



## Review

## Role and mechanism of angiotensin-converting enzyme 2 in acute lung injury in coronavirus disease 2019

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**Abstract**

Coronavirus disease 2019 is a major threat to public health globally. Though its pathogenesis has not been fully elucidated, angiotensin-converting enzyme 2 (ACE2) has been recently identified as a receptor for the entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into the cell. Here, we aimed to clarify the potential role of ACE2 in SARS-CoV-2-induced acute lung injury and its underlying mechanism. As a receptor for coronavirus, ACE2 mediates the entry of SARS-CoV-2 into cells in a similar way as for severe acute respiratory syndrome coronavirus (SARS-CoV). The high binding affinity of SARS-CoV-2 to ACE2 correlates with its efficient spread among humans. On the other hand, ACE2 negatively regulates the renin-angiotensin-aldosterone system (RAAS) primarily by converting angiotensin II to angiotensin 1–7, which exerts a beneficial effect on coronavirus-induced acute lung injury. Human recombinant ACE2 has been considered as a potential therapy for SARS-CoV-2 by blocking virus entry and redressing the imbalance of RAAS in SARS-CoV-2 infection. The level of ACE2 expression can be upregulated by treatment with an ACE inhibitor (ACEI) or angiotensin II type 1 receptor blocker (ARB). To date, no evidence shows that ACEIs or ARBs increase the susceptibility and mortality of patients infected with SARS-CoV-2, and hence, it is not advisable to discontinue such drugs in patients with cardiovascular disease.

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Up until March 22, 2020, coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in 292,142 confirmed infections and 12,784 deaths in 171 countries and regions.<sup>1</sup> COVID-19 is currently a significant threat to global public health.

SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA virus belonging to the genus *Betacoronavirus* and subgenus *Sarbecovirus*. Currently, this

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virus is believed to originate from bats and is transmitted to humans through an intermediate host before human-to-human transmission, which results in the spread of the disease.<sup>2</sup> The gene sequence of SARS-CoV-2 shares 79% and 50% similarity with that of severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus, respectively, and it is highly infectious.<sup>3</sup> Infection with SARS-CoV-2 is characterized by fever, fatigue, and cough, but some patients also develop myalgia, dyspnea, and gastrointestinal symptoms, such as diarrhea, nausea, and vomiting. Severe dyspnea and hypoxemia occurs within one week of onset in severely affected patients, which rapidly progresses to acute respiratory distress syndrome (ARDS) (in 41.8% of patients), shock, multi-organ failure, or even death (in 21.9% of patients).<sup>4</sup> Lung autopsies of patients who had severe COVID-19 showed alveolar epithelial cell detachment, pulmonary interstitial lymphocyte infiltration, and hyaline membrane formation, which suggested the development of ARDS. Multinucleated syncytial cells and atypical enlarged pneumocytes could be observed in the alveoli, which are indicators of virus-infected cells; however, virus inclusion bodies were not detected.<sup>5</sup>

Angiotensin-converting enzyme 2 (ACE2), an important metalloproteinase, plays a key role in SARS-CoV-2 infection.<sup>2</sup> ACE2 not only mediates viral infection but also exerts important regulatory effects on the renin-angiotensin-aldosterone system (RAAS) and participates in the development of acute lung injury (ALI) after infection. Intervention measures that target ACE2 may improve the prognosis of patients with COVID-19. This paper comprehensively describes ACE2 structure and function, regulation of ACE2 expression, and its role in viral infection and ALI.

## ACE2 overview

ACE2 was discovered in 2000 as a homolog of angiotensin-converting enzyme (ACE).<sup>6,7</sup> ACE2 is a type I transmembrane protein comprised of 805 amino acids. ACE2 contains two domains, the amino-terminal and the carboxy-terminal domain. The amino-terminal catalytic domain contains one active site, a zinc metalloprotein domain that shares 41.8% sequence identity with ACE.<sup>6–8</sup> The gene that encodes ACE2 is located on the X-chromosome at Xp22.2.<sup>9</sup> ACE2 is expressed in the human heart, kidneys, lungs, liver, testes, and intestines.<sup>7</sup> In the heart and large vessels, ACE2 is localized on the surface of endothelial and smooth

muscle cells. In the kidneys, ACE2 is mainly expressed on the apical membrane of proximal tubular epithelial cells and colocalized with ACE.<sup>10</sup> In the lungs, ACE2 is mainly located on type II pneumocytes, or may be found on type I pneumocytes and airway epithelial cells.<sup>11</sup>

## Function of ACE2

The functions of ACE2 can be broadly classified into two types: protease and protease-independent functions.

### Protease function

ACE2 is a metalloproteinase and its active domain is exposed on the cell surface to facilitate the catalysis of circulating polypeptides. ACE2 can cleave angiotensin I into inactive angiotensin 1–9 (Ang 1–9) peptide,<sup>6,7</sup> which is further converted by ACE or other peptidases into angiotensin 1–7 (Ang 1–7) peptide.<sup>6</sup> Moreover, ACE2 can efficiently metabolize angiotensin II (Ang II) into Ang 1–7, which binds to Mas receptor (Mas), a G-protein coupled receptor, to exert vasodilation and anti-proliferation effects.<sup>12</sup> It is well established that Ang II has two G-protein-coupled receptors, the angiotensin II type 1 receptor (AT1R) and angiotensin II receptor type 2 receptor (AT2R). Ang II exerts its biological functions and is involved in pathogenesis of various diseases mainly through AT1R. The activation of ACE/Ang II/AT1R pathway leads to vasoconstriction, proliferation, fibrosis and inflammation, which may ultimately result in progressive tissue damage.<sup>10</sup> The effects of Ang 1–7 counterbalance the deleterious effects of the ACE/Ang II/AT1R pathway<sup>13–16</sup> (Fig. 1). Ang 1–7 exerts multiple cardioprotective effects, including anti-inflammatory, anti-thrombotic, anti-fibrotic, anti-arrhythmic, and natriuretic effects. It has also been shown to inhibit cardiac hypertrophy and plaque formation, and ameliorate vascular dysfunction.<sup>17</sup> The ACE2/Ang 1–7/Mas pathway has shown beneficial effects in many animal models of hypertension, heart failure, ischemic cardiomyopathy, and non-ischemic cardiomyopathy.<sup>18</sup> In the lungs, the Ang 1–7 peptide inhibits inflammatory cell infiltration and regulates the release of anti- and pro-inflammatory cytokines to alleviate lung inflammation. In addition, it also improves oxygenation, reduces airway hyperresponsiveness, decreases alveolar epithelial cell apoptosis, and inhibits fibroblast proliferation and migration, goblet cell metaplasia, and airway remodeling. The ACE2/Ang 1–7/Mas pathway has been shown to exert protective

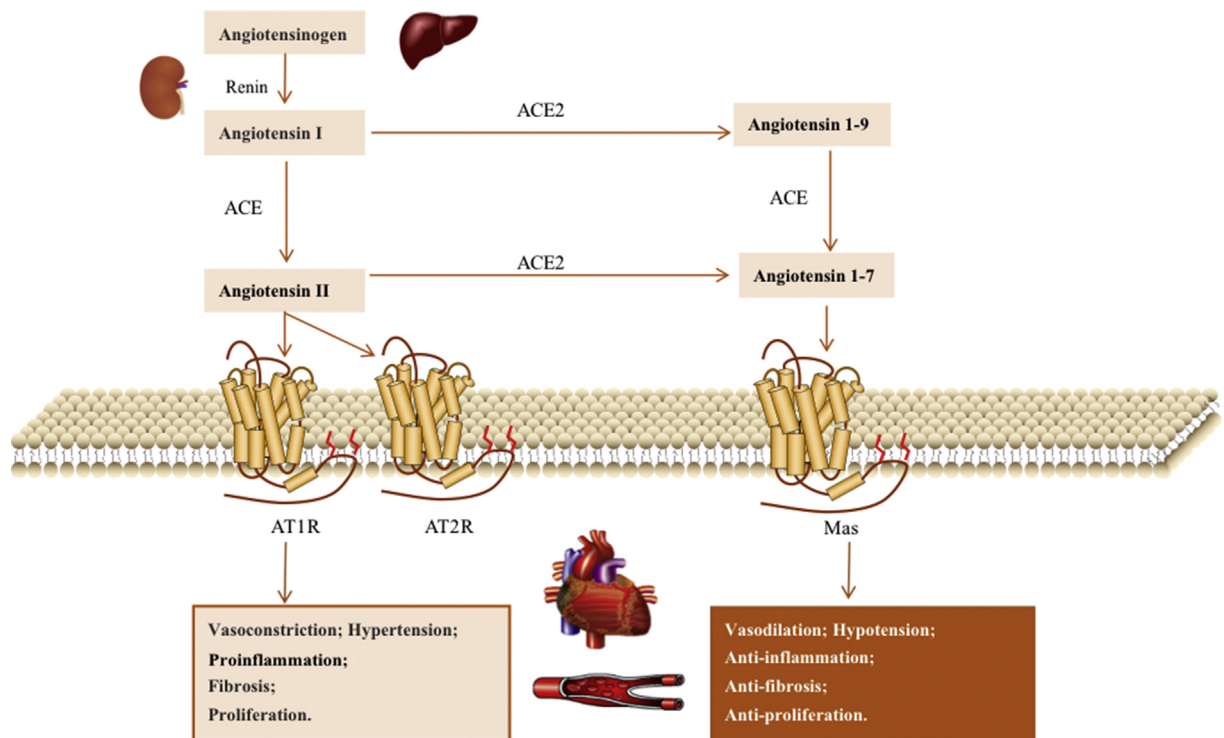


Fig. 1. Role of ACE2 in the renin-angiotensin-aldosterone system (RAAS). AT1R: Angiotensin II type 1 receptor; AT2R: Angiotensin II type 2 receptor; Mas: Mas receptor; ACE: Angiotensin-converting enzyme; ACE2: Angiotensin-converting enzyme 2.

effects on the lungs in animal models of ARDS, asthma, pulmonary fibrosis, and pulmonary hypertension.<sup>19</sup> In addition, *in vitro* experiments have shown that ACE2 cleaves apelin-13 to remove one amino acid from the C-terminal,<sup>12</sup> which affects the hypotensive effects of apelin-13.

#### Protease-independent function

In addition to protein cleavage, ACE2 has other important biological functions. In 2003, ACE2 was confirmed to be a receptor for SARS-CoV.<sup>20</sup> ACE2 mediates infection of this virus, which is independent of its protease activity. Structural analysis shows that the spike protein of SARS-CoV comes in contact with the apex of subunit I in the catalytic domain of ACE2 without involvement of subunit II and does not block the active site. Once the receptor binding domain (RBD) of the SARS-CoV spike protein binds to ACE2, the extracellular domain of ACE2 is cleaved and the transmembrane domain migrates into the cell, mediating further fusion between virus particles and host cells<sup>21</sup> (Fig. 2).

ACE2 also participates in amino acid absorption in the intestine. In the intestine, ACE2 binds to the B<sup>0</sup>AT1 amino acid transporter<sup>22</sup> to mediate neutral amino acid

absorption. This amino acid transport function is not associated with the protease activity of ACE2.

#### Regulation of ACE2 expression

##### Regulation of ACE2 expression by pathophysiological status

ACE2 mRNA and protein levels were shown to be downregulated in the kidneys of mouse models of hypertension and diabetes.<sup>9,23</sup> Zisman et al<sup>24</sup> observed that the ACE2 protein level was significantly increased in failing human hearts with idiopathic dilated cardiomyopathy. Brake et al showed elevated ACE2 protein levels in the lung tissues of patients with chronic obstructive pulmonary disease and smokers with normal lung function.<sup>25</sup> Kuba et al<sup>26</sup> demonstrated that ACE2 protein expression was significantly decreased in a mouse model of ALI. Koitka et al<sup>27</sup> discovered that ACE2 mRNA and protein levels were significantly decreased in rats with kidney disease or rats that underwent subtotal nephrectomy. However, the precise molecular mechanism of ACE2 regulation remains unclear. Some transcriptional and post-transcriptional mechanisms have been investigated. ACE2 was recognized as a target gene for hepatocyte nuclear

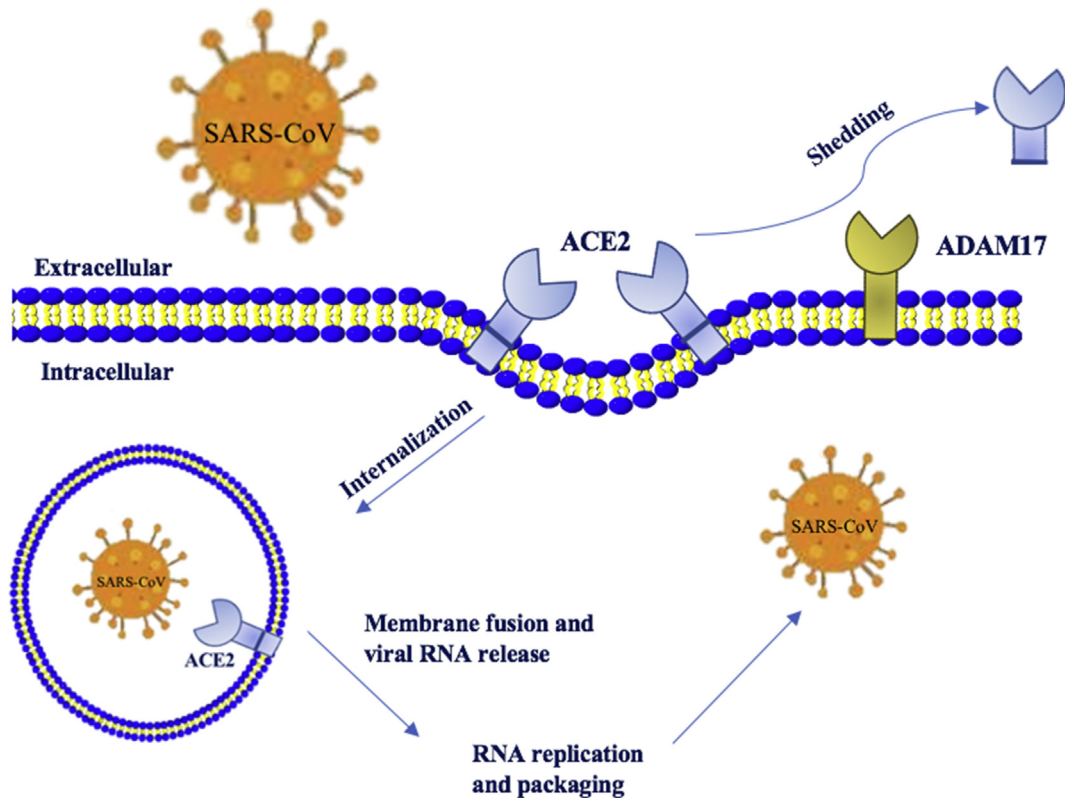


Fig. 2. Process by which SARS-CoV utilizes ACE2 for cell entry. SARS-CoV: Severe acute respiratory syndrome coronavirus; ACE2: Angiotensin-converting enzyme 2; ADAM17: A disintegrin and metalloprotease 17.

factor 1 $\beta$  (HNF-1 $\beta$ ), as binding sites for HNF-1 $\beta$  were identified in the promoter regions of *ACE2*.<sup>28</sup> Accumulation of hypoxia-inducible factor-1 $\alpha$ , a crucial transcription factor, was found to decrease *ACE2* mRNA and protein levels at the later stages of hypoxia.<sup>29</sup> Energy stress has been shown to regulate *ACE2* mRNA expression by activating adenosine monophosphate kinase, following which sirtuin 1 binds to the promoter region of *ACE2* and enhances its transcription.<sup>18</sup> Furthermore, microRNA-421 was shown to bind with the 3'-untranslated region of *ACE2* transcript and reduce *ACE2* protein level post-transcriptionally.<sup>30</sup>

#### Regulation of *ACE2* expression by drugs

RAAS inhibitors, including ACE inhibitors (ACEIs) and AT1R antagonists, can upregulate *ACE2* mRNA levels. Ishiyama et al<sup>31</sup> employed a rat model of myocardial infarction to assess the effects of myocardial infarction on myocardial *ACE2* mRNA expression. The results showed that myocardial infarction did not cause significant changes in the myocardial *ACE2* mRNA level; however, when the rats were treated with the AT1R antagonists, losartan and olmesartan, for 28

days, the cardiomyocyte *ACE2* mRNA level increased by 97% and 42%, respectively. This suggested that the cardioprotective effects of AT1R antagonists on myocardial infarction were partially mediated by *ACE2*. Subsequent studies showed that myocardial *ACE2* mRNA levels were significantly increased when lisinopril, losartan, and a combination of these two drugs were administered to rats continuously for 12 days. In addition, losartan monotherapy or combined therapy with lisinopril has been shown to significantly increase myocardial *ACE2* activity in rats.<sup>32</sup> Keidar et al<sup>33</sup> showed that mineralocorticoid (aldosterone) inhibition could inhibit oxidative stress through the Ang II/nuclear factor- $\kappa$ B signaling pathway, thereby increasing macrophage *ACE2* mRNA levels in patients with heart failure. Interferon- $\gamma$  and interleukin-4 could reduce *ACE2* mRNA levels and *ACE2* expression in epithelial cells *in vitro* to decrease susceptibility to SARS-CoV.<sup>34</sup> Additionally, Tikoo et al<sup>35</sup> showed that *ACE2* protein but not mRNA expression was down-regulated in the rabbit heart and kidneys in a high-cholesterol diet-induced atherosclerosis model. However, atorvastatin treatment increased *ACE2* protein

expression in the heart and kidneys, but only increased ACE2 mRNA level in the heart. Furthermore, enhanced occupancy of histone H3 acetylation mark on the promoter region of *ACE2* was observed in the heart after atorvastatin treatment, suggesting that epigenetic regulation might be involved.

#### *Other regulatory mechanisms of ACE2-shedding and internalization*

In 2003, ACE2 was confirmed to be a receptor for SARS-CoV. After the spike protein of SARS-CoV comes in contact with ACE2, the complete molecular structure or transmembrane domain of ACE2 and the virus are endocytosed (internalized) using clathrin. Under the effects of trypsin and furin, the spike proteins are further activated to induce membrane fusion, and the virus RNA is released into the cytoplasm, thereby infecting the cell.<sup>20</sup> In addition, cleavage of the extracellular domain of ACE2 (shedding) can occur under the action of the transmembrane protein, a disintegrin and metalloprotease 17 (ADAM17), to release the catalytic domain to the extracellular space.<sup>36</sup> Although the physiological function of shedding has not been fully elucidated, studies have shown that it is associated with virus entry into cells and virus replication.<sup>37</sup> In brief, internalization and shedding decrease ACE2 expression on the cell surface.

#### **ACE2 mediates SARS-CoV-2 infection**

The whole genome sequence of SARS-CoV-2 was successfully determined by next-generation sequencing.<sup>2</sup> A comparison with the genome sequences of previously detected coronaviruses showed that SARS-CoV-2 was closely related to two bat SARS-like viruses (88% similarity): bat-SL-CoVZC45 and bat-SL-CoVZXC21. Evolutionary analysis showed that SARS-CoV-2 belonged to the subgenus *Sarbecovirus* in the genus *Betacoronavirus*. A comparison of the spike protein sequences between SARS-CoV-2 and SARS-CoV showed that the similarity of the entire spike protein sequence was 76%–78%, RBD was 73%–76%, and receptor binding motif (RBM) was 50%–53%.<sup>38</sup> The high sequence similarity in spike proteins between these two viruses suggested that they utilized the same receptor, ACE2. Further analysis showed that ACE2 not only mediated SARS-CoV-2 infection in humans, but also resulted in human-to-human transmission. The RBD of SARS-CoV-2 interacted with ACE2 through its polar residues.<sup>39–41</sup> Shang et al<sup>42</sup> showed that the Asn 487 and Ala 475 residues in the RBM of SARS-CoV-2 formed an extra

hydrogen bond in the main chain compared with that in SARS-CoV. This resulted in a tighter conformation in the ridge structure and the Ala475-containing loop approached ACE2. In addition, structural changes were observed in two virus-binding hotspots at the RBD/ACE2 interface, which increased binding interface stability. Therefore, the affinity between the RBD of SARS-CoV-2 and ACE2 was significantly higher than that between the RBD of SARS-CoV and ACE2.<sup>43</sup> The higher affinity of SARS-CoV-2 for ACE2 enabled easier human-to-human transmission.<sup>44</sup> Hoffmann et al<sup>45</sup> performed *in vitro* experiments and showed that SARS-CoV-2 used host cell proteases to cleave the spike protein at the S1/S2 and S2' sites, thereby activating it, and then utilized ACE2 as a receptor to enter target cells. Both SARS-CoV-2 and SARS-CoV used transmembrane serine protease 2 to activate their spike proteins. Unlike in SARS-CoV, a furin cleavage site has been shown to be present between the S1/S2 subunits in SARS-CoV-2.<sup>39</sup> Bao et al<sup>46</sup> showed that SARS-CoV-2 could infect transgenic mice expressing human ACE2 to cause interstitial pneumonia, and the spike protein of SARS-CoV-2 colocalized with ACE2 receptors on alveolar epithelial cells. These results show that ACE2 acts as a receptor for SARS-CoV-2 and mediates viral infection of cells.

#### **Role of ACE2 in ALI and potential implication in SARS-CoV-2-induced ALI**

##### *Role of RAAS in the pathophysiology of ALI*

ARDS is the most severe form of ALI. Severe lung infection, aspiration, sepsis, and trauma can cause diffuse alveolar damage. Its pathological presentation includes increased capillary permeability, characterized by the presence of neutrophils, macrophages, and protein-rich fluid in the alveolar cavity, and formation of hyaline membranes.<sup>47,48</sup> Since the discovery of SARS-CoV-induced ARDS in 2003, the role of RAAS in ARDS and ALI has attracted widespread attention. In an animal model of ALI induced by acid aspiration, the Ang II levels in the mouse lungs and plasma were significantly increased, and ACE inactivation decreased Ang II levels. The latest study by Liu et al<sup>49</sup> showed that Ang II levels were significantly elevated in patients infected with SARS-CoV-2. In addition, ALI was shown to be less severe in complete *Ace* knockout (*Ace*<sup>−/−</sup>) mice, followed by partial *Ace* knockout (*Ace*<sup>+/-</sup>) mice, which suggested that the expression level of ACE was associated with the severity of lung injury. Furthermore, lung injury was found to be less severe in *AT1a* receptor

knockout mice than that in wild-type mice.<sup>50</sup> In another study, the expression level of Ang II in mice was increased after injection of recombinant SARS spike protein, and treatment with an AT1R antagonist alleviated acute respiratory distress in these mice.<sup>26</sup> A cohort study showed that insertion/deletion (I/D) polymorphism in *ACE* was associated with ARDS susceptibility and prognosis. The proportion of ARDS patients with the D/D genotype was higher than that of control individuals with the D/D genotype, and the mortality rate of ARDS patients with the D/D genotype was higher than that of ARDS patients with the I/I genotype.<sup>51</sup> These results showed that ACE/Ang II/AT1R might mediate ALI pathogenesis.

#### *Reduction of ACE2 exacerbates lung injury through ACE/Ang II/AT1R and ACE2/Ang 1–7/Mas disequilibrium*

SARS-CoV infection or injection with recombinant SARS spike protein was shown to significantly decrease the expression of ACE2 in the lungs of mice and *in vitro*-cultured cells.<sup>26</sup> Reduced ACE2 expression exacerbated ALI induced by various etiologies, such as coronavirus infection.

In an animal model of ALI induced by acid aspiration and sepsis, the clinicopathological presentation of *Ace 2* knockout mice was worse than that of wild-type mice, and the mortality rate was increased.<sup>50</sup> To further validate the effects of ACE2 in ALI, Imai et al<sup>50</sup> injected recombinant human ACE2 (rhACE2) into these mice. The results showed that treatment with rhACE2 significantly alleviated lung injury induced by acid aspiration. This phenomenon was present in both *Ace2* knockout and wild-type mice. However, injection of rhACE2 without catalytic activity could not alleviate acid aspiration-induced ALI. These results suggest that ACE2 with catalytic activity has a protective role in ALI.

In an acid aspiration-induced ALI model, the Ang II levels in the lungs and plasma of *Ace 2* knockout mice were found to be significantly higher than those in wild-type mice. However, treatment with rhACE2 alleviated lung injury and simultaneously decreased Ang II expression, and treatment with an AT1R antagonist or ACE inactivation alleviated ALI in *Ace2* knockout mice.<sup>50</sup> These results suggested that ACE2 negatively regulated ACE/Ang II/AT1R to alleviate ALI.

Besides type I and II pneumocytes and vascular endothelial cells, SARS-CoV can also infect pulmonary epithelial progenitor cells through ACE2, thereby affecting lung tissue repair after ALI.<sup>52</sup>

## **Potential therapeutic value of ACE2 in COVID-19**

There is currently no specific treatment for COVID-19. As the imbalance between ACE and ACE2 plays an important role in SARS-CoV-2-induced ALI, interventions targeting ACE2 may improve the prognosis of patients with severe COVID-19. In various animal models of ALI, administration of rhACE2 has been shown to improve distribution of pulmonary blood flow, lung function, and elastance, and attenuate edema formation, hypoxemia, and pulmonary hypertension.<sup>50,53</sup> Moreover, rhACE2 was found to be capable of binding to the coronavirus directly. Li et al<sup>20</sup> showed that a soluble form of ACE2 could bind the S1 domain of the SARS-CoV spike protein in Vero E6 cells with a high affinity. Hofmann et al<sup>54</sup> proved that the soluble ACE2 ectodomain dose-dependently blocked SARS-CoV spike-induced pseudovirus infection of 293T cells. Recent *in vitro* experiments by Monteil et al showed that rhACE2 dose-dependently decreased SARS-CoV-2 growth in Vero E6 cells by a factor of 1000–5,000, and effectively inhibited SARS-CoV-2 infection of human blood vessel and kidney organoids *in vitro*, which suggested that rhACE2 could block the early entry of SARS-CoV-2 into host cells.<sup>55</sup> In addition, previous studies have validated the safety of rhACE2 in healthy volunteers and patients with ARDS without any adverse effects on hemodynamic parameters.<sup>56–58</sup> Currently, the application of rhACE2 in patients with severe COVID-19 is in the trial phase ([Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04287686) #NCT04287686). Activators of ACE2, such as diminazene aceturate have been shown to increase the enzymatic activity of ACE2 and might exert protective effects on SARS-CoV-2-induced ALI.<sup>59</sup> Besides, ACEIs and AT1R antagonists can be used to inhibit the effect of the ACE/Ang II/AT1R axis to alleviate lung injury. According to two recent observational studies, there is no evidence as yet that ACEIs or AT1R antagonists enhance the risk of SARS-CoV-2 infection.<sup>60,61</sup> Similarly, Ang 1–7 peptide may be used for its protective effects on lung tissues through Mas.<sup>62</sup>

## **Summary**

Although there is few direct evidence of SARS-CoV-2-induced ALI, we speculate that SARS-CoV-2 and SARS-CoV have a similar pathogenesis owing to their high homology. An in-depth understanding of the pathogenesis of SARS-CoV-2 will help in the development of targeted interventions to control the COVID-19 epidemic. ACE2 is not only a receptor that

mediates coronavirus infection, but is also an important RAAS enzyme that regulates virus infection-induced ALI. Although increased ACE2 expression may elevate the risk of coronavirus infection, it may also have protective effects against ALI. However, the effects of enhanced ACE2 expression (such as RAAS inhibition) on SARS-CoV-2 susceptibility and patient outcomes are not fully understood, and further basic and clinical studies are urgently required.

### Conflicts of interest

None.

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