K4), has been positively associated with *ACE2* expression in the lungs.[26] In contrast, enhancer of zeste homolog 2 (EZH2) catalyzes H3K27 trimethylation (H3K27me3) to suppress *ACE2* expression in human embryonic stem cells.[27] Finally, miRNAs represent a post-transcriptional control nidus through translational repression of *ACE2*. Based on predicted miRNA binding domains on the 3' untranslated region (UTR), *in silico* modeling identified miR-125a-5p, miR-200c, miR-200b and miR-429 as putative regulators of *ACE2*.[28] In cell lines, miR-421 reduced ACE2 protein levels and activity, and miRNA-143 inversely correlated with ACE2 activity in a study following aerobic exercise training in humans.[29,30]

Targeted degradation and proteolytic shedding control ACE2 protein levels (**Fig. 2**). The fact that no correlation exists between *ACE2* mRNA expression and ACE2 activity in certain organs highlights the impact of post-translational ACE2 processing.[11] In the development of pulmonary arterial hypertension (PAH), murine double minute 2 (MDM2), an E3 ubiquitin ligase, targets ACE2 for proteasomal degradation by ubiquitination.[5] Conversely, AMP-activated protein kinase (AMPK) phosphorylates ACE2's intracellular domain to stabilize its membrane expression.[5,31] At the cell surface, a disintegrin and metalloproteinase-17 (ADAM17) mediate ectodomain shedding of membrane-bound ACE2, which is then detectable as soluble ACE2 in the plasma.[6,32] Moreover, controlling ACE2 translocation to the plasma membrane may represent another mechanism of protein level regulation, which begins with correct conformational folding and N-glycosylation at the endoplasmic reticulum and subsequent membrane-directed transport. However, the accessory proteins required for this tightly-regulated process have yet to be elucidated. Thus, multiple transcriptional, post-transcriptional, and post-translational regulators control ACE2 levels in different cell types in a sex-specific and plausibly age-dependent

manner.[11,20] Pathological states, such as heart failure, hypertension, chronic kidney disease, diabetes, and obesity, can further modify ACE2 levels.[6,8,33]

ACE2 as the critical entry receptor for SARS-CoV and SARS-CoV-2

In 2003, ACE2 was recognized as a candidate cell-surface receptor for the newly identified SARS-CoV *in vitro*, and shown to be essential for SARS-CoV infections *in vivo* using *Ace2* knockout mice.[34] Shortly after the discovery of SARS-CoV-2 as the causative agent of COVID-19, ACE2 was shown to serve as its entry receptor in cell culture studies.[1] These findings were subsequently confirmed by genetic studies in human organoids and *Ace2* mutant mice infected with novel mouse-adapted SARS-CoV-2 strains.[35,36] Thus, all genetic experiments came to the same conclusion: ACE2 is the indispensable receptor for SARS-CoV and SARS-CoV-2 infections *in vitro* and *in vivo*.

SARS-CoV and SARS-CoV-2 belong to the betacoronavirus genera, most likely originating in bats.[37] The *S* gene, which encodes for the spike protein, is the most frequent site for recombination to enhance receptor binding affinities for ACE2, suggesting evolutionary adaptation of the receptor-binding domain (RBD) as a critical event for cross-species transmission (and immune evasion).[37] ACE2 orthologs are highly conserved across vertebrate species and are even identified in insects, reflective of ACE2's cardinal physiological activities.[38] Evolutionary conversation of ACE2 amino acid residues that bind to the SARS-CoV and SARS-CoV-2 RBDs explains the broad host tropism, thereby driving zoonotic transmission and viral evolution of SARS-CoV-2. Both *in silico* and *in vivo* analyses have confirmed the susceptibility of many domestic animals, livestock, and wildlife, including cats, dogs, ferrets, hamsters, and sheep, but not rodents or pigs, to the original Wuhan strain of SARS-CoV-2, carrying significant implications for agriculture and animal conservation.[38,39] However, the Omicron variants have now expanded their tropism to infect rodents, a testament to the molecular adaptability of coronaviruses to multiple host species.[40]

Although certain clades of sarbecoviruses appear to have lost the ability to engage ACE2 through substitutions and deletions in the ACE2-binding region, all SARS-related coronaviruses bind to ACE2 with varying affinities.[41] As a natural reservoir for SARS coronaviruses, bat species demonstrate extensive *ACE2* polymorphisms at an accelerated evolutionary pace compared to other mammalian lineages due to the ongoing selection pressures between the viral spike protein

and ACE2.[42] In contrast, *ACE2* variations are present, but exceedingly rare, in humans at the population level, though structural and functional evaluations suggest specific human *ACE2* variants may enhance or disrupt spike protein binding.[38,42]

Alterations in ACE2 glycosylation can substantially influence spike recognition and binding. For example, molecular dynamics simulations predicted that the glycan at amino acid position N90 partially shields the RBD interface to reduce SARS-CoV-2 binding, whereas the ACE2 N322 glycan interacts with a conserved region of the spike RBD to strengthen its binding affinity.[43] Moreover, the spike protein is also heavily glycosylated, and these glycans contribute to ACE2 binding, stable spike protein expression, virion assembly[44], and antibody evasion (**Fig. 3A**).[45] Interestingly, one interaction with the spike glycans is mediated via ACE2 N546, which is absent in three out of every 10,000 humans due to naturally occurring SNPs.[46] In accordance, SNPs in *ACE2* that alter its expression or glycosylation states are associated with differences in COVID-19 severity, suggesting a potential link to SARS-CoV-2 pathogenesis.[46] Detailed understanding of the spike-ACE2 glycan interactions is critical for developing ACE2-directed therapies and tissue-specific delivery of COVID-19 vaccines, as glycosylation of the spike protein shields from immune recognition and thereby affects the specificity and amounts of antibodies generated following vaccination.[45]

Ubiquitous ACE2 distribution facilitates SARS-CoV-2 infections

SARS-CoV-2's viral tropism and diverse extra-pulmonary manifestations are dependent on *ACE2* tissue distribution and expression. Specific to the infective process and primary transmission, *ACE2* expression shows a progressive reduction from the nasal epithelium towards the lower respiratory tract, which correlates with patterns of viral infectivity.[47] *ACE2's* mRNA expression is high in nasal goblet and ciliated cells and remains detectable in lower airway basal, ciliated, club, and type II alveolar cells.[8] While the lower respiratory tract and pneumocytes serve as primary replication sites for SARS-CoV, SARS-CoV-2 efficiently replicates in upper respiratory tract tissues, which contributes to its more efficient transmission and infection dynamics compared to SARS-CoV.[47]

SARS-CoV-2 has evolved to utilize multiple accessory host proteases, including cathepsin L, cathepsin B, furin, and, in particular, TMPRSS2 (transmembrane protease serine 2) for spike protein priming and cellular infection (**Movie 1**).[6] Furin cleavage dominates in the trans-Golgi

network and facilitates spike protein priming to enhance viral infectivity[48]; however, some evidence points to furin also localizing to the cell membrane to facilitate cellular entry.[48,49] Importantly, the Omicron BA.1 variant appears to be largely independent of TMPRSS2 cleavage, which drives endosomal-dominated viral entry, making therapeutic inhibition of this protease largely ineffective against this variant.[50] Proteases required for spike priming and activation are meaningful drug targets, already explored in COVID-19 trials, for instance, using the non-specific protease inhibitor camostat (www.clinicaltrials.gov number NCT04353284).

COVID-19 initially manifests as a respiratory disease following nasopharyngeal SARS-CoV-2 infection. From there, SARS-CoV-2 can spread to lung alveoli resulting in the development of a distinct pneumonia that reflects the cell type-specific expression of ACE2 and accessory molecules such as priming proteases. Deep lung damage can subsequently lead to the spread of SARS-CoV-2 to other tissues, clinically observed as a multi-organ disease in more severe COVID-19 cases.[6,51] A meta-analysis of single-cell RNA-sequencing datasets revealed that *ACE2*-expressing cells are highly abundant in intestinal enterocytes, proximal kidney tubules, cardiomyocytes, fibroblasts and vascular smooth muscle cells, and defined endothelial cells and pericytes, including cells of the choroid plexus constituting parts of the blood-brain barrier.[9] These *ACE2*-expressing cells constitute a potent landing site for the systemic spread of coronaviruses, contributing directly and indirectly (immune activation, blood clotting alterations, vascular damage, etc.) to COVID-19's diverse symptoms.

ACE2 exists in two forms – a full-length form that is membrane-bound and a shorter soluble form that is shed into bodily fluids and circulates in the blood in minimal amounts. Both forms contain the same sequence in the catalytic domain, but soluble ACE2 lacks the transmembrane domain necessary for anchoring it to the cell membrane.[2] Pathological induction of ADAM17 during SARS-CoV-2 infection through direct viral mechanisms or the ensuing inflammatory response triggers aberrant proteolytic cleavage of ACE2 into the circulation, thereby reducing its tissue expression and blocking its local counter-regulatory effects on peptide cascades.[52] Activation of the Ang II pathway through the angiotensin II type 1 receptor (AT_1R) and tumor necrosis factor (TNF) receptor signaling upregulates ADAM17 proteolytic activity, leading to a detrimental positive feedback loop in infected tissues.[32,53] Furthermore, stimulation of p38 MAPK by inflammation and cellular stress directly promotes ADAM17-mediated ectodomain ACE2 shedding.[32] Persistently elevated soluble ACE2, reflective of putative sustained

pathological ADAM17 activation and hyperinflammation, is associated with increased severity, mortality, and incidence of acute myocardial injury in COVID-19.[51] Pharmacological ADAM17 inhibition led to improved lung histology and reduced neutrophil and macrophage recruitment in an inflammatory lung injury model mimicking COVID-19's effects.[54] However, ADAM17-mediated shedding of TNF receptor 1 (TNFR1) limits inflammation in the context of sepsis, demonstrating a protective role of this enzyme.[55] Collectively, these findings provide a molecular bridge between virus-induced inflammation and cellular stress with dysregulation of membrane-bound ACE2 contributing to multi-organ pathologies and disease severity following SARS-CoV and SARS-CoV-2 infections.

Emergence of SARS-CoV-2 variants and ACE2 binding

SARS-CoV-2 contains an evolutionary conserved homotrimeric spike protein optimized for binding to human ACE2. Receptor recognition is mediated through the 21 kDa RBD of the S1 subunit interacting with the extracellular N-terminal domain of ACE2. The SARS-CoV-2 S1 subunit shares a great degree of structural homology with the original SARS-CoV, yet exhibits an approximately 5-10 times higher binding affinity to human ACE2 (**Fig. 3B**).[56] Structure-guided sequence alignment has revealed substitution of five amino-acid residues directly involved in ACE2 binding from the SARS-CoV RBD.[57] These key substitutions translate into greater electrostatic complementarity by forming additional hydrogen bonds, hydrophobic, and salt-bridge interactions, explaining the increased binding affinity.[57]

Selection pressures from natural infections, drug treatments, and vaccine-mediated immunity promote fitness advantages from random mutations acquired through antigenic drifts in coronaviruses. The RBD is exposed at the viral surface to facilitate receptor recognition and engagement; therefore, it serves as a primary target for serum-neutralizing antibodies and undergoes constant selection pressures (**Fig. 3C-D**). B.1.1.7 (Alpha, α), alongside B.1.351 (Beta, β) and P.1 (Gamma, γ) variants, all independently acquired the N501Y mutation in the spike protein RBD through convergent evolution[58], conferring enhanced binding affinity to ACE2.[59] Mutations in K417 and E484 residues of Beta and Gamma RBD elicit additional resistance against antibody neutralization beyond those afforded by the N501Y mutation[60], without discernable effects on ACE2-binding affinity.[59] The next phase of the COVID-19 pandemic saw the emergence of the B.1.617.2 (Delta, δ) variant in late 2020, rapidly surpassing other existing variants of

concern.[60] Structural analysis revealed two key mutations: L452R and T478K in the Delta RBD, which are peripheral to the RBD-hACE2 interface.[61] These Delta RBD mutations, alongside the substantial antigenic surface modifications to the N-terminal domain, were likely driven by increased selective pressures from immune recognition. The B.1.617.2.1 (Delta plus, δ+) variant contains an additional K417N mutation in the absence of the compensatory N501Y mutation, resulting in reduced affinity for ACE2, which may explain why Delta plus contributed significantly less towards global SARS-CoV-2 genomic diversity.[61] Thus, RBD mutations that compromise ACE2 binding appear to be associated with consequential fitness trade-offs.

This balancing act between maintaining strong ACE2 binding while affording escape from immune recognition is most striking with the B.1.1.529 (Omicron) variant, which was first reported in November 2021 and swept across the globe at an unprecedented rate. In total, 37 genetic alterations are present on the Omicron spike protein, with 15 mutations mapping to the RBD (**Fig. 3C-D**).[62] Most studies found the Omicron RBD exhibited increased ACE2 binding affinities, affirming the importance of preserving ACE2 binding for variant propagation.[59] Subsequently, the Omicron variants BA2, BA3, and in particular BA4 and BA5 emerged.[63] Omicron further carries two mutations, also observed in mouse-adapted SARS-CoV-2 viruses[40], enabling binding to rodent ACE2 and the potential to "jump" into rodent populations.[62] Notably, many more SARS-CoV-2 variants continue to emerge, including BW.1 (BA.5.6.2.1), which is an Omicron subvariant descending from the BA.5.6.2 lineage, or Lineage XB, which originated from a recombination event between viruses of the B.1.631 and B.1.634 lineages.[64] More recently, XBB.1.5 has overtaken other Omicron subvariants to become the dominant circulating strain, an effect attributed to its substantially stronger binding affinity to human ACE2 through the acquired S486P mutation.[65] Taken together, all studied SARS-CoV-2 variants bind to ACE2, in most cases with increased affinity and avidity, providing a molecular correlate for enhanced infectivity and transmissibility. Thus, although SARS-CoV-2 variants readily escape antibody-mediated immunity from vaccines and after natural infections[63,66], it appears that all known and future SARS-CoV-2 variants cannot escape binding to ACE2.

ACE2 in organ injury and post-acute sequelae of COVID-19 or long COVID

The devastating toll of SARS-CoV-2 infections extends beyond the infectious phase. Notably, recovering patients six months post-infection showed a stepwise increase in mortality risk and

development of new pulmonary, nervous system, metabolic, gastrointestinal, and cardiovascular diseases largely following acute COVID-19 severity, known as "long COVID".[67] The clinical definition of long COVID varies, but the World Health Organization (WHO) describes it as individuals having ongoing symptoms impacting everyday function for three months since a probable or confirmed SARS-CoV-2 infection with symptoms lasting at least two months that cannot be explained by an alternative diagnosis.[68] Several contributing mechanisms have been proposed for long COVID pathogenesis, including vascular dysfunction, chronic immune activation, autoantibodies, and sustained dysregulation of tissue ACE2.[69,70] Indeed, older age, female sex, elevated body mass index, and pre-existing conditions such as hypertension and diabetes, are recognized and validated risk factors for developing long COVID, potentially related to the differential expression and regulation of ACE2 under these conditions.[71] Moreover, developing cardiovascular complications is a significant concern in long COVID, where ACE2 dysregulation during the acute COVID-19 phase has been associated with increased mortality and acute myocardial injury.[51,72] Below, we will outline the known tissue-protective functions of ACE2 in the context of COVID-19 pathogenesis by disease category.

ACE2 in respiratory disease

ACE2 deficiency is implicated in the pathogenesis of acute lung injury (ALI) and acute respiratory disease syndrome (ARDS), pulmonary arterial hypertension (PAH), and pulmonary fibrosis.[73,74] Catalytically active recombinant ACE2 alleviated pulmonary injury and vascular damage[73], decreased lung fibrosis, arterial remodeling, and improved right ventricular performance in PAH.[75] Loss of ACE2 worsened ALI in mice associated with increased immune infiltration, altered pulmonary architecture, and enhanced vascular permeability.[73] Conversely, genetic inactivation of *Ace* or *Agtr1a*, AT_1R blockade using losartan, and recombinant human ACE2 (rhACE2) mitigated these lung pathologies, suggesting that pulmonary protection by ACE2 is in part elicited through limiting canonical RAS activation.[73] Additionally, lentiviral-mediated ACE2 overexpression attenuated inflammation and lung injury by reducing phosphorylation of p38 MAPK and NF-kB, whereas selective inhibition of the Ang-(1-7) receptor Mas nullified these effects.[76] Another role of pulmonary ACE2 in ALI is through the proteolytic inactivation of des-Arg^9 bradykinin to limit proinflammatory cytokine expression and vascular leakage[12], highlighting how RAS-independent ACE2 functionality mitigates vascular and pulmonary injuries.

Treatment of multiple cell types, organoids, or mice *in vivo* with recombinant spike or RBD protein from SARS-CoV and SARS-CoV-2 led to ACE2 downregulation from the cell surface with worsening pulmonary function and exacerbation of lung pathologies.[34,77,78] Reduced ACE2 expression contributes to increased severity and pathogenesis of acute and chronic lung injury through enhanced RAS or bradykinin system activation (and a possible deregulation of other peptide cascades).[34,73] ACE2 downregulation is also observed independent of direct viral interaction in pathogenic avian influenza A H7N9 virus infections or non-infectious acid injury, leading to greater disease severity and elevation in Ang II levels.[79] In comparison, less pathogenic human influenza viruses and coronaviruses causing limited non-severe upper-respiratory tract infections, including HCoV-NL63, apparently do not alter ACE2 levels.[52,78] Thus, in addition to blocking spike binding to prevent SARS-CoV-2 infections, the ACE2 axis could be temporally manipulated to mitigate SARS-CoV-2 induced lung pathologies, representing a potential dual mechanism for therapeutic intervention.[34,73] In support of these findings, reconstituting ACE2 enzymatic activity independent of spike binding alleviated pulmonary symptoms of SARS-CoV-2 infections in animal models.[80]

ACE2 in cardiovascular disease

Multiple studies have linked ACE2 deficiency to pathological states in hypertension, myocardial infarction, atherosclerosis, dilated cardiomyopathy, diastolic dysfunction, and heart failure.[81-83] In post-mortem autopsy heart tissues from patients who succumbed to SARS-CoV, 35% of heart samples had detectable viral SARS-CoV, associated with increased fibrosis, inflammation, and reduced myocardial ACE2[84], These findings were recently corroborated in hamsters infected with SARS-CoV-2 and autopsied human heart samples from COVID-19 patients.[85] Acute and chronic cardiac injuries related to functional and structural abnormalities, including arrhythmias, microthrombi formation, myocarditis, and heart failure, are prevalent in patients with severe COVID-19.[86] Endothelial and microvascular injuries in the heart, lungs, or brains of COVID-19 patients leads to increased risk of thrombosis, including acute coronary syndrome, ischemic stroke, pulmonary embolism, and ischemic limbs.[86,87] Increased and sustained inflammatory cytokine levels throughout COVID-19's clinical course also contribute to systemic vascular injury in patients, particularly when ACE2 is compromised.[4,88]

Cardiovascular complications, such as myocarditis and pericarditis, are infrequently reported in individuals vaccinated with spike mRNA-based vaccines.[89] Of note, the spike protein is detectable in the plasma up to 14 days after mRNA vaccination[90], and it has been proposed, albeit controversially, that spike exosomes exist up to 4 months after spike mRNA vaccines.[91] Whether spike-mediated ACE2 downregulation, as observed in multiple experimental paradigms *in vitro* and *in* vivo[52,77,84], or a deregulated RAS might contribute to such vaccine side effects and long-term symptoms remains undetermined. Experimentally, SARS-CoV-2 can directly infect stem cell-derived human blood vessel organoids.[35] Moreover, SARS-CoV-2 infects human cardiomyocytes, an interaction critically dependent on ACE2.[92] In fact, multiple cardiac cell types harbour ACE2 and are susceptible to direct SARS-CoV-2 infection. Cardiac infection in concert with aberrant immune activation, increased thrombus formation, and dysregulated vascular homeostasis results in acute cardiac pathologies. Whether long-term ACE2 deregulation due to inflammation or viral remnants contributes to frequently observed cardiovascular long COVID symptoms and rare incidences of vaccine-induced myocarditis needs formal proof. Nevertheless, the crucial protective functions of ACE2 in the cardiovascular system cannot be understated, since the first phenotype discovered in *Ace2* mutant animals was that of impaired heart function.[7,83]

ACE2 in renal disease

In the kidney of humans and other mammalian species, ACE2 is highly expressed in the proximal tubules and, to a lesser extent, in glomerular endothelial cells and podocytes.[10] Genetic inactivation of *Ace2* exacerbates kidney injury, while administration of soluble ACE2 is renal-protective.[93] For instance, *Ace2* knockout mice exhibit worsened renal injury in an ischemia-reperfusion model of acute kidney injury (AKI), associated with increased immune cell infiltration.[94] Furthermore, local RAS activation contributes to the development of diabetic nephropathy, the leading cause of chronic kidney disease. Global *Ace2* knockout in the Akita type 1 diabetes mouse model worsened albuminuria, renal fibrosis, and glomerular hypertrophy, which was rescued by systemic substitution with soluble ACE2.[95] In human diabetic kidney disease, ACE2 renal loss correlates with markers of increased severity, suggesting enhanced renal susceptibility to Ang II-mediated damage.[96] Elevated urinary ACE2 levels have been consistently and independently associated with aminoaciduria and a greater risk of developing AKI in patients with COVID-19.[97]

SARS-CoV-2 can directly infect tubular kidney epithelial cells and stem cell engineered human kidney organoids. This process is genetically dependent on *ACE2* expression and inhibited by soluble ACE2.[35] ACE2 protein levels and SARS-CoV-2 replication are also increased in human kidney organoids exposed to oscillatory glucose to model diabetic conditions, and deletion of ACE2 completely abolished SARS-CoV-2 infection.[98] Thus, ACE2 in kidney cells, which is dysregulated in pathological conditions such as hypertension, obesity, and diabetes, favours SARS-CoV-2 infections and kidney injury associated with increased morbidity and mortality in patients with COVID-19.[99]

ACE2 in gastrointestinal disease

ACE2, TMPRSS2, and *FURIN* are highly expressed at the luminal surface of intestinal enterocytes.[10,100] In murine models, *Ace2* deficiency in experimental colitis leads to inflammation and alterations in the intestinal microbiome[19], and has been associated with dysbiosis and a reduction in circulating angiogenic cells - hematopoietic cells with reparative function.[101,102] Patients with inflammatory bowel disease exhibit reduced *ACE2* expression in the inflamed ileum, which is associated with a more severe colitis phenotype.[103] Functionally, intestinal ACE2 controls intestinal amino acid homeostasis via its collectrin-homology domain, independent of its carboxypeptidase activity. Hartnup disease is a rare autosomal recessive disorder caused by mutations in the *SLC6A19* gene encoding B^0AT1 and results in impaired amino acid transport in the small intestine and kidneys.[18,104] Collectrin was identified as the missing subunit that stabilizes the surface expression of the B^0AT1 transporter in the kidneys, required for the reabsorption of amino acids from the urine.[17] In comparison, ACE2 is essential to stabilize B^0AT1 at the brush border membrane of enterocytes.[19,104] Consequently, ACE2 controls dietary uptake of essential amino acids such as tryptophan, which is involved in local and systemic immunity.[105] Disease-causing variants of B^0AT1 have impaired interaction with ACE2, resulting in reduced surface expression and, thus, reduced amino acid uptake.[104] Therefore, ACE2 is necessary for intestinal amino acid transport and intestinal microbiome homeostasis. By inference, malabsorption of neutral amino acids might occur in COVID-19 and thereby contribute to dysbiosis and disease severity.[102] Indeed, alterations in the microbiome have been reported in SARS-CoV-2 infected mice and patients with COVID-19.[106] In human small intestinal organoids, SARS-CoV-2 can directly infect and replicate in enterocytes resulting in apoptosis, thus propagating local and

systemic illness.[100,107] Infection of intestinal organoids has been demonstrated to be critically dependent on ACE2, but not other candidate receptors.[108] SARS-CoV-2 viral RNA and, rarely, infectious particles are present in patients' stool, suggesting the plausibility of fecal-oral transmission as occurs in bats.[109] Consequently, SARS-CoV-2 RNA has also been detected in the intestine and stool samples of patients seven months following the initial infection[110], implying that the intestinal epithelium might be a reservoir for SARS-CoV-2 contributing to long COVID symptomology.[110] Moreover, up to 44% of COVID-19 patients reported gastrointestinal symptoms at 90 days after discharge[111], and in a prospective cohort study, long-term symptoms were associated with diarrhea during the acute phase.[112] Finally, wastewater screening for SARS-CoV-2 variants is a staple of epidemiological studies and early detection.[113]

ACE2 in metabolic disease

Metabolic disorders such as diabetes or obesity predispose patients with COVID-19 to adverse outcomes, and ACE2 is altered in such pathologic conditions in experimental animal models and humans. Therefore, targeting systemic and tissue RAS may improve clinical outcomes in diabetic patients infected with SARS-CoV-2.[114] Furthermore, increased SARS-CoV-2 viral loads in diabetic kidney organoids have been demonstrated to be critically dependent on elevated ACE2 protein levels in hyperglycemic states.[98] Variability of ACE2 levels in diabetes may be dependent on the organ sampled, method of detection, and disease duration.[115] Due to existing gut dysbiosis and an overactive RAS resulting from ACE2 dysregulation, obesity and diabetes can also complicate COVID-19.[101,116] Obesity and diabetes predispose individuals to an excessive proinflammatory response, insulin resistance, reduced vascular repair, and impaired amino acid absorption leading to their association with an increased risk of morbidity and mortality in COVID-19.[114] Moreover, it has been reported that pancreatic islet cells, as well as adipocytes, could serve as sites of viral replication for SARS-CoV-2.[117] In fact, SARS-CoV-2 infection of adipocytes induces adipose tissue dysfunction, leading to altered adipokine secretion and insulin resistance, further exacerbating COVID-19 severity.[118]

ACE2 in neurological disease

A local RAS exists within the brain and is associated with neurological diseases, including neurogenic hypertension, inflammation, stroke, and neurodegenerative disorders.[119] Canonical

RAS activation leading to vascular dysfunction has also been linked to aging and vascular dementia.[120,121] Specifically, Ang II-induced AT_1R activation generates superoxide by increasing the expression of, and activating NADPH oxidase in the cerebral vasculature while reducing the bioavailability of the potent vasodilator nitric oxide (NO). The production of reactive oxygen species also generates peroxynitrite that results in tissue damage.[120] Thus, cerebral *ACE2* deficiency results in impaired Ang II inactivation and contributes to aging-related blood vessel alterations and mental decline.[120,122] Furthermore, reduced ACE2 activity is associated with cognitive impairment and increased hallmarks of Alzheimer's disease[123], and enhancing ACE2 activity in transgenic mice has been shown to reduce amyloid plaque burden and prevent cognitive decline.[124]

Regarding COVID-19, SARS-CoV-2 neurotropism was initially suggested from reports of diminished smell and taste perception in the acute infectious phase. Direct SARS-CoV-2 invasion of the olfactory mucosa, cornea, and conjunctiva supports the nasal and ophthalmic routes of infection. These infection patterns correlate with the widespread regional expression of *ACE2*, particularly in defined neurons, astrocytes, pericytes, and smooth muscle cells of the cerebral vasculature and the choroid plexus.[125,126] Therefore, a putative mechanism of viral dissemination involves olfactory nerve projections following infection of the nasal mucosa and sustentacular cells.[125,126] Additional potential mechanisms of viral neuroinvasion occur in regions with high vascular permeability and an abundance in ACE2 and TMPRSS2. Examples include the choroid plexus and pituitary and median eminence of the hypothalamus, as well as in conditions with compromised blood-brain barrier integrity, such as diabetes and hypertension.[126] Finally, additional routes of viral neuroinvasion might include the carotid body and possibly the aortic body, clusters of oxygen, carbon dioxide, and pH chemoreceptive cells that increase respiratory drive in response to hypoxia.[127] ACE2 is detected in blood vessels and glomus cells of the carotid body, and infection or injury of this chemosensory organ may contribute to the silent hypoxemia observed in patients with COVID-19.[128]

Apart from direct CNS infections, systemic immune responses to SARS-CoV-2 and thromboembolisms can lead to microvascular injuries.[126] Although largely undetermined, emerging signs and symptoms of the neurological post-acute sequelae of SARS-CoV-2 infection (long COVID) include cognition and mood disorders, dysautonomia, pain syndromes, and exercise intolerance.[129] Further mechanistic insights are urgently required to delineate the contribution of

ACE2-mediated direct neuroinvasion or systemic inflammation to chronic neurological complications of COVID-19.[126]

Soluble ACE2 as a disease marker

Studies have found that male sex, advanced age, smoking, higher blood pressure, and body mass index are associated with elevated soluble ACE2 and increased risk for severe COVID-19.[130,131] In a study including 10,753 participants, higher soluble ACE2 concentrations in the serum were independently associated with a greater risk of all-cause mortality and incidences of myocardial infarction, diabetes, and heart failure beyond traditional cardiac risk factors.[130] Moreover, in prospective studies of patients with heart failure, atrial fibrillation, and coronary artery disease, elevated soluble ACE2 activity was associated with adverse clinical outcomes.[132-134] Similarly, the relative ratios of Ang II to Ang-(1-7) effector peptides in the RAS, that are regulated by ACE2 activity, predicted outcomes in patients with heart failure.[135] These observations suggest a pathological link between elevated circulating soluble ACE2 and the loss of protection against RAS activation in local tissues, mediated by downregulation of membrane-bound ACE2. Although persistent elevation in plasma ACE2 activity is associated with COVID-19 mortality and severity, ACE2 activity is substantially attenuated at 8-month post-infection and has not been associated with long COVID symptoms.[136] However, ACE2 autoantibodies that can directly inhibit ACE2 enzymatic activity are present in the plasma of severe COVID-19 patients[137], which may explain the findings of lowered plasma ACE2 activity in prolonged disease sequelae.

Controversy has existed since the pandemic's nascence regarding whether the systemic RAS is pathologically active in COVID-19. Several clinical trials are underway to assess the protective effects of ACE inhibitors, AT_1R antagonists (ARBs), AT_2R agonists, Ang-(1-7) peptides, and activators of the Ang-(1-7) receptor Mas, or the bradykinin system in COVID-19 (www.clinicaltrials.gov numbers NCT04332666, NCT04605887, NCT04472728, and NCT04924660). Due to the dominant respiratory involvement of acute SARS-CoV-2 infection and the central role of pulmonary endothelial ACE in generating systemic Ang II from Ang I, plasma Ang II levels are actually lowered in acute COVID-19.[51,138] In agreement with these findings, early clinical trials demonstrated no benefits of AT_1R blockade or Ang-(1-7) therapy.[139,140] However, whether modulation of the RAS or other ACE2-regulated peptide

cascades can be utilized to alleviate long COVID-associated complications warrants further investigation.

ACE2 and COVID-19 vaccines

Blocking the spike-RBD interaction with ACE2 is the foundation of COVID-19 vaccine development and neutralization strategies for SARS-CoV-2 (**Fig. 4**). Not excluding other immune cells, all vaccines, irrespective of their underlying technologies, rely on establishing humoral immunity against the spike protein, thereby preventing viral interaction with ACE2. Profiling of 127 representative antibodies from mRNA vaccine recipients revealed that 98% targeted the RBD, indicating a predominant role in preventing ACE2 interaction for vaccine-mediated SARS-CoV-2 neutralization.[60] Therefore, the principle to block the RBD-ACE2 interaction to prevent and alleviate COVID-19 has now been validated in 13 billion vaccine administrations, the largest vaccination campaign in history.[141] Similarly, convalescent serum therapies and all approved and under-development antibodies, nanobodies, or engineered small interfering molecular therapeutics rely on the principle of blocking the spike-ACE2 interaction.[142] Thus, based on the COVID-19 vaccination campaigns and treatments, ACE2 has become one of the therapeutically most targeted molecules in human history.

Universal prevention and treatment strategy for SARS-CoV-2 variants

Antibody escape and the emergence of variants make it paramount to design universal therapeutic and preventative strategies, not only against all current and future SARS-CoV-2 variants, but also for global preparedness against other coronaviruses with pandemic potential (**Fig. 4**). Spike protein binding to membrane-bound ACE2 is essential for all known and, by extension, all future SARS-CoV-2 variants to ensure infection.[143,144] To this end, multiple efforts are underway to exploit human ACE2 decoys as a universal prevention and treatment strategy (**Movie 2**). The safety profile of soluble ACE2 infusion has already been demonstrated in phase 1 healthy volunteers[145] and phase 2 clinical trials.[146,147] Administration of soluble ACE2 leads to prompt systemic conversion of Ang II to Ang-(1-7), associated with a corresponding reduction in inflammatory and oxidative stress biomarkers.[146-148] In human blood vessel and kidney organoids, ACE2 decoys neutralize SARS-CoV-2 and drastically reduced viral replication.[35] This treatment works by orders of

magnitude better against the Alpha, Beta, Gamma, Delta, and Omicron variants, due to their enhanced affinity/avidity for ACE2 binding.[149]

These beneficial effects have been reproduced in hundreds of independent studies with variations in soluble ACE2, such as an addition of immunoglobulin crystallizable fragment (Fc-IgG) or albumin-binding domain fusions to ACE2 to promote longer-lasting neutralizing effects.[150,151] In hamsters and mouse models, inhaled or nasally instilled ACE2 decoys nearly completely blocked SARS-CoV-2 infections and disease.[36,152] This provides a proof-of-concept for the application of ACE2 decoys as a universal prevention strategy, particularly during the early stages of infection. Intriguingly, ACE2 decoys also appear to improve the accessibility of antibodies to otherwise cryptic antigenic regions of the spike protein, indicating that such combination therapies might be of unique benefit.[153] Moreover, ACE2-mimicking small molecules and biomimetic peptides[154], oligomeric versions of ACE2 to enhance avidity[155], ACE2-expressing microspheres[156], engineered decoy ACE2 receptors that improve delivery and neutralization capacity[157, 158], or ACE2-based nanodecoys[142,159] can sequester SARS-CoV-2 in different cell lines and human organoids in a dose-dependent manner while also alleviating and preventing SARS-CoV-2 infections in multiple experimental animal models *in vivo*.

In principle, early intervention results in better outcomes for SARS-CoV-2 infections. However, early treatment needs to be made accessible and feasible for medical personnel at the frontlines of COVID-19 patient care and those at high risk for severe disease, namely, elderly patients, those with co-morbidities or those exposed to novel variants. To accomplish this, multiple efforts are underway worldwide to develop inhalable versions of soluble ACE2 and ACE2 mimics to prevent or alleviate early SARS-CoV-2 infections[36], including an effort to add ACE2 to chewing gum to de-bulk SARS-CoV-2.[160] Because all ACE2-centered approaches to limit viral infectivity leverage the essentiality of ACE2 binding, they are expected to work for all SARS-CoV-2 variants.

Conclusion

In summary, ACE2 is the critical entry receptor for all known and, by extension, all future SARS-CoV-2 variants, placing ACE2 at the heart of the COVID-19 pandemic. As an evolutionarily ancient protein that is conserved across animals and bacteria, ACE2 protects multiple organs and contributes to physiological homeostasis via its enzyme and amino acid transport function in humans, which explains the route of transmission and multi-organ injury during acute SARS-CoV-

2 infection and long COVID. A fundamental understanding of the essentiality of ACE2 provides a rationale for ACE2-centered approaches to universally prevent and treat COVID-19. These therapeutic strategies now need to be tested in clinical trials as part of the effort to combat the ongoing pandemic.

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Author Contributions

G.Y.O., K.W., A.V., M.J.K, and J.M.P. wrote, revised and approved the final version of the manuscript.

Declaration of Interests

J.M.P. is named on the original patent (2002) using ACE2 as a therapeutic, including lung failure, and is a shareholder of Apeiron Biologics and JLP Health that are developing ACE2 as therapy. All remaining authors have nothing to disclose.

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Figures

Figure 1. Expression, structure, and enzymatic function of ACE2.

A. *ACE2* is predominantly expressed in the small intestine, adipose tissue, kidney, heart, respiratory system, and, to a limited extent, in brain from bulk RNA sequencing studies (red). Findings are corroborated by single cell RNA (scRNA) sequencing data to identify cell-type specific expression in each organ. High-confidence data sets were used to determine $ACE2^{high}$ (blue) and $ACE2^{low}$ (grey) cell types. **B.** The canonical RAS results in the generation of Angiotensin II (Ang II), which acts on the AT1 receptor (AT_1R) to promote vasoconstriction, inflammation, and fibrosis. ACE2 deactivates Ang II to Ang-(1-7), activating the Mas receptor to promote vasodilation and inhibit fibrosis or inflammation. ACE2 further regulates the apelin pathway to convert active apelin 17, pyr-apelin 13, and apelin 13 into apelin 16, pyr-apelin 12, and apelin 12, respectively. Finally, ACE2 is involved in deactivating des-arg9 bradykinin, which acts on the B1 receptor (B1R) to potentiate inflammation and vasodilation. **C.** The N-terminal domain of ACE2 resides on the extracellular leaflet of the plasma membrane, with a C-terminal, or collectrin-like, transmembrane domain. The N-terminal domain harbors the catalytic site of ACE2. The non-enzymatic collectrin domain of ACE2 functions as a chaperone protein for the amino acid transporter B^0AT1. The ACE2- B^0AT1 complex exists as a dimer of heterodimers and is involved in the absorption of neutral amino acids in the small intestine. Taken from Yan *et al* without modification.[57] https://www.science.org/doi/10.1126/science.abb2762.

Figure 2. Transcriptional, post-transcriptional, and post-translational regulation of ACE2.

Nuclear translocation of GATA transcription factors and binding to the ACE2 promoter increases *ACE2* expression, though the precise GATA family members have yet to be identified. Agonists binding to the androgen receptor results in nuclear translocation and association with *ACE2* enhancer elements. Assembly of the enhanceosome complex, consisting of BRD4, MED1, and other accessory proteins, connects to the promoter bound RNA polymerase machinery (consisting of TATA-binding protein (TBP), TBP-associated factors (TAFs), and additional factors), initiating transcription. Epigenetic control of *ACE2* involves includes methylation and acetylation of histone tails, which repress and promote gene transcription, respectively. Lysine-specific histone demethylase 5B (KDM5B) upregulates *ACE2* expression by removing a methyl group from lysine 4 (K4) of the histone H3 tail. In contrast, enhancer of zeste homolog 2 (EZH2) catalyzes the trimethylation of lysine 27 (K27) and represses *ACE2* expression. DNA methyltransferases facilitate hypermethylation at CpG4 and CpG5 within the *ACE2* promoter. Circles represent the addition of a methyl group, whereas triangles represent acetylation. Post-transcriptional control by miRNAs, namely miR-421 and miR-143, target *ACE2* mRNA transcripts for degradation or prevent their translation. At the post-translational level, fine-tuning of ACE2 membrane protein levels is mediated by phosphorylation, ubiquitination and proteolytic cleavage, and glycosylation. Murine double minute 2 (MDM2), an E3 ubiquitin ligase, ubiquitinates and targets ACE2 for proteasomal degradation. AMP-activated protein kinase (AMPK)-mediated phosphorylation stabilizes ACE2 at the plasma membrane and prevents ubiquitination. Finally, ADAM17 cleaves and releases ACE2 from the cell membrane.

Figure 3. SARS-CoV-2 variants and ACE2 binding.

A. Non-glycosylated (left) and glycosylated (right) SARS-CoV-2 spike protein. The spike protein is coloured in light blue and glycans in dark blue. Adapted from Casalino *et al.*[44]; https://pubs.acs.org/doi/10.1021/acscentsci.0c01056. Further permissions related to the material excerpted should be directed to the ACS. **B.** Comparison between SARS-CoV and SARS-CoV-2 spike protein subunits and binding to human ACE2. Key amino acid residues for ACE2 binding are highlighted by red stars. **C.** 3D rendering of the spike protein heterotrimer with a subset of the single amino acid mutations defining the Omicron variant of concern (green), and the mutated regions used in experiments in the cited study (grey). Red indicates a single RBD. The glycan modifications of the spike protein are depicted in yellow. Adapted from Monteil *et al.*[149] **D**. Relative binding affinities for ACE2 between SARS-CoV, wildtype SARS-CoV-2 and the SARS-CoV-2 B.1.1.7, B.1.351, P.1, B.1.617.2, and B.1.1.529 variants of concern from surface plasmon resonance analyses.[59] Key amino acid alterations in the RBD of variants of concern are depicted by blue boxes. In general, the SARS-CoV-2 variants of concerns tend to have increased binding affinity towards human ACE2 compared to the wildtype SARS-CoV-2. Global vaccination distribution data related to the date of designation for variants of concern were obtained from the Our World in Data COVID-19 vaccination database[141] (Accessed February 9, 2022), highlighting vaccine-mediated immunity and growing natural immunity in driving the selection pressure for novel SARS-CoV-2 variants to escape immune surveillance.

Figure 4. ACE2 decoys as therapeutic interventions for COVID-19.

Limiting viral infectivity is a key prevention and therapeutic strategy. Vaccine- induced antibodies, soluble recombinant ACE2, engineered ACE2 decoys, or peptide mimetic can neutralize the spike protein binding to ACE2 (left). Following SARS CoV-2 exposure, the viral spike protein attaches

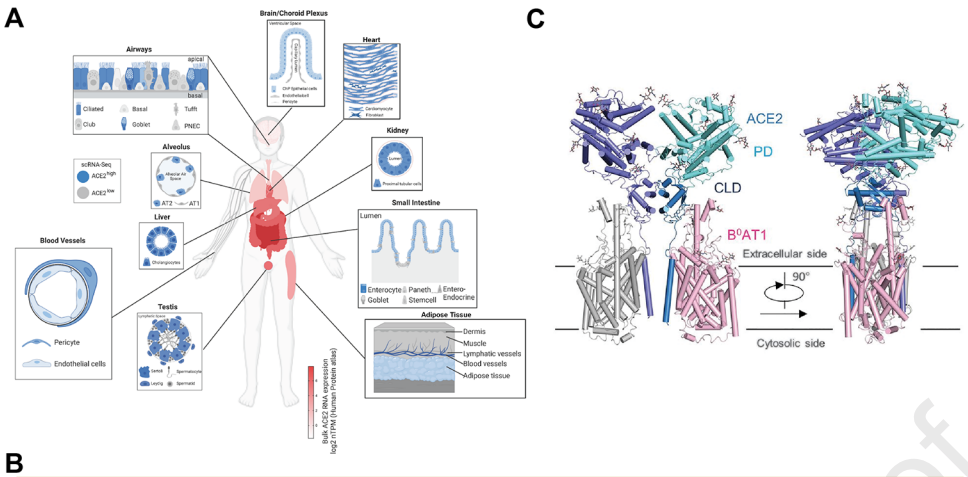
to ACE2. After processing of the spike protein by proteases such as the transmembrane serine protease 2 (TMPRSS2), which is not required for Omicron, the viral membrane fuses directly with the target cell membrane and releases the viral single-stranded RNA (ssRNA) into the cytosol, which represents the cell surface entry process for SARS-CoV-2. Alternatively, the entire viral particle is endocytosed, followed by acidification of the endosome for S2' cleavage by cathepsins, allowing the fusion of the viral and intracellular membranes to release the viral RNA. The ssRNA is subsequently replicated and translated into viral proteins, which are then assembled into mature, infectious viral particles through the secretory pathway and pre-processed by furin at the S1-S2 junction prior to exocytosis.

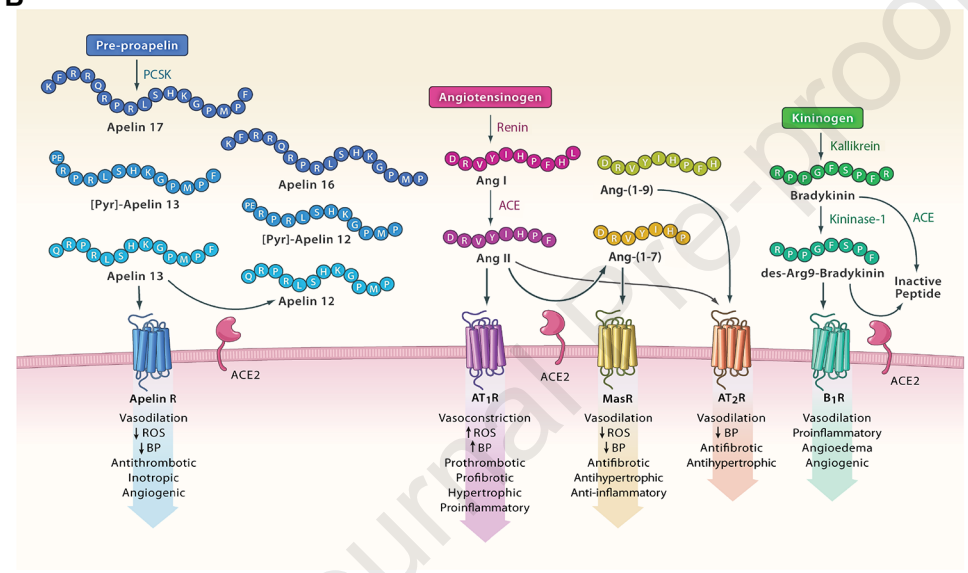
Movie 1. SARS-CoV-2 docking and infection in association with ACE2.

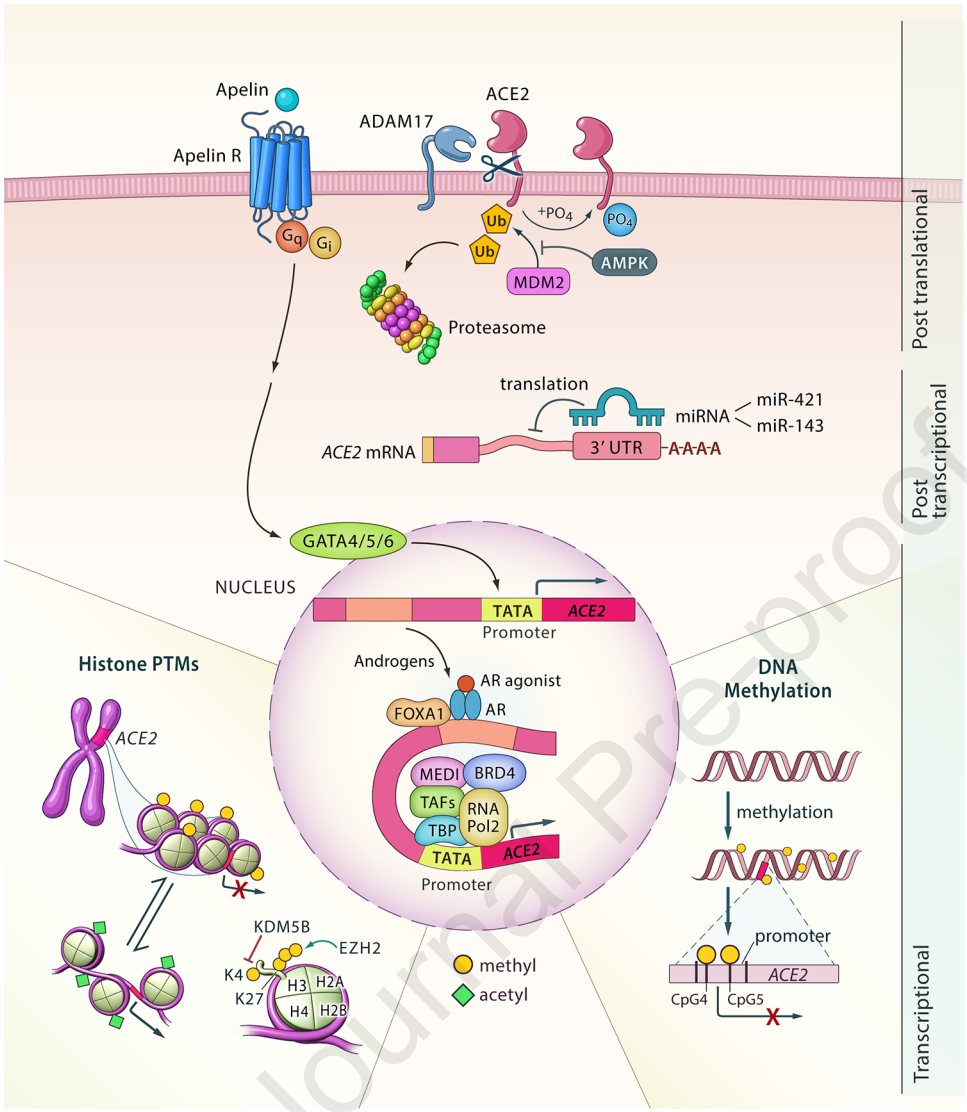
After binding of the SARS-CoV-2 spike protein to membrane bound ACE2 (white), spike is cleaved by host proteases, in particular TMPRSS2 (green) facilitating fusion of the virus with the cell membrane and clathrin-mediated endocytosis. See Figure 4 for a detailed description. Furin (purple) cleavage likely dominates in the intracellular compartment; however, furin (and possibly other proteases) may also facilitate viral entry at the cell membrane despite being dispensable for this process.

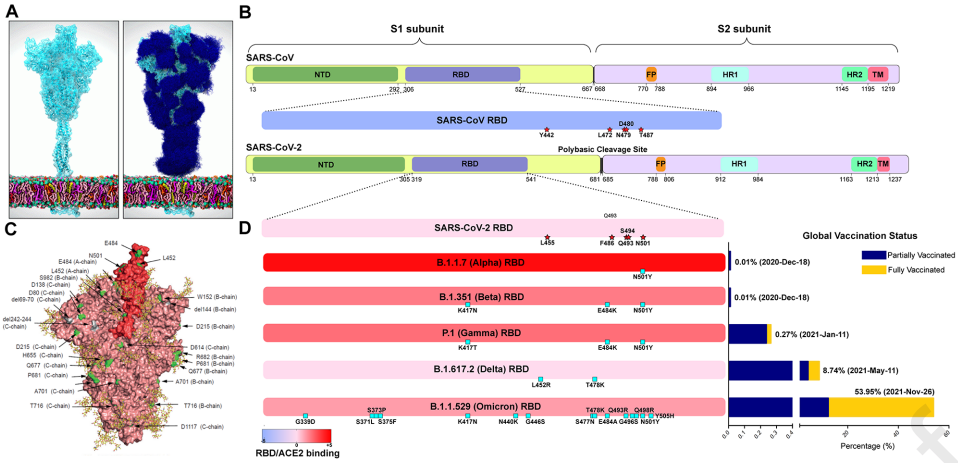
Movie 2. ACE2 decoy viral neutralization strategy.

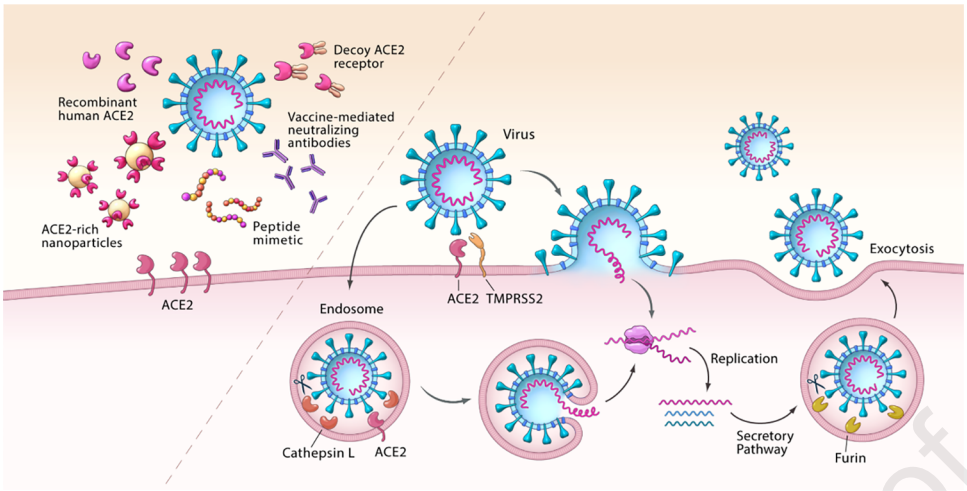
SARS-CoV-2 variants are visualized depicted by different colours of their spike protein. Despite mutations that facilitate immune escape, the variants can not escape the binding of ACE2 (white). Soluble ACE2 decoys (ivory) bind to all SARS-CoV-2 spike variants and thereby blind SARS-CoV-2 variants to find membrane bound ACE2 and hence prevent cellular infection. See Figure 4 for a detailed description.











ACE2 plays fundamental roles in human physiology and disease. This review summarizes ACE2's functions, highlights its relationship with SARS-CoV-2, describes implications for long COVID, and provides a framework for developing universal therapeutic strategies against current and future SARS-CoV-2 variants by exploring the ACE2 pathway and interfering with the spike-ACE2 interaction.