

# Evaluation of the structural development potential of receptors based on SARS-CoV-2 infection

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**Abstract.** Since 2019, the coronavirus has posed a huge threat to human health and the environment. Many scholars have since started studying the coronavirus and its branches, including SARS-CoV-2. SARS-CoV-2 is a positive stranded RNA virus composed of four main proteins and several auxiliary proteins that encapsulate genetic material. It mainly mediates virus invasion through the binding of S protein to host cell receptors, determining the tissue and host preference of the virus. ACE2 is a receptor required for SARS-CoV-2 to enter cells, which is consistent with the epidemiological risk of severe diseases observed in Chinese cardiovascular disease and hypertension patients. This article will explore the potential development of SARS-CoV-2 receptor ACE2 in terms of structure.

**Keywords:** ACE2, SARS-CoV, S-Glycoprotein, Receptor Binding, subunit.

## 1. Introduction

Coronavirus Disease 19 (COVID-19) is caused by the newly defined Severe Acute respiratory Syndrome 2 (SARS-CoV-2). Like other coronaviruses, its genome has a positive envelope of about 30 kb; Coronaviruses mainly infect birds and mammals [1]. The COVID-19 enters host cells by utilizing a transmembrane glycoprotein (S), which protrudes from the virus surface and facilitates its entry [2]. S is composed of two functional subunits that have distinct roles: the S1 subunit connects to the host cell receptor, while the S2 subunit facilitates the fusion between the virus and the cell membrane [3]. For all COVID-19, S is still cleaved by the host's protease at a location called s2', which is the elevation of the fusion peptide [4]. Due to the extensive changes of irreversible configuration, this slope considered to be membrane fusion by activating proteins. Therefore, the process of infecting susceptible cells with COVID-19 is intricate and involves a combined action of receptor binding and protein S proteolysis to induce viral cell fusion.

Ace2 has extracellular carboxypeptidase domain is located on the cell membrane of various epithelial cells who is a transmembrane protein. Ace1 and ace2 are composed of different gene codes and account for 40% of the amino acid sequence in the catalytic region. Ace1 is used to catalyze angiotensin, which tightens blood vessels and raises blood pressure, and ace2 can convert angiotensin to angiotensin1-9, thereby increasing the dilation of blood vessels and thus reducing blood pressure. ACE2 is a receptor required for SARS-CoV-2 to enter cells, which is consistent with the epidemiological risk of severe diseases observed in Chinese patients who have cardiovascular disease and hypertension. In typical virtual receptors, there are cross-cutters that link spaces to spaces, spaces and channels. ACE2's model suggests that most of the molecules are part of the carbon dioxide, or most of the molecules are part of the carbon dioxide [5].

Up to now, the host range and intermediate host of SARS-CoV-2 are still uncertainly, and our understanding of the mechanism of action of receptor ACE2 is still limited. The aim of this article is to assess the structural capability of the receptor ACE2 in facilitating SARS-CoV-2 infection, with the goal of offering theoretical backing for the treatment of such infections.

Given that ACE2 serves as a receptor for SARS-CoV-2, we opted to examine the structural feasibility of developing a receptor that is resistant to infection by SARS-CoV-2.

## 2. SARS-CoV-2 and SARS-CoV

SARS refers to a respiratory symptom observed by medical observation and presented by infected individuals, generally referring to infectious atypical pneumonia. This is an acute respiratory infection, named SARS. SARS-CoV is a scientific name named after the "SARS" virus by the WHO in 2003. In 2020, the novel coronavirus that triggered the global pneumonia epidemic in recent years was named SARS CoV-2. This name reflects the heredity and similarity between 2019 COVID-19 and coronal SARS CoV that led to the SARS outbreak that year.

Orders, families, subfamilies, genera, and species are used to categorize viruses, with "species" serving as the smallest classification unit in the virus classification scheme. So, when two viruses are classified as members of the same "species," it means that they are very similar to one another. According to recent studies, SARS-like coronavirus species and new coronavirus both belong to the coronaviridae family of coronaviruses. In the evolutionary tree, the 2019 new coronavirus and the SARS coronavirus are next to the SARS virus (which gave rise to "SARS" in 2003) and the SARS-like virus.

To distinguish it from the SARS coronavirus, the ICTV designated the novel coronavirus (2019 nCoV) as SARS CoV-2 two years ago. The disease brought on by this virus is officially known as COVID-19, the WHO added at the same time. This naming denotes a distinction between the names of viruses and the diseases they cause, necessitating separate naming. SARS-CoV-2 and the SARS coronavirus are quite similar, yet they need to be recognized by separate nomenclature. The coronavirus research team concluded that SARS and new coronavirus are not the same virus and differ in several key ways. The virus is unique, yet it also belongs to the same type and shares similarities with the SARS virus.

The COVID-19 outbreak in 2019 belongs to the same species as the well-known SARS coronavirus, but it does not mean that SARS CoV-2 is the offspring of SARA-CoV, and they have many differences. Although novel coronavirus is highly similar to SARS coronavirus, it is not directly mutated from SARS coronavirus, and its specific source is still unclear. In addition, there are also some differences between the characteristics of novel coronavirus and SARS coronavirus. For example, novel coronavirus is more infectious, but the mortality rate is not as high as SARS coronavirus infection in general. According to the current data, the proportion of severe patients with SARS coronavirus is higher, and the mortality rate is about 10%, while the mortality rate of novel coronavirus after infection is about 1%~2%. Novel coronavirus is more infectious, which makes the number of cases infected by SARS coronavirus far higher than that infected by SARS coronavirus.

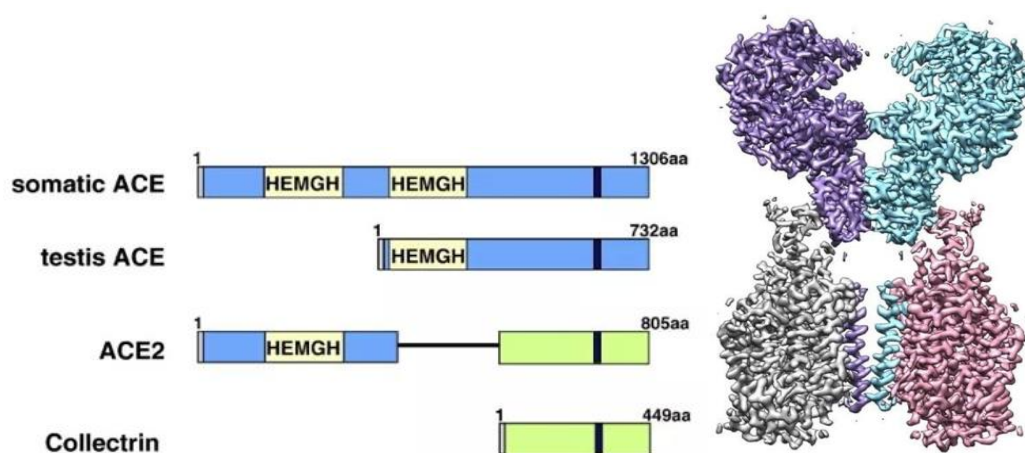
The conserved domain of novel coronavirus shares more than 90% homology with the seven gene fragments of SARS virus. According to this standard, although COVID-19 and SARS virus belong to the same "species", there are still 10% of the sequence differences that cannot be ignored, which proves that the two viruses are different in microcosm.

Although both belong to the coronavirus category and can cause respiratory infectious diseases with similar symptoms after infection. However, severe cases of SARS-CoV-2 infection manifest as septic shock, coagulation dysfunction and difficult to correct metabolic acidosis; The sequelae of severe SARS coronavirus patients after treatment are pulmonary fibrosis. In all respects, novel coronavirus and SARS coronavirus are similar but not identical viruses [6].

## 3. Gene expression profile and structure of ACE2

The gene ACE2, also known as ACEH, encodes a protein that is a member of the angiotensin converting enzyme family and shares a great deal of similarities with human converting enzymes. This secreted protein functions as a receptor for the human coronavirus HCoV-NL63, the human severe acute respiratory syndrome coronavirus SARS-CoV, and SARS-CoV-2. It catalyzes the conversion of angiotensin I into angiotensin. As is widely known, ACE2 is expressed in a variety of human organs, and given that it is expressed differently in different cells and organs, it is possible that it controls fertility as well as renal, cardiovascular, and other functions.

With 805 amino acids and a single extracellular catalytic domain, ACE2 is a type I transmembrane glycoprotein. The His Glu Met Gly Hiss (HEMGH) motif, seen in many peptidases, is an active zinc binding motif that is present in both of the homologous domains of the human ACE gene (Fig 1), which is located on chromosome 17. The amino terminal catalytic domain and the carboxyl terminal domain are the two structural domains of ACE2 [7].



**Figure 1.** Two or more references

In humans, two different types of ACE isoenzymes have been described. One is a rich form of somatic cell cells found on the surface of the pulmonary endothelium and the brush marginal membrane of the kidney, intestine, placenta and choroid plexus, and the other is a form of ACE production found only in the testis. These two subtypes of ACE are membrane encapsulated proteins that act as exoenzymes to hydrolyze circulating peptides on the cell surface.

## 4. Current theoretical basis

### 4.1. Theory 1

As early as 2003, the SARS pandemic threatened the world, and ACE2 is a functional receptor of the pathogenic pathogen SARS coronavirus. The peptidase action of ACE2 is unnecessary for SARS virus to enter host cells, as cells expressing non-catalytic active mutants of ACE2 still allow SARS virus infection.

The transmembrane domain of ACE2 is internalized while the outer domain of the SARS coronavirus cleaves it, causing additional fusion between the virus particles and the host cell [8]. The transmembrane domain of ACE2 therefore plays a role in the movement of the SARS coronavirus receptor complex from the cell membrane to the cytoplasm during SARS coronavirus infection, even though the precise process is yet unknown.

SARS-CoV binds to ACE2 in a Clathrin protein dependent manner and internalizes it to enter cells. Membrane fusion is activated by protease Spike (Fig 2), and viral RNA is released into the cytoplasm, thus causing SARS infection [9]. The transmembrane protease cleaves the extracellular near membrane region of ACE2 and releases the catalytic active Ectodomain into the extracellular environment. It is not yet clear whether this ACE2 cleavage contributes to the onset of SARS.

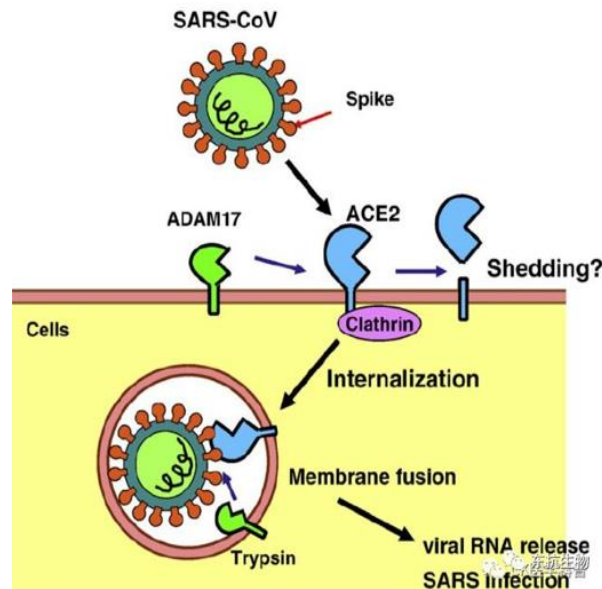


Figure 2. The mechanism of ACE2.

#### 4.2. Theory II

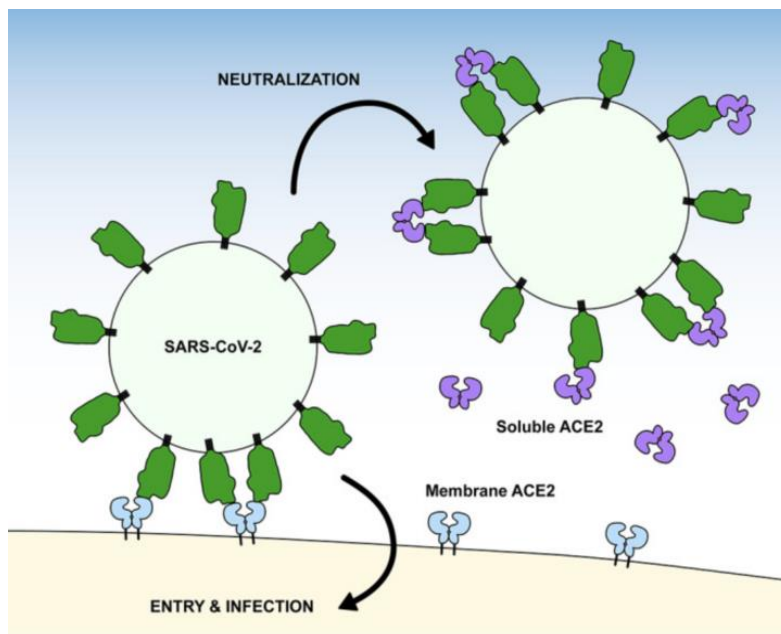
To identify the type of cells that may be infected by COVID-19, scientists led a study. Through single cell RNA sequencing technology, multiple tissues of humans, rhesus monkeys and mice were analyzed. It was found that the gene encoding ACE2 would increase expression in epithelial cells stimulated by interferon (IFN). Early studies confirmed that SARS CoV-2 would bind to ACE2 receptor and enter human cells through a protease called TMPRSS2. This means that SARS-CoV-2 can use the up regulation of ACE2 driven by IFN to enhance the invasion ability and promote more cells to be infected by COVID-19 [10].

The role of ACE2 in SARS CoV-2 infection is complex. One way it helps SARS CoV-2 penetrate human cells is by acting as a receptor. On the other hand, ACE2 plays a peptidase dependent protective role in acute lung injury caused by virus infection (including SARS CoV, H7N9, and H5N1), and this molecular mechanism may also be applicable to COVID-19. Alveolar epithelial type II cells expressing ACE2 provide a variety of genes related to virus replication, which can promote the replication of coronavirus.

Therefore, it is worth studying whether the increase in ACE2 expression is positively correlated with viral infection. But like IFN, ACEIs/ARBs will also increase the expression level of ACE2. Many researchers were worried about the clinical treatment of hypertension patients.

#### 5. Treatment studies of ACE2 in SARS-CoV-2

Viral entry is facilitated by the interaction between viral spikes (trimer complexes of protein S) and ACE2 mediates viral entry. When the RBD located in the S1 unit attaches to ACE2, When ACE2 binds to the RBD in the S1 subunit, the spike protein hydrolyzes into the two subunits S1 and S2, causing S1 to shed, fusion peptides in S2 to become exposed, and the fusing of the viral envelope with the host membrane, Multiple S epitopes, including RBD, can be targeted by antibodies to disrupt ACE2 conjugation or prevent membrane fusion [11]. Another strategy is to infect Vero-E6 cells with different amounts of SARS coronavirus type 2 after successful culture in Vero E6 cells by SARS-CoV-2, purify viral RNA from the cells as markers of replication, and analyze by qRT-PCR. Cells infected within 1 h in the presence of hrsACE2, then washed and incubated in the absence of hrsACE2, were found to significantly inhibit Vero-E6 cells 15 hours after infection. It accomplishes this by competing for receptor binding sites on the Spike protein by employing ACE2 itself as a soluble decoy receptor (Fig 3) [12].



**Figure 3.** Soluble decoy receptor.

It further facilitates cell entrance by attaching to the C-terminal of the S1 subunit after processing. The primary benefit of utilizing a soluble decoy entry receptor like hrsACE2 is that the virus has a restricted mutation mechanism to escape without also compromising its affinity for the anchored form of the promembrane. Although there are several clinical uses for soluble decoy receptors, no medication has yet been approved to treat viruses [13].

## 6. Studies of ACE2 in other disease

Existing studies have shown that increased ACE2 prevents tumor growth and epithelial-mesenchymal transition in preclinical models of cancers such as gallbladder cancer, non-small cell lung cancer, and hepatocellular carcinoma [14] [15] [16]. For CCRC, treatment typically involves targeting VEGF [17], although resistance to this approach has been observed. Combining angiotensin signaling inhibitors with therapy has shown improved survival rates, and overexpression of ACE2 has been found to hinder the formation of tumor colonies. The cleavage product of ACE2, Angiotensin-(1-7), when combined with VEGF inhibitors, has a stronger antitumor effect than using VEGF inhibitors alone, thus proving to be an effective treatment for ccRCC. Elevated Ace2 activity has been observed in individuals with cardiovascular disease as compared to the general population. Furthermore, increased circulating ACE2 activity in individuals with cardiovascular disease suggest an unfavorable prognosis for their cardiovascular health. The lack of ACE2 encourages the progression of Ang II-induced vascular inflammation and cardiovascular disease associated with activation of the JNK pathway. To shed light on the development of cardiovascular disease, researchers identified Ace2 expression at injury locations in atherosclerosis as well as in both normal and atherosclerotic internal mammary and radial arteries. Subsequent Research has demonstrated that ACE2 is present in the neointima, mesentery, neovascular micro vessels, and chorioallantois vessels.

## 7. Limitations and future development

It's not possible to predict whether hrsACE2 would still be able to prevent SARS-CoV-2 from entering host cells during advanced stages of the disease. Lung organoids, which are the main target organ of COVID-19, have not been studied. Besides, there are many external influencing factors that have not been considered. The function of hrsACE2 in the later phases of infection in vivo and in vitro must be clarified in more investigations to answer these problems.

## 8. Conclusion

ACE2 is an important receptor target for SARS-CoV-2 and a key player in the pathogenesis of COVID-19 due to its ability to enable viral entry into target cells. The affinity of ACE2 for the receptor binding domain (RBD) of the SARS-CoV spiking glycoprotein is substantially higher than that for the SARS-CoV spiking glycoprotein itself. Proteolysis is thus used to make the viral spiking into two subunits and generate the RBD, triggering conformational changes to prevent ACE2 binding or membrane fusion. The second approach utilizes ACE2 as a soluble decoy receptor to compete with spike proteins. HrsACE2 has been shown in vitro to be able to reduce the growth of SARS-CoV-2 viruses in cell culture, artificial blood vessels and renal organoids. Nowadays, Soluble wild-type ACE2 (sACE2) has been quickly repurposed as an antiviral for SARS-CoV-2. Soluble ACE2 (hrsACE2) is abundantly expressed in not only the pulmonary and gastrointestinal epithelium but also in the vascular endothelium all over the body. However, the extent to which each region is impacted by spike protein entry remains unclear. Additionally, it's worth noting that decoy receptors can potentially pose additional safety risks due to their potential for inducing an immune response.

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