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ACE2, B⁰AT1, and SARS-CoV-2 spike protein: Structural and functional implications
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Abstract
The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has emerged as a public health crisis and led to tremendous economic devastation. The spike protein (S) of SARS-CoV-2 hijacks the angiotensin converting enzyme 2 (ACE2) as a receptor for virus entry, representing the initial step of viral infection. S is one of the major targets for development of the antiviral drugs, antibodies, and vaccines. ACE2 is a peptidase that plays a physiologically important role in the renin–angiotensin system. Concurrently, it also forms dimer of heterodimer with the neutral amino acid transporter B⁰AT1 to regulate intestinal amino acid metabolism. The symptoms of COVID-19 are closely correlated with the physiological functions of ACE2. In this review, we summarize the functional and structural studies on ACE2, B⁰AT1, and their complex with S of SARS-CoV-2, providing insights into the various symptoms caused by viral infection and the development of therapeutic strategies.

Introduction
The emergence and continued presence of SARS-CoV-2 variants highlight the need to develop effective interventions against COVID-19 pandemic. S of SARS-CoV-2 [1,2] is responsible for receptor recognition and membrane fusion, similar to that of SARS-CoV-1, which has caused the severe acute respiratory syndrome pandemic in 2002–2003 [3]. During assembly of the virus, S is cleaved into the S1 and S2 subunits by furin or furin-like proprotein convertase [4]. The S1 subunit binds to the viral entry receptor ACE2 through the receptor binding domain (RBD) [5] (Figure 1). Then, S undergoes second protease processing to release the membrane fusion peptide by transmembrane protease serine 2 (TMPRSS2) [6,7] or carhepsin L [8] on S² cleavage site to mediate the fusion of the virus and host cell membrane [9]. The interaction between S and ACE2 is the important step of viral infection, therefore making it the critical target for developing the small molecule drugs, neutralizing antibodies and vaccines [10,11].

The lung damage followed by pulmonary fibrosis and chronic impairment of lung function is one typical symptom for SARS-CoV-2 infection [12]. In addition to its role as the receptor for SARS-CoV-2 invasion, ACE2 is a peptidase belonging to the renin–angiotensin system that controls vasoconstriction and blood pressure in human [13]. The ace2 gene knockout mice showed that the downregulation of ACE2 significantly increases angiotensin II (Ang II) levels in the lungs and plasma, causing acute lung failure [14]. In addition, Ang II levels in the lung tissues of the mice were significantly increased after treatment with acid and SARS-CoV-1 Spike-Fc (a fusion protein of SARS-CoV-1 S with the Fc portion of human IgG1), and lung damage induced by this treatment could be attenuated by blocking Ang II receptor type 1 (AT1R) using its inhibitor [15]. These results collectively suggest that SARS-CoV-2 infection is closely correlated with the primary physiological function of ACE2. Besides, ACE2 forms heterodimers with the intestinal transporter B⁰AT1 to mediate the uptake of neutral amino acids, which provides an important insight into enterocyte infection with SARS-CoV-2 [16,17].

In the past few years, many studies have reported on SARS-CoV-2 and its receptor, ACE2. In this review, we will focus on the physiological functions and structural information of ACE2, B⁰AT1 and their complex with S of SARS-CoV-2, which can help us understand the
mechanism of SARS-CoV-2 infection and its symptom
determinants to develop effective therapeutic and
prophylactic strategies.

ACE2 and renin–angiotensin system
ACE2, encoded by a gene located on the X chromosome,
was discovered in 2000 as a protein homolog of ACE [13,18]. Multiple studies have shown that ACE2 is highly expressed in many tissues, including the small intestine, thyroid, kidney, heart, testis and adipose tissue, and expressed at low levels in the blood, spleen, muscle, brain, and bone marrow [19–21]. ACE2 is a type I transmembrane (TM) glycoprotein with a full length of 805 amino acid residues that can be divided into two parts: the N-terminal catalytic domain (also known as the peptidase domain, PD) and the C-terminal collectrin-like domain (CLD) [13,22]. PD of ACE2, which shares 42% sequence homology with the N-terminal domain of ACE, contains a zinc ion in its active site. CLD of ACE2 contains a single TM helix and has approximately 48% sequence homology with collectrin, which does not contain a PD [22].

ACE2 plays an important role in the renin–angiotensin system [23,24] (Figure 1). Both ACE2 and ACE have peptidase activity, but their substrates and cleavage mechanisms are different [25]. ACE is a dipeptidyl peptidase that releases a dipeptide from the C-terminal of its substrate per digestion reaction, while ACE2 cleaves one amino acid. The crystal structure of ACE2 [25] shows that Arg273 forms a salt bridge with the C-terminal of the substrate. But in ACE, it is replaced with the smaller amino acid Glu, which explains the difference in substrate specificity between ACE and ACE2.

To be exact, ACE converts Ang I to Ang II, which function is to constrict blood vessels and raise blood pressure. ACE2 cleaves Ang II to Ang-(1–7), the role of which is to relax blood vessels and lower blood pressure. ACE2 can also convert Ang I into Ang-(1–9), and ACE or other peptidases will then convert Ang-(1–9) into Ang-(1–7) (Figure 1). ACE2 much more efficiently cleaves Ang II into Ang-(1–7) than Ang I into Ang-(1–9) [26]. Therefore, ACE2 plays a role in lowering blood pressure in the renin–angiotensin system. In
addition, Ang-(1–7) mediates various effects, including vasodilatation, anti-inflammatory, anti-oxidation and so on [27], by binding the G protein-coupled receptor Mas [28], making Ang-(1–7) a promising therapeutic target for cardiovascular disease [29]. In summary, ACE2 negatively regulates the level of Ang II and maintains the balance with ACE to control local homeostasis to protect the lung, kidney, and cardiovascular system [14].

ACE2 and B₀AT1 complex

ACE2 is reported to be a molecular chaperone of the neutral amino acid transporter B₀AT1 in small intestine, which is also known as SLC6A19 that belongs to the neurotransmitter and amino acid co-transporter SLC6 family [16,30]. The slc6a19 gene is located on the chromosome 5 and was cloned in 2004 because its mutation causes Hartnup disorder, an autosomal recessive condition that leads to aminoaciduria and eventually to symptoms like photosensitive rash, cerebellar ataxia and emotional instability [31–33]. It is also called B₀AT1 due to the properties of system B₀ which mediates the Na⁺-dependent neutral amino acid transporter [32]. B₀AT1 has a total length of 634 amino acid residues, including its N-terminal and C-terminal on the intracellular side, as well as 12 TM helices arranged as a LeuT-fold [17]. The plasma membrane location of B₀AT1 requires the chaperone of some proteins, represented by ACE2 in the small intestine or collectrin in the kidney [16,30].

It was reported that ACE2-B₀AT1 complex was involved in immunoregulation by controlling amino acid homeostasis, antimicrobial peptide expression and ecological regulation of intestinal microbes [34–37]. Further research showed that this complex affects the composition of the gut microbiota through its role in amino acid transport, which may explain why amino acid malnutrition in Hartnup disease can lead to diarrhea and intestinal inflammation [35,36]. The ace2 gene knockout mice showed a high susceptibility to intestinal inflammation and diarrhea, which could be reversed by dietary tryptophan or its metabolite nicotinamide, which are necessary for the biosynthesis of nicotinamide adenine dinucleotide (phosphate) via the kynurenine pathway [37]. The Hartnup diseases patients show symptoms of the skin and psychiatric disorders that are also ameliorated by nicotinamide supplementation [36]. The similarity between these studies is related to the weakened function of B₀AT1 that transports neutral amino acids, suggesting that ACE2 is essential for the expression and stability of B₀AT1 in the small intestine [16,30,37].

The high-resolution cryo-EM structure of the ACE2-B₀AT1 complex revealed that ACE2 and B₀AT1 form a heterodimer, and this heterodimer further forms a dimer through the ACE2-mediated dimerization interfaces (Figure 2) [17]. ACE2 has two dimerization interfaces, one of which is mediated by weaker interactions in PD and can be disrupted, inducing a conformational change of ACE2 from a closed conformation to an open conformation [17]. Another interface is mediated by CLD with extensive polar interactions. The properties of the ACE2 dimeric interfaces suggest that ACE2 can form a dimer independently in the absence of B₀AT1. Besides, B₀AT1 adopts a typical LeuT-fold, whose TM7 helix region extends to the extracellular and binds CLD of ACE2 (Figure 2), suggesting that ACE2 can regulate the transport activity of B₀AT1 through this interface.

ACE2 as SARS-CoV-2 receptor

ACE2 is the entry receptor for SARS-CoV-2 [5,7], as well as other coronaviruses such as SARS-CoV-1 [38] and NL63 [39]. The virion particles of SARS-CoV-2 are irregularly spherical with dozens of S randomly arranged on the surface, which can bind to ACE2 [17,40–42] and then bring viral genetic material into cells through membrane fusion at cell surface or later at endosome after endocytosis [6–9]. RBD of S has two conformations, “up” and “down” [1,2], of which only the “up” conformation can bind the receptor (Figure 1). A binding assay showed that SARS-CoV-2 binds ACE2 more strongly than SARS-CoV-1 does [1]. The interface between ACE2 and RBD of SARS-CoV-2 is similar to that of SARS-CoV-1, which is mainly involved in polar interactions. The extended loop region of RBD spans the α1 helix of ACE2 like an arch bridge (Figure 2) [17]. Besides, the functional study and cryo-EM structure of the extracellular domain of S (S-ECD) and the ACE2-B₀AT1 complex showed that each ACE2 monomer in the ACE2 dimer can bind an S (Figure 2) [17,43,44]. Structures of S of SARS-CoV-2 in different states in complex with ACE2 showed that PD of ACE2 binds S with a consistent interface and triggers the conformational change of S1 region to activate RBD. The uncleaved and trypsin-digested S-ECD alone exhibits an almost identical conformation, but the trypsin-digested S-ECD can be bound by more molecules of PD of ACE2 [44]. To be noticed, S has great structural flexibility and can form complex with ACE2 in various conformations [45,46]. RBD of SARS-CoV-2 variants tends to be in “up” state, so it more easily binds ACE2, which exhibits stronger infectivity. Interestingly, previous studies showed that the cleavage of the C-terminal segment of ACE2, especially residues 697 to 716, by proteases such as TMPRSS2 can enhance the S-driven viral entry [47,48]. In the ACE2-B₀AT1 complex structure, the residues 697–716 of ACE2 form helices in CLD and map to the dimeric interface. The presence of B₀AT1 might block the access of TMPRSS2 to the cutting site on ACE2 (Figure 1). These findings revealed the structural basis for the activation of S during infection.
and led to the development of specific peptide drugs [49] against SARS-CoV-2.

In addition, SARS-CoV-2 is a zoonotic pathogen, which can infect a variety of animals [50]. The cryo-EM structure of the complex of cat ACE2 and RBD of SARS-CoV-2 shows that cat ACE2 and human ACE2 bind the virus in a similar manner [50].

**The different forms of ACE2**

There are two forms of ACE2: the full-length form, which exists on the cell membrane, and the soluble form (sACE2), which is generated by enzymes cleavage [51]. ACE2 is cleaved by the metalloprotease ADAM17 [52] or the serine protease TMPRSS2, and then released into the blood. The cleavage patterns and functions of the two enzymes are different: only ACE2 cut by TMPRSS2
enhances SARS-CoV-1 infection [48]. Expression of TMPRSS2 inhibits the shedding of ACE2 by ADAM17. In many lung diseases, treatment with recombinant ACE2 can prevent blood vessel and lung damage. sACE2 has enzymatic activity and the ability to bind SARS-CoV-2, which can prevent the virus from invading host cells and spreading [53]. Recombinant sACE2 molecules can reduce viral load and prevent SARS-CoV-2 infection in blood vessels and kidney organoids [54]. An artificially designed trimeric ACE2 molecule eliminated the symmetry mismatch with trimeric S, and greatly enhanced the affinity between ACE2 and S [55]. The trimeric ACE2 molecule induces three RBDs of S to open state, which has an excellent ability to neutralize viruses [55]. Antibodies targeting to ACE2 can compete with viruses to bind ACE2, so they can be used for antiviral prevention and treatment [56]. However, a recent study found the secretory form of ACE2 can mediate the endocytosis of SARS-CoV-2 by the interaction between S and sACE2 or sACE2-vasopressin through AT1 or AVPR1B, respectively [57]. The concrete mechanism of soluble form of ACE2 requires further investigation.

Relationship with intestinal diseases
Gastrointestinal symptoms, including nausea, vomiting, anorexia, abdominal discomfort, diarrhea, are one class of the symptoms of COVID-19 infection [58,59]. SARS-CoV-2 can be detected in the stool samples and rectal swabs of COVID-19 patients, suggesting the invasion of digestive tract by this virus [60,61]. These findings are supported by an assay for SARS-CoV-2 infection with human small intestinal organoids [62]. In addition, previous studies have shown that intestinal inflammation and diarrhea occur in ace2 gene knockout mice and Hartnup disease patients caused by B0AT1 mutation [35–37]. These results collectively support the hypothesis that intestinal ACE2 engagement by S of SARS-CoV-2 might negatively regulate the absorption of neutral amino acids in the small intestine of COVID-19 patients, leading to diarrhea and intestinal inflammation. Other studies have shown that SARS-CoV-2 was detected in the small intestine, but small intestine infection appeared to have an attenuating effect on SARS-CoV-2-associated inflammation and a reduction in mortality in COVID-19 patients [59,63,64]. Further studies are required due to individual differences and limited case numbers.

Conclusion
ACE2 plays a major role in the renin—angiotensin system as a peptidase, and participates in the absorption and metabolism of amino acids as a molecular chaperone of B0AT1, thus related to the intestinal inflammation. Over the past two years, ACE2 has attracted much attention as the entry receptor of SARS-CoV-2. The physiological functions and the tissue expression and distribution of ACE2 are one of keys to understanding the symptoms of COVID-19. The interaction interface between receptor and virus is an important target for developing drugs to inhibit viral invasion and alleviate infection symptoms. A variety of potential drugs to block virus binding receptors are being developed, including small molecules, peptides, and a variety of potent neutralizing antibodies. In addition, therapeutic strategies such as supplementation with essential amino acids, soluble ACE2 [53,54] or Ang-(1–7) [27–29] have been proposed and should be considered.

Conflict of interest statement
Nothing declared.

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