Angiotensin-converting enzyme 2 (ACE2): SARS-CoV-2 receptor and RAS modulator

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Abstract  The coronavirus disease 2019 (COVID-19) outbreak is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Angiotensin-converting enzyme 2 (ACE2) was rapidly identified as the critical functional receptor for SARS-CoV-2. ACE2 is well-known as a counter-regulator of the renin-angiotensin system (RAS) and plays a key role in the cardiovascular system. Given that ACE2 functions as both a SARS-CoV-2 receptor and a RAS modulator, the treatment for COVID-19 presents a dilemma of how to limit virus entry but protect ACE2 physiological functions. Thus, an in-depth summary of the recent progress of ACE2 research and its relationship to the virus is urgently needed to provide possible solution to the dilemma. Here, we summarize the complexity and interplay between the coronavirus, ACE2 and RAS (including anti-RAS drugs). We propose five novel working modes for functional receptor for SARS-CoV-2 infection and the routes of ACE2-mediated virus entering host cells, as well as its regulatory mechanism. For the controversy of anti-RAS drugs application, we also give theoretical analysis and discussed for drug application. These will contribute to a deeper understanding of the complex mechanisms of underlying the relationship between the virus and ACE2, and provide guidance for virus intervention strategies.

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1. Introduction

The coronavirus disease 2019 (COVID-19) outbreak is caused by a novel coronavirus, which was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Globally, as of 16 May 2020, there have been 4,396,392 confirmed COVID-19 cases and 300,441 deaths, which has already posed a great threat to global public health security. Although respiratory symptoms are predominant, multi-organ dysfunction occurs in response to SARS-CoV-2 infections, including acute cardiac and kidney injuries, arrhythmias and liver function abnormalities. At present, most patients with COVID-19 have a good prognosis; however, patients with underlying disorders have greater severity and higher mortality. 25.2% of all patients had at least one underlying disease, particularly, hypertension (14.9%) and coronary heart disease (2.5%). Therefore, integrated treatment for COVID-19 patients with underlying diseases is key to reduce mortality.

SARS-CoV-2 has been shown to share the same functional receptor, angiotensin-converting enzyme 2 (ACE2), with severe acute respiratory syndrome coronavirus (SARS-CoV)\(^\text{1-5}\). ACE2 is a carboxypeptidase and a negative regulator of the renin-angiotensin system (RAS) through balancing its homology angiotensin-converting enzyme (ACE). ACE mediates angiotensin (Ang) II production to activate RAS that plays a key role in cardiovascular diseases, especially hypertension. Thus, ACE inhibitor (ACEI) is used widely for treatment of hypertension, which reduces Ang II levels. Since ACE2 is a homologue of ACE, disputes have arisen about whether ACEI can upregulate ACE2 and thus the risk and severity of coronavirus infection increase. It calls into question whether to continue using of ACEI in virus-infected patients with hypertension. At present, some experts suggest that COVID-19 patients with hypertension should stop using ACEI.\(^\text{6,7}\) On the other hand, other experts believe that not only does ACEI not increase the risk of SARS-CoV-2 infection, but ACEI could reduce lung injury and cardiovascular damage in COVID-19 patients.\(^\text{9}\) Thus, a comprehensive evaluation of ACEI treatment in COVID-19 patients is of great significance since it may reduce the severity and mortality in this epidemic. Importantly, there are about 245 million people suffering from hypertension in China and approximately 1 billion patient worldwide.\(^\text{10,11}\) The safe use of ACEI is critical for the hypertensive patients with or at high risk of SARS-CoV-2 infection. Meanwhile, the changes in the use of anti-hypertensive drugs can also affect the personal finances of patients and the national financial burdens associated with hypertension and infection. Here, we summarize the latest insights into the complexity and interplay between coronavirus, ACE2 and RAS (including anti-RAS drugs), which will contribute to a deeper understanding of the complex mechanisms of the virus and provide a theoretical basis for guiding clinical decision-making.

2. Characteristics of SARS-CoV-2

SARS-CoV-2 is the seventh human coronavirus, belonging to the Betacoronavirus genus. Betacoronaviruses are enveloped, single-stranded RNA viruses that infect wild animals, herds and humans.\(^\text{4}\) Currently, seven different coronavirus strains are known to infect humans, including HCoV-229E (229E), HCoV-OC43 (OC43), SARS-CoV, HCoV-NL63 (NL63), HCoV-HKU1 (HKU1), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2.\(^\text{12}\) 229E, OC43, NL63 and HKU1 have low pathogenicity and generally cause mild respiratory symptoms, which are similar to the common cold.\(^\text{12}\) Both SARS-CoV and MERS-CoV cause serious respiratory diseases.\(^\text{13,14}\) SARS-CoV-2 appears to have greater infectivity when compared with SARS-CoV and MERS-CoV.

3. The functional receptors of SARS-CoV-2

The virus initiates its infection process mainly by binding to functional receptors on the membrane of a host cell. The functional receptor determines the invasion and spread of viruses and the clinical symptoms of patients.\(^\text{6,15,16}\) Therefore, the functional receptor is a key factor for the prevention and treatment of viral diseases. Here, we summarize the major functional receptors of the seven known human coronaviruses (Table 1).\(^\text{17-25}\) Since the outbreak of SARS-CoV-2, ACE2 was rapidly identified as the functional receptor of SARS-CoV-2. However, there are many biological phenomena related to virus receptors that cannot be explained by ACE2, suggesting that the functional receptor for the SARS-CoV-2 is not completely clear. Based on the existing evidence, we propose several working modes of functional receptors for SARS-CoV-2, including monomer receptor, homodimer receptor, alternative receptors, co-receptors and transmissive receptor (Fig. 1).

### Table 1 The functional receptor of seven human coronavirus.

| HCoV   | Year | Disease                        | Functional receptor | Ref. |
|--------|------|--------------------------------|--------------------|------|
| SARS-CoV-2 | 2019 | Corona virus disease          | ACE2, CD147, (?)   | 5, 17|
| MERS-CoV | 2012 | Middle-East respiratory syndrome | DPP4               | 18, 19|
| HKU1   | 2005 | Acute respiratory tract infection | 9-O-Ac-Sia         | 20, 21|
| NL63   | 2004 | Acute lower respiratory tract infection in children | ACE2               | 22, 23|
| SARS-CoV | 2002 | Severe acute respiratory syndrome | ACE2, CD209L, DC-SIGN | 24--26|
| OC43   | 1967 | Common cold                    | 9-O-Ac-Sia         | 20, 27|
| 229E   | 1966 | Common cold                    | APN                | 28   |

ACE2, angiotensin-converting enzyme 2; DPP4, dipeptidyl peptidase 4; CD209L (also called L-SIGN), liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing integrin; 9-O-Ac-Sia, acetylated sialic acid; APN, aminopeptidase.

? other unknown functional receptors.
trimer on the virus surface with each monomer harboring a receptor binding domain (RBD) that interacts with a particular receptor on the host cell. Furthermore, sequence analysis showed that SARS-CoV-2’s S-protein RBD was more similar to SARS-CoV’s RBD than MERS-CoV’s. Next, based on the computer-guided homology modeling method, the RBD of the SARS-CoV-2 S-protein supported a strong interaction with human ACE2 molecules. In addition, biological research showed that HeLa cells expressing ACE2 were more susceptible to SARS-CoV-2 infection while cells without ACE2 were not. Thus, those results suggested that ACE2 was the functional receptor of SARS-CoV-2.

Excitingly, the structures of SARS-CoV-2 and ACE2 were obtained by cryo-electron microscopy (cryo-EM) quickly. Firstly, the cryo-EM structure of the SARS-CoV-2 S-protein was determined in the prefusion conformation. The predominant state of the S-protein trimer has one of the three RBDs rotated up in a receptor-accessible conformation. The biophysical and structural evidence showed that SARS-CoV-2’s S-protein bound ACE2 with higher affinity than the SARS-CoV S-protein (approximately 10- to 20-fold), which might explain why SARS-CoV-2 is more contagious than SARS-CoV. Then, the cryo-EM structure of full-length human ACE2 in complex with neutral amino acid transporter B^AT1 was revealed. Immediately, the crystal structure of the RBD of SARS-CoV-2 S-protein bound with ACE2 was determined. This series of work strongly confirmed that ACE2 was the functional receptor of SARS-CoV-2.

Notably, the structure of full-length human ACE2 in complex with B^AT1 revealed a novel mode that SARS-CoV-2 binds to ACE2 in a homodimer manner (Fig. 1B). In this mode, B^AT1 might be a key factor in determining the formation of an ACE2 homodimer. Thus, these results suggest that SARS-CoV-2 binds with ACE2 by two working modes: i) SARS-CoV-2 binds directly with an ACE2 monomer without the B^AT1 protein (Fig. 1A) and ii) SARS-CoV-2 binds with ACE2 homodimer when with the existing of B^AT1 protein (Fig. 1B). However, the binding affinity and biological function of these two different modes are still unclear.

3.2. The potential receptors of SARS-CoV-2

ACE2 is widely expressed across a variety of organs and its expression is higher in many organs (such as heart, kidney, etc.) than that in lung. However, the lung is the major organ infected by SARS-CoV-2. In addition, although ACE2 knockout mice showed a significant decrease in SARS-CoV infection, it did not completely prevent virus infection from occurring. Those data suggested that there could be other receptors involved in a viral invasion. More recently, CD147, a transmembrane glycoprotein that belongs to the immunoglobulin superfamily, was identified as a receptor for SARS-CoV-2. Interestingly, previous studies have shown that CD147 played a key role in SARS-CoV invasion into host cells, while CD147 antagonistic peptides have an inhibitory effect on SARS-CoV. These results further suggested that CD147 might be a novel receptor for SARS-CoV-2. Another possible receptor is CD209L (L-SIGN), a type II transmembrane glycoprotein in the C-type lectin family, which has been identified as the receptor of SARS-CoV from in vitro studies. Thus, given that SARS-CoV-2 is similar to SARS-CoV, CD209L could be another potential receptor for SARS-CoV-2. Therefore, besides ACE2, there are several other potential receptors for SARS-CoV-2. SARS-CoV-2 might invade cells through an alternative receptor mode (Fig. 1C) or a co-receptors mode (Fig. 1D). Interestingly, viruses are very tricky, as they can also infect host cells in a detour or bait-and-switch strategy. For example, SARS-CoV can first attach to the surface of dendritic cells (DC)
through the DC-SIGN receptor, which is a DC-specific intercellular adhesion molecule-3-grabbing non-integrin, a homologue of CD209L. Then the virus is presented to host cells by the DC cells and binds to ACE2 of host cell, which eventually leads to infection. Similarly, SARS-CoV-2 might also use this subterfuge to infect a host cell. Since the DC-SIGN receptor plays only a transmitting role, it could be called the “transmissive receptor” (Fig. 1E).

In summary, we propose five distinctive working modes of SARS-CoV-2 receptors: monomer receptor, homodimer receptor, alternative receptors, co-receptors and transmissive receptor. The five modes are distinct but also interconnected. For example, i) the monomer receptor may be the basis of other modes; ii) the transmissive receptor could be another form of a co-receptor and involved in other receptor modes; iii) different modes may be switched at different pathological stages, or iv) multiple receptor modes may coexist in the same organ. These potential interconnections raise an important implication for diversity and complexity of SARS-CoV-2 infection. Here, five working modes of receptors enrich the theoretical basis of SARS-CoV-2 receptor, which may provide novel insights into the prevention and treatment of viral diseases.

4. Characteristics of ACE2

4.1. The structure of ACE2

ACE2 was discovered as a homologue of ACE in 2000. It plays a physiological and pathological role in cardiovascular, renal, intestinal and respiratory systems. ACE2 is a type I transmembrane protein, which consists of 805 amino acids with an extracellular N-terminal domain and a short intracellular C-terminal tail (Fig. 2). The N-terminal domain has one active, a site-zinc metallopeptidase domain (HEMGH domain), which has 41.8% sequence homology with the amino domain of ACE. This domain is essential for both formation of the vasodilator peptide Ang (1–7) and SARS-CoV-2 S-protein binding. The C-terminal domain of ectodomain of ACE2 shares 48% homology with collectrin, which is crucial for the interaction with the neutral amino acid transporter B^AT1.

4.2. The characteristics of ACE2 expression

4.2.1. The localization of ACE2 in cells

ACE2 is mainly expressed on the cell-surface with little localization in the cytoplasm. Under normal conditions, ACE2 is not easy to internalize. However, after SARS-CoV infection, ACE2 was shown to be internalized into cytoplasm upon virus binding. In addition to the membrane-bound form, the soluble form of ACE2 can be detected in the plasma and urine through its extracellular domain shedding. A disintegrin and metallopeptidase domain 17 (ADAM17) and type II transmembrane serine proteases (TMPRSS2) are considered as the proteases for ACE2 proteolytic cleavage. In 2004, Hamming et al. investigated the localization of the ACE2 protein in human organs by immunohistochemistry and ACE2 protein expression was found in various organs, including oral and nasal mucosa, nasopharynx, lung, stomach, small intestine, colon, skin, lymph nodes, thymus, bone marrow, spleen, liver, kidney, and brain. More recently, single-cell RNA-seq datasets revealed that the abundance of ACE2 expression from high to low is in the ileum, heart, kidney, bladder, respiratory tract, lung, esophagus, stomach and liver. Here, we present a map of ACE2 distribution and expression in major organs based on the previous results and the BioGPS website (http://biogps.org) (Fig. 3, left panel).

4.2.2. The distribution of ACE2 in organs

Identifying the distribution of ACE2 in organs has major implications for understanding the pathogenesis and treatment options for SARS-CoV-2. ACE2 was initially only detected in the heart, kidneys and testes. Then, ACE2 mRNA expression was revealed in virtually all organs (72 human tissues) by real-time PCR. Given that ACE2 is the functional receptor of SARS-CoV-2, its expression is associated with the organic vulnerability to SARS-CoV-2. Indeed, the tissue expression of ACE2 shows some correlation with the sites of SARS-CoV-2 infection. For example, ACE2 protein is abundantly expressed in the lung and heart which are the vulnerable organs for SARS-CoV-2 infection. However, the level of ACE2 expression is not completely related with the vulnerability to SARS-CoV-2. Here, we present a comparative map of ACE2 expression and vulnerability to SARS-CoV-2 in different organs (Fig. 3). Based on the comparative map, the ACE2 expression is the highest in ileum, but the ileum is not the most vulnerable organ, suggesting other complicated mechanisms might be involve in virus infection. The possible mechanisms for inconsistency between ACE2 expression and virus vulnerability include: i) There might be other unknown receptor-mediated virus infection; ii) ACE2 alone is not enough to mediate virus infection, which needs another co-receptor’s assistance (such as angiotensin II type 2 receptor (AT2R), a potential receptor for SARS-CoV-2); iii) The function of the ACE2 receptor is regulated by some protein as yet unidentified. For


Receptor-mediated endocytosis, including both clathrin-dependent pathways and caveolae-dependent pathways, is the most important mechanism for virus internalization. Clathrin-dependent endocytosis is considered the primary endocytic route for the coronavirus. The infection of both SARS-CoV and MERS-CoV have been shown by clathrin-dependent endocytosis. As an alternative to clathrin, caveole-dependent endocytosis has been also described as the endocytic route for some viruses, such as simian virus 40 (SV40). However, the caveole-dependent endocytosis does not seem to involve ACE2 endocytosis after SARS-CoV infection. Additionally, lipid raft-dependent endocytosis was found in SARS-CoV as a novel pathway, which is both clathrin- and caveole-independent, may constitute a specialized high capacity endocytic pathway for lipids and fluids. Therefore, it is reasonable to assume that SARS-CoV-2 binds to ACE2 and might be able to either initiate clathrin-dependent and/or non-caveole lipid raft-dependent endocytosis to enter host cell (Fig. 4A), but these need to be further verified by biological experiments.

5.1. TMPRSS2-mediated membrane fusion

In addition to ACE2-mediated virus endocytosis, TMPRSS2-mediated direct membrane fusion is another important way for SARS-CoV-2 entry. Unlike the route that ACE2 binds with SARS-CoV-2 to mediated virus endocytosis together, during the process of TMPRSS2-mediated membranes fusion, ACE2 plays a role in arresting and fixing the SARS-CoV-2 at the surface. After the viruses arrested and fixed, TMPRSS2 induces direct membrane fusion between virus and host cell (Fig. 4B). Notably, the route of TMPRSS2-mediated membrane fusion is also crucial for SARS-CoV and MERS-CoV entry.

5.2. The regulation of ACE2-mediated entering host cells

The proteolytic shedding of a transmembrane protein like ACE2 can result in release of the soluble extracellular domain (ectodomain) from the membrane and a fragment that remains bound to the membrane. The shedding is an important regulatory mechanism to control the function and distribution of membrane proteins, which can terminate the function of a full-length membrane protein or release the biologically active ectodomain to activate the membrane protein. In addition, the shedding contributes to membrane protein endocytosis. ACE2 shedding can increase SARS-CoV entry, but the exact molecular mechanism responsible for increased virus entry is unclear at present. Some researchers have suggested that ACE2 shedding promoted ACE2-mediated virus internalization and increased virus uptake into target cells. Here, we summarize two distinct modes for ACE2 shedding: ADAM17-dependent shedding and TMPRSS2-dependent shedding (Fig. 5). ADAM17, a disintegrin and metalloproteinase, has an important established role in the regulation of ACE2 shedding. ADAM17 functions in the shedding of ACE2 via arginine and lysine residues within ACE2 amino acids 652 to 659 (Fig. 5). SARS-CoV S-protein binding facilitates ADAM17-dependent ACE2 shedding and has been shown to induce viral entry into the cell. On the other hand, there is also another research has suggested that only type II transmembrane serine proteases TMPRSS2, but not ADAM-17, promotes SARS-CoV entry via ACE2 shedding. Different from ADAM17, TMPRSS2 requires arginine and lysine residues within ACE2 amino acids 697 to 716 for ACE2 cleavage. Notably, the neutral amino acid transporter B0AT1 might inhibit TMPRSS2-dependent

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**Figure 3** The comparative map of ACE2 expression and vulnerability to SARS-CoV-2 in different organs. ACE2 distribution in major organs is shown in the left panel. The vulnerability to SARS-CoV-2 in different organs is shown in the right panel. Different levels of ACE2 expression (left) or vulnerability to SARS-CoV-2 (right) is shown by color scale. ACE2, angiotensin-converting enzyme 2.
ACE2 shedding (Fig. 5B) since B^60AT1 binds ACE2 to form a protein complex. The structure of the complex of ACE2/B^60AT1 reveals that the TMPRSS2 cleavage sites (residues of 697–716) are hidden in the dimeric interface of ACE2. It indicates that B^60AT1 may block the access of TMPRSS2 to the cleavage sites on ACE2 which would result in decrease of ACE2 shedding.

In addition, the proprotein convertase furin has been also identified as an important factor for regulation of ACE2-mediated virus entering host cells. Furin mainly preactivates SARS-CoV-2 S-protein during viral packaging, and enhances virus entry into target cells. However, recent study showed that furin reduced SARS-CoV-2 entry into Vero cells. The reason for this conflicting result is not clear. In addition, ACE2 genetic polymorphisms might be involved in the regulation of SARS-CoV-2 entering host cells in different ethnic groups. The research showed that the East Asian populations have much higher allele frequencies (AFs) in the expression quantitative trait loci (eQTL) variants associated with higher ACE2 expression in tissues, suggesting different susceptibility or response to SARS-CoV-2 from different populations under the similar conditions.

In the future, the accurate regulatory mechanisms of receptor-mediated virus endocytosis and TMPRSS2-mediated membrane fusion need to be further investigated. For example, it needs to be determined whether ADAM17- or TMPRSS2-dependent shedding influences ACE2 to initiate different endocytosis pathways (either clathrin-dependent or non-caveolea lipid raft-dependent pathway). In addition, it is also needed to distinguish the roles of TMPRSS2 in membrane fusion and ACE2 shedding. Moreover, the role of ACE2 shedding in SARS-CoV-2 infection should be further investigated in vivo. ACE2 shedding not only contributes to transmembrane protein endocytosis, but also results in the release of the soluble extracellular domain (soluble ACE2). The previous study shows that the increase of ADAM-17-dependent ACE2 shedding is associated with myocardial hypertrophy and fibrosis. In addition, elevated soluble ACE2 activity is associated with severer of myocardial dysfunction and is an independent predictor of adverse clinical events. However, the question whether soluble ACE2, produced by ACE2 shedding, can bind with SARS-CoV-2 S-protein to clear away virus is confusing, which will be the future direction for research on soluble ACE2.

**Figure 4** The routes of ACE2-mediated virus entering host cells. There are two routes of ACE2-mediated virus entering host cells. (A) The first route is dependent on the ACE2-mediated virus endocytosis. There are two potential endocytic routes. One is the clathrin-dependent pathway. Another is the lipid raft-dependent pathway. Caveolea-dependent pathway does not involve ACE2-mediated endocytosis after SARS-CoV-2 binding. (B) The second route is dependent on the TMPRSS2-mediated membrane fusion. After binding with ACE2, S-protein is cleaved and activated by TMPRSS2 to promote membrane fusion. ACE2, angiotensin-converting enzyme 2; EEA1, Early endosomal autoantigen 1; S-protein, Spike protein; TMPRSS2, type II transmembrane serine proteases.
6. The effect of coronavirus on ACE2 and RAS

6.1. ACE2 and RAS

RAS plays a critical role in maintaining blood pressure homeostasis, as well as fluid and salt balance. Therefore, RAS is intimately connected to the pathophysiology of heart and kidney diseases. Production of angiotensins from angiotensinogen requires the participation and coordination of many proteases in different pathways. In classical RAS, renin from kidney acts on liver-derived angiotensinogen to generate Ang I. Subsequently, ACE cleaves Ang I into Ang II. Ang II is the main bioactive component of RAS through angiotensin II type 1 receptor (AT1R) and AT2R. The ACE-Ang II-AT1R axis is well-established to mediate vasoconstriction and increase blood pressure. Consequently, ACEI and angiotensin receptor blocker (ARB) are highly effective drugs for the treatment of hypertension.

Recent studies have discovered an ACE homologue ACE2. ACE2 differs from ACE in its physiological role, which is considered to be a negative regulator of RAS. Production of angiotensins from angiotensinogen requires the participation and coordination of many proteases in different pathways. In classical RAS, renin from kidney acts on liver-derived angiotensinogen to generate Ang I. Subsequently, ACE cleaves Ang I into Ang II. Ang II is the main bioactive component of RAS through angiotensin II type 1 receptor (AT1R) and AT2R. The ACE-Ang II-AT1R axis is well-established to mediate vasoconstriction and increase blood pressure. Consequently, ACEI and angiotensin receptor blocker (ARB) are highly effective drugs for the treatment of hypertension.

ACE2: SARS-CoV-2 receptor and RAS modulator

Figure 5 The modes for ACE2 shedding. SARS-CoV S-protein binding initiates ACE2 shedding and further induces viral entry into cell. There are two major modes of ACE2 shedding: ADAM17-dependent and TMPRSS2-dependent. The arginine and lysine residues within ACE2 amino acids 652 to 659 are essential for ACE2 cleavage by ADAM17. Different from ADAM17, the arginine and lysine residues within ACE2 amino acids 697 to 716 are essential for cleavage by TMPRSS2. However, the neutral amino acid transporter B0AT1 might inhibit TMPRSS2-dependent ACE2 shedding. ACE2, angiotensin-converting enzyme 2; ADAM17, a disintegrin and metalloproteinase domain 17; S-protein, Spike protein; TMPRSS2, type II transmembrane serine proteases.

6.2. The effect of coronavirus on ACE2 and RAS

Previous animal experiments have shown that SARS-CoV infection or recombinant SARS S-protein led to significant downregulation of ACE2 expression. In post-mortem autopsy heart tissues from 20 patients who succumbed to SARS-CoV, seven heart samples had detectable viral SARS-CoV genome. These patients were also characterized by reduced myocardial ACE2 expression, which further confirmed that ACE2 expression was decreased after virus infection.

Given that ACE2 functions as a counter-regulator of RAS, the decrease of ACE2 expression leads to the weakened ACE2-Ang (1–7)-MasR axis, mainly manifested as the increase of Ang II and decrease of vasodilator Ang (1–7) level. At present, a cohort study of 12 COVID-19 patients have partly confirmed this view. It was found that the level of Ang II in the plasma sample from SARS-CoV-2 infected patients was significantly higher than that from uninfected individuals. Moreover, the weakened ACE2-Ang (1–7)-MasR axis after SARS-CoV-2 infection also resulted in the other damage, such as myocardial fibrosis, inflammation and cardiovascular disease. These clinical symptoms have been documented in COVID-19 patients.
7. Anti-drugs ACEI/ARB and coronavirus

Since ACE2 has been identified as the functional receptor of SARS-CoV-2, some disputes have arisen on whether ACEI/ARB usage increases the risk of SARS-CoV-2 infection, whether hypertensive patients should stop using ACEI drugs or change to other anti-hypertension drugs, and/or whether ACEI/ARB affects (aggravates or reduces) the injury of target organs after SARS-CoV-2 infection. To answer these questions, the effect of ACEI/ARB on ACE2 expression and activity are two key issues. Current studies have shown that both ACEI and ARB affect the mRNA expression of ACE2. For example, ACEI drugs, lisinopril and enalapril, can increase ACE2 mRNA expression in heart and enalapril, can increase ACE2 mRNA expression in heart. However, as we know, the change of protein levels is not always consistent with the mRNA levels, sometimes even in the opposite direction. Many factors lead to the inconsistencies between mRNA and protein expression levels, including microRNA regulation, translation, post-translational modification, protein transport and degradation.

At present, it is not clear whether ACEI and ARB increase ACE2 expression at the protein level. For example, perindopril (ACEI) was able to increase hepatic ACE2 expression at the protein level under conditions of liver fibrosis. However, another ACEI drug ramipril decreased ACE2 protein expression after myocardial infarction. For ARB drugs, olmesartan up-regulated ACE2 protein expression in the carotid arteries after balloon injury. But there were no changes in ACE2 protein expression in uninjured carotid arteries in olmesartan-treated rats. Therefore, from an ACE2 expression point of view, it does not seem to be consistent support for the suggestion that ACEI/ARB will increase the risk of the SARS-CoV-2 infection by up-regulating ACE2 protein level.

ACE2 enzyme activity influences its counter-balancing role for ACE. Whether ACEI/ARB affects the activity of ACE2 becomes an important question. Current research suggests that ARB can increase the enzymatic activity of ACE2, but ACE2 was not susceptible to ACEI. For example, lisinopril (ACEI) only increased the expression of ACE2 mRNA, but did not affect actual ACE2 enzymatic activity in heart. Losartan (ARB) can not only increase the expression of cardiac ACE2 mRNA, but also significantly increase the activity of ACE2 in heart. Given that coronavirus infection results in lung injury by reducing ACE2 expression and increasing Ang II levels, losartan should have a protective effect on lung injury. It has been shown that losartan can protect against lung injury caused by coronaviruses (SARS-CoV, H7N9 and H5N1) in mice. Losartan also gave protective effect on lung injury. It has been shown that losartan can protect against lung injury caused by coronaviruses (SARS-CoV, H7N9 and H5N1) in mice. Beside, losartan also gave protection against the development of acute respiratory distress syndrome (ARDS) in rodents by decreasing production of pro-inflammatory cytokines. However, a systematic review and meta-analysis of clinical trials indicated ACEIs but not ARBs reduced the risk of pneumonia and had a protective role, especially patients with previous stroke and in Asia. Therefore, there is currently no consensus on the effect of ACEI/ARB on lung damage.

More recently, some clinical studies also suggested that there was no correlation between ACEI/ARB and the risk of COVID-19 infection. In addition, there was no evidence to support that the risk of severe COVID-19 was associated with using ACEIs and ARBs. Thus, from either theoretical analysis or clinical data, there is still no need to recommend the discontinuation of ACEIs/ARBs for hypertensive patients with or at high risk of SARS-CoV-2 infection, or change to other antihypertensive drugs.

8. Summary and prospects

ACE2 functions as both a SARS-CoV-2 receptor and RAS modulator, which presents the dilemma of how to limit virus entry while protecting its physiological function. Therefore, it is of great significance to understand fully the function and mechanism of ACE2 and the relationships among virus, ACE2 and RAS. Although ACE2 has been identified as a SARS-CoV-2 receptor, there might be other receptors or co-receptors for this virus that are yet to be discovered. In this review, we propose five working modes of functional receptors for SARS-CoV-2: monomer receptor, homodimer receptor, alternative receptors, co-receptors and transmissive receptor. We summarize the routes of ACE2 receptor-mediated virus entering host cells (ACE2-mediated virus endocytosis via clathrin-dependent pathways and non-caveolar lipid raft dependent pathways, and TMPRSS2-mediated membrane fusion upon ACE2 engagement) and its regulatory mechanism. In addition, a comparative map of ACE2 expression and vulnerability to SARS-CoV-2 in different organs was described. Moreover, the complex relationship among coronavirus, ACE2 and RAS (including anti-RAS drugs) is also summarized and discussed. These will contribute to a deeper understanding of the complex mechanisms and intervention strategies for virus infection and target organ damage. These
raise further important implications for therapeutic targets for SARS-CoV-2. Effective therapies against SARS-CoV-2 are urgently required due to the severity of the outbreak. Based on the above theoretical summary, five steps are important anti-viral targets, including 1) the binding between coronavirus and ACE2; 2) virus entry mediated by ACE2; 3) virus replication; 4) virus assembly, and 5) virus exit (Fig. 7).

One of important strategies to control viral infections is to block the initial binding of virus to its functional receptor. A number of candidate drugs have been developed for blocking the binding of S-protein and ACE2, including S-protein-based drugs and ACE2-based drugs. Neutralizing antibodies and coronavirus vaccine are typical S-protein-based drugs. A SARS-CoV-specific human neutralizing antibody, CR3022, has been shown to bind to the RBD of the SARS-CoV-2 S-protein and block the binding of the SARS-CoV-2 S-protein to ACE2. Human recombinant soluble ACE2 is typical ACE2-based drugs. It acts as scavenger for SARS-CoV-2 and inhibits SARS-CoV-2 infection.

In addition, ACE2 interference is another way to block the binding between the SARS-CoV-2 S-protein and ACE2. For example, chloroquine can impact terminal glycosylation of ACE2, thereby preventing it from binding to the S-protein and inhibiting the SARS-CoV-2 infection. Furthermore, interfering with other receptors (such as CD147) proposed in Fig. 1 also can be considered as the therapeutic targets. For example, meplazumab, an anti-CD147 humanized antibody, significantly inhibited the viruses from invading host cells.

Inhibition of ACE2 receptor-mediated virus entry is another important antiviral strategy (Fig. 7 ②). ACE2 receptor-mediated endocytosis is a complex process regulated by a variety of proteins, which are potential target for interference. For example, AP2-associated protein kinase 1 (AAK1) is a well-known positive regulator of receptor endocytosis. An inhibitor of AAK1 (baricitinib) is predicted to reduce the ability of the SARS-CoV-2 entry by inhibiting ACE2 receptor-mediated endocytosis. In addition, camostat mesylate, a TMPRSS2 inhibitor, has been shown to inhibit SARS-CoV-2 entry into cells.

Of course, in addition to the steps related to ACE2 (the binding between coronavirus and ACE2, and virus entry mediated by ACE2), virus replication has been the hotspot for antiviral drug research. Some drugs have entered clinical practice (Fig. 7 ③). For example, remdesivir, a nucleoside analogue, can incorporate into nascent viral RNA chains, resulting in pre-mature termination of virus replication. Previous study has found remdesivir can inhibit SARS-CoV and MERS-CoV replication. For SARS-CoV-2, remdesivir also have a significant inhibitory effect on virus infection. Thus, remdesivir has been recognized as a promising antiviral drug and has entered clinical practice for SARS-CoV-2. Virus assembly and virus exit (Fig. 7 ④ and ⑤) are also important targets for antiviral drugs. However, because the exact mechanism of virus assembly and exit is still unclear, there are few antiviral drugs targeting for these steps. In view of the complexity of virus-host interactions, cocktail therapy might be a better choice for COVID-19 patients.

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Author contributions

All authors researched data for the article and discussed its content. Jingwei Bian wrote the manuscript. Zijian Li designed, reviewed and edited this manuscript before submission.

Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. World Health Organization. COVID-2019 situation reports. 16 May 2020. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports.
2. Ghelawit M, Wang K, Vieveros A, Nguyen Q, Zhong J, Turner AJ, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotension system: celebrating the 20th anniversary of the discovery of ACE2. Circ Res 2020;126:1456–74.
3. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708–20.
4. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci 2020;63:457–65.
5. Yan W, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronaviruses. J Virol 2020;94:e00127-20.
6. Forrester SJ, Booz GW, Sigmund CD, Coffman TM, Kawai T, Rizzo V, et al. Angiotensin II signal transduction: an update on mechanisms of physiology and pathophysiology. Physiol Rev 2018;98:1627–738.
7. Fang L, Karakulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? Lancet Respir Med 2020;8:e21.
8. Diaz JH. Hypothesis: angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may increase the risk of severe coronavirus disease 2019 (COVID-19). J Trav Med 2020;27:taaa041.
9. Guo J, Huang Z, Lin L, Lv J. Coronavirus disease 2019 (COVID-19) and cardiovascular disease: a viewpoint on the potential influence of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers on onset and severity of severe acute respiratory syndrome coronavirus 2 infection. J Am Heart Assoc 2020;9:e16219.
10. Hu SS, Gao RL, Liu LS, Zhu ML, Wang W, Wang YJ, et al. Summary of the 2018 report on cardiovascular diseases in China. Chin Circ J 2019;34:209–20.
11. Moran AE, Wood DA, Narula J. The 2000–2016 WHF global atlas of CVD: take two. Glob Heart 2018;13:139–41.
12. Su S, Wong G, Shi W, Liu J, Lai A, Zhou J, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 2016;24:490–502.
13. Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 2003;361:1319–25.
14. Okba NM, Raj VS, Haagmans BL. Middle East respiratory syndrome coronavirus vaccines: current status and novel approaches. Curr Opin Virol 2017;23:49–58.
15. Kuba K, Imai Y, Penninger JM. Multiple functions of angiotensin-converting enzyme 2 and its relevance in cardiovascular diseases. Circ J 2013;77:301–8.
16. Greeneberg DA, Ilgenfeld R, Zabel P. Molecular mechanisms of severe acute respiratory syndrome (SARS). Respir Res 2005;6:8–23.
17. Ulrich H, Pillaia MM. CD147 as a target for COVID-19 treatment: suggested effects of azithromycin and stem cell engagement. Stem Cell Rev Rep 2020;16:344–40.
18. Lu G, Hu Y, Wang Q, Qi J, Gao F, Li Y, et al. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. Nature 2013;500:227–31.
19. Wang N, Shi X, Jiang L, Zhang S, Wang D, Tong P, et al. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. Cell Res 2013;23:986–93.
20. Hulswit R, Lang Y, Bakkers M, Li W, Li Z, Schouten A, et al. Human coronaviruses OC43 and HKU1 bind to 9-O-acetylated sialic acids via a conserved receptor-binding site in spike protein domain A. Proc Natl Acad Sci U S A 2019;116:2681–90.
21. Huang X, Dong W, Milewskas A, Golda A, Qi Y, Zhu QK, et al. Human coronavirus HKU1 spike protein uses O-acetylated sialic acid as a receptor-destroying enzyme. J Virol 2015;89:7202–13.
22. Wu K, Li W, Peng G, Li F. Crystal structure of NL63 respiratory coronavirus receptor-binding domain complexed with its human receptor. Proc Natl Acad Sci U S A 2009;106:19970–4.
23. Milewskas A, Nowak P, Owczarek K, Szczepanski A, Zarebski M, Hoang A, et al. Entry of human coronavirus NL63 into the cell. J Virol 2018;92:e01933-17.
24. Li W, Moore MJ, Vasilevsk N, Sai J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 2003;426:450–4.
25. Jeffers SA, Tussell SM, Gillin-Ross L, Hemmilta EM, Achenbach JE, Babcock GI, et al. CD209L (L-SIGN) is a receptor for severe acute respiratory syndrome coronavirus. Proc Natl Acad Sci U S A 2004;101:15748–53.
26. Yang ZY, Huang Y, Ganesh L, Leung K, Kong WP, Schwartz O, et al. pH-dependent entry of severe acute respiratory coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN. J Virol 2004;78:5642–50.
27. Tortorici MA, Walls AC, Lang Y, Wang C, Li Z, Koerhuis D, et al. Structural basis for human coronavirus attachment to sialic acid receptors. Nat Struct Mol Biol 2019;26:481–9.
28. Yeager CL, Ashmun RA, Williams RK, Cardellichio CB, Shapiro LH, Look AT, et al. Human aminopeptidase N is a receptor for human coronavirus 229E. Nature 1992;357:420–2.
29. Li F. Structure, function, and evolution of coronavirus spike proteins. Proc Natl Acad Sci U S A 2016;113:237–61.
30. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020;367:1260–3.
31. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 2020;367:1444–8.
32. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain to the ACE2 receptor. Nature 2020;581:215–20.
33. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
34. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med 2005;11:875–9.
35. Wang K, Chen W, Zhou Y, Lian J, Zhang Z, Du P, et al. SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. bioRxiv; 2020. Available from: http://doi.org/10.1101/2020.03.14.988345.
36. Chen Z, Li M, Xu J, Yu J, Wang X, Jiang J, et al. Function of HAb18G/CD147 in invasion of host cells by severe acute respiratory syndrome coronavirus. J Infect Dis 2005;191:755–60.
37. Cormier EG, Durso RJ, Tsamis F, Boussennart L, Maniv C, Olson WC, et al. L-SIGN (CD209L) and DC-SIGN (CD209) mediate
transfection of liver cells by hepatitis C virus. *Proc Natl Acad Sci U S A* 2004;101:14067–72.
38. Mummidi S, Catano G, Lam L, Hoeffe A, Telles V, Begum K, et al. Extensive repertoire of membrane-bound and soluble dendritic cell-specific ICAM-3-grabbing nonintegrin 1 (DC-SIGN1) and DC-SIGN2 isoforms. Inter-individual variation in expression of DC-SIGN transcripts. *J Biol Chem* 2001;276:33196–212.
39. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin II. *Circ Res* 2000;87:E1–9.
40. Tipnis SR, Hooper NM, Hyde R, Karran E, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* 2000;275:33238–43.
41. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Tipnis SR, et al. Collectrin, a collecting duct-specific transmembrane glyco-protein, is a novel homolog of ACE2 and is developmentally regulated and facilitates viral entry. *J Biol Chem* 2005;280:17132–9.
42. Fairweather SJ, Broer A, Subramanian N, Tumer E, Cheng Q, Lambert DW, et al. Angiotensin metabolism in renal proximal tubules, a novel angiotensin-converting enzyme-related carboxypeptidase. *Pharm Res* 2004;21:2299–301.
43. Danilczyk U, Sarao R, Remy C, Benabbs C, Stange G, Richter A, et al. Essential role for collectin in renal amino acid transport. *Nature* 2006;444:1088–91.
44. Oliveira AJ, de Farias LD, Mafra V, Cota J. The angiotensin converting enzyme 2 (ACE2), gut microbiota, and cardiovascular health. *Protein Pept Lett* 2017;24:827–32.
45. Jia H. Pulmonary angiotensin-converting enzyme 2 (ACE2) and inflammatory lung disease. *Shock* 2016;46:239–48.
46. Zhang H, Wada J, Hida K, Tsuchiyama Y, Hiragushi K, Shikata K, et al. Collectrin, a collecting duct-specific transmembrane glycoprotein, is a novel homolog of ACE2 and is developmentally regulated in embryonic kidneys. *J Biol Chem* 2001;276:17132–9.
47. Fairweather SJ, Broer A, Subramanian N, Turner E, Cheng Q, Schmoll D, et al. Molecular basis for the interaction of the mammalian amino acid transporters B^AT1 and B^AT3 with their ancillary protein collectrin. *J Biol Chem* 2015;290:24308–25.
48. Wang H, Yang P, Liu K, Guo F, Zhang Y, Zhang G, et al. SARS coronavirus entry into host cells through a novel clathrin- and caveolea-independent endocytic pathway. *Cell Res* 2008;18:290–301.
49. Inoue Y, Tanaka N, Tanaka Y, Inoue S, Morita K, Zhuang M, et al. Clathrin-dependent entry of severe acute respiratory syndrome coronavirus into target cells expressing ACE2 with the cytoplasmic ancillary protein collectrin. *J Biol Chem* 2018;293:982–91.
50. Warner AJ, Lew RA, Smith AI, Lambert DW, Hooper NM, Turner AJ. Angiotensin-converting enzyme 2 (ACE2), but not ACE, is preferentially localized to the apical surface of polarized kidney cells. *J Biol Chem* 2005;280:39353–62.
51. Lambert DW, Yasaki M, Warner AJ, Thornhill P, Parkin ET, Smith AI, et al. Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). *J Biol Chem* 2005;280:30113–9.
52. Haga S, Yamamoto N, Nakai-Murakami C, Osawa Y, Tokunaga K, Sata T, et al. Modulation of TNF-alpha-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-alpha production and facilitates viral entry. *Proc Natl Acad Sci U S A* 2008;vol. 105:7809–14.
53. Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Harmer D, et al. Soluble angiotensin-converting enzyme 2 in diabetic nephropathy. *Hypertension* 2003;41:392–7. 54. Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. * FEBS Lett* 2002;532:107–10.
55. Lamert DW, Yasaki M, Warner AJ, Thornhill P, Parkin ET, Smith AI, et al. Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome coronavirus spike protein. *J Virol* 2014;88:1293–307.
56. Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pohmann S. TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. *J Virol* 2014;88:1293–307.
57. Tselepis C, Johnston CI, Forbes JM, Burns WC, Burrell LM, Risvans J, et al. Characterization of renal angiotensin-converting enzyme 2 in diabetic nephropathy. *Hypertension* 2003;41:392–7.
de Gasparo M, Rogh M, Brink P, Wang L, Whitebread S, Bullock G, et al. Angiotensin II receptor subtypes and cardiac function. *Hypertension* 1994;15 Suppl D:98–103.

Kaschina E, Namsalock P, Unger T. AT2 receptors in cardiovascular and renal diseases. *Pharmacol Res* 2017;125(Pt A):39–47.

Paz OM, Riquelme JA, Garcia L, Jalil JE, Chiong M, Santos R, et al. Counter-regulatory renin–angiotensin system in cardiovascular disease. *Nat Rev Cardiol* 2020;17:116–29.

Williams B. Drug discovery in renin-angiotensin system intervention: past and future. *Ther Adv Cardiovasc Dis* 2016;10:118–25.

Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci* 2020;63:364–74.

Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutranen SM, Penninger JM, et al. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur J Clin Invest* 2009;39:618–25.

Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci* 2020;63:364–74.

Jessup JA, Gallagher PE, Averill DB, Brosnihan KB, Tallant EA, Chappell MC, et al. Effect of angiotensin II blockade on a new congenic model of hypertension derived from transgenic ren-2 rats. *Am J Physiol Heart Circ Physiol* 2006;291:H1266–72.

Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation* 2005;111:2605–10.

Ocananza MP, Godoy I, Jail JE, Varas M, Collantes P, Pinto M, et al. Enalapril attenuates downregulation of angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. *Hypertension* 2006;48:572–8.

Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Uprégulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. *Hypertension* 2004;43:970–6.

Huang M, Li X, Meng Y, Xiao B, Ma Q, Ying S, et al. Upregulation of angiotensin-converting enzyme (ACE) 2 in hepatic fibrosis by ACE inhibitors. *Clin Exp Pharmacol Physiol* 2010;37:1–6.

Burchill LJ, Velkoska E, Dean RG, Griggs K, Patel SK, Burrell LM. Combination renin-angiotensin system blockade and angiotensin-converting enzyme 2 in experimental myocardial infarction: implications for future therapeutic directions. *Clin Sci* 2012;123:649–58.

Igase M, Kohara K, Nagai T, Miki T, Ferrario CM. Increased expression of angiotensin converting enzyme 2 in conjunction with reduction of neo-intima by angiotensin II type 1 receptor blockade. *Hypertens Res* 2008;31:553–9.

Karram T, Abbasi A, Keidar S, Golomb E, Hochberg I, Winaver J, et al. Effects of spironolactone and eprosartan on cardiac remodeling and angiotensin-converting enzyme isoforms in rats with experimental heart failure. *Am J Physiol Heart Circ Physiol* 2005;289:H1351–8.

Ferrario CM, Jessup J, Gallagher PE, Averill DB, Brosnihan KB, Ann TE, et al. Effects of renin-angiotensin system blockade on renal angiotensin-(1–7) forming enzymes and receptors. *Kidney Int* 2005;68:2189–96.

Huang F, Guo J, Zou Z, Liu J, Cao B, Zhang S, et al. Angiotensin II plasma levels are linked to disease severity and predict fatal outcomes in H7N9-infected patients. *Nat Comm* 2014;5:3595.

Zou Z, Yan Y, Shl Y, Gao R, Sun Y, Li X, et al. Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. *Nat Comm* 2014;5:3595.

Gaddam RR, Chambers S, Bhata M, ACE and ACE2 in inflammation: a tale of two enzymes. *Inflamm Allergy Drug Targets* 2014;13:224–34.

Caldeira D, Alcaro J, Vaz-Carneiro A, Costa J. Risk of pneumonia associated with use of angiotensin converting enzyme inhibitors and angiotensin receptor blockers: systematic review and meta-analysis. *BMJ* 2012;345:e2690.

Mancia G, Rea F, Ludergnani M, Apolone G, Corrao G. Renin–angiotensin–aldosterone system blockers and the risk of Covid-19. *N Engl J Med* 2020;382:2431–40.

Reynolds HR, Adhikari S, Pulgarin C, Troxel AB, Itrrate E, Johnson SB, et al. Renin–angiotensin–aldostrone system inhibitors and risk of COVID-19. *N Engl J Med* 2020;382:2441–8.

He C, Qin M, Sun X. Highly pathogenic coronaviruses: thrusting vaccine development in the spotlight. *Acta Pharrm Sin B* 2020;10:1175–91.

Tian X, Li C, Huang A, Xia S, Lu S, Shi Z, et al. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. *Emerg Microb Infect* 2020;9:382–5.

Monteil V, Kwon H, Prado P, Hagelkrays A, Wimmer RA, Stahl M, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell* 2020;181:905–13.

Colson P, Rolain JM, Raoult D. Chloroquine for the 2019 novel coronavirus SARS-CoV-2. *Int J Antimicrob Agents* 2020;55:105923–4.

Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020;30:269–71.

Cui C, Zhang M, Yao X, Tu S, Hou Z, Jie EV, et al. Dose selection of chloroquine phosphate for treatment of COVID-19 based on a physiologically based pharmacokinetic model. *Acta Pharm Sin B* 2020;10:1216–27.

Richardson P, Griffin I, Tucker C, Smith D, Oechsle O, Phelan A, et al. Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. *Lancet* 2020;395:e30–1.

Sheahan TP, Sims AC, Graham RL, Menachery VF, Geurisnik LE, Case JB, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci Transl Med* 2017;9:eaal3653.