Advances in research on ACE2 as a receptor for 2019-nCoV

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Abstract
Currently, a novel coronavirus (SARS-CoV-2, also called 2019-nCoV) has triggered pandemic Coronavirus Disease 2019 (COVID-19), an acute infectious respiratory disease that first became epidemic in Wuhan (China) and is now spreading worldwide. Although 2019-nCoV and SARS-CoV are very similar viruses genomically and structurally, the huge number of severe cases and deaths now being caused by 2019-nCoV infections has understandably prompted intense research on the receptor used by it to enter human cells. Angiotensin converting enzyme 2 (ACE2), a functional receptor for SARS-CoV, now appears likely to mediate 2019-nCoV entry into human cells. In this review, we describe the roles performed by ACE2 as an enzymatic catalyst and as a receptor for this novel coronavirus. We also summarize the latest research pertaining to the changes noted in ACE2 expression after viral binding, and the relationships relating to virus transmission and population susceptibility to it. Lastly, we speculate on the pathogenesis of COVID-19 and provide a useful reference for drug development against this aggressive virus.

Keywords Angiotensin converting enzyme 2 · 2019-nCoV · Virus receptor · Coronavirus · Renin angiotensin system · Immune function

Introduction
The new SARS-like acute infectious respiratory disease has now reached pandemic levels. First emerging in Wuhan in December 2019 in the Hubei Province of China, it was temporarily called “novel coronavirus pneumonia (NCP)” by China’s National Health Commission. Studies have shown that the disease is caused by a novel coronavirus (CoV), officially named 2019-nCoV, and now referred to as SARS-CoV-2 by scientists [1]. The US Food and Drug Administration named this new epidemic infectious disease coronavirus disease 2019 (COVID-19). Epidemiological studies have shown that 2019-nCoV can spread rapidly through person-to-person transmission routes, which include coughs, sneezes, droplet inhalation, and other contact transmission. The disease, which is now rapidly spreading around the globe, has become an international public health emergency. The clinical symptoms of COVID-19 include fever, dry cough, fatigue, dyspnea, respiratory failure, and even death in severe cases. Although preliminary experiments by researchers or research institutions have reported that some agents may be effective against 2019-nCoV, there are currently no effective drugs targeting 2019-nCoV/SARS-CoV-2 [2].

Mammalian angiotensin-converting enzyme 2 (ACE2) was first discovered in the human lymphoma cDNA library by the Tipnis team [3] and a human heart failure ventricle cDNA library by Donoghue team [4] in 2000. During the SARS outbreak in 2003, ACE2 was identified by Li et al. [5] as a functional receptor for SARS-CoV in that it mediated the viral invasion of host cells, a finding later confirmed in animal experiments [6]. Some recent studies have suggested
that 2019-nCoV may infect host cells through the ACE2 receptor, as has already been established for SARS-CoV [7–10]. Accordingly, ACE2, as a 2019-nCoV receptor, may be a potential target for the treatment of COVID-19. In this review, the latest advances in our understanding of the roles played by ACE2 in enzyme catalysis, CoV invasion, cellular expression changes after viral–host cell binding, and its relationships with viral transmission and population susceptibility are described in the context of the pathogenesis of COVID-19. This review aims to provide a reference for the development of ACE2-targeted drugs for 2019-nCoV.

**Physiological role of ACE2**

**Discovery and characterization of ACE2**

In 1995, a protein highly homologous to mammalian somatic angiotensin-converting enzyme (ACE), was isolated and purified from *Drosophila melanogaster* embryos and named AnCE [11]. In 1996, researchers identified another Drosophila gene whose predicted translation product shared homology with mammalian testicular ACE and with AnCE and called it Ace-related (Acer), after which it was renamed ACE-like protein [12]. Insect AnCE and ACE-like protein then became classified as “ACE-like enzymes” [3].

In 2000, scientists also discovered ACE-like enzymes in mammals. Tipnis et al. [3] were the first researchers to clone a human metalloproteinase with high homology to ACE, calling it “ACE homolog (ACEH)” or “ACE related carboxypeptidase”, latterly known as “angiotensin-converting enzyme 2 (ACE2)” by Donoghue team [4]. The sequence identity between ACE2 and ACE is about 40%, with a similarity score of 61% [3], while the sequence similarity between mouse and human ACE2 is about 83% [13]. The human ACE2 gene is located on the short arm of the X chromosome and contains 18 exons. The complete human ACE2 cDNA encodes an 805 amino acid protein with a molecular weight of 120kD [3]. Membrane-associated ACE2 is a type I transmembrane protein consisting of a signal peptide at the amino terminal, a single metalloproteinase active site containing a zinc ion binding motif (i.e., HEMGH), a transmembrane domain, and a small cytoplasmic domain at the carboxyl terminus. A variety of transmembrane proteins can be cleaved by proteolytic enzymes to release the catalytically functional and free-standing extracellular domain, thereby regulating their activities [3, 14]. ACE2, like ACE, is a cleavable, extracellular enzyme located on the cell surface membrane [15]. ADAM17, a metalloproteinase family member, can cleave membrane-bound ACE2 and release its extracellular portion into the circulation as soluble ACE2 (sACE2), which lacks the transmembrane and cytoplasmic domains but retains its activity [14]. It has been shown that the activity of sACE2 is suppressed by the presence of an endogenous inhibitor in the form of a currently unknown positively charged small molecule [16].

**ACE2 catalysis and the renin–angiotensin system (RAS)**

RAS, one of the most important hormonal mechanisms for maintaining homeostasis in the human body, regulates blood pressure, fluid volume, and sodium–potassium balance. RAS, which is located in the circulation and in local tissues, is linked with many diseases such as cardio and cerebrovascular diseases, and diabetes mellitus, among others [17]. The classical RAS encompasses ACE, Ang II, the AT1 receptor, and other molecules, collectively forming the ACE–Ang II–AT1 axis. As a dicarboxypeptidase, ACE can catalyze the conversion of Ang I to Ang II, after which Ang II binds to the AT1 receptor to play roles in promoting vasoconstriction, inflammation and proliferation. The discovery of ACE2 suggests that there is another ACE2–Ang–(1–7)–Mas receptor pathway in the RAS, which is contrary to the classical pathway [18]. ACE2, a monocarboxyl peptidase, contains an N-terminal peptidase domain (PD) and a C-terminal collectrin-like domain (CLD) [4]. The N-terminal PD directly hydrolyzes Ang II into Ang-(1–7), or firstly Ang I into Ang-(1–9), which can later be hydrolyzed to Ang-(1–7) by ACE or other enzymatic molecules. Ang-(1–7) exerts the opposite effect of Ang II (i.e., vasodilation, anti-inflammation and anti-proliferation) by activating Mas receptor [18]. In fact, RAS metabolic pathways are not only limited to the two above-mentioned pathways, but some alternative pathways as well, such as Ang II formation via the chymase pathway and Ang-(1–7) formation directly from Ang I by prolyl endopeptidase or neutral endopeptidase [18] (Fig. 1).

**CoV structure and characteristics of 2019-nCoV**

CoVs are a large group of spherical, 120–160 nm in diameter, positive-sense viruses with single-stranded RNA genomes. CoV genomes, ranging from 26 to 32 kb in size, are the largest among the known RNA viruses. The RNA genomes of CoVs can serve as messenger RNAs and can be translated directly into proteins in the ribosomes of host cells [19]. To date, seven CoV types are known to infect humans, among which HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1 only cause common cold symptoms, whereas the other three types, SARS-CoV, MERS-CoV and 2019-nCoV, are highly transmissible with high fatality rates [20].

The CoV genome comprises a 5′-cap structure and 3′-poly-A tail. The 5′-terminal-located two-thirds of CoV genes mostly encode the non-structural proteins that form
polymers and perform replication and translation functions. One-third of the genome near the 3′-terminus encodes at least four main structural proteins; namely, spike (S), envelope (E), membrane (M) and nucleocapsid (N) [21]. The S protein forms radially protruding trimers on the viral envelope, which mediate virus–receptor binding and membrane fusion. So, the S protein is a key protein for determining the host range and transmission ability of CoVs [19]. The S protein is structurally divided into two functionally distinct subunits called the S1 and S2 subunits. While the S1 subunit is responsible for receptor binding and includes the N-terminal domain and C-terminal receptor binding region (RBD), the S2 subunit facilitates membrane fusion and anchors S into the viral membrane [22].

Annotation of the 2019-nCoV genome shows that it possesses 14 open reading frames (ORFs) encoding 27 proteins [23]. Although HCoV-NL63, 2019-nCoV and SARS-CoV all invade host cells via ACE2 receptors, only the latter two viruses share homology and similarity in their genome sequences [23, 24]. 2019-nCoV and SARS-CoV are both β CoVs, sharing the highest nucleotide sequence identity (79.7%) across their whole genomes [25]. The main difference between them lies in ORF1a and the gene sequence encoding the S protein. Homology modeling shows that the receptor domain of 2019-nCoV has a similar receptor-binding domain as SARS-CoV [8, 10].

Despite multiple mutations in 2019-nCoV are present in its S and M structural proteins when compared with bat-CoV, revealing that different selection pressures have been exerted on 2019-nCoV during its evolutionary history [26]. Previous studies have indicated that positions 442, 472, 479, 487, and 491 are important amino acid residues interacting with ACE2 in the SARS-CoV S protein and are considered to be crucial for the cross-species and person-to-person transmission of SARS-CoV [27]. Although the RBD domain of the S protein from 2019-nCoV is highly conserved in its sequence, as compared with SARS-CoV, amino acids at four important positions in five key residues are replacements with only Tyr491 being retained [10]. The 2019-nCoV and SARS-CoV S proteins, nevertheless, still share an almost identical 3D structure in the RBD domain. Thus, similar van der Waals forces and electrostatic properties are still predicted to occur at the interaction interfaces of both viruses, although the binding affinity of 2019-nCoV for ACE2 is significantly higher than that of SARS-CoV [10].

ACE2 as a receptor for CoVs

Identification of ACE2 as a viral receptor in the human host

During the SARS outbreak in 2003, Li et al. [5] were the first research group to identify ACE2 as a functional receptor for SARS-CoV. They first found that 293T cells transfected with ACE2 formed multinucleated syncytia in cells expressing the S protein. They also found that SARS-CoV could replicate effectively in non-susceptible cells transfected with ACE2, while an anti-ACE2 antibody was found to block viral replication in E6 Vero cells. The above in vitro experiments led them to conclude that ACE2 is a functional receptor for SARS-CoV, and mediates viral entry into host cells.

Three in vivo experiments using humanized ACE2 transgenic (hACE2) mice infected with SARS-CoV were reported in 2007. Under the regulation of CAG-complex promoters, including cytomegalovirus immediate-early (CMV-IE) enhancers and chicken β-actin promoters, the hACE2 gene was expressed in the lungs, small intestines, and livers (at the mRNA level) of AC70 transgenic mice. The mice showed clinical manifestations such as weight loss after being attacked by virus, and all died within 8 days [28]. Under the control of the cytokeratin 18 promoter, the hACE2 gene was expressed in alveolar epithelial cells and the liver, kidneys and gastrointestinal epithelial cells of another transgenic mouse, and rapid death also occurred after viral infection [29]. The transgenic mouse with the endogenous mACE2 promoter was found to have hACE2 expression in its lungs, heart, kidneys and intestines at both the mRNA and protein levels. Dissimilar to the above two mice showing
lethal SARS-CoV infections, none of the hACE2 transgenic mice challenged with SARS-CoV died; however, the viral titers in their lung tissues had significantly increased above normal and serious pathological changes in their lung tissues and multiple organ damage was apparent, thereby establishing a susceptible animal model for SARS-CoV [30].

Recently, some researchers have shown that ACE2 can be used as a 2019-nCoV receptor using molecular models and in vitro experiments. Zhou et al. [7] first performed viral transfection experiments using HeLa cells expressing or not expressing ACE2. The results showed that 2019-nCoV could only enter HeLa cells expressing human ACE2, thus confirming that 2019-nCoV is likely the cell receptor for 2019-nCoV [7]. Another study demonstrated that expression of human or bat ACE2 in non-susceptible BHK-21 cells can enable 2019-nCoV and SARS-CoV to enter cells, and that anti-serum raised against human ACE2 can block SARS-S and 2019-nCoV S-driven cell entry [31]. A genomic characterization and homology modeling study on the origins of 2019-nCoV revealed the existence of structural similarities in the receptor binding domains of SARS-CoV and 2019-nCoV [8]. In other research on the potential receptor for 2019-nCoV, the sequence of 2019-nCoV RBD, including its receptor-binding motif that directly contacts ACE2, was found to be similar to that of SARS-CoV [9].

ACE2-binding affinity has been shown to be one of the most important factors determining the infectivity of SARS-CoV [9]. Recently, Yan et al. [32] analyzed the interface between ACE2 and 2019-nCoV-RBD and SARS-RBD complexes to reveal variations that may determine the different affinities of the RBDs in the two related viruses. Some researchers have used molecular models to show that the 2019-nCoV RBD penetrates the deep hydrophobic pocket of ACE2, resulting in a stronger interaction with ACE2 than SARS-CoV [33]. Wrapp et al. [34] analyzed the structure of the 2019-nCoV S protein trimer by cryo-electron microscopy and found that the binding capacity of the S protein from 2019-nCoV to the ACE2 receptor was 10- to 20-fold higher than that of SARS-CoV. Interestingly, Coutard et al. [35] identified a peculiar furin-like cleavage site in the S protein from 2019-nCoV, possibly explaining why 2019-nCoV seems to be readily transmitted between humans.

Recently, humanized transgenic animal modeling has further demonstrated that ACE2 can mediate host cell invasion by 2019-nCoV. In an experiment where hACE2 transgenic mice were infected with 2019-nCoV, viral antigens were observed in bronchial epithelial cells, alveolar epithelial cells and macrophages in the transgenic mice, while no viral antigens were found in their infected wild-type counterparts [36]. Therefore, it is speculated that like SARS-CoV, 2019-nCoV infects host cells via the mediating effects of its S protein and ACE2 receptors on the surfaces of human cells.

As a point of reference, HCoV-NL63, which was discovered in the Netherlands in 2004, shares no structural homology with SARS-CoV, but also utilizes the ACE2 receptor when invading host cells. However, the crystal structure has showed that the HCoV-NL63 RBD domain binds to a narrower region of ACE2, involving fewer amino acids, which would lead to a weaker interaction. However, NL63-CoV does not have as aggressive transmission as SARS-CoV or the newer-discovered 2019-nCoV, so its pathogenicity is weak and any relevant research on it is scarce [24].

The ACE2 receptor mediates viral entry into cells

ACE2 as a receptor may mediate the entry of CoV into host cells in two independent ways (Fig. 2).

The first way involves ACE2-receptor-mediated clathrin-dependent endocytosis. The RBD of the virus is recognized by the extracellular PD of ACE2 mainly through polar residues. When CoV is connected to ACE2, the ACE2 extracellular domain controlling the catalytic effect is cleaved off by specific proteases, such as metalloproteinase ADAM17, and the transmembrane domain is internalized. Next, with the assistance of clathrin [37], viral particles and host cells fuse, and the intracellular structure of ACE2 aids viral transport from the cell membrane to the cytoplasm. In vitro studies have shown that ADAM17 inhibitors can attenuate virus replication [38]. Although the physiological function of ACE2 extracellular shedding has not been fully elucidated, it has been shown to be associated with virus invasion and replication [38, 39]. sACE2 retains catalytic activity and can partially block SARS-CoV binding to receptors on target cells, and one in vitro study has shown that sACE2 could block the association between the S1 domain of SARS-CoV and E6 Vero cells and reduce viral replication [5]. Cells expressing ACE2 non-catalytic mutants can still be infected by SARS-CoV, and the enzymatic activity of ACE2 did not contribute to S-protein-mediated infection by this virus [40], indicating that the peptidase action of ACE2 is not necessary for viral entry into host cells. Elsewhere, structural analysis has shown that the metallopeptidase domain of ACE2 can be divided into two subdomains (I and II), with the CoV S protein contacting the top of subdomain I of the ACE2 catalytic domain, but the S protein does not contact subdomain II or seal the peptidase activity sites in ACE2 [41].

The second way involves ACE2-receptor-mediated transmembrane serine protease 2 (TMPRSS2)-dependent membrane fusion. One study found that ACE2-mediated viral invasion involves TMPRSS2, which is employed for S protein priming and activation of membrane fusion processes [42], and TMPRSS2s priming role has recently been confirmed for 2019-nCoV [31]. When the SARS-S protein binds to ACE2, processing by TMPRSS2 is thought to allow fusion at the cell surface or upon uptake into cellular vesicles.
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but before virion transport into cell endosomes. TMPRSS2 has also been found to compete with the ADAM17 metalloprotease for ACE2 processing, but only cleavage by TMPRSS2 was found to enhance SARS-S protein-driven entry [42]. The molecular mechanism underlying protease-enhanced cell entry and the potential role of ACE2 cleavage upon SARS-S protein activation are unclear [42]. Research using the 2019-nCoV S protein pseudovirus system and different inhibitors has shown that 2019-nCoV enters 293/hACE2 cells mainly by endocytosis, and that phosphatidylinositol 3-phosphate 5-kinase, two pore channel subtype 2, and cathepsin L are critical for viral entry. Another study revealed that 2019-nCoV S protein can trigger syncytia in 293/hACE2 cells independent of an exogenous protease [43].

Recent resolution of the full-length structure of human ACE2 using cryo-electron microscopy by Yan et al. [32] indicated that ACE2 exists as a dimer with both “open” and “closed” conformations. Conversion of the two conformations is achieved by rotation of the PD domains in ACE2, and both conformations contain the mutual recognition interface with CoVs. Further studies have shown that ACE2 dimers can accommodate two S protein trimers through each ACE2 monomer [44]. ACE2 is also a chaperone protein for membrane transportation of the amino acid transporter B0AT1 (also called SLC6A19) [45], which mediates the uptake of neutral amino acid-like substances into intestinal cells in a sodium-dependent manner. These findings suggest that B0AT1 may play a regulatory role in intestinal infection with some CoVs [44]. Further research indicates that the presence of B0AT1 may block TMPRSS2s access to the cutting site on ACE2; therefore, the role played by the ACE2/B0AT1 dimer structure in conformational binding of the 2019-nCoV S protein appears to affect the viral invasion of host cells. Whether B0AT1 can inhibit 2019-nCoV infection by blocking ACE2 cleavage remains an unanswered question [44].

Changes in ACE2 expression after binding to CoV

ACE2 gene expression is regulated at both the transcriptional and post-transcriptional levels by a variety of mechanisms. Ang II/AT1 can upregulate ACE2 mRNA and protein expression in human cardiac fibroblasts via the ATTGGA promoter sequences in the − 516 to − 481 regions of the gene [46]. While Ang II/AT1 promotes extracellular ACE2 internalization followed by lysosomal degradation [47], ADAM17 cleaves membranes bearing ACE2, thereby releasing sACE2 into the circulation [14]. Furthermore, microRNAs such as miR-421 can mediate ACE2 gene silencing in cardiac fibroblasts and a variety of human tissue cell lines [48]. Thus, these mechanisms of expression and activity regulation of ACE2 play an important role in cardiovascular and other diseases.
Interestingly, one study has suggested that ACE2 expression is downregulated after viral infection. In their 2005 study, Kuba et al. [6] showed that SARS-CoV infection and SARS-CoV S proteins reduce the expression of ACE2 in lungs (but not ACE), lead to increased Ang II levels, signaling through the AT1 receptor, increased pulmonary vascular permeability, and acute lung injury induction. Notably, ACE2 is not only a receptor for viral invasion of host cells during SARS-CoV infection, but viral binding to it deregulates its protective effect on the lungs. This may explain why SARS-CoV is more lethal in humans than other CoVs [49]. Oudit et al. [50] demonstrated that SARS-CoV was also strongly equipped to infect the heart and modulate ACE2 expression in this organ. Pulmonary infections with SARS-CoV in wild-type mice subsequently led to downregulation of myocardial ACE2 mRNA and loss of ACE2 protein in an ACE2-dependent manner. Furthermore, the presence of SARS-CoV in the heart in patients who died from SARS was associated with macrophage infiltration and myocardial damage in association with decreased myocardial ACE2 protein expression. Collectively, these findings suggest that myocardial ACE2 downregulation may be an underlying pathophysiological mechanism for SARS-associated heart disease.

Recently, some researchers have speculated that 2019-nCoV causes ACE2 downregulation by binding to the ACE2 receptor, and they have proposed strategies to treat COVID-19 infections with ACE2 as the target, which involves using the TMPRSS2 inhibitor to prevent CoV S protein-ACE2 binding [31, 51], blocking the ACE2 receptor using anti-ACE2 antibodies or peptides, or using sACE2 to attenuate viral invasion through competitively binding with 2019-nCoV, thereby protecting against lung injury through its unique enzymatic properties [51]. Supporting such strategies is the finding from Wang et al. [52], who showed that, individually, SARS-CoV or 2019-nCoV S proteins can interact with ACE2 on the surface of host cells and become separately internalized together with ACE2 through endocytosis, resulting in reduced surface ACE2 expression [53]. This endocytic event also upregulates ADAM17 activity, which, in turn, cleaves ACE2 on cell membrane surfaces, perpetuating the loss of the ACE2 protective effect in tissue RAS. Loss of ACE2 leads to imbalance of RAS homeostasis and increased Ang II levels, causing damage to the organism through AT1 receptors, while also upregulating ADAM17 activity in a well-characterized positive feedback loop leading to further cleavage of cell surface attached ACE2 [52]. One study reported that the level of Ang II in the plasma of 2019-nCoV-infected patients is significantly higher than in uninfected individuals, a finding linearly correlated with viral load and the degree of lung injury [54]. ADAM17 also mediates the liberation of the membrane bound precursors of tumor necrosis factor α (TNFα), interferon (IFN)-γ, and IL-4 (interleukin-4) proinflammatory cytokines into the circulation [39], which also downregulates ACE2 expression on the cell surface and decreases ACE2 mRNA levels. Soluble recombinant human ACE2 (rhACE2) inhibits viral invasion of host cells by competitive binding with 2019-nCoV, limits

![Fig. 3 The proposed mechanism underlying 2019-nCoV-induced downregulation of cell surface ACE2 expression. CoV interacting with ACE2 becomes internalized together with ACE2 through endocytosis, which upregulates ADAM17-mediated proteolytic cleavage of ACE2. Loss of ACE2 leads to accumulation of angiotensin II, which also increases ADAM17 activity through AT1 receptors. rhACE2 inhibits viral invasion of host cells by competitive binding to CoV with membrane ACE2, limits the activities of Ang II, and increases protective Ang-(1–7) levels.](image-url)
the activities of Ang II, and increases protective Ang-(1–7) levels [52, 53] (Fig. 3). Elsewhere, Monteil et al. [56] found that rhACE2 can prevent 2019-nCoV from infecting human blood vessel organoids and human kidney organoids engineered via the induction of pluripotent stem cells and addition of rhACE2 also showed no cellular toxicity. However, because it has only been shown that rhACE2 can block the early stages of 2019-nCoV infections, this finding requires confirmatory clinical evidence.

However, some researchers have the opposite viewpoint, suggesting that ACE2 expression is upregulated by viral infection. A team at Shandong University (China) searched databases for cells and tissues known to be stimulated by viral infections or inflammatory cytokines, and they clearly showed that ACE2 can be significantly upregulated by virus infection and inflammatory cytokine stimulation [57]. The ACE2 gene is responsive to viral infections, and its expression can be stimulated by a variety of viruses. When upregulated, ACE2 is more receptive to viral invasion and transmission, while its induction by inflammatory cytokines also implies that the “cytokine storm” caused by 2019-nCoV not only damage host tissues, but may also accelerate viral spread [57]. Their analysis also showed that there are multiple transcription factor binding sites related to immune and cytokine responses in the promoter region of ACE2. It is speculated that the activated immune system induces the expression of a variety of cytokines, including IFN, after viral infection, which promotes the transcription and expression of ACE2 by activating downstream signaling pathways such as the JNK (c-Jun N-terminal kinase) pathway. Ziegler et al. [58] showed that IFNs increase ACE2 in human nasal epithelia and lung tissue, and ACE2 belongs to a large family of interferon-stimulated genes (ISGs), and suggested that 2019-nCoV may exploit the ACE2-mediated tissue-protective response to provide further cellular targets for entry.

In conclusion, the changes occurring in the expression level of ACE2 and its regulatory mechanism after the S protein of CoV binds to the ACE2 receptor remain unclear. If viral invasion downregulates ACE2 via adaptive protective mechanisms in the host this will inevitably cause RAS homeostasis impairment, thereby weakening the protective effect of ACE2 and leading to acute lung injury. If, on the other hand, ACE2 is upregulated, that will provide more receptors for viral entry and cause more viruses to invade host cells. Therefore, either upregulation or downregulation of ACE2 may have adverse consequences for the body. Perhaps during the period when cells are fighting the virus, ACE2 expression may be continuously changing (i.e., either up- or downregulated), and while human immune functioning is particularly important at this time, only those with robust immune functioning can defeat the virus (Fig. 4).

Fig. 4 Changes of ACE2 expression and key role of immune function after 2019-CoV infection. Invasion of 2019-nCoV to host cells induces nonspecific reactions of local tissue, releasing cytokines such as TNF-α, IFNs, and so on. The expression of ACE2 may be downregulated by endocytosis and TNF-α. At this point, although the viral invasion decreases, the local RAS is imbalance, reducing its anti-inflammatory effect. The expression of ACE2 may be upregulated by IFNs, enhancing the anti-inflammatory effect of local RAS, but also providing more entry for virus. Therefore, when cells and viruses fight, the human cellular and humoral immune function is particularly important. The insufficient or excessive immune responses will destroy their own tissue cells as a result of virus replication or cytokine storm. Only moderate immunity can clear viruses and restore cells.
ACE2 expression in different organs and viral transmission pathways

ACE2 is expressed in many different tissues at different levels, with an organ-specific distribution. It is highly expressed in the heart (coronary vascular endothelial cells, vascular smooth muscle, and cardiomyocytes, among other cells), kidneys (renal vessels and renal tubular epithelial cells) and testes. It is also found in the gastrointestinal tract and lungs [3, 59]. ACE2 expression levels and expression patterns in different tissues may be critical to the susceptibility, symptom manifestations, and outcome of infection with COVID-19. Therefore, it is hugely important to explore the expression characteristics of ACE2 in human tissues and its relationship with virus transmission, as well as determining the possible infection pathways for understanding the pathogenesis and treatment options for COVID-19.

The distributional expression of ACE2 in human tissues was reported as early as the SARS outbreak in 2003. It has been shown that the epithelial expression of ACE2 in lung and small intestine provided possible routes for SARS-CoV entry, and ACE2 is also expressed in vascular endothelium [60]. During their recent analysis of COVID-19, based on 43,134 cells derived from normal lung tissue of eight adult donors by single-cell RNA sequencing (RNA-Seq), the team at Tongji University Medical College (China) determined that more than 80% of ACE2 in the lungs was distributed on the surface of type II alveolar epithelial cells [61]. Other researchers found that ACE2 mRNA is mainly distributed in the small intestine, colon, duodenum, kidneys, testes and gallbladder by searching The Human Protein Atlas Database, but its expression level was low in the lungs, suggesting that some cells may upregulate ACE2 expression under certain conditions [33].

That ACE2 is expressed in different tissue types suggests that CoV infections may also involve the intestines, liver, kidneys, testes and other organs [62]. Previous studies have found that the proportion of patients infected SARS-CoV with diarrhea was as high as 70% [63]. A recent case report showed the presence of 2019-nCoV in the feces from a patient who initially presented with diarrhea symptoms [64], and ACE2 has been found to be highly expressed in the upper esophagus, stratified epithelial cells, and absorptive enterocytes from the ileum and colon, findings consistent with the possibility of a fecal–oral transmission route [33, 65]. Liver dysfunction or injury in patients with NCP may be caused by the inappropriate use of antiviral drugs or systemic inflammatory reactions [66]. Recently, Zhao et al. [66] showed that 2019-nCoV could infect cholangiocytes expressing human-specific ACE2 using the human liver ductal organoids model, with resultant barrier and bile acid transport function impairment. Thus, liver damage in COVID-19 patients might partly result from direct cholangiocyte injury and consequent bile acid accumulation during 2019-nCoV infection.

Acute kidney injury has also been seen in patients with 2019-nCoV infections [67]. RNA-Seq has shown that ACE2 is abundantly expressed in various kidney proximal tubule cell subtypes. In addition to renal injury from host immune responses, renal injury may be caused by the virus directly attacking target cells where ACE2 is expressed [68]. Some researchers have indicated that 2019-nCoV may enter renal tubular cells by binding to ACE2, thereby inducing cytotoxicity and renal dysfunction [69]. One place where ACE2 is highly expressed is the testes, mainly in the spermatogonia, Sertoli cells and interstitial cells. Although there are no studies to establish whether 2019-nCoV infection causes testicular damage, some experts have highlighted the importance of evaluating reproductive function in recovered male patients, especially young ones [69].

It has been reported that 2019-nCoV was present in blood from patients with COVID-19, suggesting that infection sometimes may be systemic [70]. Guo et al. [71] analyzed the single cell RNA-Seq data for normal whole lung tissue samples and fibrotic lung tissue samples and found that ACE2 was mainly expressed in the arterial vascular cells of fibrotic lungs. The first destination of pulmonary circulation outflow is the heart, and the failing human heart has a higher percentage of ACE2-expressing cardiomyocytes. Therefore, it is assumed that 2019-nCoV may attack the heart through the blood flow, which may explain the high incidence of cardiac injury in critically ill patients [71, 72]. The 31% incidence of thrombotic complications in ICU patients with COVID-19 infections is remarkably high [73]. In short, the flow of virus into the blood stream is a key step for its spread to other organs, body fluids and excreta, and provides the blood transmission pathways for 2019-nCoV.

In conclusion, in addition to the respiratory, digestive and genitourinary systems, the blood system may also be a potential route of infection for 2019-nCoV. More recent researches [74, 75] revealed that ACE2 and TMPRSS2 were co-expressed in uveal surface cells and nasal epithelium which could therefore serve as a portal of initial infection and transmission. Thus, it is important to clarify the mechanism of virus transmission for public health administration departments to formulate the best health policies regarding the prevention of 2019-nCoV infections.

ACE2 in different species and populations and susceptibility to 2019-nCoV infection

ACE2 gene variation in different species and virus susceptibility

The world is currently in a severe emergency state regarding 2019-nCoV, and the analysis of ACE2 in different
species can help with providing a warning system for the cross-species transmission of 2019-nCoV.

Variation in the ACE2 gene sequence in different species is closely linked with viral susceptibility. During the SARS outbreak, Li et al. [76] used pseudovirus to infect 293T cells transfected by human, mouse or rat ACE2. Their results showed that mouse and rat ACE2-transfected cells were both less efficiently infected by pseudovirus than human ACE2-transfected cells, and the infection efficacy was significantly enhanced by incubation with human ACE2-transfected mouse 3T3 cells. In human ACE2, amino acid sites 30–41, 82–84 and 353–357 were shown to be involved in the interaction with the SARS-CoV S protein, with residues 31, 35, 38, 82 and 353 being the key positions [27]. Based on the latter residue positions, some researchers explored the effect of ACE2 variation in different species on their susceptibility to the virus. Holmes et al. [77] identified variation at the M82N position in rat ACE2 compared with that in the human ortholog, which contains a large N-linked glycan at this position. The same study found that a K353H mutation resulted in the lack of a hydrophobic pocket. In mouse ACE2, there are M82S and K353H mutations. Therefore, ACE2 mutations in rat and mouse ultimately lead them to lack susceptibility to SARS-CoV. Moreover, research on rhesus monkeys experimentally infected with a pathogenic SARS-CoV strain showed that there are natural non-synonymous changes in the rhesus ACE2 gene, and further mutagenesis analysis showed that Y217N, a natural mutation, caused ACE2 expression to be dramatically downregulated and reduced the viral entry [78]. Another study found an enhanced interaction between civets and SARS-CoV caused by an additional region covering residues 90–93 in ACE2 [79].

A recent study on hACE2 transgenic mice and wild-type mice infected with 2019-nCoV reported that viral antigens were found in the trachea, alveolar epithelial cells and macrophages of the hACE2 mice, but not in the wild-type mice, suggesting that mice are insusceptible to 2019-nCoV [36]. Zhou et al. [7] used HeLa cells expressing human, civet, pig, Chinese chrysanthemum bat or mouse ACE2 to conduct in vitro viral infection experiments, observing that all ACE2-expressing cells except those from mouse were infected by the virus. It is worth mentioning here that some researchers have compared the amino acid sequences of ACE2 from different species and found that human and non-human primates share identical sequences in some regional residues. The above critical residues 31, 35, 38, 82 and 353 of companion animals, domestic animals and wildlife animals are relatively conserved among them, while certain ones are variable. Changes in amino acid residues may lead to lower affinity viral binding, and certainly the presence of other variable regions cannot be excluded to compensate for such changes [80]. Thus, generic variation in the ACE2 gene can influence the susceptibility of different species to CoV, which should alert us to the potential interspecies transmission of 2019-nCoV.

**ACE2 expression in different populations and viral susceptibility**

Exploring whether different populations are potentially susceptible to 2019-nCoV by analyzing ACE2 expression in different people could provide a valuable reference for effective and timely epidemic prevention. A recent RNA-Seq analysis of the ACE2 expression patterns in eight normal human lung transplant donors showed no association between the number of cells expressing ACE2 and donor age or smoking status. However, men had a higher proportion of ACE2-expressing cells than women [61], a finding highly consistent with an epidemiological investigation showing that most patients diagnosed with 2019-nCoV infection were male [81]. Asian males were also found to have a higher percentage of ACE2-expressing cells than white and African Americans in one study [61]. But in the current situation, the original epidemic has become a global outbreak, not only in Asia, but in Europe and America also. One study that analyzed large-scale transcriptomic datasets of normal lung tissue found no significant differences in ACE2 gene expression between race, age, or sex, whereas in smokers, ACE2 gene expression was upregulated [82, 83]. Elsewhere, ACE2 genes were found to be expressed in specific cell types related to smoking history and location. ACE2 was reported to be actively expressed in the goblet cells of smokers and club cells of non-smokers in the bronchial epithelium [83]. In the lungs, ACE2 expression is abundant in the remodeled type II alveolar epithelial cells of smokers. But the reasons for such differences in ACE2 expression related to tobacco smoking are obscure [83]. Some researchers have reported that ACE2 expression decreases with increasing age, and because ACE2 is on the X chromosome its level is higher in women than in men. The ACE2 level is low in men and the elderly, but this pattern does not match the characteristic of severely ill COVID-19 patients being mostly elderly males. Therefore, whether or not ACE2 expression is high or low is not a crucial factor affecting the prognosis of patients with COVID-19 [84].

Some researchers have investigated the genetic backgrounds of different populations through coding-region variants in ACE2 in terms of its diversity and variability, which may affect its function. Quantitative trait locus variations that may affect ACE2 expression were analyzed using the GTEx database (https://www.gtexportal.org/home/datasets), which enabled the genomic characteristics of ACE2 in different populations to be compared, and the results indicated that ACE2 expression may also show potential differences

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among different groups and races in Asia. However, there is no genomic evidence to support the existence of ACE2 mutants in different populations that can resist its binding to the CoV S protein [85]. Recently, Othman et al. [86] found that none of the eight ACE2 mutants they investigated had disrupted interactions with the 2019-nCoV RBD; hence, they proposed that the genetic polymorphism they investigated for ACE2 had a marginal effect on the affinity with 2019-nCoV RBD. Nevertheless, this research area would benefit from further in-depth investigations.

Recent studies on the epidemiological characteristics of COVID-19 have revealed that people with chronic diseases such as hypertension, diabetes, chronic obstructive lung disease and coronary heart disease are more likely to have serious infections [1, 87, 88]. Applying statistical methods to collect clinical cases, we previously found that the serum concentration levels of ACE2 were significantly elevated in patients with essential hypertension [89]. Recently, Chen et al. [90] showed that the level of myocardial ACE2 mRNA and protein in patients with heart failure had increased significantly above normal levels by RNA-Seq. Another study analyzed lung transcriptome samples from more than 700 COVID-19 patients with comorbidities, and found that ACE2 was highly expressed in these patients, as compared with the control group, and that ACE2 may be regulated by histone modifying enzymes, including KDM5B (lysine-specific demethylase). This finding suggests that patients with such comorbidities may have higher chances than normal of developing severe COVID-19 [91]. In conclusion, whether ACE2 is up- or downregulated, patients with underlying diseases may have more imbalanced RAS homeostasis, lower immunity and greater susceptibility to 2019-nCoV. Hence, more attention should be paid to this population to reduce their infection risk and disease severity.

**Summary and perspectives**

It has been suggested that 2019-nCoV, like SARS-CoV, invades host cells through ACE2 receptors, but there are also CD209L co-receptors for SARS-CoV [92]. Some scholars noted that ACE2 is widely expressed across a variety of organs and is expressed moderately but not highly in lung, which, however, is the major infected organ [93]. In addition to the factors that connect the respiratory system with the external environment, is there any receptor other than ACE2 that mediates the infection of 2019-nCoV into the host? One study using molecular model fitting may have an answer to this in showing that Ang II receptor type 2 (AT2), a G-protein-coupled receptor, is abundantly expressed in lung tissues and may be another receptor for 2019-nCoV [93]. AT2 plays an antagonistic role with AT1 in the RAS system. Invasion of host cells by 2019-nCoV affects the expression of ACE2, and if ACE2 is re-incorporated into cells with AT2 as a possible co-receptor, it would accelerate the imbalance of the RAS system, worsening the situation for patients. It remains unclear whether the AT2 co-receptor leads to a more serious imbalance between the classical ACE–AngII–AT1 receptor axis and the new ACE2–Ang–(1–7)–Mas homeostasis in the RAS system.

Clinical studies have shown that "cytokine storms", or the massive release of inflammatory factors, occur in critically ill patients, causing bodily tissue damage and multiple organ failure in critical patients [87]. CoV invades host cells through ACE2, which changes ACE2 expression and leads to increased levels of Ang II, a proinflammatory factor. Ang II contributes to "cytokine storms". Recently, studies have shown that 2019-nCoV directly affects the human spleen and lymph nodes by infecting tissue-resident CD169+ macrophages. 2019-nCoV also triggers macrophages to produce IL-6, a proinflammatory cytokine [94]. 2019-nCoV may directly infect lymphocytes, especially T cells, leading to lymphopenia and impaired antiviral responses. But the lymphocytes lack ACE2 expression, and whether alveolar macrophages can phagocytose the viral particles, transferring to lymphocytes remains unknown [95]. The internal mechanism by which the immune system and RAS system interact and regulate each other when 2019-nCoV infects host cells is unclear, and this lack of clarity needs to be solved urgently.

In effect, ACE2 plays a dual role in COVID-19 infections by first acting as a receptor for 2019-nCoV, and then as a protective molecule in the RAS system. It is currently unclear whether ACE2 acts as a human friend or foe during infection with 2019-nCoV. This review has attempted to summarize the up-to-date research on ACE2 in CoV disease, mainly focusing on CoV invasion, changes in the expression of ACE2 after viral binding, the effects of 2019-nCoV distribution on viral transmission, and genetic susceptibility to 2019-nCoV in humans. It is hoped that the information in this review will provide a clearer picture of the pathogenic mechanism of 2019-nCoV, and act as a useful reference on ACE2 as a potential therapeutic target for COVID-19.

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**Compliance with ethical standards**

**Conflict of interest** The authors report no conflicts of interest in this work.
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